Design and Synthesis of Small Molecules and Nanoparticle Conjugates for Cell Type-Selective Delivery

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Design and Synthesis of Small Molecules and Nanoparticle Conjugates for Cell Type-Selective Delivery

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<td>alanine</td>
</tr>
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<td>aspartic acid</td>
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<td>Au</td>
<td>gold</td>
</tr>
<tr>
<td>AuNPs</td>
<td>gold nanoparticles</td>
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<tr>
<td>BDAC</td>
<td>benzylidimethylammonium chloride hydrate</td>
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<tr>
<td>BODIPY</td>
<td>dipyrrromethene boron difluoride</td>
</tr>
<tr>
<td>Bp</td>
<td>biphenyl</td>
</tr>
<tr>
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</tr>
<tr>
<td>BSA</td>
<td>bovine serum albumin</td>
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<td>BzD</td>
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xii
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<tr>
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<td>BzOH</td>
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</tr>
<tr>
<td>C</td>
<td>carbon</td>
</tr>
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<td>CDCl₃</td>
<td>deuterated chloroform</td>
</tr>
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<td>CD₃OD</td>
<td>deuterated methanol</td>
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<tr>
<td>Cys, C</td>
<td>cysteine</td>
</tr>
<tr>
<td>°C</td>
<td>degrees Centigrade</td>
</tr>
<tr>
<td>CH₂Cl₂</td>
<td>dichloromethane</td>
</tr>
<tr>
<td>CLSI</td>
<td>Clinical Laboratory Standard Institute</td>
</tr>
<tr>
<td>cm⁻¹</td>
<td>reciprocal centimeter (SI unit for wavenumber)</td>
</tr>
<tr>
<td>CTAB</td>
<td>cetyltrimethylammonium bromide</td>
</tr>
<tr>
<td>Cu</td>
<td>copper</td>
</tr>
<tr>
<td>CuI</td>
<td>copper iodide</td>
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<td>doublet</td>
</tr>
<tr>
<td>dd</td>
<td>doublet of doublet</td>
</tr>
<tr>
<td>DDQ</td>
<td>2,3-dichloro-5,6-dicyano-p-benzoquinone</td>
</tr>
<tr>
<td>DHN</td>
<td>dihydroxyl naphthalene</td>
</tr>
<tr>
<td>DIPEA</td>
<td>N,N-diisopropylethylamine</td>
</tr>
<tr>
<td>DMAP</td>
<td>N,N-dimethylaminopyridine</td>
</tr>
<tr>
<td>DMF</td>
<td>N,N-dimethylformamide</td>
</tr>
<tr>
<td>DMP</td>
<td>dimethoxylphenyl</td>
</tr>
<tr>
<td>DMSO</td>
<td>dimethylsulfoxide</td>
</tr>
<tr>
<td>DNA</td>
<td>deoxyribonucleic acid</td>
</tr>
<tr>
<td>EC₅₀</td>
<td>half maximal effective concentration</td>
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EDCI 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride
EPR enhance permeability and retention
ESI electrospray ionization
Et$_3$N triethylamine
EtOAc ethyl acetate
FAB fast-atom bombardment
g gram
Gly, G glycine
H proton
h hour
H$_2$O water
HAT histone acetyltransferase
HCl hydrochloric acid
HDAC histone deacetylase
HDACi histone deacetylase inhibitors
HDLP histone deacetylase-like protein
His histidine
HIV human immunodeficiency virus
HOBT hydroxylbenzyltriazole
HPLC high performance liquid chromatography
HRMS High Resolution Mass Spectroscopy
Hz hertz
I$_2$ diatomic iodine

xiv
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<tr>
<td>IC&lt;sub&gt;50&lt;/sub&gt;</td>
<td>half maximal inhibitory concentration</td>
</tr>
<tr>
<td>J</td>
<td>coupling constant</td>
</tr>
<tr>
<td>KBr</td>
<td>potassium bromide</td>
</tr>
<tr>
<td>KCN</td>
<td>potassium cyanide</td>
</tr>
<tr>
<td>LHRH</td>
<td>luteinizing hormone-releasing hormone</td>
</tr>
<tr>
<td>LiOH•H&lt;sub&gt;2&lt;/sub&gt;O</td>
<td>lithium hydroxide hydrate</td>
</tr>
<tr>
<td>M</td>
<td>molar concentration</td>
</tr>
<tr>
<td>m</td>
<td>multiple, multiplet</td>
</tr>
<tr>
<td>MALDI</td>
<td>matrix-assisted laser desorption/ionization</td>
</tr>
<tr>
<td>Me</td>
<td>methyl</td>
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<td>methanol</td>
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<tr>
<td>mg</td>
<td>milligram</td>
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<tr>
<td>MIC</td>
<td>minimal inhibitory concentration</td>
</tr>
<tr>
<td>min</td>
<td>minutes</td>
</tr>
<tr>
<td>mL</td>
<td>milliliter</td>
</tr>
<tr>
<td>mmol</td>
<td>millimole</td>
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<tr>
<td>MS</td>
<td>mass spectroscopy</td>
</tr>
<tr>
<td>Ms</td>
<td>mesylate, mesyl</td>
</tr>
<tr>
<td>MsCl</td>
<td>Methanesulfonyl chloride</td>
</tr>
<tr>
<td>N&lt;sub&gt;3&lt;/sub&gt;</td>
<td>azide or azido</td>
</tr>
<tr>
<td>Na</td>
<td>sodium</td>
</tr>
<tr>
<td>NaH</td>
<td>sodium hydride</td>
</tr>
<tr>
<td>NaN&lt;sub&gt;3&lt;/sub&gt;</td>
<td>sodium azide</td>
</tr>
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</table>
NaNO₂  sodium nitrite
NaOAc  sodium acetate
Nap    naphthyl
NH₄OAc ammonium acetate
NH₂OH  hydroxylamine
NH₄OH  ammonium hydroxide
NIAID  National Institute of Allergy and Infectious Disease
NIR    near-infrared region
NLS    nuclear localization signaling
nm     nanometer
nM     nanomolar
NMM    N-methylmorpholine
NMR    Nuclear Magnetic Resonance
PEG    polyethylene glycol
Ph     phenyl
Phe    phenylalanine
PK     pharmacokinetic
PMB    para-methoxylbenzyl
PNB    para-nitrobenzyl
ppm    parts per million
prep TLC preparatory thin-layer chromatography
Pro, P proline
Py     pyridyl
PyBzOH
PyP
q
Quin
RES
RGD
RME
RNA
rpm
rt
s
S
SAHA
SAR
SERS
SH
siRNA
SPR
SPS
SV
t
T
TBAF

pyridylbenzylhydroxyl
pyridylphenyl
quartet
Quinyl
reticuloendothelial system
arginine-glycine-aspartic acid
receptor-mediated endocytosis
ribonucleic acid
revolution per minute
room temperature
singlet
sulfur
suberoylanilide hydroxamic acid
structure activity-relationship
surface-enhanced Raman scattering
thiol
small interference ribonucleic acid
surface plasmon resonance
solid phase synthesis
simian virus
triplet
threonine
tetrabutylammonium fluoride
TBTA  tris-(benzyltriazolylmethyl)amine
\( \tau \)-BuPh\(_2\)SiCl  tert-butyldiphenylsilylchlorane
TFA  trifluoroacetic acid
TfN\(_3\)  triflic azide
THF  tetrahydrofuran
Thp  thiopyl
TIPS  triisopropylsilane
TLC  thin-layer chromatography
TMSCl  trimethylsilylchlorane
TOF  time of flight
Tol  Tolyl
TSA  trichostatin A
Tyr  tyrosine
\( \mu \)g  microgram
\( \mu \)M  micromolar
\( \mu \)m  micromole
UV-vis  ultraviolet-visible spectroscopy
ZBG  zinc-binding group
Zn\(^{2+}\)  zinc ion
Inhibition of histone deacetylases inhibitors (HDACi) hold great promise in cancer therapy due to their demonstrated ability to arrest proliferation of nearly all transformed cell types. However, small molecule HDACi, especially the aliphatic hydroxamates, suffers from low oral bioavailability. More importantly, these HDACi non-selectively inhibits various HDAC isoforms leading to reduction of their in vivo potency and production of toxic side effect. Of the several structurally distinct small molecules HDACi reported, macrocyclic depsipeptides have the most complex recognition cap-group moieties and present an excellent opportunity for the modulation of the selectivity and biological activities of HDACi. Unfortunately, the structure–activity relationship (SAR) studies for this class of compounds have been impaired largely because most macrocyclic HDACi known to date are comprised of complex peptide macrocycles. I document in this thesis the discovery of a new class of macrocyclic HDACi based on the macrolide antibiotics skeletons. SAR studies revealed that these compounds displayed both linker-length and macrolide-type dependent HDAC inhibition activities with IC$_{50}$ in low nanomolar range. In addition, these non-peptide macrocyclic HDACi are more selective against HDAC 1 and 2 relative to HDAC 8, another class I HDAC isoform, and hence have subclass HDAC isoform selectivity as well as producing selective toxicity to cancer cells.

Some of the challenges facing conventional therapies, as described above, are poor bioavailability and intrinsic toxicity. These have seriously compromised the therapeutic efficacy of many otherwise beneficial drugs. Nanoscopic systems that alter
the pharmacological and therapeutic properties of molecules are being designed to
overcome some of these limitations. Research efforts in this area have resulted innovative
nanostructures, such as gold nanoparticles (AuNPs), that remain relatively nontoxic to
cells with enhancement in bioavailability. Additionally, AuNPs possess unique optical
and electrical properties, making it possible to track its intracellular trafficking and
localization. These unique properties make AuNPs especially attractive for biological
and medical applications.

The delivery of AuNPs takes advantage of pathophysiological conditions and
anatomical changes within diseased tissues, compared to normal tissues, to achieve site-
specific and targeted delivery. However, efficient delivery of AuNPs into a living system
requires overcoming natural biological barriers such as the cell membrane and the
reticuloendothelial system (RES). For specific tumor targeting, AuNPs face additional
challenges from receptor specificity and intra-tumor barriers. Several investigators have
grafted different delivery platforms onto AuNPs surface to attempt cellular selectivity,
internalization, and localization within heterogeneous population of cancer cells in solid
tumors. I document in this thesis our efforts of conjugating peptidyl-based delivery
platforms onto AuNPs that selectively entered and identified the nucleus of HSC oral
cancer cells under optical microscopy.

In continuation of the general theme of molecular targeting, we are investigating
potential transformation of β-lactam antibiotics, such as penicillins, for facile conjugation
to assorted cell permeable peptides and peptoids that may potentially enable bacterial
cell-selectivity. We synthesized various 6-aryltriazolylpenicillanic acids using a facile,
high yielding synthetic route. Preliminary biological studies indicated retention of
antibacterial activity in several of these 6-aryltriazolylpenicillanic acids. The observed antibacterial activity of 6-azidopenicillanates represents an important step toward the development of cell permeable peptide-conjugated penicillins.

Finally, biomacromolecules, such as peptides and oligonucleotides, undoubtedly play important roles in molecular- and cell-targeting. However, current peptide and oligonucleotide synthesis, derived from solid phase method, usually required initial loading of lead monomer onto solid support or resin using special coupling protocols that can often be difficult and time-consuming. We have developed a modified solid-supported azide capable of facile, rapid loading of lead monomer as well as retaining the innate synthetic property of the unmodified solid supports. This led to a milder, flexible methodology for synthesizing oligonucleotides and targeting peptides, such as nuclear localization signaling (NLS) peptides, investigated in the research herein described.
CHAPTER 1

Histone Deacetylase, Gold Nanoparticles, and Cancer Therapy

1.1 Histone Deacetylase and Tumorigenesis

The proper control of double-stranded DNA supercoil is essential for normal functioning of all living cells.¹ Three functionally related enzymes that maintain the structural integrity of the DNA supercoil are topoisomerase, histone acetyltransferases (HATs) and histone deacetylases (HDACs). In both prokaryotes and eukaryotes, the function of topoisomerases is to relieve the supercoiling and torsional stress of DNA helix caused by replication, transcription, and chromatin remodeling and recombination. In eukaryotes, HATs, and HDACs are two functionally opposing enzymes that tightly regulate the chromatin structure and function via sustenance of equilibrium between the acetylated- and deacetylated-states of nucleosomal histones.¹,² HATs acetylate histones, thereby gradually loosen the chromatin structure and aid transcription through increasing accessibility of gene promoter regions to transcription factors, regulatory complexes and the RNA polymerase.²,³ HDACs deacetylate histones, resulting in positively charged, hypoacetylated histones, which bind tightly to the phosphate backbone of DNA, thus inducing gene-specific repression of transcription.¹⁻³ Aberrations in intracellular histone acetylation-deacetylation equilibrium have been linked to the repression of a subset of gene resulting in tumorigenesis and are implicated in a number of malignant diseases.² The aberrant activity of HDACs has been shown to sustain the abnormal transcriptional silencing of tumor-suppressor-gene expression leading to the development and maintenance of tumor cells.³
1.1.1 Classical HDACs Biology

The eukaryotic HDACs are categorized as class I (HDAC-1, -2, -3, and -8); class II (HDAC-4, -5, -6, -7, -9, and -10); and class IV (HDAC-11) with Zn$^{2+}$ ion at the base of their catalytic core. Class III HDACs are identified as NAD-dependent proteases for their deacetylase function.$^{1-4}$ The aberrant HDAC activity leading to tumorigenesis has been linked to HDACs class I and II.$^{5, 6}$ The specific involvement of each HDAC isoform in tumor cells varied from one isoform to another. For examples, siRNA knockout of HDAC-1, and -2 confirmed their involvement in apoptosis and proliferation of transformed cells.$^{7, 6b-c}$ However, the exact cellular function of HDACs is still far from being completely elucidated. Nevertheless, inhibiting HDAC activity should lead to chromatin decondensation and overall increasing in gene transcription.$^{5, 8}$

**Figure 1-1.** Crystal structure of (a) HDLP and (b) HDAC-8 presented in secondary structural motif viewed in PYMOL. Zinc ion is shown as gray ball.$^{3}$

Structural information essential for rational design of isoform selective class I and II HDACs inhibitors is scarce. To date, only the crystal structure of histone deacetylase-
like protein (HDLP, bacterial homologue to human class I HDAC), HDAC-7, and human HDAC-8 are available (Figure 1-1). Overall architecture of HDLP and HDAC-8 active site is conserved relative to other class I and II HDACs. In HDLP, the active site is composed of a hydrophobic tunnel about 14 Å deep (Figure 1-2). The amino acid residues, Pro 22, Phe141, Phe198, Leu265, His180, Gly140, and Tyr297, formed the wall of the tunnel. The Zn$^{2+}$ ion at the base of the tunnel is coordinated with adjacent amino acid residues (Asp168, His170, and Asp258) and water molecules to hydrolyze the acetylated lysine of the N-terminal tail of core histones. In HDAC8, the only architectural difference is the replacement of leucine with methionine among the amino acid residues that constructed the wall of the active site. In addition, HDAC-8 active site adopted a different shape and is only 12 Å deep, which can be used to gain isoform selectivity for the design of HDAC inhibitors (Figure 1-2).
Proposed mechanism for the hydrolysis of N-acetylated lysine residue required that the carbonyl group of the N-acetyl amide bond of the acetylated lysine residue first coordinate to the Zn$^{2+}$ ion. More importantly, the coordination brings the acetylated lysine in close proximity with the water molecule at the base of the active site. The Zn$^{2+}$ ion then polarized the carbonyl group making the carbonyl carbon a better electrophile.\(^3\) The nucleophilicity of the water molecule is increased by both hydrogen bonding to His 131 as well as coordination to the Zn$^{2+}$ ion and therefore, leading to the hydrolysis of the carbonyl carbon to form a tetrahedron oxyanion intermediate. The intermediate is stabilized by two oxygen-zinc interactions and hydrogen bonding to the Tyr297 hydroxyl group.\(^3\) Oxyanion reformed the carbonyl group leading to the cleavage the carbon-nitrogen bond of the intermediate. The free nitrogen then accepts a proton from His132 yielding the free lysine residue and the acetate group (Figure 1-3).\(^3\) Understanding the

![Figure 1-3. The proposed mechanism for the deacetylation of acetylated lysine residue in HDLP active site.](image)
overall structure and mechanism of the class I and II HDACs active site has led to the development of many HDAC inhibitors with few in clinical trials. In fact, HDAC inhibition has been clinically validated with approval of suberoylanilide hydroxamic acids for the treatment of cutaneous T cell lymphoma.\textsuperscript{8}

1.1.2 Pharmacology of HDAC Inhibitors

Because of their demonstrated ability to arrest proliferation of nearly all transformed cell types\textsuperscript{8}, HDAC inhibitors (HDACi) hold great promise as agents of choice, either as stand alone therapeutics or in combination with others, in the fight against the cancer scourge. More importantly, majority of these HDAC inhibitors displayed relative low toxicity profile because a broad variety of transformed cell are more sensitive to HDACi-induced apoptosis than normal cells.\textsuperscript{1} However, they suffer from short half-life and low oral bioavailability. In addition, most of the HDAC inhibitors non-selectively inhibit various isoforms of HDACs leading to reduced \textit{in vivo} potency.\textsuperscript{5,8} Hence, selective inhibition against specific HDAC isoforms in conjunction with improving pharmacokinetic profile are important issues that must be addressed toward HDAC inhibitors.

1.2 Gold in Medicine

Chrysotherapy, the use of gold in medicine, has been practiced since antiquity. Ancient cultures such as those in Egypt, India, and China used gold to treat diseases such as smallpox, skin ulcers, syphilis, and measles.\textsuperscript{9-13} Presently, gold is in use in medical
devices including pacemakers and gold plated stents,\textsuperscript{14, 15} for the management of heart disease; middle ear gold implants,\textsuperscript{16} and gold alloys in dental restoration.\textsuperscript{17, 18} Because of their photo-optical distinctiveness, biocompatibility, and enhanced permeability and retention (EPR) effect, nanoscopic gold or gold nanoparticles (AuNPs) have also proven to be powerful tools in various nanomedicinal and nanomedical applications, such as bioimaging and photothermal therapy,

Bioimaging is achieved through the generation of colorimetric contrast between different cells/sub-cellular organelles by imaging agents. The use of AuNPs as imaging or contrast agents eliminate most of the vulnerabilities (photobleaching, low quantum yields, broad emission window, and nonselective localization in extravascular space) of the conventional imaging agents (lanthanide chelates and organic fluorophores).\textsuperscript{19-21} In addition, the colorimetric contrast observed within the AuNPs treated cells could be controlled by size,\textsuperscript{22-24} shape\textsuperscript{25-27} or even surface modification\textsuperscript{28, 29} of the AuNPs due to a phenomenon called surface plasmon resonance (SPR).\textsuperscript{21} When excited, the SPR of AuNPs could scatter and/or absorb light in the visible or the near-infrared (NIR) spectrum,\textsuperscript{30} an extremely useful property for \textit{in vivo} optical imaging.

Photothermal therapy is a less invasive experimental technique that holds great promise for the treatment of cancer and related disease conditions.\textsuperscript{31} It combines two key components: (i) light source, specifically lasers with a spectral range of 650-900 nm\textsuperscript{31} for deep tissue penetration, and (ii) optical absorbing AuNPs which transform the optical irradiation into heat on a picosecond time scale, thereby inducing photothermal ablation.\textsuperscript{32, 33} Recent developments have shown that the spectral signature of AuNPs could be tailored or tuned by altering their shape or size. El-Sayed and co-workers have
demonstrated that gold nanorods have a longitudinal absorption band in the NIR on account of their SPR oscillations and are effective as photothermal agents.\textsuperscript{31} Other gold nanostructures such as gold nanoshells,\textsuperscript{34} gold nanocages,\textsuperscript{32} and gold nanospheres\textsuperscript{35} have also demonstrated effective photothermal destruction of cancer cells and tissue. However, efficient \textit{in vivo} targeting of AuNPs to heterogeneous population of cancer cells and tissue still requires better selectivity and non-cytotoxicity to surrounding normal cells.

\textbf{1.2.1 Selective Delivery of AuNPs}

Efficient delivery of AuNPs into a living system requires overcoming natural biological barriers such as the cell membrane and the reticuloendothelial system (RES). For specific tumor targeting, AuNPs face additional challenges from receptor specificity and intra-tumor barriers. Potential approach for optimizing AuNPs delivery is particle size reduction ("true nanometer scale") or acquisition of surface modification. For example, large AuNPs are quickly opsonized by blood and eliminated by the RES in mammalian cells.\textsuperscript{36-39} To bypass RES, antibiofouling agents such as thiol-derivatized poly-ethylene glycol (PEG-SH) have been grafted onto AuNPs surface as secondary coating. It has been observed that this secondary coating could delay RES clearance to liver from 0.5 hour to 72 hours in a mice model, an approximately 150 folds improvement compared to the unmodified CTAB-capped AuNPs.\textsuperscript{40} Several investigators have grafted different delivery platforms onto AuNPs surface to attempt cellular selectivity, internalization, and localization within heterogeneous population of cancer cells in solid tumors. These delivery platforms generally consist of macromolecules such as proteins and peptides or small molecules such as folic acid and paclitaxel. Several of
these platforms have shown very promising results in delivering AuNPs into solid tumors for therapeutic or imaging applications.\textsuperscript{41-45}

The sustained fascination of the scientific community with AuNPs research have been facilitated by significant strides in many fronts including availability of a plethora of methods for the production and functionalization of AuNPs of various shapes and sizes. It is now possible to control particle sizes at nanometer resolution. Improved understanding of molecular targeting in biology has furnished several ligands that have been successfully used for specific delivery of AuNPs. With information accruing from proteomics studies on various diseases, one expects that many more ligands will be made available for AuNPs targeted delivery.

1.3 References


CHAPTER 2
TARGETED HISTONE DEACETYLASE INHIBITION

2.1 Aliphatic Hydroxamates

Histone deacetylase inhibitors (HDACi) have become promising anticancer agents in recent years. They have shown ability to block angiogenesis and cell cycling, as well as initiate differentiation and apoptosis.\(^1,2\) Histone deacetylase (HDAC) inhibition has recently been clinically validated as a new therapeutic strategy for cancer treatment with the FDA approval of suberoylanilide hydroxamic acid (SAHA) for the treatment of cutaneous T cell lymphoma.\(^3\) Intense research activities are ongoing in pharmaceutical and academic laboratories toward improving the pharmacokinetic and therapeutic indices of current HDACi. The classic pharmacophore for HDACi consists of three distinct structural motifs: the zinc-binding group (ZBG), a hydrophobic linker, and a recognition cap group (Figure 2-1).\(^4\) The X-ray structures of a bacterial HDAC homolog, histone deacetylase-like protein (HDLP), bound to SAHA or Trichostatin A (TSA) and,

![Figure 2-1. Small molecule HDACi - (a) selected examples of acyclic HDACi; (b) pharmacophoric model of HDACi](image-url)
more recently, human HDAC8 and HDAC7, confirmed that the ZBG interacts with a Zn\(^{2+}\) ion at the base of a channel-like active site.\(^{4,6}\) The hydrophobic linker efficiently presents the ZBG to the active site by filling the channel while the cap-group at the other end of the linker makes contacts with amino acid residues at the rim of the channel.

The common ZBG of HDACi is the hydroxamate moiety. The structural modifications of the hydroxamate ZBG have been modestly successful; yielding isosteres such as benzamide, \(\alpha\)-ketoesters, electrophilic ketones, mercaptoamide and phosphonates.\(^{4,7}\) Hence, the cap group presents an alternative opportunity to discover potent and more selective HDACi. In this regard, recent work by Schreiber and co-workers has led to the identification of cap group-modified agents that display differential inhibition against specific HDAC sub-types.\(^{8,9}\)

In a prototypical HDACi, the cap group could be linked to the aliphatic linker group through either hydrogen bonding accepting or donating groups such as keto- and amide- groups (Figure 2-1). The apparent lack of preference for H-bond donor or acceptor in the linking-moiety presents an opportunity to incorporate other more synthetically accessible and pharmacokinetically desirable moieties that may help simplify the molecular design and synthesis of novel HDACi. We proposed that a 1, 2, 3-triazole ring could act as a linking-moiety which joins the cap group to the linker group in a HDACi. The triazole ring could serve two purposes: (1) it could facilitate stronger cap group interactions with the amino acid side chains at the entrance of the HDAC active site; (2) it could serve as an isostere to the pharmacokinetically and toxicologically disadvantageous groups such as amide and ketone. We report here the synthesis and
structure activity relationship for HDACi incorporating 1, 2, 3-triazole as the cap group linking moiety.

2.1.1 Molecular Design of SAHA-like Triazole-Linked HDAC Inhibitors

The key reaction in the synthesis of the proposed HDACi is the Cu(I)-catalyzed Huisgen cycloaddition between azides and terminal alkynes (click chemistry). Click chemistry was popularized by elegant works from Sharpless and Medal’s laboratories, and has become a tool for the construction of various complex macromolecules and rapid identification of small molecules with interesting biological activities. To directly assess the effect of triazole ring as a cap group linking moiety in simple linear aliphatic-hydroxamate HDACi, we first synthesized a series of SAHA-like hydroxamates 4a-d. These compounds link the aromatic surface recognition cap group to the aliphatic zinc binding hydroxamate moiety via 1, 2, 3-triazole ring.

Scheme 2-1. Synthesis of aryltriazolylhydroxamate 4a-d. Conditions: (a) CuI, Hunig’s base, THF, rt; (b) NH$_2$OH$_{(aq)}$, KCN, 1:1 THF/MeOH, rt.

Cu(I)-catalyzed Huisgen cycloaddition between phenylacetylene 1 and known azido esters 2a-d, followed by treatment of the intermediate esters 3a-d with 50% aqueous
hydroxylamine\textsuperscript{15} furnished the desired hydroxamic acids 4a-d in good to excellent yields (Scheme 2-1). Similarly, the synthesis of analogs with other aromatic surface recognition cap groups is achieved starting from the corresponding aryl alkynes and azido esters (Scheme 2-2). Aryl alkynes, 5g, 5i, 5j, 5l, 5p, and 5q, that were not commercially available were synthesized from the corresponding aldehydes and carboxylic acids (through the intermediacy of aldehyde) using the Bestmann-Ohira Reagent.\textsuperscript{16-18}

\[ \text{R}_2 \equiv \text{a} \rightarrow \]

\[
\begin{align*}
5a: & \quad \text{R}_2 = \text{Anil} & 5k: & \quad \text{R}_2 = \text{Bz} \\
5b: & \quad \text{R}_2 = 3-\text{Py} & 5l: & \quad \text{R}_2 = \text{PyP} \\
5c: & \quad \text{R}_2 = 4-\text{Py} & 5m: & \quad \text{R}_2 = 4-\text{ Tol} \\
5d: & \quad \text{R}_2 = 2-\text{Py} & 5n: & \quad \text{R}_2 = 3-\text{ Tol} \\
5e: & \quad \text{R}_2 = \text{Thp} & 5o: & \quad \text{R}_2 = 2-\text{Quin} \\
5f: & \quad \text{R}_2 = \text{Nap} & 5p: & \quad \text{R}_2 = 2-\text{bp} \\
5g: & \quad \text{R}_2 = 2-\text{bp} & 5q: & \quad \text{R}_2 = 7-\text{Quin} \\
5h: & \quad \text{R}_2 = 4-\text{bp} & 5r: & \quad \text{R}_2 = 4-\text{Anis} \\
5i: & \quad \text{R}_2 = 3-\text{bp} & 5s: & \quad \text{R}_2 = 3-\text{Anis} \\
5j: & \quad \text{R}_2 = 2,6-\text{DMP} & 5t: & \quad \text{R}_2 = 2-\text{Anis} \\
6a: & \quad n = 3, R_1 = \text{Me}, R_2 = \text{Anil} & 6n: & \quad n = 3, R_1 = \text{Me}, R_2 = \text{Thp} \\
6b: & \quad n = 4, R_1 = \text{Me}, R_2 = \text{Anil} & 6o: & \quad n = 3, R_1 = \text{Me}, R_2 = 4-\text{bp} \\
6c: & \quad n = 3, R_1 = \text{Me}, R_2 = 3-\text{Py} & 6p: & \quad n = 3, R_1 = \text{Me}, R_2 = 3-\text{bp} \\
6d: & \quad n = 3, R_1 = \text{Me}, R_2 = 4-\text{Py} & 6q: & \quad n = 4, R_1 = \text{Me}, R_2 = 3-\text{bp} \\
6e: & \quad n = 3, R_1 = \text{Me}, R_2 = 2-\text{Py} & 6r: & \quad n = 3, R_1 = \text{Me}, R_2 = 2-\text{bp} \\
6f: & \quad n = 4, R_1 = \text{Me}, R_2 = 2-\text{Py} & 6s: & \quad n = 3, R_1 = \text{Me}, R_2 = \text{PyP} \\
6g: & \quad n = 3, R_1 = \text{Me}, R_2 = 4-\text{tol} & 6t: & \quad n = 4, R_1 = \text{Me}, R_2 = \text{PyP} \\
6h: & \quad n = 3, R_1 = \text{Me}, R_2 = 3-\text{tol} & 6u: & \quad n = 3, R_1 = \text{Me}, R_2 = \text{Nap} \\
6i: & \quad n = 3, R_1 = \text{Me}, R_2 = 2-\text{Anis} & 6v: & \quad n = 4, R_1 = \text{Me}, R_2 = \text{Nap} \\
6j: & \quad n = 3, R_1 = \text{Me}, R_2 = 4-\text{Anis} & 6w: & \quad n = 3, R_1 = \text{Me}, R_2 = 2-\text{Quin} \\
6k: & \quad n = 3, R_1 = \text{Me}, R_2 = 3-\text{Anis} & 6x: & \quad n = 3, R_1 = \text{Me}, R_2 = 7-\text{Quin} \\
6l: & \quad n = 3, R_1 = \text{Me}, R_2 = 2-\text{Anis} & 6y: & \quad n = 2, R_1 = \text{Me}, R_2 = \text{Bz} \\
6m: & \quad n = 3, R_1 = \text{Me}, R_2 = 2,6-\text{DMP} & &
\end{align*}
\]

\[ \text{b} \rightarrow \]

\[
\begin{align*}
7a: & \quad n = 3, R_2 = \text{Anil} & 7n: & \quad n = 3, R_2 = \text{Thp} \\
7b: & \quad n = 4, R_2 = \text{Anil} & 7o: & \quad n = 3, R_2 = 4-\text{bp} \\
7c: & \quad n = 3, R_2 = 3-\text{Py} & 7p: & \quad n = 3, R_2 = 3-\text{bp} \\
7d: & \quad n = 3, R_2 = 4-\text{Py} & 7q: & \quad n = 4, R_2 = 3-\text{bp} \\
7e: & \quad n = 3, R_2 = 2-\text{Py} & 7r: & \quad n = 3, R_2 = 2-\text{bp} \\
7f: & \quad n = 4, R_2 = 2-\text{Py} & 7s: & \quad n = 3, R_2 = \text{PyP} \\
7g: & \quad n = 3, R_2 = 4-\text{tol} & 7t: & \quad n = 4, R_2 = \text{PyP} \\
7h: & \quad n = 3, R_2 = 3-\text{tol} & 7u: & \quad n = 3, R_2 = \text{Nap} \\
7i: & \quad n = 3, R_2 = 2-\text{Anis} & 7v: & \quad n = 4, R_2 = \text{Nap} \\
7j: & \quad n = 3, R_2 = 4-\text{Anis} & 7w: & \quad n = 3, R_2 = 2-\text{Quin} \\
7k: & \quad n = 3, R_2 = 3-\text{Anis} & 7x: & \quad n = 3, R_2 = 7-\text{Quin} \\
7l: & \quad n = 3, R_2 = 2-\text{Anis} & 7y: & \quad n = 2, R_2 = \text{Bz} \\
7m: & \quad n = 3, R_2 = 2,6-\text{DMP} & &
\end{align*}
\]

**Scheme 2-2.** Synthesis of aryltriazolylhydrazamate 7 for SAR studies. Conditions: (a) 2b-d, Cul, Hunig’s base, THF, rt, (b) NH\textsubscript{2}OH\textsubscript{(aq)}, KCN, 1:1 THF/MeOH, rt.
2.1.2 Structure Activity-Relationship Studies of SAHA-like Triazole-linked HDAC Inhibitors

To establish whether 1, 2, 3-triazole ring is a suitable moiety for connecting the surface recognition cap group to the aliphatic zinc binding hydroxamate in a prototypical HDACi, we initially synthesized simple SAHA-like linear aliphatic-hydroxamates 4a-d. Subsequent in vitro evaluation in Fluor de lys assay\(^\text{19}\) reveals some interesting features about the anti-HDAC activity of these compounds. Compound 4a, an analog with three methylene spacers separating the triazole ring and the hydroxamate moiety, has no detectable anti-HDAC activity. Conversely, compounds 4b-d displayed spacer-length dependent HDAC inhibitory activities with 4c and 4d, analogs with five- and six-methylene spacers, being most potent (Table 2-1). This preliminary result shows that the anti-HDAC activities of these compounds track with, and thus confirm the early observation about the optimal spacer length for TSA- and SAHA-like anti-HDAC hydroxamates.\(^4,\text{20}\) More importantly, a head-to-head comparison reveals that incorporation of triazole ring potentiates HDAC inhibition. For example, compound 4c, the closest analog of SAHA, is about 4-fold more active than SAHA.

Interestingly, there is no clear trend in activity between the five- or six-methylene group spacer groups. For the simple phenyl substituted compounds, the six-methylene linked compound 4d is slightly more active than the five-methylene compound 4c (Table 2-1). However, the introduction of N, N-dimethylamino moiety to the para position of the cap group, similar to the substitution pattern on TSA (Fig. 2-1a), led to a five-methylene linked compound that is about 25-fold more active than the corresponding six-methylene compound in the Fluor de lys assay (Table 2-2, compare 7a and 7b). Because of this cap
Table 2-1. *In vitro* inhibition data for aryltriazolylhydroxamates 4. TSA and SAHA with IC<sub>50</sub> values of 5 nM and 65 nM, respectively, were used as controls.

![Image](305x618 to 310x654)

<table>
<thead>
<tr>
<th>Compound</th>
<th>n</th>
<th>IC&lt;sub&gt;50&lt;/sub&gt; (nM)&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>4a</td>
<td>1</td>
<td>N.D. &lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>4b</td>
<td>2</td>
<td>110.0</td>
</tr>
<tr>
<td>4c</td>
<td>3</td>
<td>14.2</td>
</tr>
<tr>
<td>4d&lt;sup&gt;c&lt;/sup&gt;</td>
<td>4</td>
<td>9.6</td>
</tr>
</tbody>
</table>

<sup>a</sup> Data represent mean values of at least three independent experiments in *Fluor de Lys* assay, performed by William Guerrant.

<sup>b</sup> N.D. = Not Determinable.


The group dependent potency of the five- and six-methylene spacer group, we prepared a series of aromatic and heteroaromatic derivatives consisting of either spacer group in order to further explore the SAR of these compounds. This exercise results in compounds that display potent HDAC inhibiting activity which trends with both the hydrophobicity and the substitution pattern of the aromatic ring. Relative to compounds 4c and 4d, nitrogen substitution into the phenyl ring did not improve the potency of the simple phenyl substituted compounds (Table 2-2, compounds 7c-f). However, the HDAC inhibitory activity of these pyridine derivatives is dependent on the ring location of the nitrogen atom with the 2-pyridyl derivative 7e being more active than the corresponding 3-pyridyl and 4-pyridyl analogs 7c and 7d respectively. Additionally, homologous
analogs 7e and 7f, differing by a methylene group, show a chain length dependency similar to that observed with 4c and 4d. Similarly, methyl-substituted compounds 7g-i have ring ortho-position substitution preference. However, para-substitution is preferred when the substituent is a methoxy group (Table 2-2, compare compounds 7j-l with 7g-i). In fact, the activity of para-methoxylated compound 7j is enhanced compared to the

Table 2-2. *In vitro* inhibition data for aryltriazolylhydroxamates 7a.

<table>
<thead>
<tr>
<th>Compound</th>
<th>R-</th>
<th>n</th>
<th>IC&lt;sub&gt;50&lt;/sub&gt; (nM)&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Compound</th>
<th>R-</th>
<th>n</th>
<th>IC&lt;sub&gt;50&lt;/sub&gt; (nM)&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>7a</td>
<td></td>
<td>3</td>
<td>4.3</td>
<td>7n</td>
<td></td>
<td>3</td>
<td>31.7</td>
</tr>
<tr>
<td>7b&lt;sup&gt;c&lt;/sup&gt;</td>
<td></td>
<td>4</td>
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<td></td>
<td>3</td>
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<tr>
<td>7c&lt;sup&gt;c&lt;/sup&gt;</td>
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<td>3</td>
<td>287.2</td>
<td>7p</td>
<td></td>
<td>3</td>
<td>1.9</td>
</tr>
<tr>
<td>7d&lt;sup&gt;c&lt;/sup&gt;</td>
<td></td>
<td>3</td>
<td>112.5</td>
<td>7q&lt;sup&gt;c&lt;/sup&gt;</td>
<td></td>
<td>4</td>
<td>5.4</td>
</tr>
<tr>
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<td>3</td>
<td>67.6</td>
<td>7r</td>
<td></td>
<td>3</td>
<td>162.6</td>
</tr>
<tr>
<td>7f&lt;sup&gt;c&lt;/sup&gt;</td>
<td></td>
<td>4</td>
<td>23.9</td>
<td>7s&lt;sup&gt;c&lt;/sup&gt;</td>
<td></td>
<td>3</td>
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</tr>
<tr>
<td>7g&lt;sup&gt;c&lt;/sup&gt;</td>
<td></td>
<td>3</td>
<td>43.4</td>
<td>7t&lt;sup&gt;c&lt;/sup&gt;</td>
<td></td>
<td>4</td>
<td>16.6</td>
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<tr>
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<tr>
<td>7i&lt;sup&gt;c&lt;/sup&gt;</td>
<td></td>
<td>3</td>
<td>17.4</td>
<td>7v&lt;sup&gt;c&lt;/sup&gt;</td>
<td></td>
<td>4</td>
<td>15.3</td>
</tr>
</tbody>
</table>
In addition to SAHA, TSA with IC$_{50}$ value of 5 nM was used as control. aVishal Patil and Patience Green also contributed to the synthesis of aryltriazolylhydroxmates 7. bData represent mean values of at least three independent experiments in Fluor de Lys assay, performed by William Guerrant. cCompounds made by Vishal Patil. These are included to clarify the SAR. See Chen et al, Bioorg. & Med. Chem., 2008, 16, 4839-4853 for detailed synthetic protocol. dN.D. = Not Determinable.

reference compound 4c. A similar para-substitution preference has been observed with methoxy-substituted SAHA-like HDACi in which the surface recognition cap group is linked to the aliphatic zinc binding hydroxamate through a ketone moiety.$^{21}$ The para-position preference may be due to the increased steric bulk of the methoxy group. To further investigate this possibility, we synthesized bisortho methoxy-substituted compound 7m. We observed an attenuation of the anti-HDAC activity of compound 7m relative to 7l. This observation confirmed the influence of steric constraints at the ortho position on the potency of SAHA-like compounds.

Cap groups consisting of fused six-six ring systems such as naphthalenes and quinolines also furnished compounds with potent HDAC inhibitory activities. Parallel to the activity of pyridine derivatives 7c-e, the 2-quinoline analog 7w is more active than the 7-quinoline analog 7x. The enhanced potency of the five-methylene linked, 6-
methoxynaphthalene capped compound 7u relative to a corresponding six-methylene analog 7v further underscored the preference of the larger cap groups for a five-methylene spacer. Furthermore, biphenyl compounds 7o-t display varying anti-HDAC activity that is dependent on the relative position of the triazole ring and the ring heteroatomic substitution pattern. In general, unsubstituted biphenyl compounds show preference for the meta-placement of the triazole ring and a five-methylene spacer (Table 2-2, compare compounds 7o-r).

Finally, the location of the triazole ring influences anti-HDAC activity. Compounds 4c and 7y are two isomeric compounds with the same number of carbon atoms separating the cap group and the hydroxamate moiety. Compound 4c, which has the triazole ring directly attached to the phenyl cap group is several orders of magnitude more potent than 7y whose triazole ring is separated from the cap group by a methylene group. This result is an indirect evidence that the triazole ring is indeed an active participant in the interaction of this class of compound with the HDAC active site.

2.1.3 SAHA-like Triazole-linked HDAC Inhibitors Docking Results

To obtain information on the structural basis of the observed disparity in the HDAC inhibitory activity of these compounds, we performed molecular docking using a validated molecular dock program (AutoDock)\textsuperscript{22-24} Docking analysis was performed on histone deacetylase-like protein (HDLP).\textsuperscript{5} We chose to use the HDLP structure because it shared conserved active site residues with class I HDACs. Additionally, direct docking experiments using this structure or HDAC1 homology model built from the same HDLP structure have given docking results that are essentially of the same quality and also
**Figure 2-2.** Molecular docking of aryltriazolylhydroxamates 7o (orange), 7p (green), 7u (magenta), and SAHA (cyan) to HDLP using Autodock 3.05\textsuperscript{19-21} and viewed in PYMOL. Upper picture: surface of HDLP near the active site; lower picture: side view of the triazolylhydroxamics 7o, 7p, 7u, and SAHA coordinating to zinc with amino acid residues in and near the active sites.\textsuperscript{19-21}
agreed with experimentally obtained data. Either of these approaches has been extensively used in the literature to interrogate the binding interaction of HDAC inhibitors at the protein active site.\textsuperscript{22b,c} The crystal structures of HDLP alone and bound to two known HDACi, SAHA and TSA, are currently available in the public domain.\textsuperscript{5} We performed docking studies with AutoDock 3.05 as described by Lu \textit{et al.}\textsuperscript{24} Independent docking of SAHA (IC\textsubscript{50} = 65 nM), 4c (IC\textsubscript{50} = 14 nM), 7o (IC\textsubscript{50} = 52 nM), 7p (IC\textsubscript{50} = 1.9 nM), and 7u (IC\textsubscript{50} = 1.8 nM) into HDLP revealed that these compounds have preferences for two different binding pockets at the protein surface (Fig. 2-2). Previous investigation have shown that there are four possible binding pockets on the HDLP surface whose interactions with the aromatic cap groups could enhance the inhibitor binding ability.\textsuperscript{23} Compounds 7o and 7u bind within the binding pocket designated pocket 1 while SAHA, 4c and 7p bind within pocket 2. The 1,4-biphenyl ring of 7o adopts a co-planar geometry in order to fit within pocket 1. Presumably the stacking interactions in pocket 1 favor a co-planar geometry of the cap group, an inference that may be supported by the binding of 7u, an analog with a flat fused six-six ring, within pocket 1. To adopt the observed conformation, a sharp kink is introduced into the methylene spacer group portion of 7o. The consequence of this kinked structure is a twist in the orientation of the critical hydroxamate moiety of 7o, pulling its hydroxamate-group farther from the active site Zn\textsuperscript{2+} ion, compared to that of 7p and SAHA. This potentially nullified any positive effects derived from the cap group interaction with pocket 1 and may proffer explanation about the reduced potency of 7o compared to structurally related 7p. Unlike 7o, the 1,3-biphenyl ring of 7p preferred a non-planar geometry with the biphenyl ring protruding deeper into the binding pocket 2 where it hydrophobically
Table 2-3. Cell Growth Inhibitory data for lead compounds.

<table>
<thead>
<tr>
<th>Aryltriazolylhydroxamates 7</th>
<th>IC$_{50}$ (nM)</th>
<th>EC$_{50}$ (µM)$^a$</th>
<th>EC$_{50}$ (µM)$^b$</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image1" alt="Molecule 7u" /></td>
<td>1.8</td>
<td>3.95</td>
<td>2.6</td>
</tr>
<tr>
<td><img src="image2" alt="Molecule 7v" /></td>
<td>15.3</td>
<td>7.76</td>
<td>3.99</td>
</tr>
<tr>
<td><img src="image3" alt="Molecule 7s" /></td>
<td>2.3</td>
<td>4.72</td>
<td>3.07</td>
</tr>
<tr>
<td><img src="image4" alt="Molecule 7w" /></td>
<td>2.1</td>
<td>2.25</td>
<td>2.46</td>
</tr>
<tr>
<td><strong>SAHA</strong></td>
<td>65.0</td>
<td>2.12</td>
<td>2.11</td>
</tr>
</tbody>
</table>

SAHA was used as a control for these experiments. EC$_{50}$ values were determined from $^a$trypan blue exclusion data and $^b$MTS assay (Progmega), in DU-145 prostate cancer cell line, performed by William Guerrant. $^c$Compounds made by Vishal Patil. These are included to clarify the SAR. See Chen et al, *Bioorg. & Med. Chem.*, 2008, 16, 4839-4853 for detailed synthetic protocol.
interacted further with the pocket residues. This extra interaction could explain the higher potency of \textit{7p} compared to \textit{4c} and SAHA.

\textbf{2.1.4 Cell Growth Inhibition Studies of SAHA-like Triazole-linked HDAC Inhibitors}

To preliminarily screen for the whole cell activity of compounds described in this study, we tested the effect of the exposure of selected compounds on the viability of DU-145, a human prostate cancer cell line known to respond to HDACi.\textsuperscript{25} We evaluated compounds \textit{7s}, \textit{7u}, \textit{7v} and \textit{7w} with SAHA as a reference, using both the MTS test (a colorimetric method) and trypan blue exclusion, to qualitatively and quantitatively measure the effect of compound exposure to cell viability.\textsuperscript{26, 27} We obtained an EC\textsubscript{50} value of 2.12 µM for SAHA, a value in close agreement with the reported EC\textsubscript{50} in DU 145 under similar experimental conditions.\textsuperscript{28} Compounds \textit{7s}, \textit{7u}, \textit{7v} and \textit{7w} also inhibit the proliferation of DU 145 in a dose-dependent manner, with EC\textsubscript{50} values ranging from 2.2 to 8.0 µM (Table 2-3). These results validate the suitability of the triazole ring as a linking moiety in the design of SAHA-like HDACi.

\textbf{2.2 Nonpeptide Macro cyclic HDAC Inhibitors}

To date, several structurally distinct small molecules HDACi have been reported including aryl hydroxamates, benzamides, short-chain fatty acids, electrophilic ketones and macro cyclic-peptides (Scheme 3-1).\textsuperscript{4, 7, 29, 30} All HDACi so far reported fit into the class HDAC three-motif pharmacophoric model.\textsuperscript{4} Of these HDACi, macro cyclic-peptides have the most complex recognition cap-group moieties and present an excellent opportunity for the modulation of the biological activities of HDACi. Although cyclic peptide HDACi possess potent HDAC inhibition activity (nanomolar range), their broad
application in cancer therapy currently remains largely unproven. One promising exception, **FK-228** (Figure 2-3), is currently in phase II study for the treatment of cutaneous T-Cell lymphoma.\(^{31}\)

![Chemical Structures](image)

**Figure 2-3.** Structure of various HDACi and macrolide antibiotics (a) Selected examples of acyclic HDACi; (b) Representative examples of Cyclic-peptide HDACi; (c) Representative examples of Macrolide Antibiotics.

The dearth of clinically effective cyclic-peptide HDACi may be in part due to development problems characteristic of large peptides, most especially poor oral
bioavailability. In addition to retaining the pharmacologically disadvantaged peptidyl-backbone, they offer only limited opportunity for side-chains modifications.\textsuperscript{32} Identification of non-peptide macrocyclic HDACi will offer a new class of macrocyclic HDACi with potentially more favorable drug-like properties. Furthermore, this will aid comprehensive SAR studies and further enhance our understanding of the roles of specific interactions between the enzyme outer rim and inhibitor cap-groups in HDACi activity and selectivity. Herein we report the discovery of a new class of potent, non-peptide macrocyclic HDACi derived from the macrolide macrocyclic ring structures.

\textit{2.2.1 Molecular Design of Nonpeptide Macrocyclic HDAC Inhibitors}

Macrolides are glycosylated polyketide antibiotics that have been in use for over 50 years for the treatment of respiratory tract infections. Additionally, macrolides have elicited other non-antibiotic effects, including anti-inflammatory and immunomodulatory effects that make them promising candidates for the management of diseases of chronic airway inflammation.\textsuperscript{33, 34} More recently, macrolides derived from 6-$O$-methylerthromycin A ring have been reported to serve as nonpeptidic surrogate for the peptide backbone of macrocyclic peptide luteinizing hormone-releasing hormone (LHRH) receptor antagonists.\textsuperscript{35} Drawing inspiration from the peptidomimetic property of macrolides, we hypothesize that an appropriate substitution of the cyclic peptide moiety of a prototypical cyclic-peptide HDACi with macrolide skeletons will generate a new class of potent non-peptide macrocyclic HDACi.

To test our hypothesis, we first sought a SAHA-macrolide conjugate which incorporated the 15-membered azalide ring of azithromycin as the macrolide template.
Azithromycin skeleton is an attractive choice due to its excellent pharmacokinetic (PK) profile and ease of chemical transformation of key moieties on the skeleton. Our design approach is to attach the HDAC inhibiting group to a macrolide moiety that is remote from the macrocyclic ring. We anticipate that this will minimize the potential steric clash at the HDAC active site that might result from the introduction of the azithromycin skeleton, a macrocyclic ring not optimized to bind HDAC enzyme. NMR structure and 3D model revealed that the 3’-tertiary amine of the desosamine sugar and the 4”-OH of the cladinose sugar met our design requirement. We opted for the former group because of the well established facile transformations of the tertiary amine moiety. Almost all modifications of this moiety have resulted in the attenuation of the antibacterial activity of this class of compound. Furthermore, coupling of such desosamine modifications with cladinose sugar removal will result in compounds devoid of antibacterial activity, a property of the parent macrolides undesirable to our goals. Toward this end, we synthesized compound 15, which incorporates a SAHA-like moiety (Scheme 2-3) into the 3’-tertiary amine of azithromycin, as a prototype molecule. Compound 15 was synthesized from azithromycin 12 and methyl 8-chloro-8-oxooctanoate 8 through a five-step synthetic route as shown in Scheme 2-3. To gain some preliminary insights into the roles of key macrolide moieties in anti-HDAC activity of 15, we synthesized compound 17, an analog of 15 lacking the cladinose sugar. Acid promoted removal of cladinose sugar according to published protocol quantitatively yielded compound 16, which was converted to hydroxamate 17 using the same protocol for the synthesis of 15 from 14 (Scheme 2-3). We then tested for the HDAC inhibition activity of 15 and 17 using a cell free kit assay (Fluor de Lys), and found that both
compounds caused a concentration dependent inhibition of HDACs 1 and 2 from HeLa cell nuclear extract (Table 2-4). In fact, both compounds have identical anti-HDAC activity, with IC\textsubscript{50} values in low nanomolar range. Conversely, compound 14, the methyl ester precursor of 15, is completely inactive in this assay. This result suggests that the 15-membered azalide ring of azithromycin macrolide is a suitable non-peptidyl surrogate for the macrocyclic peptide moiety of a typical cyclic-peptide HDACi. Additionally, the binding orientations of these compounds at the HDAC active site may be such that the hydroxamate group is efficiently presented to chelate the active site Zn\textsuperscript{2+} ion while the cladinose moiety is oriented away from the enzyme’s outer rim.

We then initiated structure activity relationship studies on 15 and 17 to optimize the HDAC binding affinity of these compounds. Preliminarily, we focused on the effects of the modification of linker-cap group connection moiety, macrolide skeleton, and linker length on anti-HDAC activity. We used AutoDock program\textsuperscript{22} and the crystal structure of a histone deacetylase-like protein (HDLP)\textsuperscript{5} to guide our structural optimization. In Table 2-4. In vitro HDAC inhibition (IC\textsubscript{50}) and isoform selectivity of nonpeptide macrocyclic HDACi.

<table>
<thead>
<tr>
<th>Compound</th>
<th>HDAC 1/2 (nM)</th>
<th>HDAC 8 (nM)</th>
<th>Isoform Selectivity\textsuperscript{a}</th>
</tr>
</thead>
<tbody>
<tr>
<td>14</td>
<td>N.D.\textsuperscript{c}</td>
<td>N.T.\textsuperscript{d}</td>
<td>- \textsuperscript{b}</td>
</tr>
<tr>
<td>15</td>
<td>107.10</td>
<td>6,680</td>
<td>62</td>
</tr>
<tr>
<td>17</td>
<td>109.80</td>
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<td>23a</td>
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<tr>
<td>23c</td>
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<td>72</td>
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<tr>
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<td>10.56</td>
<td>1,020</td>
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</tr>
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<td>38</td>
</tr>
<tr>
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<td>N.D.\textsuperscript{c}</td>
<td>- \textsuperscript{b}</td>
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<td>56</td>
</tr>
<tr>
<td>31h</td>
<td>223.36</td>
<td>N.D\textsuperscript{c}</td>
<td>- \textsuperscript{b}</td>
</tr>
<tr>
<td>SAHA</td>
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<td>1,860</td>
<td>29</td>
</tr>
</tbody>
</table>

IC\textsubscript{50} values were determined from the Fluor de Lys assay, performed by William Guerrant. Each data is obtained from three independent experiments. \textsuperscript{a}calculated by dividing the IC\textsubscript{50} of HDAC 8 with the IC\textsubscript{50} of HDAC 1/2. \textsuperscript{b}undeterminable. \textsuperscript{c}N.D. = not determinable. \textsuperscript{d}N.T. = not tested
section 2.1, we demonstrated the suitability of a 1,2,3-triazole ring as an alternative linker-cap group connection moiety in SAHA-like HDACi. In addition to serving as an alternative connection moiety with a potentially favorable pharmacokinetic properties, the triazole ring is a more synthetically tractable group, and has been adopted in the construction of active-site directed chemical probes for profiling HDAC activities in proteomes and live cells. Its incorporation into our design could facilitate a facile, high yielding synthesis of the triazole-based SAHA-macrolide conjugates and thus provide solution to the problems of low yields that currently plagued the synthesis of the amide-based compounds 15 and 17 (Scheme 2-3).

Accordingly, we proceeded to test the compatibility of the triazole ring with the anti-HDAC activity of 15 and 17. We prepared compounds 23a and 23b, analogs of 15 and 17 respectively, having the amide moiety connecting the cap group and the linker region substituted with a 1, 2, 3-triazole ring (Scheme 2-4a). The reaction of 4-ethynylbenzyl mesylate with 18 led to the desired desosamine alkylation product 19 in very good yields. Cu(I) catalyzed cycloaddition reaction of alkyne 19 with O-silyl azido-hydroxamate 21 gave the desired cycloadduct 22 in good yields. Similarly, the reaction of alkynes 19 and 20 with azido ester 25 gave the desired cycloadducts 26a and 26b respectively. The deprotection of the silyl group of compound 22 is accomplished with TBAF treatment, leading to desired hydroxamate 23a in good yields. However, difficulties were encountered in separating 23a from the TBAF reagent. In much the same manner as in the synthesis of 15 or 17 (Scheme 2-3), the conversion of methyl ester 26a and 26b to the corresponding hydroxamate is dogged by low yields. Subsequently, we found that a direct Cu(I) catalyzed cycloaddition between unprotected azido-
Scheme 2-4. SAR studies on nonpeptide macrocyclic HDACi (a) Synthesis of triazole-linked nonpeptide macrocyclic HDACi based on azithromycin 15-membered ring; (b) Synthesis of triazole-linked nonpeptide macrocyclic HDACi based on clarithromycin 14-membered ring.
hydroxamate 24 and alkyne 19 or 20 in anhydrous, degassed THF under exclusion of oxygen uneventfully gave the desired hydroxamates (Scheme 2-4a).

### 2.2.3 Nonpeptide Macroyclic HDAC Inhibitors Docking Results

HDAC inhibition studies revealed that the triazolyl-compounds 23a and 23b have virtually identical anti-HDAC activity as the amide-compounds 15 and 17 (Table 2-4). This result is in contrast with our observation on simple aliphatic hydroxamates where the introduction of the triazole ring led to an enhancement of anti-HDAC activity. To gain insights on the molecular interactions between these non-peptide macrocyclic HDACi and HDAC active site, we performed molecular docking analysis of 23b on HDLP, using AutoDock program, as previously described. The obtained docked structure indicated interesting molecular surface complementarities between the macrolide skeleton of 23b and the HDAC outer rim. Previous investigations have shown that there are four possible binding pockets on the HDLP surface whose interactions with the HDACi cap groups could enhance the inhibitor binding ability. Compound 23b adopts a docked structure that placed the macrolide macrocyclic ring in binding pockets 1 and 3 (Figure 2-4a). In addition, the hydrophobic components of the macrolide ring optimally interact with the hydrophobic residues in pockets 1 and 3 while the hydrophilic hydroxyl groups are oriented away from the pocket’s hydrophobic residues. Compared to the structure of SAHA, the hydroxamate moiety of 23b is farther oriented from the active site Zn$^{2+}$ ion (Figure 2-4b). Based on the preceding observation, we inferred that optimization of the linker region could result in compounds with enhanced HDAC affinity due to a better presentation of the hydroxamate moiety to the catalytic Zn$^{2+}$ ion.
Subsequent docking analyses with analogs of 23b having varied methylene-linker lengths revealed that 23d, a C7-linker compound, optimally interacts with the Zn$^{2+}$ ion (Figure 2-4c). Interestingly, compound 31d, an analog of 23d in which the 15-membered azithromycin ring has been substituted with the 14-membered clarithromycin ring, has a slight preference for the enzyme. A closer analysis of the docked structures of 23d and 31d revealed that the C12 - C14 region of the larger 15-membered ring is about 0.5Å

**Figure 2-4.** Docked structures of SAHA, 14- and 15-membered macrocyclic HDACi in the active site of HDLP. (a) Super-position of the low energy conformation of 23b (blue) and SAHA (yellow) revealed the pocket binding preferences of inhibitors at the HDLP surface. Ball and stick model of the orientation of the hydroxamate group of SAHA and 23b (b); 23b and 23d (orange) (c) with respect to the active site Zn$^{2+}$ ion (grey ball). (d) Relative orientation of the macrocyclic rings of 23d and 31d (yellow) with respect to Phe 338 at the HDLP surface.
closer to the phenyl ring of Phe338 that defines one of the hydrophobic pockets at the enzyme outer rim compared to that of the 14-membered compound (Figure 2-4d). This might compromise the binding affinity of the 15-membered compounds relative to the 14-membered analogs.

To experimentally test these *in silico* observations, we synthesized compounds 16c-h and 24a-h, 14- and 15-membered non-peptide macrocyclic hydroxamates respectively (Fig. 2-4a and 2-4b). Results from the Fluor de Lys assay on these compounds revealed HDAC inhibition activities that essentially paralleled the *in silico* prediction (Table 2-4). The compounds displayed both linker-length and macrolide-type dependent HDAC inhibition activities. For compounds derived from the same macrolide ring, an increase in the linker length from C₆ to C₇ conferred a better anti HDAC activity. Further linker length increase did not improve HDAC inhibition activity; in fact such increase is detrimental to function in some cases. For compounds with C₆ and C₇ linkers, a head-to-head comparison between 14- and 15-membered macrolides revealed that the 14-membered compounds are about 2-5 folds better HDACi than their 15-membered counterparts (Table 2-4, see 23c and 31c for example). However, this preference dissipated with increase in linker length. This is presumably due to a relief of steric clash between the macrocyclic ring and the phenyl ring of Phe338 at the enzyme outer rim, conferred by the longer linkers.

### 2.2.4 HDAC Isoform- and Cell-Selectivity Studies of Nonpeptide Macro cyclic HDAC Inhibitors

To obtain preliminary evidence for the HDAC isoform selectivity of the macrocyclic HDACi described herein, we tested their HDAC8 inhibition activity. We
chose HDAC8 because it is in the same sub-class as HDACs 1 and 2, the principal HDACs contained in the HeLa cell nuclear extract used in assay kit employed in this study. There are very few examples of HDACi that are selective for HDAC isoforms within the same class; hence this choice should permit a quick, yet rigorous assessment of HDAC isoform selectivity of our compounds. Compared to SAHA, all non-peptide macrocyclic hydroxamates tested are more selective for HDAC1/2. This observation is in agreement with the literature reports which suggest that complex head groups tend to promote isoform selectivity.\textsuperscript{4,9} However, C\textsubscript{7}-linked, 14-membered compounds 31c and 31d are several folds more selective than their 15-membered counterparts (Table 2-4). Although HDLP and HDAC8 shared similar amino acid sequences and topology at the active site, observation from the analysis of X-ray data however revealed significant inhibitors specific changes in the enzyme active site topology of HDAC8.\textsuperscript{6b} Our docking analysis operates in the rigid receptor mode and it is incapable of capturing such crystallographically observed ligand induced conformational changes. Nevertheless, we performed molecular docking analysis of 23b on HDAC8 structure reported by Somoza \textit{et al.}\textsuperscript{6b} In contrast to its docked structure on HDLP, the orientation of 16b which has a chance of making any interaction with the active site Zn\textsuperscript{2+} ion is that which adopted a closed conformation nestled atop of the entrance to the enzyme active site. In this conformation, the linker group wrapped around the macrocyclic ring to orient the hydroxamate moiety toward the Zn\textsuperscript{2+} ion, albeit much farther away to make any stabilizing interaction (Figure 2-5). An alternative lower energy conformation of 23b oriented the hydroxamate moiety away from the Zn\textsuperscript{2+} ion. It is therefore possible that the observed isoform selectivity may be due to the inability of the nonpeptide macrocyclic
Figure 2-5. Docking structure of 23d in the active site of HDAC8. (a) Surface representation of docked Structure of 23d at the entrance of HDAC8 active site. (b) Ball and stick model of the orientation of the hydroxamate group of 23d with respect to the active site Zn$^{2+}$ ion (grey ball).
hydroxamates herein described to efficiently induce active site conformational changes that facilitate HDAC8 specific inhibitor association with the enzyme active site.\textsuperscript{41a} Alternatively, HDAC8 activity has been observed to depend on the sequence of its peptide substrate\textsuperscript{22b}, hence it is also conceivable that the extent of enzyme inhibition, and consequently isoform selectivity, may depend on the substrate used in the inhibition study.

To further test for HDAC isoform selectivity, we investigated the effect of selected macrocyclic HDACi on the deacetylase activity of HDAC6, a representative member of class II HDAC. For this preliminary study, we chose compounds \textit{23b} and \textit{23d}, and \textit{31c} and \textit{31d}, the most potent representative 15- and 14-membered macrocyclic HDACi respectively (Table 2-4). Cell free HDAC6 inhibition assay was performed as recommended by the supplier.\textsuperscript{19} Unlike SAHA which equally inhibit HDAC 1/2 and HDAC6, we observed that the macrocyclic HDACi still displayed significant preference for HDAC1/2 (Table 2-5). This observation is in agreement with the literature reports which suggest that complex head groups tend to promote isoform selectivity.\textsuperscript{4, 41c}

\begin{table}[h]
\centering
\caption{In vitro HDAC inhibition (IC\textsubscript{50}) and isoform selectivity of nonpeptide macrocyclic HDACi.}
\begin{tabular}{|l|c|c|c|}
\hline
\textbf{Compound} & \textbf{HDAC 1/2 (nM)} & \textbf{HDAC 6 (nM)} & \textbf{Isoform Selectivity}\textsuperscript{a} \\
\hline
23c & 13.9 & 78.0 & 6 \\
\hline
23d & 10.6 & 117.4 & 11 \\
\hline
31c & 4.1 & 89.3 & 22 \\
\hline
31d & 1.9 & 148.5 & 78 \\
\hline
SAHA & 65.0 & 85.5 & 1 \\
\hline
\end{tabular}
\end{table}

IC\textsubscript{50} values were determined using a cell free kit assay.\textsuperscript{19} Each data is obtained from two independent experiments, performed by William Guerrant. \textsuperscript{a}calculated by dividing the IC\textsubscript{50} of HDAC 6 with the IC\textsubscript{50} of HDAC 1/2
To screen for the whole cell activity of compounds described in this study, we studied their effect on the viability of SK-MES-1 (human NSCLC cell line), NCI-H69 (human SCLC cell line), DU-145 (a human prostate cancer cell line,) and non-transformed human primary lung fibroblasts and mammary epithelial cell lines. The non-transformed cell lines were investigated to obtain evidence for compound selective toxicity.

### Table 2-6. Cell growth inhibition data for nonpeptide macrocyclic HDACi.

<table>
<thead>
<tr>
<th>Compound</th>
<th>SKMES 1 (µM)</th>
<th>NCI-H69 (µM)</th>
<th>DU-145 (µM)</th>
<th>Lung fibroblast (µM)</th>
<th>HMEC (µM)</th>
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</thead>
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<td>N.T. a</td>
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<td>N.T. a</td>
<td>N.T. a</td>
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<tr>
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<td>N.T. a</td>
<td>&lt;25</td>
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<td>N.T. a</td>
</tr>
<tr>
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</tr>
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</tr>
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<td>2.67</td>
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<tr>
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<td>1.45</td>
<td>1.12</td>
<td>&gt;10</td>
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</tr>
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</table>

EC$_{50}$ values were determined from trypan blue exclusion data. Each data is obtained from a duplicate of four simultaneous experiments, performed by William Guerrant. aN.T. = not tested.

Drug concentrations necessary for 50% inhibition of cell viability (EC$_{50}$) were quantitatively measured using trypan blue exclusion$^{27}$, as previously described.$^{39}$ Table 3-2 shows the EC$_{50}$ values of each compound. The EC$_{50}$ values obtained for SAHA are in close agreement with the reported values under similar experimental conditions.$^{28}$

39
Macrocyclic methyl ester 14, the precursor to compound 15 (Scheme 2-3), has no effect on cell viability. This result may not be unexpected since compound 14 has no HDAC inhibition activity (Table 2-4). However, all macrocyclic hydroxamates inhibit the proliferation of all transformed cells studied. Most importantly, compounds 23b, 31c, and 31d are at least twice as potent as SAHA in DU-145 cells (Table 2-6). Gratifyingly, none of the macrocyclic hydroxamates tested shows any growth inhibitory effects on the normal human primary lung fibroblast and mammary epithelial cell lines at concentrations in excess of 10 μM. This data showed that the macrocyclic compounds are selectively toxic to the transformed cells, a trait that tracks with that of many HDACi.

### 2.2.5 Design and Synthesis of Isotopically Labeled Nonpeptide Macroyclic HDAC Inhibitors for In Vivo Studies

Macrolide, especially 15-membered azithromycin, are known to selectively accumulate in target organs such as the lungs, tonsil and cervix.\(^\text{42}\) It is therefore conceivable that the nonpeptide macrocyclic HDACi could possess targeted anti-cancer activity due to selective tissue distribution conferred by the appended macrolide moiety. To test this possibility, we designed and synthesized two classes of isotopically labeled analogs of 15-membered macrocyclic HDACi compounds 32a-b and 34c-d. Compounds 32a-b are \(^{15}\text{N}\)-labeled analogs of compounds 23a and 23c, respectively, while 34c-d are O-3’-\(^{14}\text{C}\)-acetylated derivatives of 23a and 23c, respectively. The synthesis of 32a-b followed essentially the same route described for the synthesis of 23a and 23c except that the corresponding \(^{15}\text{N}\)-azide 24a and 24b were used in the cycloaddition reaction with 4’-ethynylbenzyl azithromycin 19 (Scheme 2-5). The synthesis of \(^{14}\text{C}\)-acetylated compounds 34c-d was analogously accomplished using O-3’-\(^{14}\text{C}\)-acetylated 4’-
ethynylbenzyl azithromycin 33 (Scheme 2-5). The isotopically-labeled compounds 32a-b and 34c-d are currently being used as probes to establish the organ distribution profile of the nonpeptide macrocyclic HDACi in healthy Balb c mice.

Scheme 2-5. Synthesis of isotopically labeled nonpeptide macrocyclic HDACi. This indicated the location of the isotopic $^{14}$C- or $^{15}$N-labels.

2.3 Nuclear Targeting HDAC Inhibitors

As mentioned before, most of the HDACi, including SAHA, non-selectively inhibit the deacetylase activity of class I/II HDAC enzymes. This broad HDAC inhibition is associated with reduced potency and toxic side effects. So far, several attempts aimed
at identifying isoform selective HDACi have been modestly successful, resulting in very few HDACi that are only partially isoform selective.\textsuperscript{43} Because HDACs 1 and 2, the primary targets for the anticancer activity of HDACi, are exclusively localized in the nucleus,\textsuperscript{2b, 44} we propose that an alternative approach to isoform selective HDACi is the development of a strategy for nuclear delivery and localization of HDACi.

\textbf{2.3.1 Design and Synthesis of Nucleus Localization Signal peptide-HDAC Inhibitors Conjugates}

To test our hypothesis, we first sought peptide-HDACi conjugates that are capable of crossing both the plasma and nuclear membrane.\textsuperscript{45} Most of the nuclear membrane-penetrating peptides described in the literatures are derived from viral sources. Common examples include the Simian virus nuclear localization peptides (NLS)\textsuperscript{45-47}, HIV 1 Tat-protein derived peptides\textsuperscript{48}, and peptides derived from adenovirus fiber protein. NLS peptides are peptides utilized by viruses to cross many cellular membranes especially the nuclear membrane. In addition, we reasoned that NLS, with lysine-enriched sequences, could act as a substrate-mimetic to HDAC by mimicking the N-terminal tail lysine residues of the core histones. Hence, NLS-HDACi conjugates could be ideal “near-substrate” inhibitors of nuclear HDAC 1 and 2.

Our design approach is to attach an HDAC inhibition group, such as our triazole-based SAHA-like aliphatic-hydroxamates \textit{4}, directly to the NLS peptides through another triazole ring. This very simple, initial design could facilitate a facile, high yielding synthesis of the proposed NLS-HDACi conjugates. Accordingly, we prepared compounds \textit{40a-c} having a second 1,2,3-triazole ring connecting a NLS-derived peptide.
Scheme 2-6. Synthesis of NLS-HDACi conjugates. Conditions: (a) 4-ethynylaniline, CuI, DIPEA, THF, (b) NaNO\textsubscript{2}, NaN\textsubscript{3}, 17.2\% HCl\textsubscript{(aq)}, (c) LiOH•H\textsubscript{2}O, THF/H\textsubscript{2}O, (d) o-tritylhydroxylamine, EDCI, HOBT, NMM, DMF, (e) alkyne-terminated NLS peptides, CuI, TBTA, DIPEA, THF/DMF, (f) 90:5:5 TFA/TIPS/Phenol
to the cap group of the triazole-based aliphatic hydroxamates (Scheme 2-6). The Cu(I)-catalyzed reaction of 4-ethynylaniline with azido esters 2c-e gave the desired cycloadduct 35a-c in excellent yields.\textsuperscript{10,39} The diazotization of aryl amine 35a-c by treatment with
sodium nitrite and sodium azide led to the desired azido derivative 36a-c in good yields. However, a portion of azido derivatives 36 was hydrolyzed into carboxylic acid giving a mixture of both ester and carboxylic acid derivatives. To hydrolyze the rest of the ester, lithium hydroxide hydrates were added to the mixture giving a complete conversion to the azido carboxylic acid derivative 37 in excellent yields. The reaction of acid 37a-c with O-trityl hydroxylamine gave the desired O-trityl azido-triazolylhydroxamates 38a-c that were subsequently coupled to the alkyne-terminated N-Boc protected NLS peptide PCS-37689-PI. The resulting cycloadducts 39a-c were then subjected to TFA treatment to remove all protecting groups yielding the desired NLS-HDACi conjugates 40a-c in quantitative yield.

**Table 2-7.** Preliminary *in vitro* inhibition data for NLS-HDACi conjugates 40. TSA and SAHA with IC50 values of 5 nM and 65 nM, respectively, were used as controls.

<table>
<thead>
<tr>
<th>Compound</th>
<th>n</th>
<th>IC50 (nM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>40a</td>
<td>1</td>
<td>36.0</td>
</tr>
<tr>
<td>40b</td>
<td>2</td>
<td>14.0</td>
</tr>
<tr>
<td>40c</td>
<td>3</td>
<td>24.0</td>
</tr>
</tbody>
</table>

Data represent mean values of at least three independent experiments in *Fluor de Lys* assay, performed by Josh Canzoneri.

We then tested the HDAC inhibition activity of 40a-c using a cell free kit assay *(Fluor de Lys)*.19 We found that the the NLS-HDACi conjugates displayed potent HDAC
inhibition activities that is somewhat linker-length dependent against HDAC 1 and 2 from HeLa cell nuclear extract (Table 2-7). An increase in the linker length from C₆ to C₇ conferred a better anti-HDAC activity. Investigation of HDAC isoform selectivity and whole cell anti-proliferative activities of the NLS-HDACi conjugates is currently underway. Also, we are interested in probing the intracellular trafficking of these NLS-HDACi conjugates. The next section discusses the design and synthesis of fluorescent NLS and HDACi probes that will enable us to accomplish this objective.

2.3.2 Design and Synthesis of Fluorescent Tagged Nuclear Localization Signal Peptides and HDAC Inhibitors

To monitor the intracellular trafficking of the NLS-HDACi conjugates, we first sought NLS and HDACi that are independently labeled with a fluorescent tag. NLS is known to facilitate nuclear localization of assorted ligands.⁴⁵-⁴⁷ A demonstration of nuclear localization with the fluorescently tagged NLS could be used to inferred nuclear localization of the unlabeled NLS-HDACi. Similarly, fluorescently tagged HDACi could shed more light on the intracellular distribution of the unmodified HDACi. We chose a BODIPY-based dye⁴⁹ as the fluorescent tag for the proposed labeled NLS and HDACi. The application of BODIPY dyes in bioimaging is well-established. They

![Scheme 2-7. Synthesis of alkyne BODIPY 42.](image)
provide sharp fluorescence peak with high quantum yields. Their relative insensitivity to polarity and pH of their environment and stability in physiological conditions has led to their wide use in both proteins and DNA labeling. The synthesis of NLS-BODIPY

**Scheme 2-8.** Synthesis of NLS-BODIPY 47. Conditions: (a) 4-azidobenzyl alcohol, CuI, DIPEA, THF, (b) MsCl, Et$_3$N, CH$_2$Cl$_2$, (c) NaN$_3$, DMF, 65°C, (d) alkyne-terminated NLS peptides, CuI, TBTA, DIPEA, THF/DMF, (e) 90:5:5 TFA/TIPS/Phenol
was accomplished as shown in scheme 2-8. Alkyne BODIPY 42 was synthesized from alkynyl aldehyde 41 and 2,4-dimethylpyrrole by adapting literature protocols (Scheme 2-7). Cu(I)-catalyzed cycloaddition reaction between 42 and 4-azidobenzyl alcohol gave triazole 43, which was subsequently transformed to azido-triazole 45 through mesylate 44. The Cu(I)-catalyzed cycloaddition of azido-triazole 45 and alkyne-terminated N-Benzocarbamate protected NLS peptide PCS-37689-PI gave the desired cycloadduct 46 in good yields. The deprotection of the NLS side chain was accomplished with TFA treatment, leading to desired NLS-BODIPY 47 in quantitative yields (Scheme 2-8). Similarly, HDACi-BODIPY 49 was synthesized by the Cu(I)-catalyzed cycloaddition between the O-trityl azido-triazolylhydroxamate 38a and alkyne BODIPY 42, followed by deprotection with TFA treatment, to give the unprotected cycloadduct 49 in good yields (Scheme 2-9). Bioimaging experiments with HDACi-BODIPY 49 and NLS-BODIPY 47 are currently ongoing.

Scheme 2-9. Synthesis of HDACi-BODIPY 49. Conditions: (a) CuI, TBTA, DIPEA, THF/DMF, (b) 90:5:5 TFA/TIPS/Phenol
2.4 Conclusion

We have established that 1, 2, 3-triazole ring is suitable as a surface recognition cap group linking-moiety in SAHA-like HDACi. The structure-activity relationship of the resulting triazole-linked hydroxamates displays a cap group dependent preference for either five- or six-methylene spacer groups. We identified compounds that are several folds more potent than SAHA. A subset of these compounds also inhibited the proliferation of DU-145 cells. Due to their anticipated resistance to intracellular peptidases, these triazole-linked HDACi may display improved \textit{in vivo} activity relative to the common amide based inhibitors.

More significantly, we have identified a new class of non-peptide HDACi derived from the macrocyclic skeletons of clinically useful macrolides. These compounds will enable a molecular description of the interaction between the HDAC enzyme outer rim and the inhibitors’ macrocyclic cap-groups thereby further aid our understanding of the roles of this interaction in inhibitors binding affinity and possibly HDAC isoform selectivity. In addition, due to the selective tissue distribution that may be conferred by the appended macrolide moiety, some of these HDACi are anticipated to have targeted anti-cancer activity. Specifically, compounds incorporating azithromycin skeleton could be selectively accumulated in the lungs\textsuperscript{42}, thereby possess lung-selective anti-cancer activity. The prospect of tissue-specific HDACi delivery is a particularly enticing alternative to isoform selective HDACi and could lead to the identification of new chemotherapeutic agents for use in targeted cancer therapy applications. Efforts are underway in our lab to profile the tissue distribution of the new class of the macrocyclic HDACi described here. Additionally, efforts are in progress to investigate the
intracellular trafficking of NLS-HDAC conjugates and the consequence of nuclear accumulation on the anti-proliferative activity of HDACi.

2.5 General Procedure and Experimental

2.5.1 General Procedure

4-bromobutyric acid, 5-bromovaleric acid, 6-bromohexanoic acid, 7-bromoheptane-nitrile, 8-bromoocitanoic acid, ethyl 6-bromohexanoate, ethyl 7-bromoheptanoate, methyl 10-bromodecanoate, 4-ethynylbenzyl alcohol, sodium azide-\(^{15}\text{N}_1\), and acetic anhydride-carbonyl-\(^{14}\text{C}\) (0.25 mCi) were purchased from Sigma Aldrich. Clarithromycin and azithromycin were purchased from Greenfield Chemical and Pfizer, respectively. 9-bromononanoic acid was purchased from Karl Industries Inc. Alkyne-terminated \(N\)-Boc NLS peptide (PCS-\textbf{37689-PI}) was synthesized by solid phase method at Peptide International, Louisville, Kentucky.

Common reaction solvents were either high performance liquid chromatography (HPLC) grade or American Chemical Society (ACS) grade, and used without further purification. HDAC Fluorimetric Assay kit and recombinant HDACs were procured from BIOMOL® International, PA. Analtech silica gel plates (60 F\(_{254}\)) were used for analytical TLC, and Analtech preparative TLC plates (UV 254, 2000 \(\mu\)m) were used for purification. UV light was used to examine the spots. 200-400 Mesh silica gel was used in column chromatography. Nuclear magnetic resonance (NMR) spectra were recorded on a Varian-Gemini 400 magnetic resonance spectrometer. \(^1\text{H}\) NMR spectra were recorded in parts per million (ppm) relative to the peak of CDCl\(_3\), (7.24 ppm), CD\(_3\)OD (3.31 ppm), DMSO-d\(_6\) (2.49 ppm) or acetone-d\(_6\) (2.04 ppm). \(^{13}\text{C}\) spectra were recorded
relative to the central peak of the CDCl$_3$ triplet (77.0 ppm), CD$_3$OD (49.0 ppm), DMSO-d$_6$ septet (39.7 ppm), or acetone-d$_6$ (2.04 ppm) and were recorded with complete hetero-decoupling. High-resolution mass spectra were recorded at the Georgia Institute of Technology mass spectrometry facility in Atlanta. Melting points were recorded on a MelTemp II and are uncorrected.

Methyl bromoalkanoates 1a-d, azido alkylesters, $^{15}$N-labeled azido alkylester, 4-ethynylbenzyl methylsulfonate 18, and alkynl aldehyde 41 were synthesized by adapting literature protocol. The Bestmann-Ohira reagent was prepared as described by Ghosh et al.17 Aryl alkynes that we could not obtain from commercial sources were synthesized using the Bestmann-Ohira reagent as described by Chen et al.51 Tris-(benzyltriazolylmethyl)amine (TBTA) is prepared by using the procedure described by Chan et al.52 6-Azido-O-silyl hexahydroxamate 21 was prepared from the corresponding azido carboxylic acid, t-BuPh$_2$SiCl and NaH, according to the procedure described by Muri et al.53

2.5.2 SAHA-Like Triazole-Linked HDACi Experimental

**Methyl 4-(phenyl)triazolylbutanoate (3a).** Methyl 4-azidobutanoate 2a (0.125 g, 0.87 mmol) and phenylacetylene (0.21 mL, 1.92 mmol) were dissolved in anhydrous THF (10 mL) and stirred under argon at room temperature. Copper (I) iodide (0.011 g, 0.07 mmol) and Hunig’s base (0.1 mL) were then added to the reaction mixture, and stirring continued for 24 h. The reaction mixture was diluted with CH$_2$Cl$_2$ (20 mL) and washed with 1:4 NH$_4$OH/saturated NH$_4$Cl (3 x 30 mL) and saturated NH$_4$Cl (30 mL). The organic layer was dried over Na$_2$SO$_4$ and concentrated *in vacuo*. The crude product was
purified by flash chromatography (silica, gradient 2:1; 3:2 Hexane/EtOAc) to give 117 mg (55%) of 3a as white solid. $^1$H-NMR (CDCl$_3$, 400MHz) δ 2.24 (2H, m), 2.36 (2H, t, $J$ = 6.4 Hz), 3.65 (3H, s), 4.45 (2H, t, $J$ = 6.8 Hz), 7.28-7.33 (1H, m), 7.37-7.41 (2H, m), 7.75 (1H, s), 7.78-7.81 (2H, m); $^{13}$C NMR (CDCl$_3$, 100 MHz) δ 25.5, 30.4, 49.2, 51.8, 119.5, 125.5, 127.9, 128.6, 130.3, 147.6, 172.5; HRMS (FAB, thioglycerol) calcd for [C$_{13}$H$_{15}$N$_3$O$_2$ + H]$^+$ 246.1242, found 246.1245.

**Methyl 5-(phenyl)triazolylpentanoate (3b).** Reaction of methyl 5-azidopentanoate 2b (0.211 g, 1.34 mmol) and phenylacetylene (0.3 mL, 2.79 mmol) within 24 h as described for the synthesis of 3a, followed by flash chromatography (silica, 1:1 Hexane/EtOAc), gave 180 mg (52%) of 3b as white solid. $^1$H NMR (CDCl$_3$, 400 MHz) δ 1.66-1.70 (2H, m), 1.97-2.01 (2H, m), 2.36 (2H, t, $J$ = 7.2 Hz), 3.65 (3H, s), 4.40 (2H, t, $J$ = 6.8 Hz), 7.31 (1H, t, $J$ = 8.0 Hz), 7.40 (2H, t, $J$ = 8.4 Hz) 7.74 (1H, s), 7.81 (2H, d, $J$ = 8.4 Hz); HRMS (FAB, thioglycerol) calcd for [C$_{14}$H$_{17}$N$_3$O$_2$ + H]$^+$ 260.1399, found 260.1386.

**Methyl 6-(phenyl)triazolylhexanoate (3c).** Reaction of methyl 6-azidohexanoate 2c (0.075 g, 0.43 mmol) and phenylacetylene (1.0 mL, 0.87 mmol) within 24 h as described for the synthesis of 3a, followed by prep TLC (silica, 1:1 Hexane/EtOAc) gave 94 mg (79%) of 3c as white solid. $^1$H NMR (CDCl$_3$, 400 MHz) δ 1.26-1.33 (2H, m), 1.56-1.64 (2H, m), 1.84-1.91 (2H, m), 2.24 (2H, t, $J$ = 7.2 Hz), 3.58 (3H, s), 4.30 (2H, t, $J$ = 7.2 Hz), 7.26 (1H, t, $J$ = 7.2 Hz), 7.35 (2H, t, 7.6 Hz), 7.74 (1H, s), 7.77 (2H, d, $J$ = 7.6 Hz); $^{13}$C NMR (CDCl$_3$, 100 MHz) δ 23.9, 25.6, 29.7, 33.3, 49.7, 51.3, 117.4, 125.3, 127.8, 128.5, 130.4, 147.3, 173.5.
Methyl 6-(4-anilyl)triazolylhexanoate (6a). Reaction of methyl 6-azidohexanoate \(2c\) (0.075 g, 0.44 mmol) and 4-ethynyl-\(N,N\)-dimethylaniline \(5a\) (131 mg, 0.90 mmol) within 24 h as described for the synthesis of \(3a\), followed by prep TLC (silica, 1:1 Hexane/EtOAc) gave 114 mg (83%) of \(6a\) as pale yellowish solid. \(^1\)H NMR (CDCl\(_3\), 400 MHz) \(\delta\) 1.30-1.36 (2H, m), 1.60-1.66 (2H, m), 1.87-1.93 (2H, m), 2.26 (2H, t, \(J = 7.2\) Hz), 2.93 (6H, s), 3.61 (3H, s), 4.30 (2H, t, \(J = 7.2\) Hz), 6.72 (2H, d, \(J = 8.8\) Hz), 7.58 (1H, s), 7.66 (2H, d, \(J = 9.2\) Hz); \(^{13}\)C NMR (CDCl\(_3\), 100 MHz) \(\delta\) 24.0, 25.8, 29.8, 33.5, 40.3, 49.8, 51.4, 112.3, 117.9, 118.7, 126.4, 148.0, 150.2, 173.7.

Methyl 6-(2,6-dimethoxyphenyl)triazolylhexanoate (6m). Reaction of methyl 6-azidohexanoate \(2c\) (0.15 g, 0.88 mmol) and 2-ethynyl-1,3-dimethoxybenzene \(5j\) (0.16 g, 0.99 mmol) within 24 h as described for the synthesis of \(3a\), followed by flash chromatography (silica, gradient 2:1; 1:1; 1:2; Hexane/EtOAc) gave 114 mg (39%) of \(6m\) as a white solid. \(^1\)H-NMR (CDCl\(_3\), 400MHz) \(\delta\) 1.38-1.46 (2H, m), 1.66-1.73 (2H, m), 1.95-2.02 (2H, m), 2.33 (2H, t, \(J = 7.2\) Hz), 3.66 (3H, s), 3.80 (6H, s), 4.40 (2H, t, \(J = 7.2\) Hz), 6.65 (2H, d, \(J = 8.4\) Hz), 7.29 (1H, t, \(J = 9.2\) Hz), 7.68 (1H, s); \(^{13}\)C-NMR (CDCl\(_3\), 100MHz) \(\delta\) 24.1, 25.8, 29.8, 33.5, 49.6, 51.2, 55.7, 103.8, 108.4, 123.7, 129.2, 139.3, 157.8, 173.2; HRMS (FAB, thioglycerol) calc for [C\(_{17}\)H\(_{23}\)N\(_3\)O\(_4\) + H]\(^+\) 334.1766, found 334.1776.

Methyl 6-(2-thiopyl)triazolylhexanoate (6n). Reaction of methyl 6-azidohexanoate \(2c\) (0.2 g, 1.16 mmol) and 2-ethynylthiophene \(5e\) (0.1 mL, 1.02 mmol) within 24 h as described for the synthesis of \(3a\), followed by flash chromatography (silica, gradient 2:1;
1:1 Hexane/EtOAc) gave 209 mg (74%) of 6n as a white solid. $^1$H-NMR (CDCl$_3$, 400MHz) δ 1.24-1.31 (2H, m), 1.54-1.62 (2H, m), 1.81-1.89 (2H, m), 2.22 (2H, t, $J = 7.2$ Hz), 3.57 (3H, s), 4.28 (3H, t, $J = 5.4$ Hz), 7.29 (1H, dd, $J = 4.8, 2.8$ Hz), 7.38 (1H, dd, $J = 4.8, 1.2$ Hz), 7.59 (1H, dd, $J = 3.2, 1.2$ Hz), 7.63 (1H, s); $^{13}$C-NMR (CDCl$_3$, 100MHz) δ 24.0, 25.7, 29.8, 33.5, 49.8, 51.3, 119.1, 120.5, 125.4, 125.9, 131.6, 143.4, 173.2; HRMS (FAB, thioglycerol) calc for [C$_{13}$H$_{17}$N$_3$O$_2$S + H]$^+$ 280.1244, found 280.1223.

**Methyl 6-(4-biphenyl)triazolylhexnoate (6o).** Reaction of Methyl 6-azidohexanoate 2c (0.2 g, 1.17 mmol) and 4-ethynylbiphenyl 5h (0.317 g, 1.75 mmol) within 24 h as described for the synthesis of 3a, followed by flash chromatography (silica, gradient 2:1; 1:1 Hexane/EtOAc) to give 90 mg (22%) of 6o as a pale yellowish solid. $^1$H-NMR (CDCl$_3$, 400MHz) δ 1.31-1.39 (2H, m), 1.61-1.69 (2H, m), 1.89-1.95 (2H, m), 2.28 (2H, t, $J = 7.2$ Hz), 3.62 (3H, s), 4.35 (2H, t, $J = 7.2$ Hz), 7.32 (1H, t, $J = 8.0$ Hz), 7.41 (2H, t, $J = 8.0$ Hz), 7.58-7.63 (4H, m), 7.76 (1H, s), 7.87 (2H, d, $J = 8.0$ Hz); $^{13}$C-NMR (CDCl$_3$, 100MHz) δ 24.1, 25.9, 29.9, 33.6, 49.9, 51.4, 119.4, 125.7, 126.6, 127.1, 128.5, 129.4, 140.1, 140.4, 147.0, 173.3; HRMS (FAB, thioglycerol) calc for [C$_{21}$H$_{23}$N$_3$O$_2$ + H]$^+$ 350.1868, found 350.1885.

**Methyl 6-(3-biphenyl)triazolylhexnoate (6p).** Reaction of methyl 6-azidohexanoate 2c (0.2 g, 1.16 mmol) and 3-ethynylbiphenyl 5i (0.32 g, 1.79 mmol) within 24 h as described for the synthesis of 3a, followed by flash chromatography (silica, gradient 2:1; 1:1 Hexane/EtOAc) gave 300 mg (74%) of 6p as a white solid. $^1$H-NMR (CDCl$_3$, 400MHz) δ 1.27-1.35 (2H, m), 1.58-1.65 (2H, m), 1.85-1.93 (2H, m), 2.25 (2H, t, $J = 7.6$ Hz), 3.57 (3H, s), 4.28 (3H, t, $J = 5.4$ Hz), 7.29 (1H, dd, $J = 4.8, 2.8$ Hz), 7.38 (1H, dd, $J = 4.8, 1.2$ Hz), 7.59 (1H, dd, $J = 3.2, 1.2$ Hz), 7.63 (1H, s); $^{13}$C-NMR (CDCl$_3$, 100MHz) δ 24.0, 25.7, 29.8, 33.5, 49.8, 51.3, 119.1, 120.5, 125.4, 125.9, 131.6, 143.4, 173.2; HRMS (FAB, thioglycerol) calc for [C$_{13}$H$_{17}$N$_3$O$_2$S + H]$^+$ 280.1244, found 280.1223.
Methyl 6-(2-biphenyl)triazolylhexanoate (6r). Reaction of methyl 6-azidohexnoate 2c (0.16 g, 0.93 mmol) and 2-ethynylbiphenyl 5g (0.11 g, 0.62 mmol) within 24 h as described for the synthesis of 3a, followed by flash chromatography (silica, gradient 2:1; 1:1 Hexane/EtOAc) gave 161 mg (75%) of 6r as a white solid. $^1$H-NMR (CDCl$_3$, 400MHz) $\delta$ 1.09-1.17 (2H, m), 1.49-1.57 (2H, m), 1.61-1.69 (2H, m), 2.21 (2H, t, $J = 7.6$ Hz), 3.59 (3H, s), 4.09 (2H, t, $J = 6.8$ Hz), 6.35 (1H, s), 7.16-7.18 (2H, m), 7.24-7.34 (5H, m), 7.39 (2H, t, $J = 7.2$ Hz); $^{13}$C NMR (CDCl$_3$, 100 MHz) $\delta$ 24.0, 25.6, 29.6, 33.4, 49.5, 51.3, 121.8, 126.9, 127.4, 127.5, 128.0, 128.2, 128.8, 128.9, 129.7, 139.8, 141.3, 145.8, 173.2; HRMS (FAB, thioglycerol) calc for [C$_{21}$H$_{23}$N$_3$O$_2$ + H]$^+$ 350.1868, found 350.1877.

Methyl 6-(6-methoxynapthalyl)triazolylhexanoate (6u). Reaction of methyl 6-azidohexnoate 2c (0.2 g, 1.16 mmol) and 2-ethynyl-6-methoxynaphthalene 5f (0.32 g, 1.76 mmol) within 24 h as described for the synthesis of 3a, followed by flash chromatography (silica, gradient 2:1; 1:1; 1:2; 0:1 Hexane/EtOAc) gave 284 mg (70%) of 6u as a white solid. $^1$H-NMR (CDCl$_3$, 400MHz) $\delta$ 1.32-1.39 (2H, m), 1.61-1.69 (2H, m), 1.87-1.99 (2H, m), 2.28, (2H, t, $J = 7.2$ Hz), 3.62 (3H, s), 3.89 (3H, s), 4.36 (2H, t, $J = 6.8$ Hz), 7.32 (1H, t, $J = 7.2$ Hz), 7.39-7.46 (3H, m), 7.52 (1H, d, $J = 8.0$ Hz), 7.62 (2H, d, $J = 7.6$ Hz), 7.77 (1H, d, $J = 7.6$ Hz), 7.82 (1H, s), 8.08 (1H, s); $^{13}$C-NMR (CDCl$_3$, 100 MHz) $\delta$ 23.9, 25.7, 29.7, 33.4, 49.7, 51.2, 119.5, 123.9, 124.1, 126.3, 126.7, 127.0, 128.3, 128.8, 130.8, 140.2, 141.2, 147.0, 173.2; HRMS (FAB, thioglycerol) calc for [C$_{21}$H$_{23}$N$_3$O$_2$ + H]$^+$ 350.1868, found 350.1870.
Representative Procedure for Conversion of Methyl Ester to Hydramic Acid. 4-(phenyl)triazolylbutahydroxamic acid (4a). To a solution of methyl 4-(phenyl)triazolylbutanoate 3a (0.05 g, 0.204 mmol) in 1:1 THF (1.5 mL) and methanol (1.5 mL) was added aqueous hydroxylamine (0.13 mL, 2.11 mmol) and KCN (0.004 g, 0.062 mmol), and the stirring continued for 24 h. The reaction was diluted with EtOAc (30 mL) and washed with saturated NaHCO₃ (2 x 30 mL) and saturated brine (30 mL). The organic layer was dried over Na₂SO₄ and concentrated in vacuo to give 40 mg (80%) of 4a as white solid. ¹H NMR (DMSO-d₆, 400 MHz) δ 2.98 (2H, t, J = 7.2 Hz), 2.04-2.11 (2H, m), 4.39 (2H, t, J = 6.8 Hz), 7.31 (1H, t, J = 8.0 Hz), 7.82 (2H, d, J = 8.4 Hz), 8.55 (1H, s); ¹³C NMR (CDCl₃, 100 MHz) δ 24.2, 25.9, 30.0, 33.6, 50.0, 51.5, 55.2, 105.6, 119.0, 119.2, 124.0, 124.1, 125.7, 127.1, 128.7, 129.4, 134.0, 147.6, 157.5, 173.4; HRMS (FAB, thioglycerol) calcd for [C₂₀H₂₃N₅O₃ + H]⁺ 354.1817, found 354.1819.

5-(phenyl)triazolypentahydroxamic acid (4b). Reaction of methyl 5-(phenyl)triazolypentanoate 3b (0.06 g, 0.231 mmol) and aqueous hydroxylamine (0.15 ml, 2.44 mmol) within 24 h as described for the synthesis of 4a, gave 30 mg (50%) of 4b as white solid. ¹H NMR (DMSO-d₆, 400 MHz) δ 1.45-1.49 (2H, m), 1.80-1.84 (2H, m), 1.98 (2H, t, J = 6.8 Hz), 4.38 (2H, t, J = 6.8 Hz), 7.30 (1H, t, J = 8.0 Hz), 7.42 (2H, t, J =
7.2 Hz), 7.81 (2H, d, J = 7.6 Hz), 8.54 (1H, s), 8.67 (1H, s), 10.3 (1H, s); $^{13}$C NMR (CDCl$_3$, 100 MHz) $\delta$ 22.1, 29.2, 31.5, 49.1, 121.0, 124.8, 127.5, 128.6, 130.6, 146.0, 168.3; HRMS (FAB, thioglycerol) calcd for [C$_{13}$H$_{16}$N$_4$O$_2$ + H]$^+$ 261.1351, found 261.1354; M.P. = 151.0-152.0 °C.

6-(phenyl)triazolylhexahydroxamic acid (4c). Reaction of methyl 6-(phenyl)triazolylhexanoate 3c (0.15 g, 0.522 mmol) and aqueous hydroxylamine (0.37 ml, 6.03 mmol) within 24 h as described for the synthesis of 4a, gave 79 mg (55%) of 4c as white solid. $^1$H NMR (DMSO-$d_6$, 400 MHz) $\delta$ 1.18-1.26 (2H, m), 1.48-1.55 (2H, m), 1.81-1.88 (2H, m), 1.93 (2H, t, J = 7.2 Hz), 4.36 (2H, t, J = 7.2 Hz), 7.31 (1H, t, J = 7.2 Hz), 7.42 (2H, t, J = 6.8 Hz), 7.82 (2H, d, J = 8.0 Hz), 8.56 (1H, s), 8.68 (1H, s), 10.3 (1H, s); $^{13}$C NMR (CDCl$_3$, 100 MHz) $\delta$ 24.5, 25.4, 29.3, 32.0, 49.3, 121.0, 124.8, 127.5, 128.6, 130.6, 146.0, 168.6; HMS (FAB, thioglycerol) calcd for [C$_{14}$H$_{18}$N$_4$O$_2$ + H]$^+$ 275.1508, found 275.1513.

6-(4-anilyl)triazolylhexahydroxamic acid (7a). Reaction of methyl 6-(4-anilyl)triazolyl-hexanoate 6a (0.1 g, 0.303 mmol) and aqueous hydroxylamine (0.6 ml, 9.78 mmol) within 24 h as described for the synthesis of 4a, gave 50 mg (53%) of 7a as white solid. $^1$H NMR (CD$_3$OD, 400 MHz) $\delta$ 1.20-1.26 (2H, m), 1.48-1.56 (2H, m), 1.79-1.86 (2H, m), 1.93 (2H, t, J = 6.8 Hz), 2.917 (6H, s), 4.32 (2H, t, J = 6.8 Hz), 6.76 (2H, d, J = 8.8 Hz), 7.63 (2H, d, J = 8.8 Hz), 8.32 (1H, s), 8.66 (1H, s), 10.3 (1H, s); $^{13}$C NMR (DMSO-$d_6$, 100 MHz) $\delta$ 25.3, 26.3, 30.2, 32.8, 50.0, 112.9, 119.4, 119.9, 126.6, 147.4,
HRMS (FAB, thioglycerol) calcd for [C$_{16}$H$_{23}$N$_4$O$_2$ + H]$^+$ 318.1930, found 318.1929; M.P. = 165.0-166.0 °C.

**6-(2,6-dimethoxyphenyl)triazolylhexahydroxamic acid (7m).** Reaction of methyl 6-(2,6-dimethoxyphenyl)triazolylhexanoate 6m (0.1 g, 0.30 mmol) and aqueous hydroxylamine (0.4 mL, 6.52 mmol) within 24 h as described for the synthesis of 4a, gave 90 mg (90%) of 7m as a white solid. $^1$H-NMR (DMSO-$d_6$, 400MHz) $\delta$ 1.33-1.41 (2H, m), 1.63-1.71 (2H, m), 1.92-1.99 (2H, m), 2.10 (2H, t, $J$ = 7.2 Hz), 3.76 (6H, s), 4.42 (2H, t, $J$ = 6.8 Hz), 6.71 (2H, d, $J$ = 8.4 Hz), 7.32 (1H, t, $J$ = 8.4 Hz), 7.96 (1H, s); $^{13}$C-NMR (DMSO-$d_6$, 100MHz) $\delta$ 26.1, 27.0, 31.0, 33.5, 51.0, 55.2, 104.9, 108.9, 126.1, 131.1, 140.7, 148.9, 159.4, 172.3; HRMS (FAB, thioglycerol) calc for [C$_{16}$H$_{22}$N$_4$O$_2$ + H]$^+$ 335.1719, found 335.1724; M.P. = 98.0-100.0 °C.

**6-(2-thiopyl)triazolylhexahydroxamic acid (7n).** Reaction of methyl 6-thiopyl-triazolylhexanoate 6n (0.1 g, 0.35 mmol) and aqueous hydroxylamine (0.2 mL, 3.26 mmol) within 24 h as described for the synthesis of 4a, gave 54 mg (55%) of 7n as a white solid. $^1$H-NMR (DMSO-$d_6$, 400MHz) $\delta$ 1.17-1.24 (2H, m), 1.47-1.55 (2H, m), 1.79-1.86 (2H, m), 1.92 (2H, t, $J$ = 7.2 Hz), 4.35 (3H, t, $J$ = 6.8 Hz), 7.48 (1H, d, $J$ = 5.2 Hz), 7.62 (1H, dd, $J$ = 5.2, 3.2 Hz), 7.80 (1H, d, $J$ = 2.8 Hz), 8.42 (1H, s), 8.68 (1H, s), 10.36 (1H, s); $^{13}$C-NMR (DMSO-$d_6$, 100MHz) $\delta$ 24.4, 25.3, 29.3, 31.9, 49.1, 120.3, 120.7, 125.4, 126.8, 131.8, 142.4; HRMS (FAB, thioglycerol) calc for [C$_{12}$H$_{16}$N$_4$O$_2$S + H]$^+$ 281.1072, found 281.1087; M.P. = 148.0-149.0 °C.
6-(4-biphenyl)triazolylhexahydroxamic acid (7o). Reaction of methyl 6-(4-biphenyl)-triazolylhexanoate 6o (0.09 g, 0.258 mmol) and aqueous hydroxylamine (0.2 mL, 3.26 mmol) within 24 h as described for the synthesis of 4a, gave 35 mg (39%) of 7o as a white solid. $^1$H-NMR (DMSO-$d_6$, 400MHz) δ 1.50-1.60 (2H, m), 1.87 (2H, t, $J = 7.2$ Hz), 1.94 (2H, t, $J = 7.6$ Hz), 2.30 (2H, t, $J = 7.6$ Hz), 4.39 (2H, t, $J = 6.8$ Hz), 7.36 (1H, t, $J = 7.2$ Hz), 7.46 (2H, t, $J = 7.6$ Hz), 7.72 (4H, q, $J = 17.6$, 8.0 Hz), 7.921 (2H, t, $J = 8.0$ Hz), 8.61 (1H, s), 10.3 (1H, s); HRMS (FAB, thioglycerol) calc for [C$_{20}$H$_{22}$N$_4$O$_2$ + H]$^+$ 351.1828, found 351.1864. [Note: Strong aggregation prevent collection of $^{13}$C NMR.]

6-(3-biphenyl)triazolylhexahydroxamic acid (7p). Reaction of methyl 6-(3-biphenyl)-triazolylhexanoate 6p (0.12 g, 0.34 mmol) and aqueous hydroxylamine (0.4 mL, 6.52 mmol) within 24 h as described for the synthesis of 4a, gave 73 mg (61%) of 7p as a white solid. $^1$H-NMR (DMSO-$d_6$, 400MHz) δ 1.18-1.21 (2H, m), 1.48-1.51 (2H, m), 1.81-1.85 (2H, m), 1.92 (2H, t, $J = 6.4$ Hz), 4.36 (2H, t, $J = 6.8$ Hz), 7.36 (1H, t, $J = 6.0$ Hz), 7.44-7.54 (3H, m), 7.59 (1H, d, $J = 8.0$ Hz), 7.68 (2H, d, $J = 7.2$ Hz), 8.06 (1H, s), 8.59 (1H, s), 8.85 (1H, br s), 10.48 (1H, s); $^{13}$C-NMR (DMSO-$d_6$, 100MHz) δ 24.9, 25.8, 29.7, 32.5, 50.0, 121.9, 123.6, 124.5, 126.6, 127.0, 128.0, 129.3, 130.0, 131.4, 140.0, 141.1, 146.5, 169.7; HRMS (FAB, thioglycerol) calc for [C$_{20}$H$_{22}$N$_4$O$_2$ + H]$^+$ 351.1821, found 351.1832; M.P. = 101.0-102.0 °C.

6-(2-biphenyl)triazolylhexahydroxamic acid (7r). Reaction of methyl 6-(2-biphenyl)-triazolylhexanoate 6r (0.1 g, 0.28 mmol) and aqueous hydroxylamine (0.4 mL, 6.52
mmol) within 24 h as described for the synthesis of 4a, gave 42 mg (43%) of 7r as a white solid. $^1$H-NMR (DMSO-$d_6$, 400MHz) δ 0.85-1.01 (2H, m), 1.37-1.43 (2H, m), 1.56-1.59 (2H, m), 1.88 (2H, t, $J = 7.2$ Hz), 4.15 (2H, t, $J = 6.4$ Hz), 7.00 (1H, s), 7.12 (2H, d, $J = 6.8$ Hz), 7.32 (4H, d, $J = 5.2$ Hz), 7.40-7.46 (2H, m), 7.76 (1H, d, $J = 8.0$ Hz), 8.79 (1H, br s), 10.44 (1H, s); $^{13}$C-NMR (DMSO-$d_6$, 100MHz) δ 24.9, 25.6, 29.7, 32.5, 49.5, 123.2, 127.5, 128.0, 128.5, 128.6, 129.1, 129.2, 129.4, 130.4, 140.4, 141.1, 145.6, 169.5; HRMS (FAB, thioglycerol) calc for [C$_{20}$H$_{22}$N$_4$O$_2$ + H]$^+$ 351.1821, found 351.1842; M.P. = 115.0-117.0 °C.

6-(6-methoxynapthaly)triazolylhexahydroxamic acid (7u). Reaction of methyl 6-(6-methoxynapthalenyl)triazolylhexanoate 6u (0.095 g, 0.27 mmol) and aqueous hydroxylamine (0.2 mL, 3.26 mmol) within 24 h as described for the synthesis of 4a, gave 31 mg (33%) of 7u as a white solid. $^1$H-NMR (DMSO-$d_6$, 400MHz) δ 1.23-1.27 (2H, m), 1.51-1.57 (2H, m), 1.85-1.97 (4H, m), 2.28, 3.88 (3H, s), 4.40 (2H, t, $J = 6.4$ Hz), 7.19 (1H, d, $J = 10.4$ Hz), 7.33 (1H, s), 7.90 (3H, dd, $J = 22.4$, 9.2 Hz), 8.30 (1H, s), 8.63 (2H, d, $J = 12.0$ Hz), 10.32 (1H, s); $^{13}$C-NMR (DMSO-$d_6$, 100MHz) δ 24.5, 25.4, 29.3, 32.0, 49.3, 55.1, 105.8, 118.9, 120.9, 123.1, 123.9, 125.7, 127.1, 128.3, 129.2, 133.6,146.2,157.1; HRMS (FAB, thioglycerol) calc for [C$_{19}$H$_{22}$N$_4$O$_3$ + H]$^+$ 355.1809, found 355.1806; M.P. = 166.0-167.0 °C.

2.5.3 Nonpeptide Macrocyclic HDACi Experimental

Synthesis of Methyl 8-(4-(hydroxymethyl)phenylamino)-8-oxooctanoate (10). To a solution of (4-aminophenyl)methanol 9 (0.314 g, 2.500 mmol) in anhydrous pyridine (5
mL) was added chlorotrimethylsilane (0.32 mL, 2.500 mmol) at room temperature and stirring continued for 2 h. The reaction was cooled in ice bath to 0°C and to the mixture was added methyl 8-chloro-8-oxooctanoate 8 (0.32 mL, 2.200 mmol) and a catalytic amount of DMAP. The reaction was allowed to warm to room temperature and stirring continued overnight. Water (5 mL) and 1 M TBAF in tetrahydrofuran (THF) (0.25 mL, 0.250 mmol) were added and stirring continued for additional 30 min. EtOAc (50 mL) and 1N HCl (30 mL) were added, the two layers were separated, the organic layer was washed with 1N HCl (30 mL) and saturated brine (30 mL) and dried over Na₂SO₄. Solvent was evaporated off and the crude was purified by preparative TLC, eluting with EtOAc/hexanes 2:1 to give 275 mg (43 %) compound 10 as a yellow-white solid. 

1H-NMR (DMSO-d₆, 400 MHz) δ 1.26 (4H, m), 1.47-1.56 (4H, m), 2.26 (4H, m), 3.55 (3H, s), 4.40 (2H, d, J = 5.6 Hz), 5.06 (1H, t, J = 5.6 Hz), 7.19 (2H, d, J = 8.8 Hz), 7.51 (2H, d, J = 8.8 Hz), 9.79 (1H, s); 13C NMR (DMSO-d₆, 100MHz) δ 24.3, 24.9, 28.2, 28.3, 33.2, 36.3, 51.2, 62.6, 118.8, 126.9, 136.9, 138.0, 171.2, 173.4; HRMS (FAB, thioglycerol) calc for [C₁₆H₂₃NO₄ + H]⁺ 294.1705, found 294.1652

4'-Desmethyl azithromycin (13). To a solution of azithromycin (2.000 g, 2.548 mmol) and sodium acetate (1.78 g, 21.500 mmol) in 80% aqueous methanol (30 mL) at 90 °C was added iodine (0.700 g, 2.756 mmol) in three batches within 5 min. The reaction was maintained at pH 8-9 by addition of 1M NaOH (2 ml, once at 10 min of reaction time) and stirring continued for 3 h. The reaction was poured into cold water containing 5% sodium thiosulfate (80 mL) and extracted with CH₂Cl₂ (2 x 40 mL). The aqueous layer was basified with concentrated NH₄OH, extracted with 10 % MeOH in CH₂Cl₂ (3 x 40
mL) and the organic layer was dried over dried over Na₂SO₄. Solvent was evaporated off to give 1.57 g of compound 13 as an off white solid (>90 % purity, TLC: CHC₂Cl₂/MeOH/ NH₄OH 12:1:0.1). The crude 13 was used without further purification.

**Azithromycin-arylalkyl methyl ester (14).** To a solution of compound 10 (0.175 g, 0.597 mmol) in CH₂Cl₂ (7 mL) and triethylamine (Et₃N) (0.24 mL, 1.800 mmol) was added mesyl chloride (0.10 mL, 1.200 mmol) at 0°C and the reaction was allowed to warm to room temperature. Stirring continued for 1 h during which TLC revealed a quantitative conversion into a higher Rₜ product. CH₂Cl₂ (40 mL) and saturated sodium bicarbonate (30 mL) were added, the two layers were separated. The organic layer was washed with sodium bicarbonate (2 x 30 mL), saturated brine (30 mL) and dried over Na₂SO₄. Solvent was evaporated off to give compound 11 as white solid.

A mixture of 4’-desmethylazithromycin 13 (0.315 g, 0.430 mmol) and crude compound 11, in anhydrous DMSO (7 mL) and Hunig’s base (0.7 mL) was stirred at 85°C for 1.5 h. The reaction was cooled and diluted with EtOAc (60 mL) and washed with saturated NaHCO₃ (40 mL) and saturated brine (40 mL). The organic layer was dried over Na₂SO₄, concentrated in vacuo and the crude was purified by preparative TLC, eluting with EtOAc/hexanes/ Et₃N 3:2:0.1 to give 152 mg (35 %) of compound 14 as a white solid. ¹H-NMR (CDCl₃, 400MHz) δ 0.78-0.82 (7H, m), 0.94-1.03 (11H, m), 1.09-1.32 (22H, m), 1.34-1.58 (5H, m), 1.59- 1.73 (4H, m), 1.77-2.01 (5H, m), 2.12 (3H, s), 2.20-2.28 (8H, m), 2.42-2.53 (2H, m), 2.54-2.71 (3H, m), 2.87-3.01 (2H, m), 3.11 (3H, s), 3.25-3.33 (3H, m), 3.42 (2H, m), 3.54-3.65 (6H, m), 3.97 (1H, m), 4.18 (1H, m), 4.36 (1H, d, J = 6.8 Hz), 4.61 (2H, m), 5.03 (1H, d, J = 4.4 Hz), 7.14 (2H, d, J = 8.4 Hz), 7.42
(2H, d, $J = 8.4$ Hz), 7.73 (1H, s), 8.97 (1H, bs); $^{13}$C-NMR (CDCl$_3$, 100MHz) $\delta$ 7.5, 9.1, 11.3, 14.8, 16.2, 16.9, 18.2, 20.5, 21.2, 21.4, 21.5, 21.9, 24.6, 25.3, 26.7, 27.5, 28.7, 29.6, 33.9, 34.7, 36.2, 36.7, 37.3, 39.1, 41.9, 42.2, 45.1, 48.5, 49.3, 51.4, 57.3, 62.2, 64.3, 65.4, 68.5, 69.9, 70.5, 72.7, 73.5, 73.8, 74.1, 77.7, 77.9, 83.4, 94.4, 102.6, 119.5, 129.0, 134.2, 137.0, 171.0, 173.8, 178.4 MS; HRMS (FAB, mnba) calc for [C$_{53}$H$_{91}$N$_3$O$_{15}$ + H]$^+$ 1010.6529, found 1010.6450

Azithromycin-arylalkyl hydroxamic acid (15). To a solution of compound 14 (0.050 g, 0.050 mmol) in 1:1 THF/MeOH (2 mL) was added hydroxylamine (50 % in H$_2$O) (0.07 mL, 1.260 mmol) and a catalytic amount of KCN. The mixture was stirred at room temperature for 24 h. The reaction was partitioned between 5 % MeOH in CH$_2$Cl$_2$ (30 mL) and saturated sodium bicarbonate (25 mL), the two layers were separated and the aqueous layer was extracted with 5 % MeOH in CH$_2$Cl$_2$ (2 x 20 mL). The combined organic layer was washed with saturated brine (40 mL) and dried over Na$_2$SO$_4$. Solvent was evaporated off and the crude was purified by preparative TLC, eluting with CH$_2$Cl$_2$/MeOH/NH$_4$OH 10:1:0.1 to give 26 mg (52 %) compound 15 as brown-white solid. $^1$H-NMR (CD$_3$OD, 400MHz) $\delta$ 0.87-0.92 (6H, m), 1.03-1.12 (12H, m), 1.17-1.37 (m), 1.43-1.69 (m), 1.75-1.88 (6H, m), 1.99 (4H, m). 2.08 (3H, m), 2.13-2.19 (3H, m), 2.24-2.41 (13H, m), 2.54 (1H, d, $J = 11.2$ Hz), 2.75-2.80 (4H, m), 3.00 (1H, d, $J = 9.6$ Hz), 3.19 (4H, m), 3.47-3.51 (1H, m), 3.60-3.78 (6H, m), 4.14-4.22 (3H, m), 4.50 (1H, d, $J = 7.2$ Hz), 5.02 (1H, d, $J = 4.8$ Hz), 7.29 (2H, d, $J = 8.0$ Hz), 7.49 (2H, d, $J = 8.4$ Hz); $^{13}$C-NMR (Acetone-d$_6$, 100MHz) $\delta$ 7.6, 9.7, 11.6, 14.3, 15.3, 17.4, 18.9, 21.7, 21.9, 22.2, 22.5, 26.0, 26.1, 27.3, 27.9, 30.9, 32.6, 33.2, 35.5, 36.5, 36.8, 37.6, 43.0, 43.1, 46.1, 49.6, 62
Desclasinose azithromycin-arylalkyl hydroxamate (17). A mixture of compound 14 (0.050 g, 0.050 mmol) in 0.25 N HCl (15 mL) was stirred at room temperature for 20 h and poured into EtOAc (20 mL). The two layers were separated and the aqueous layer was washed with EtOAc (2 x 20 mL), basified with concentrated NH₄OH and then extracted with 5 % MeOH in CH₂Cl₂ (2 x 30 mL). The combined organic layer was washed with saturated brine (30 mL) and dried over Na₂SO₄. Solvent was evaporated off to give compound 16 which was used for the next reaction without further purification.

To a solution of compound 16 in 1:1 THF/MeOH (2 mL) was added hydroxylamine (50 % in H₂O) (0.05 mL, 0.790 mmol) and a catalytic amount of KCN. The mixture was stirred at room temperature for 24 h. The reaction was partitioned between 5 % MeOH in CH₂Cl₂ (30 mL) and saturated brine (20 mL), the two layers were separated and the organic layer was dried over Na₂SO₄. Solvent was evaporated off and the crude was purified by preparative TLC, eluting with CH₂Cl₂/ MeOH/NH₄OH 9:1:0.1 to give 7 mg (16 %) compound 17 as brown-white solid. ¹H-NMR (CD₃OD, 400MHz) δ 0.78 (3H, m), 0.85 (3H, d, J = 7.2 Hz), 0.91 (3H, d, J = 8.0 Hz), 0.99 (3H, s), 1.08-1.14 (10H, m), 1.18-1.79 (20H, m), 1.87 (2H, m), 1.98 (2H, t, J = 7.4 Hz), 2.05-2.13 (2H, m), 2.17 (3H, s), 2.25 (2H, t, J = 7.4 Hz), 2.52-2.65 (4H, m), 2.95 (1H, bs), 3.24 (m), 3.37-3.56 (6H, m), 3.67 (1H, d, J = 13.2 Hz), 4.53 (2H, d, J = 7.6 Hz), 7.19 (2H, d, J = 8.4
Hz), 7.39 (2H, d, $J = 8.4$ Hz); HRMS (EI) cale for $[C_{44}H_{76}N_4O_{12} + H]^+$ 853.5538, found 853.5488

4'-Ethynylbenzyl azithromycin (19). To a solution of 4'-desmethylazithromycin 13 (0.940 g, 1.280 mmol) in anhydrous DMSO (15 ml) was added Hunig’s base (1.5 ml) and 4-ethynylbenzyl methanesulfonate 18 (0.380 g, 1.800 mmol). The reaction mixture was heated with stirring under argon at 85°C for 2 h. The reaction was cooled and diluted with EtOAc (100 mL) and washed with saturated NaHCO$_3$ (3 x 60 mL) and saturated brine (60 mL). The organic layer was dried over Na$_2$SO$_4$ and concentrated in vacuo. The crude product was purified by flash chromatography (silica, 12:1:0.05 CH$_2$Cl$_2$/MeOH/conc. NH$_4$OH) to give 850 mg (78%) of 19 as a brown-white solid. $^1$H-NMR (CDCl$_3$, 400MHz) $\delta$ 0.86 (6H, m), 0.99 (3H, d, $J = 7.6$ Hz), 1.05 (7H, m), 1.11-1.32 (19H, m), 1.36-1.55 (2H, m), 1.68-1.77 (2H, m), 1.81-2.07 (4H, m), 2.22 (3H, s), 2.27-2.31 (4H, m), 2.51 (2H, m), 2.65-2.75 (2H, m), 2.87 (1H, bs), 2.98 (1H, t, $J = 9.8$ Hz), 3.04 (1H, s), 3.10 (3H, s), 3.29-3.34 (2H, m), 3.40-3.47 (2H, m), 3.58 (1H, d, $J = 6.8$ Hz), 3.65 (1H, s), 3.74 (1H, d, $J = 13.2$ Hz), 3.99 (1H, m), 4.21 (1H, dd, $J = 2$ Hz, 4.4 Hz), 4.38 (1H, d, $J = 7.2$ Hz), 4.65 (1H, dd, $J = 2.8$ Hz, 10 Hz), 5.06 (1H, d, $J = 4.4$ Hz), 7.23 (2H, d, $J = 8$ Hz), 7.41 (2H, d, $J = 8$ Hz); $^{13}$C-NMR (CDCl$_3$, 100MHz) $\delta$ 7.5, 9.2, 11.3, 14.9, 16.3, 18.3, 21.3, 21.4, 21.6, 22.0, 26.8, 27.6, 29.6, 34.7, 36.3, 36.9, 42.0, 42.3, 45.2, 49.2, 57.7, 62.3, 63.7, 65.4, 68.5, 70.0, 70.6, 72.7, 73.5, 73.8, 74.2, 77.1, 77.9, 78.0, 83.4, 83.7, 94.5, 102.6, 120.8, 128.5, 132.0, 139.6, 178.3; HRMS (FAB, mnba) cale for $[C_{46}H_{76}N_2O_{12} + H]^+$ 849.5476, found 849.5411
Descladinose-4’-ethynylbenzyl azithromycin (20). A solution of 4’-ethynylbenzyl azithromycin 19 (0.12 g, 0.14 mmol) in 0.25 N HCl (15 mL) was stirred at room temperature for 20 h and poured into EtOAc (20 mL). The two layers were separated and the aqueous layer was washed with EtOAc (2 x 20 mL), basified with concentrated NH₄OH and then extracted with 5 % MeOH in CH₂Cl₂ (2 x 30 mL). The combined organic layer was washed with saturated brine (30 mL) and dried over Na₂SO₄. Solvent was evaporated off to give 89 mg (91%) of descladinose compound 20 as a white solid.

1H-NMR (CDCl₃, 400MHz) δ 0.80-1.52 (16H, m), 1.65-1.90 (2H, m), 1.97 (3H, s), 2.20-2.30 (6H, m), 2.42-2.69 (3H, m), 2.77 (1H, s), 2.89 (1H, s), 3.02 (1H, s), 3.18 (1H, s), 3.30-3.37 (3H, m), 3.49-3.63 (6H, m), 3.72 (2H, d, J = 10.6 Hz), 3.84 (1H, s), 3.89 (2H, s), 4.04 (3H, q, J = 14.4, 7.2 Hz), 4.41 (1H, d, J = 7.2 Hz), 4.64 (1H, d, J = 10.8 Hz), 7.15 (2H, d, J = 8.0 Hz), 7.37 (2H, d, J = 8.4 Hz); 13C NMR (CDCl₃, 100 MHz) δ 7.7, 7.9, 10.9, 14.2, 16.1, 20.9, 21.0, 21.2, 25.8, 26.6, 29.2, 35.9, 36.4, 37.0, 40.5, 44.5, 57.6, 60.3, 62.4, 65.3, 69.8, 70.4, 70.9, 73.0, 74.1, 75.4, 79.4, 83.3, 94.9, 106.4, 120.8, 128.2, 131.9, 139.2, 171.0, 177.2; HRMS (FAB, thioglycerol) calc for [C₃₈H₆₂N₂O₉ + H]⁺ 691.4533, found 691.4513

Azithromycin-N-benzyltriazolyl-O-silyl-hexahydroxamate (22). 4’-Ethynylbenzyl azithromycin 19 (0.045 g, 0.050 mmol) and 6-azido-O-silyl hexahydroxamate 21 (0.060 g, 0.146 mmol) were dissolved in anhydrous THF (5 mL) and stirred under argon at room temperature. Copper (I) iodide (0.010 g, 0.050 mmol), Hunig’s base (0.5 mL) and tris[(1-benzyl-1H-1,2,3-triazol-4-yl)methyl]amine, (TBTA) (0.016 g, 0.030 mmol) were then added to the reaction mixture, and stirring continued for 2 h. The reaction mixture was
diluted with CH₂Cl₂ (40 mL) and washed with 1:4 NH₄OH/saturated NH₄Cl (2 x 30 mL) and saturated NH₄Cl (30 mL). The organic layer was dried over Na₂SO₄ and concentrated in vacuo. The crude product was purified by prep TLC (silica, 12:1:0.1 CH₂Cl₂/MeOH/conc. NH₄OH) to give 38 mg (60%) of silyl protected compound 22 as a brown-white solid. ¹H-NMR (CDCl₃, 400MHz) δ 0.82-1.52 (39H, m) 1.71-2.04 (13H, m), 2.21 (3H, s), 2.28 (6H, s), 2.44-2.57 (2H, m), 2.65-2.70 (2H, m), 2.96 (1H, br s), 3.09 (2H, s), 3.31-3.35 (2H, m), 3.40-3.48 (6H, m), 3.56-3.60 (1H, m), 3.76 (1H, d, J = 13.2 Hz), 4.00 (1H, br s), 4.20 (3H, br s), 4.39 (1H, d, J = 6.8 Hz), 4.64 (1H, d, J = 9.2 Hz), 5.09 (1H, br s) 7.29-7.41 (10H, m), 7.64-7.83 (5H, m); ¹³C NMR (CDCl₃, 100 MHz) δ 7.4, 9.1, 11.3, 14.7, 16.3, 18.2, 21.3, 21.4, 21.5, 22.0, 26.7, 27.5, 29.6, 29.7, 29.9, 34.6, 36.2, 36.9, 42.2, 45.2, 49.3, 49.9, 57.6, 62.4, 64.1, 65.4, 68.5, 69.9, 70.5, 72.7, 73.5, 74.1, 77.7, 77.9, 83.4, 94.3, 102.7, 119.2, 125.5, 127.6, 129.0, 135.5, 147.1, 178.4; HRMS (ESI) calcd for [C₆₈H₁₀₆N₆O₁₄Si + H]+ 1259.7609, found 1259.7570

Methyl azithromycin-N-benzyltriazolylhexanoate (26a). 4’-Ethynylbenzyl azithromycin 19 (0.045 g, 0.053 mmol) and azido-ester 25 (0.014 g, 0.080 mmol) were dissolved in anhydrous THF (5 mL) and stirred under argon at room temperature. Copper (I) iodide (0.010 g, 0.053 mmol), and Hunig’s base (0.05 mL) were then added to the reaction mixture, and stirring continued for 12 h. The reaction mixture was diluted with CH₂Cl₂ (30 mL) and washed with 1:4 NH₄OH/saturated NH₄Cl (3 x 25 mL) and again with saturated NH₄Cl (25 mL). The organic layer was dried over Na₂SO₄ and concentrated under vacuum. The crude product was purified by preparative TLC, eluting with Hexane/EtOAc/Et₃N 3:2:0.1 to give 50 mg (92%) of 26a as a white-brown solid.
\( ^1H\)-NMR (CDCl\(_3\), 400MHz) \( \delta \) 0.82-0.90 (3H, m), 0.98 (3H, d, \( J = 7.6 \) Hz), 1.05-1.52 (24H, m), 1.60-1.74 (6H, m), 1.80-2.06 (9H, m), 2.22-2.37 (9H, m), 2.56 (3H, m), 2.67 (3H, m), 2.95 (2H, t, \( J = 9.8 \) Hz), 3.07 (3H, m), 3.29-3.34 (1H, m), 3.46 (3H, m), 3.54 (1H, d, \( J = 6.8 \) Hz), 3.61 (3H, s), 3.68 (1H, bs), 3.77 (1H, m), 3.97 (1H, m), 4.18 (1H, m), 4.34-4.38 (3H, m), 4.69 (1H, m), 5.06 (1H, d, \( J = 4 \) Hz), 7.32 (2H, d, \( J = 6.4 \) Hz), 7.73-7.75 (3H, m); \(^{13}\)C-NMR (CDCl\(_3\), 100MHz) \( \delta \) 8.7, 9.2, 11.3, 14.2, 14.7, 16.5, 18.2, 21.4, 21.5, 22.2, 24.2, 25.9, 26.6, 27.3, 29.7, 30.0, 33.6, 34.6, 36.4, 36.9, 42.4, 45.3, 45.8, 49.3, 50.0, 51.5, 57.7, 63.9, 65.5, 68.6, 69.4, 70.5, 72.7, 73.8, 74.2, 77.2, 77.6, 78.0, 83.4, 94.4, 102.7, 119.3, 125.5, 129.1, 129.4, 147.2, 173.4, 178.1. HRMS (FAB, mnba) calc for [C\(_{53}\)H\(_{89}\)N\(_5\)O\(_{14}\) + H]\(^+\) 1020.6484, found 1020.6430

**Methyl descladinoseazithromycin-N-benzyltriazolylhexanoate (26b).** Compound 20 (0.080 g, 0.115 mmol) and azido-ester 25 (0.030 g, 0.173 mmol) were dissolved in anhydrous THF (5 mL) and stirred under argon at room temperature. Copper (I) iodide (0.010 g, 0.053 mmol), and Hunig’s base (0.05 mL) were then added to the reaction mixture, and stirring continued for 12 h. The reaction mixture was diluted with CH\(_2\)Cl\(_2\) (30 mL) and washed with 1:4 NH\(_4\)OH/saturated NH\(_4\)Cl (3 x 25 mL) and again with saturated NH\(_4\)Cl (25 mL). The organic layer was dried over Na\(_2\)SO\(_4\) and concentrated in vacuo. The crude product was purified by preparative TLC, eluting with Hexane/EtOAc/Et\(_3\)N 3:2:0.1 to give 65 mg (65%) of 26b as a white-brown solid. \(^1\)H-NMR (CDCl\(_3\), 400MHz) \( \delta \) 0.79-0.86 (6H, m), 1.00-1.07 (6H, m), 1.17-1.26 (m), 1.42-1.51 (m), 1.55-1.72 (6H, m), 1.80-1.94 (6H, m), 2.00-2.05 (2H, m), 2.1 (3H, s), 2.23-2.27 (4H, m), 2.33 (3H, s), 2.47 (1H, d, \( J = 10.4 \) Hz), 2.58-2.72 (5H, m), 3.32-3.41 (3H, m), 67
3.52-3.73 (6H, m), 3.92-4.00 (2H, m), 4.34 (2H, t, $J = 7.0$ Hz), 4.41 (1H, d, $J = 7.6$ Hz), 4.69 (1H, d, $J = 10.8$ Hz), 7.24 (2H, d, $J = 8.4$ Hz), 7.71(2H, d, $J = 8$ Hz), 7.73 (1H, s);
$^{13}$C NMR (CDCl$_3$, 100MHz) $\delta$ 7.7, 7.9, 8.7, 10.9, 16.1, 16.1, 20.9, 21.2, 24.2, 25.8, 25.9, 26.6, 29.2, 29.6, 30.0, 33.6, 36.0, 36.3, 37.1, 42.0, 44.5, 45.8, 50.0, 51.5, 57.7, 62.6, 65.1, 69.9, 70.4, 73.1, 74.1, 75.3, 79.4, 94.8, 106.4, 119.3, 125.5, 128.9, 129.6, 138.2, 147.1, 173.4, 177.2. HRMS (EI) calc for [C$_{45}$H$_{75}$N$_5$O$_{11}$ + H]$^+$ 862.5541, found 862.5566

Representative Procedure for Conversion of Methyl or Ethyl Ester to Hydroxamic Acid. 6-Azidohexahydroxamic acid (24a). To a solution of ethyl 6-azidohexanoate (1.00 g, 5.840 mmol) in 1:1 THF (5 mL) and anhydrous methanol (5 mL) was added aqueous hydroxylamine (4 mL, 70.100 mmol) and KCN ( 0.070 g, 1.170 mmol), and the stirring continued for 24 h. The reaction was diluted with EtOAc (30 mL) and washed with saturated NaHCO$_3$ (2 x 30 mL) and saturated brine (30 mL). The organic layer was dried over Na$_2$SO$_4$ and concentrated in vacuo to give 797 mg (80%) of 24a as white solid. $^1$H-NMR (DMSO-d$_6$, 400MHz) $\delta$ 1.24-1.30 (2H, m), 1.45-1.53 (4H, m), 1.93 (2H, t, $J = 7.2$ Hz), 3.29 (2H, t, $J = 6.8$ Hz), 8.65 (1H, s), 10.3 (1H, s); $^{13}$C NMR (CDCl$_3$, 100 MHz) $\delta$ 24.8, 26.1, 28.4, 32.6, 51.1, 171.4; HRMS (ESI) calc for [C$_6$H$_{12}$N$_4$O$_2$ + H]$^+$ 173.0947, found 173.0983

7-Azidoheptahydroxamic acid (24b). Reaction of ethyl 7-azidoheptanoate (1.00 g, 5.400 mmol) and aqueous hydroxylamine (4 ml, 70.100 mmol) within 24 h as described for the synthesis of 24a, gave 820 mg (82%) of 24b as white solid. $^1$H NMR (DMSO-d$_6$, 400 MHz) $\delta$ 1.23-1.32 (4H, m), 1.45-1.52 (4H, m), 1.92 (2H, t, $J = 7.6$ Hz), 3.29 (2H, t, $J$
= 7.2 Hz), 8.63 (1H, br s), 10.3 (1H, br s); $^{13}$C NMR (CDCl$_3$, 100 MHz) δ 25.1, 26.2, 28.5, 28.6, 32.7, 51.2, 171.6; HRMS (FAB, thioglycerol) calc for [C$_7$H$_{14}$N$_4$O$_2$ + H]$^+$ 187.1195, found 187.1163

8-Azidoctahydroxamic acid (24c). Reaction of methyl 8-azidoctanoate (0.580 g, 2.910 mmol) and aqueous hydroxylamine (2.49 ml, 37.800 mmol) within 24 h as described for the synthesis of 24a, gave 432 mg (74%) of 24c as white solid. $^1$H NMR (CDCl$_3$, 400 MHz) δ 1.33-1.38 (6H, m), 1.54-1.63 (4H, m), 2.13 (2H, t, $J$ = 7.6 Hz), 3.24 (2H, t, $J$ = 6.8 Hz), 8.85 (1H, br s); $^{13}$C NMR (CDCl$_3$, 100MHz) δ 25.5, 26.7, 29.0, 29.1, 29.2, 33.1, 51.6, 172.0; HRMS (FAB, thioglycerol) calc for [C$_8$H$_{16}$N$_4$O$_2$ + H]$^+$ 201.1351, found 201.1352

9-Azidononahydroxamic acid (24d). Reaction of methyl 9-azidononanoate (0.290 g, 1.370 mmol) and aqueous hydroxylamine (1.18 ml, 17.900 mmol) within 24 h as described for the synthesis of 24a, gave 225 mg (77%) of 24d as white solid. $^1$H NMR (CDCl$_3$, 400 MHz) δ 1.30-1.40 (8H, m), 1.56-1.61 (4H, m), 2.13 (2H, t, $J$ = 7.2 Hz), 3.25 (2H, t, $J$ = 8); $^{13}$C NMR (CDCl$_3$, 100MHz) δ 25.6, 26.8, 29.0, 29.2, 29.3, 33.1, 51.6, 172.2; HRMS (FAB, thioglycerol) calc for [C$_9$H$_{18}$N$_4$O$_2$ + H]$^+$ 215.1508, found 215.1529

10-Azidodecahydroxamic acid (24e). Reaction of methyl 10-azidodecanoate (0.300 g, 1.310 mmol) and aqueous hydroxylamine (1.13 ml, 17.100 mmol) within 24 h as described for the synthesis of 24a, gave 254 mg (84 %) of 24e as white solid. $^1$H NMR (CDCl$_3$, 400 MHz) δ 1.24-1.39 (8H, m), 1.54-1.65 (4H, m), 2.12 (2H, t, $J$ = 7.6 Hz), 3.24
(2H, t, J = 7.2 Hz), 9.0 (1H, br s); $^{13}$C NMR (CDCl$_3$, 100MHz) δ 25.6, 26.9, 29.0, 29.2, 29.3, 29.4, 29.5, 33.2, 51.7, 172.2; HRMS (FAB, thioglycerol) calc for [C$_{10}$H$_{20}$N$_4$O$_2$ + H]$^+$ 229.1665, found 229.1666

6-$^{15}$N-Azidohexahydroxamic acid (24f). Reaction of ethyl 6-$^{15}$N-azidohexanoate (0.210 g, 1.129 mmol) and aqueous hydroxylamine (0.8 mL, 13.548 mmol) within 24 h as described for the synthesis of 24a, gave 80 mg (38%) of 24f as clear oil. $^1$H-NMR (DMSO-d$_6$, 400MHz) δ 1.22-1.30 (2H, m), 1.44-1.52 (4H, m), 1.92 (2H, t, J = 6.4 Hz), 3.28 (2H, t, J = 6.8 Hz), 8.63 (1H, s), 10.3 (1H, s); HRMS (ESI) calc for [C$_6$H$_{12}$N$_3$O$_2$ $^{15}$N + Na+ H]$^{2+}$ 196.0828, found 196.0824

7-$^{15}$N-Azidoheptahydroxamic acid (24g). Reaction of ethyl 7-$^{15}$N-azidoheptanoate (0.372 g, 1.860 mmol) and aqueous hydroxylamine (0.7 mL, 22.32 mmol) within 24 h as described for the synthesis of 24a, gave 162 mg (44%) of 24g as clear oil. $^1$H NMR (DMSO-d$_6$, 400 MHz) δ 1.18-1.34 (4H, m), 1.44-1.58 (4H, m), 1.91 (2H, t, J = 6.4 Hz), 3.29 (2H, t, J = 6.8 Hz), 8.64 (1H, s), 10.3 (1H, s); HRMS (FAB, thioglycerol) calc for [C$_7$H$_{14}$N$_3$O$_2$ $^{15}$N + H]$^+$ 188.1165, found 188.1172

Azithromycin-N-benzylltriazolylhexahydroxamic acid (23a)

**Method A:** To a solution of compound 26a (0.040 g, 0.040 mmol) in 1:1 THF/MeOH (3 mL) was added hydroxylamine (50% in H$_2$O) (0.03 mL, 0.540 mmol) and a catalytic amount of KCN. The mixture was stirred at room temperature for 24 h. The reaction was
partitioned between 5 % MeOH in CH₂Cl₂ (30 mL) and saturated sodium bicarbonate (25 mL), the two layers were separated and the aqueous layer was extracted with 5 % MeOH in CH₂Cl₂ (2 × 20 mL). The combined organic layer was washed with saturated brine (40 mL) and dried over Na₂SO₄. Solvent was evaporated off and the crude was purified by preparative TLC, eluting with CH₂Cl₂/MeOH/NH₄OH 10:1:0.1 to give 6.5 mg (16 %) of compound 23a as a brown-white solid.

**Method B:** 4’-Ethynylbenzyl azithromycin 19 (0.100 g, 0.109 mmol) and 6-azidohexahydroxamic acid 24a (0.081 g, 0.117 mmol) were dissolved in anhydrous THF (5 mL) and stirred under argon at room temperature. Copper (I) iodide (0.011 g, 0.070 mmol) and Hunig’s base (0.5 mL) were then added to the reaction mixture, and stirring continued for 4 h. The reaction mixture was diluted with CH₂Cl₂ (40 mL) and washed with 1:4 NH₄OH/saturated NH₄Cl (3 × 30 mL) and saturated NH₄Cl (30 mL). The organic layer was dried over Na₂SO₄ and concentrated *in vacuo*. The crude product was purified by prep TLC (silica, 12:1:0.1 CH₂Cl₂/MeOH/conc. NH₄OH) to give 71 mg (59%) of 23a as a brown-white solid.

**Method C:** To a solution of silyl protected compound 22 (0.025 g, 0.020 mmol) in THF (1 mL) was added 1 M TBAF in THF (0.030 mL, 0.030 mmol) and the mixture was stirred at room temperature for 2 h during which TLC revealed a near quantitative conversion to a lower Rf product. The reaction was partitioned between CH₂Cl₂ (30 mL) and saturated NH₄Cl (25 mL), the two layers were separated and the organic layer dried over Na₂SO₄ and concentrated *in vacuo*. The crude product was purified by prep TLC.
(silica, 12:1:0.1 CH$_2$Cl$_2$/MeOH/Et$_3$N) to give 15 mg (73%) of 23a as a brown-white solid. \(^1\)H-NMR (Acetone-d$_6$, 400MHz) $\delta$ 0.83-0.92 (6H, m), 1.02 (3H, d, $J$ = 7.6 Hz), 1.08-1.11 (8H, m), 1.14 (3H, d, $J$ = 7.6 Hz), 1.18 (3H, d, $J$ = 6 Hz), 1.24-1.29 (15H, m), 1.33-1.47 (3H, m), 1.54 (1H, dd, $J$ = 4.8 Hz, 15.2 Hz), 1.60-1.69 (5H, m), 1.80-2.01 (m), 2.06-2.12 (1H, m), 2.18-2.24 (1H, m), 2.26 (3H, s), 2.28-2.31 (1H, m), 2.35-2.41 (4H, m), 2.51 (1H, d, $J$ = 10 Hz), 2.65-2.96 (m), 3.12 (3H, s), 3.22-3.29 (1H, m), 3.47 (1H, m), 3.54-3.69 (6H, m), 3.81 (1H, d, $J$ = 13.2 Hz), 4.11 (1H, m), 4.24 (1H, m), 4.45 (3H, t, $J$ = 7.0 Hz), 4.50 (1H, d, $J$ = 6.8 Hz), 4.75 (1H, d, $J$ = 7.2 Hz), 4.97 (1H, d, $J$ = 5.2 Hz), 7.42 (2H, d, $J$ = 8.0 Hz), 7.84 (2H, d, $J$ = 8.0 Hz), 8.35 (1H, s). \(^{13}\)C NMR (CDCl$_3$, 100 MHz) $\delta$ 6.5, 8.7, 11.5, 14.4, 16.7, 17.7, 21.3, 21.6, 21.8, 24.6, 25.7, 26.7, 27.0, 29.2, 29.6, 29.9, 33.0, 34.5, 35.6, 36.7, 41.8, 42.7, 45.3, 49.3, 50.0, 53.4, 57.8, 62.6, 63.4, 65.9, 68.6, 69.3, 70.4, 72.6, 73.3, 73.8, 77.8, 78.3, 78.4, 83.4, 94.5, 102.8, 119.6, 125.7, 129.3, 129.7, 138.6, 147.4, 171.3, 178.4; HRMS (ESI) calcd for [C$_{52}$H$_{88}$N$_6$O$_{14}$+ H]$^+$ 1021.6437, found 1021.6409; M.P. = 127.0-130.0 °C.

Desclasinose azithromycin-N-benzyltriazolylhexahydroxamic acid (23b).

**Method A:** To a solution of compound 26b (0.040 g, 0.050 mmol) in 1:1 THF/MeOH (3 mL) was added hydroxylamine (50% in H$_2$O) (0.04 mL, 0.540 mmol) and a catalytic amount of KCN. The mixture was stirred at room temperature for 24 h. The reaction was partitioned between 5% MeOH in CH$_2$Cl$_2$ (30 mL) and saturated sodium bicarbonate (25 mL), the two layers were separated and the aqueous layer was extracted with 5% MeOH in CH$_2$Cl$_2$ (2 x 20 mL). The combined organic layer was washed with saturated brine (40 mL) and dried over Na$_2$SO$_4$. Solvent was evaporated off and the crude was purified by
preparative TLC, eluting with CH$_2$Cl$_2$/MeOH/NH$_4$OH 10:1:0.1 to give 9.0 mg (23%) of compound 23b as brown-white solid.

**Method B:** Reaction of descladinose compound 20 (0.134 g, 0.188 mmol) and 6-azidohexahydroxamic acid 24a (0.130 g, 0.755 mmol) within 8 h, according to the protocols of Method B described for the synthesis of compound 23a, followed by prep TLC (silica, 10:1:0.1 CH$_2$Cl$_2$/MeOH/conc. NH$_4$OH) gave 73 mg (43%) of 23b as a brown-white solid. $^1$H-NMR (Acetone-d$_6$, 400MHz) δ 0.82-0.90 (9H, m), 1.02 (3H, d, $J = 7.2$ Hz), 1.07 (3H, s), 1.09 (3H, d, $J = 6.8$ Hz), 1.18-1.23 (m), 1.28 (3H, s), 1.31-1.39 (m), 1.46-1.56 (4H, m), 1.61-1.65 (3H, m), 1.80-1.99 (7H, m), 2.05-2.11 (2H, m), 2.18-2.21 (1H, m), 2.24 (3H, s), 2.25-2.29 (1H, m), 2.35 (3H, s), 4.47 (1H, d, $J = 9.2$ Hz), 2.61-2.67 (1H, m), 2.70-2.77 (1H, m), 3.30-3.34 (1H, m), 3.41 (1H, m), 3.52-3.65 (5H, m), 3.81 (1H, d, $J = 13.2$ Hz), 4.44 (2H, t, $J = 7.0$ Hz), 4.59 (1H, d, $J = 7.6$ Hz), 4.87 (1H, dd, $J = 1.8$ Hz, 11.0 Hz), 7.43 (2H, d, $J = 8.4$ Hz), 7.83 (2H, d, $J = 8.4$ Hz), 8.34 (1H, s); $^{13}$C NMR (CDCl$_3$, 100 MHz) δ 7.3, 7.9, 10.7, 16.0, 16.2, 20.9, 21.1, 24.4, 25.6, 25.7, 26.5, 29.0, 29.6, 29.7, 35.8, 36.3, 36.8, 42.1, 44.4, 50.0, 57.9, 62.7, 64.0, 69.8, 70.5, 70.8, 73.3, 74.1, 75.0, 79.5, 94.8, 106.5, 120.0, 125.8, 129.2, 129.5, 138.4, 147.3, 170.5, 177.5; HRMS (ESI) calcd for [C$_{44}$H$_{74}$N$_6$O$_{11}$ + H]$^+$ 863.5494, found 863.5544; M.P. = 109.0-112.0 °C.

**Azithromycin-N-benzyltriazolylheptahydroxamic acid (23c).** Reaction of 4'-ethynylbenzyl azithromycin 19 (0.134 g, 0.158 mmol) and 7-azidoheptahydroxamic acid 24b (0.125 g, 0.672 mmol) within 4 h, according to the protocols of Method B described for the synthesis of compound 23a, followed by prep TLC (silica, 12:1:0.1
CH$_2$Cl$_2$/MeOH/conc. NH$_4$OH) gave 93 mg (56%) of 23c as a brown-white solid. $^1$H-NMR (CDCl$_3$, 400MHz) $\delta$ 0.81-1.51 (30H, m), 1.54-1.65 (6H, m), 1.70-2.14 (9H, m), 2.20-2.38 (9H, m), 2.46-2.56 (2H, m), 2.60-2.70 (2H, m), 3.00 (3H, s), 3.31 (2H, t, $J = 8.8$ Hz), 3.38-3.54 (6H, m), 3.60 (1H, s), 3.78 (1H, d, $J = 12.8$ Hz), 3.98-4.20 (2H, m), 4.36 (3H, d, $J = 7.2$ Hz), 4.49 (1H, d, $J = 7.2$ Hz), 5.11 (1H, d, $J = 4.0$ Hz), 7.32 (2H, d, $J = 7.6$ Hz), 7.73 (1H, s), 7.75 (2H, d, $J = 7.6$ Hz); $^{13}$C NMR (CDCl$_3$, 100 MHz) $\delta$ 6.6, 8.8, 11.5, 14.4, 16.6, 17.7, 21.3, 21.6, 21.8, 25.1, 26.0, 26.7, 27.1, 28.2, 29.2, 29.6, 30.0, 33.1, 34.5, 35.7, 36.7, 41.8, 42.7, 45.3, 49.3, 50.3, 50.7, 57.9, 62.7, 63.0, 65.8, 68.6, 69.4, 70.4, 72.6, 73.2, 73.8, 77.8, 78.1, 78.2, 83.5, 94.4, 102.8, 119.3, 125.7, 129.4, 129.7, 138.4, 147.4, 171.3, 178.4; HRMS (ESI) calcd for [C$_{53}$H$_{90}$N$_6$O$_{14}$ + H]$^+$ 1035.6587, found 1035.6628; M.P. = 115.0-119.0 0°C.

Descladinose azithromycin-N-benzylltriazolyheptahydroxamic acid (23d). Reaction of descladinose-4’-ethynylbenzyl azithromycin 20 (0.130 g, 0.188 mmol) and 7-azidoheptahydroxamic acid 24b (0.130 g, 0.755 mmol) within 8 h, according to the protocols of Method B described for the synthesis of compound 23a, followed by prep TLC (silica, 10:1:0.1 CH$_2$Cl$_2$/MeOH/conc. NH$_4$OH) gave 78 mg (47%) of 23d as a brown-white solid. $^1$H-NMR (CDCl$_3$, 400MHz) $\delta$ 0.66-2.32 (42H, m), 2.47 (2H, d, $J = 10.8$ Hz), 2.63-2.70 (4H, m), 3.34-3.51 (6H, m), 3.62-3.69 (5H, m), 4.20-4.40 (5H, m), 4.74 (2H, br s), 7.26 (2H, br s), 7.73 (3H, br s); $^{13}$C NMR (CDCl$_3$, 100 MHz) $\delta$ 7.4, 7.9, 10.7, 16.0, 16.1, 20.9, 21.1, 25.0, 25.7, 26.5, 28.0, 28.9, 29.8, 35.8, 36.3, 36.9, 42.0, 44.4, 50.1, 57.9, 62.7, 63.9, 69.9, 70.4, 70.8, 73.3, 74.1, 75.2, 79.5, 94.9, 106.6, 119.7, 125.7,
129.2, 129.6, 138.4, 147.3, 177.5; HRMS (ESI) calcd for [C_{45}H_{76}N_{6}O_{11} + H]^+ 877.5645, found 877.5665; M.P. = 105.0-109.0 °C.

Azithromycin-N-benzyltriazolyloctahydroxamic acid (23e). Reaction of 4’-ethynylbenzyl azithromycin 19 (0.100 g, 0.120 mmol) and 8-azidooctahydroxamic acid 17e (0.047 g, 0.240 mmol) within 2.5 h, according to the protocols of Method B described for the synthesis of compound 23a, followed by prep TLC (silica, 12:1:0.1 CH_{2}Cl_{2}/MeOH/conc. NH_{4}OH) gave 72 mg (58 %) of 23e as brown-white solid. ^1H NMR (CDCl_{3}, 400 MHz) δ 0.85 (3H, t, J = 4.0 Hz), 0.87-1.22 (18H, m), 1.29 (9H, s), 1.30-2.28 (18H, m), 2.29 (6H, s), 2.30-3.00 (8H, m), 3.10 (3H, s), 3.20-3.79 (9H, m), 3.99-4.03 (1H, m), 4.35-4.40 (3H, m), 4.65 (1H, d, J = 8.0 Hz), 5.11 (1H, d, J = 4.8 Hz), 7.34 (2H, d, J = 8.0 Hz), 7.72 (1H, s), 7.77 (2H, d, J = 8.0 Hz); ^13C NMR (DMSO-d_{6}, 100MHz) δ 7.5, 9.8, 11.6, 15.4, 18.2, 19.1, 21.5, 22.1, 22.7, 25.6, 26.3, 26.6, 28.7, 29.0, 29.6, 30.2, 32.0, 32.8, 35.2, 36.4, 37.2, 42.2, 45.3, 49.2, 50.1, 58.3, 63.2, 65.4, 67.7, 70.8, 73.3, 74.2, 77.0, 78.4, 83.4, 102.8, 121.6, 125.6, 129.7, 130.0, 135.0, 147.0, 177.8; HRMS (FAB, thioglycerol) calc for [C_{54}H_{92}N_{6}O_{14} + H]^+ 1049.6749, found 1049.6648; M.P. = 110.0-123.0 °C.

Descladinose azithromycin-N-benzyltriazolyloctahydroxamic acid (23f). Reaction of descladinose-4’-ethynylbenzyl azithromycin 20 (0.100 g, 0.144 mmol) and 8-azidooctahydroxamic acid 24c (0.049 g, 0.246 mmol) within 2.5 h, according to the protocols of Method B described for the synthesis of compound 23a, followed by prep TLC (silica, 10:1:0.1 CH_{2}Cl_{2}/MeOH/conc. NH_{4}OH) gave 94 mg (73 %) of 23f as a
brown-white solid. $^1$H NMR (CDCl$_3$, 400 MHz) $\delta$ 0.84-0.91 (9H, m), 1.04-1.11 (9H, m), 1.23-1.30 (12H, m), 1.37-2.16 (14H, m), 2.2 (3H, s), 2.35 (3H, s), 2.49-2.74 (5H, m), 3.22-3.75 (6H, m), 4.10-4.12 (1H, m), 4.34-4.42 (3H, m), 4.77 (1H, d, $J = 12$ Hz), 7.30 (2H, d, $J = 7.6$ Hz), 7.79 (3H, m); $^{13}$C NMR (CDCl$_3$, 100MHz) $\delta$ 7.6, 8.2, 11.0, 14.4, 16.2, 16.4, 21.1, 21.4, 25.2, 25.9, 26.1, 26.7, 28.4, 28.8, 28.9, 29.1, 29.9, 30.2, 36.1, 36.6, 42.3, 44.6, 50.5, 51.6, 58.2, 60.6, 63.0, 64.0, 70.1, 70.7, 73.6, 74.4, 75.4, 79.7, 95.1, 106.8, 120.0, 126.0, 129.5, 129.9, 138.6, 147.6, 170.6, 177.8; HRMS (FAB, thioglycerol) calcd for [C$_{46}$H$_{78}$N$_6$O$_{11}$ + H]$^+$ 891.5806, found 891.5776; M.P. = 80.0-90.0 °C.

Azithromycin-$N$-benzyltriazolylnonahydroxamic acid (23g). Reaction of 4’-ethynylbenzyl azithromycin 19 (0.100 g, 0.120 mmol) and 9-azidononahydroxamic acid 24d (0.043 g, 0.200 mmol) within 2.5 h, according to the protocols of Method B described for the synthesis of compound 23a, followed by prep TLC (silica, 12:1:0.1 CH$_2$Cl$_2$/MeOH/conc. NH$_4$OH) gave 64 mg (51%) of 23g as brown-white solid. $^1$H NMR (CDCl$_3$, 400 MHz) $\delta$ 0.84-1.30 (30H, m), 1.33-2.26 (20H, m), 2.30 (6H, s), 2.38-2.68 (8H, m), 2.99 (3H, s), 3.32-3.84 (9H, m), 4.03-4.08 (1H, m), 4.35-4.41 (3H, m), 4.53 (1H, d, $J = 8.0$ Hz), 5.13 (1H, d, $J = 4.0$ Hz), 7.35 (2H, d, $J = 8.0$ Hz), 7.75 (1H, s), 7.78 (2H, d, $J = 8.0$ Hz); $^{13}$C NMR (DMSO-d$_6$, 100MHz) $\delta$ 6.9, 9.0, 11.6, 14.7, 16.9, 18.0, 21.6, 21.8, 22.1, 25.6, 26.4, 26.9, 27.3, 28.8, 29.0, 29.1, 29.5, 29.9, 30.4, 34.8, 36.0, 37.0, 42.1, 43.0, 45.6, 49.5, 50.5, 58.1, 63.5, 66.1, 68.8, 70.7, 72.9, 74.1, 78.1, 78.3, 78.5, 83.7, 94.4, 94.7, 103.1, 119.6, 126.0, 129.7, 130.0, 147.6, 178.7; HRMS (ESI) calcd for [C$_{55}$H$_{94}$N$_6$O$_{14}$ + H]$^+$ 1063.6900, found 1063.6861; M.P. = 117.0-133.0 °C.
Azithromycin-N-benzyltriazolyldecahydroxamic acid (23h). Reaction of 4’-ethynylbenzyl azithromycin 19 (0.100 g, 0.120 mmol) and 10-azidodecahydroxamic acid 24e (0.045 g, 0.20 mmol) within 4.5 h, according to the protocols of Method B described for the synthesis of compound 23a, followed by prep TLC (silica, 12:1:0.1 CH₂Cl₂/MeOH/conc. NH₄OH) gave 70 mg (56 %) of 23h as a brown-white solid. ¹H NMR (CDCl₃, 400 MHz) δ 0.85-1.36 (30H, m), 1.41-2.24 (22H, m), 2.28, 2.36 (6H, s), 2.33-3.10 (8H, m), 3.05 (3H, s), 3.23-3.82 (9H, m), 4.06-4.10 (1H, m), 4.36-4.41 (3H, m), 4.49 (1H, d, J = 8.0 Hz), 5.15 (1H, d, J = 4.0 Hz), 7.34 (2H, d, J = 8 Hz), 7.75 (1H, s), 7.78 (2H, d, J = 8.0 Hz); ¹³C NMR (DMSO-d₆, 100MHz) δ 66.7, 9.0, 11.7, 14.6, 16.9, 17.9, 21.6, 21.9, 22.0, 25.6, 26.4, 26.8, 27.0, 27.3, 28.8, 29.0, 21.9, 29.3, 29.5, 29.9, 30.3, 33.6, 34.9, 35.8, 37.0, 42.1, 43.0, 45.6, 49.6, 50.6, 51.6, 58.0, 62.8, 63.9, 66.2, 68.9, 69.6, 70.7, 72.9, 73.5, 74.0, 74.1, 78.2, 78.6, 83.6, 94.6, 103.0, 119.6, 126.0, 129.6, 129.9, 138.9, 147.6, 178.6; HRMS (MALDI) calc for [C₁₅₆H₉₆N₁₆O₁₄ + H]⁺ 1077.7057, found 1077.6971; M.P. = 100.0-127.0 °C.

4’-Desmethyl clarithromycin (27). To a solution of clarithromycin (3.320 g, 4.440 mmol) and sodium acetate (3.280 g, 39.900 mmol) in 80% aqueous methanol (50 mL) at 85 °C was added iodine (1.240 g, 4.890 mmol) in three batches within 5 min. The reaction was maintained at pH 8-9 by additions of 1M NaOH (2 x 3 ml, once at 10 min and 45 min of reaction time). Stirring was continued at 85 °C for 3 h and TLC analysis indicated about 90% consumption of the starting material. A solution of 5% sodium thiosulfate (120 mL) and dichloromethane (80 mL) were added and the two layers were separated. The aqueous layer was extracted with CH₂Cl₂ (60 mL), the combined organic
layers was washed with saturated brine, dried over Na$_2$SO$_4$, and concentrated in vacuo to give 2.4 g of 27, which was used without further purification.

4’-Ethynylbenzyl clarithromycin (28). To a solution of 4’-desmethyl clarithromycin 27 (2.400 g, 3.340 mmol) in anhydrous DMSO (30 ml) was added Hunig’s base (3 ml) and 4-ethynylbenzyl methanesulfonate 18 (0.920 g, 4.340 mmol). The reaction mixture was then heated with stirring under argon at 85°C for 2.5 h. The reaction was cooled and diluted with EtOAc (100 mL) and washed with saturated NaHCO$_3$ (3 x 60 mL) and saturated brine (60 mL). The organic layer was dried over Na$_2$SO$_4$ and concentrated in vacuo. The crude product was purified by flash chromatography (silica, gradient 12:1; 10:1; 8:1; CH$_2$Cl$_2$/acetone) to give 1.8 g (63%) of 28 as a brown-white solid. $^1$H-NMR (CDCl$_3$, 400MHz) $\delta$ 0.82 (3H, t, $J = 7.2$ Hz), 1.03-1.28 (18H, m), 1.37 (3H, s), 1.40-1.55 (3H, m), 1.65-1.90 (6H, m), 2.03 (1H, d, $J = 10.0$ Hz), 2.22 (3H, s), 2.30 (1H, d, $J = 15.2$ Hz), 2.40-2.60 (2H, m), 2.80-2.90 (2H, m), 2.94-3.00 (6H, m), 3.04 (1H, s), 3.09 (3H, s), 3.16 (1H, s), 3.24-3.29 (1H, m), 3.38-3.46 (3H, m), 3.59 (1H, d, $J = 6.8$ Hz), 3.70-3.75 (3H, m), 3.88-3.95 (1H, m), 4.37 (1H, d, $J = 7.2$ Hz), 4.88 (1H, d, $J = 4.4$ Hz), 5.02 (1H, dd, $J = 11.6$, 2.4 Hz), 7.23 (2H, d, $J = 12.0$ Hz), 7.42 (2H, d, $J = 8.0$ Hz); $^{13}$C NMR (CDCl$_3$, 100 MHz) $\delta$ 9.2, 10.7, 12.4, 16.1, 18.1, 18.7, 19.9, 21.1, 21.5, 29.3, 32.4, 34.8, 36.9, 37.2, 39.2, 45.0, 45.2, 49.3, 50.6, 53.4, 57.6, 63.3, 65.6, 68.5, 69.0, 70.6, 72.5, 74.2, 76.5, 77.8, 78.1, 78.2, 80.8, 95.8, 102.5, 120.9, 128.6, 132.0, 133.5, 139.4, 175.4; HRMS (ESI) calc for [C$_{46}$H$_{73}$NO$_{13}$ + H]$^+$ 848.5155, found 848.5181.
Descladinose-4’-ethynylbenzyl clarithromycin (29). To a solution of 4’-ethynylbenzyl clarithromycin 28 (0.500 g, mmol) in ethanol (20 mL) was added 1N HCl (20 mL), and stirring continued for 22 h at room temperature. The reaction mixture was basified with concentrated NH₄OH to about pH = 9. The reaction mixture was diluted with distilled water (40 mL) and extracted with EtOAc (3 x 60 mL). The combined organic layers were washed with saturated brine (40 mL), dried over Na₂SO₄, and concentrated in vacuo. The crude product was purified by flash chromatography (silica, 8:1 CH₂Cl₂/acetone) to give 320 mg (79%) of 29 as a brown-white solid. ¹H-NMR (CDCl₃, 400MHz) δ 0.82 (3H, t, J = 7.6 Hz), 1.09-1.28 (12H, m), 1.34 (3H, s), 1.40-1.55 (3H, m), 1.70-1.74 (2H, m), 1.87-1.94 (3H, m), 2.08-2.15 (6H, m), 2.54-2.66 (2H, m), 2.94-2.98 (3H, m), 3.05 (1H, s), 3.25 (1H, s), 3.31-3.42 (2H, m), 3.48-3.56 (2H, m), 3.66 (2H, d, J = 10.0 Hz), 3.82 (1H, s), 3.90 (1H, s), 4.35 (1H, d, J = 7.6 Hz), 5.14 (1H, dd, J = 10.8, 1.0 Hz), 5.14 (1H, d, J = 8.0 Hz), 7.42 (2H, d, J = 8.4 Hz); ¹³C NMR (CDCl₃, 100 MHz) δ 8.4, 10.5, 12.7, 15.3, 16.3, 17.8, 18.8, 21.4, 29.2, 35.9, 36.6, 37.5, 38.7, 44.5, 45.5, 49.6, 57.8, 65.0, 69.7, 70.1, 70.6, 74.1, 77.9, 78.9, 83.3, 88.5, 106.5, 121.0, 128.4, 132.1, 139.1, 174.7; HRMS (ESI) calc for [C₃₈H₅₉NO₁₀ + H]⁺ 690.4212, found 690.4259

Clarithromycin-N-benzyltriazolyl-O-silyl-hexahydroxamate (30). 4’-Ethynylbenzyl clarithromycin 28 (0.100 g, 0.118 mmol) and 6-azido-O-silyl hexahydroxamate 21 (0.103 g, 0.251 mmol) were dissolved in anhydrous THF (6 mL) and stirred under argon at room temperature. Copper (I) iodide (0.011 g, 0.057 mmol), TBTA (0.060 g, 0.094 mmol), and Hunig’s base (0.1 mL) were then added to the reaction mixture, and stirring continued for 24 h. The reaction mixture was diluted with CH₂Cl₂ (30 mL) and washed...
with 1:4 NH₄OH/saturated NH₄Cl (3 x 30 mL) and saturated NH₄Cl (30 mL). The organic layer was dried over Na₂SO₄ and concentrated in vacuo. The crude product was purified by prep TLC (silica, 20:1 CH₂Cl₂/MeOH) to give 147 mg (99%) of compound 30 as a brown-white solid. ¹H-NMR (CDCl₃, 400MHz) δ 0.81 (3H, t, J = 7.2 Hz), 1.03-1.51 (30H, m), 1.67-1.88 (12H, m) 2.10-2.29 (8H, m), 2.53-2.58 (3H, m), 2.82-2.90 (2H, m), 2.93-3.01 (6H, m), 3.10 (3H, s), 3.17 (2H, s), 3.28-3.32 (1H, m), 3.40-3.48 (3H, m), 3.60-3.78 (4H, m), 3.91-3.97 (1H, m), 4.22 (1H, br s), 4.41 (2H, d, J = 8.0 Hz), 4.87 (1H, d, J = 4.8 Hz), 5.03 (1H, d, J = 10.8 Hz), 7.21-7.23 (2H, m), 7.30-7.42 (10H, m), 7.64-7.76 (3H, m); ¹³C NMR (CDCl₃, 100 MHz) δ 9.1, 10.6, 12.3, 16.0, 18.0, 18.6, 19.8, 21.0, 21.3, 21.5, 26.7, 29.3, 29.6, 29.9, 34.7, 36.8, 37.1, 39.0, 39.2, 45.0, 45.2, 47.0, 49.3, 49.9, 50.5, 53.4, 54.0, 65.5, 68.5, 68.9, 70.6, 72.3, 74.1, 76.4, 77.7, 78.1, 78.2, 80.7, 95.7, 102.5, 119.3, 123.5, 125.4, 127.7, 128.4, 128.8, 129.1, 133.5, 134.4, 135.5, 143.9, 147.0, 175.4; HRMS (ESI) calcd for [C₆₈H₁₀₃N₅O₁₅Si + H]⁺ 1258.7292, found 1258.7313.

Clarithromycin-N-benzyltriazolylhexahydroxamic acid (31a).

Method A: 4’-Ethynylbenzyl clarithromycin 28 (0.100 g, 0.120 mmol) and 6-azidohexahydroxamic acid 24a (0.080 g, 0.470 mmol) were dissolved in anhydrous THF (5 mL) and stirred under argon at room temperature. Copper (I) iodide (0.011 g, 0.057 mmol) and Hunig’s base (0.5 mL) were added to the reaction mixture, and stirring continued for 2.5 h. The reaction mixture was diluted with CH₂Cl₂ (50 mL) and washed with 1:4 NH₄OH/saturated NH₄Cl (3 x 30 mL) and saturated NH₄Cl (30 mL). The organic layer was dried over Na₂SO₄ and concentrated in vacuo. The crude product was
purified by prep TLC (silica, 12:1:0.1 CH₂Cl₂/MeOH/conc. NH₄OH) to give 70 mg (58%) of **31a** as a brown-white solid.

**Method B:** To a solution of compound **30** (0.085g, 0.067 mmol) in anhydrous THF (1.5 mL) was added 1M TBAF in THF (0.1 mL, 0.100 mmol). The reaction was stirred under argon for 2 h. The reaction mixture was diluted with 5% MeOH in dichloromethane (10 mL) and washed with saturated NH₄Cl (15mL). The organic layer was dried over Na₂SO₄ and concentrated *in vacuo*. The crude product was purified on prep TLC (silica, 12:1:0.1 CH₂Cl₂/MeOH/Et₃N). The purified product was dissolved in 5% MeOH in CH₂Cl₂ (10 mL) and washed with distilled water (10 mL) and saturated brine (10 mL) to remove the last trace of associated TBAF. The organic layer was dried over Na₂SO₄ and concentrated *in vacuo* to give 26 mg (38%) of **31a** as a brown-white product.

**¹H-NMR (CDCl₃, 400MHz)** δ 0.81 (3H, t, *J* = 7.6 Hz), 1.03-1.52 (21H, m), 1.62-1.92 (12H, m) 2.04-2.29 (8H, m), 2.48-2.60 (3H, m), 2.82-2.90 (2H, m), 2.93-2.99 (6H, m), 3.09 (3H, s), 3.19 (2H, s), 3.28-3.33 (1H, m), 3.42-3.46 (3H, m), 3.60 (1H, d, *J* = 7.6 Hz), 3.70-3.80 (3H, m), 3.90-3.98 (1H, m), 4.37-4.40 (3H, m), 4.87 (1H, d, *J* = 4.8 Hz), 5.03 (1H, dd, *J* = 11.6, 2.4 Hz), 7.34 (2H, d, *J* = 7.6 Hz), 7.77 (2H, d, *J* = 7.6 Hz), 7.82 (1H, s); **¹³C NMR (CDCl₃, 100 MHz)** δ 9.1, 10.5, 12.2, 15.9, 17.9, 18.5, 19.7, 20.9, 21.2, 21.4, 24.3, 25.5, 29.4, 29.6, 34.7, 36.8, 37.1, 39.0, 39.1, 45.0, 45.1, 49.3, 49.9, 50.5, 53.3, 57.5, 63.6, 65.5, 68.5, 69.0, 70.7, 72.4, 74.2, 77.8, 78.2, 80.9, 95.9, 102.6, 119.8, 125.6, 129.4, 147.4, 175.8; HRMS (ESI) calcd for [C₅₂H₈₅N₅O₁₅ + H]⁺ 1020.6114, found 1020.6121; M.P. = 120.0-124.0 °C.
**Descladinose clarithromycin-N-benzyltriazolylhexahydroxamic acid (31b).** Reaction of descladinose-4'-ethynylbenzyl clarithromycin 29 (0.075 g, 0.109 mmol) and 6-azidohexahydroxamic acid 24a (0.040 g, 0.233 mmol) within 4 h, according to the protocols of Method A described for the synthesis of compound 31a, followed by prep TLC (silica, 10:1:0.1 CH₂Cl₂/MeOH/conc. NH₄OH) gave 47 mg (51%) of 31b as a brown-white solid. ¹H-NMR (CDCl₃, 400MHz) δ 0.79 (3H, t, J = 7.2 Hz), 1.08-1.32 (22H, m), 1.39-1.64 (3H, m), 1.71-1.81 (2H, m), 1.82-1.96 (3H, m), 2.04-2.18 (6H, m), 2.51-2.70 (4H, m), 2.92-2.98 (3H, m), 3.18-3.38 (3H, m), 3.45-3.55 (2H, m), 3.60-3.74 (3H, m), 3.81 (3H, s), 3.90 (1H, s), 4.33 (3H, br s), 5.13 (1H, d, J = 10.4 Hz), 7.29 (2H, br s), 7.74 (2H, br s); ¹³C NMR (CDCl₃, 100 MHz) δ 8.6, 10.7, 12.9, 15.5, 16.4, 18.0, 19.0, 21.5, 21.6, 29.5, 29.9, 36.1, 36.7, 37.7, 39.0, 44.7, 45.7, 49.8, 50.3, 58.2, 64.6, 70.0, 70.3, 70.9, 74.4, 78.3, 79.1, 88.5, 106.7, 120.3, 126.1, 129.6, 129.9, 147.7, 175.4; HRMS (ESI) calcd for [C₄₄H₇₁N₅O₁₂ + H]⁺ 862.5172, found 862.5155; M.P. = 104.0-108.0 °C.

**Clarithromycin-N-benzyltriazolylheptahydroxamic acid (31c).** Reaction of 4'-ethynylbenzyl clarithromycin 28 (0.130 g, 0.153 mmol) and 7-azidoheptahydroxamic acid 24b (0.105 g, 0.565 mmol) within 2.5 h, according to the protocols of Method A described for the synthesis of compound 31a, followed by prep TLC (silica, 12:1:0.1 CH₂Cl₂/MeOH/conc. NH₄OH) gave 105 mg (67%) of 31c as a brown-white solid. ¹H-NMR (CDCl₃, 400MHz) δ 0.82 (3H, t, J = 8.0 Hz), 1.04-1.52 (21H, m), 1.67-1.92 (14H, m) 2.14-2.29 (8H, m), 2.52-2.60 (3H, m), 2.82-2.90 (2H, m), 2.95-3.00 (6H, m), 3.10 (3H, s), 3.16 (2H, s), 3.27-3.32 (1H, m), 3.41-3.46 (3H, m), 3.59 (1H, d, J = 6.8 Hz), 3.69-3.79 (3H, m), 3.90-3.95 (1H, m), 4.34-4.39 (3H, m), 4.87 (1H, d, J = 4.4 Hz), 5.02
Descladinose clarithromycin-N-benzyltriazolylheptahydroxamic acid (31d). Reaction of descladinose-4'-ethynylbenzyl clarithromycin 29 (0.075 g, 0.109 mmol) and 7-azidoheptahydroxamic acid 24b (0.040 g, 0.233 mmol) within 4 h, according to the protocols of Method A described for the synthesis of compound 31a, followed by prep TLC (silica, 10:1:0.1 CH₂Cl₂/MeOH/conc. NH₄OH) gave 80 mg (84%) of 31d as a brown-white solid. ¹H-NMR (CDCl₃, 400MHz) δ 0.78 (3H, t, $J = 7.2$ Hz), 1.06-1.31 (22H, m), 1.40-1.53 (5H, m), 1.71 (2H, d, $J = 11.6$ Hz), 1.80-1.91 (3H, m), 2.01-2.20 (6H, m), 2.50-2.65 (4H, m), 2.91-2.97 (3H, m), 3.16 (1H, t, $J = 6.4$ Hz), 3.26-3.35 (2H, m), 3.42-3.54 (2H, m), 3.64-3.71 (3H, m), 3.80 (1H, br s), 3.90 (1H, br s), 4.30-4.34 (3H, m), 5.12 (1H, dd, $J = 11.6, 2.4$ Hz), 7.27 (2H, d, $J = 8.0$ Hz), 7.72 (2H, d, $J = 7.2$ Hz), 7.80 (1H, s); ¹³C NMR (CDCl₃, 100 MHz) δ 8.3, 10.3, 12.5, 15.2, 16.1, 17.6, 18.6, 21.1, 21.3, 24.8, 25.1, 25.5, 26.2, 27.8, 28.5, 29.1, 29.6, 29.7, 32.3, 35.8, 36.3, 37.4, 38.6, 44.3, 45.4, 49.5, 50.0, 51.2, 57.8, 64.2, 69.7, 69.9, 70.6, 74.1, 77.9, 78.7, 88.0, 106.3, 119.9, 125.7, 129.3, 129.5, 138.3, 147.3, 175.1; HRMS (ESI) calcd for [C₄₅H₇₁N₅O₁₂ + H]⁺ 876.5329, found 876.5301; M.P. = 85.0-89.0 °C.
**Clarithromycin-N-benzyltriazolyloctahydroxamic acid (31e).** Reaction of 4'-ethynylbenzyl clarithromycin $28$ (0.101 g, 0.120 mmol) and 8-azidooctahydroxamic acid $24c$ (0.047 g, 0.240 mmol) within 2.5 h, according to the protocols of **Method A** described for the synthesis of compound $31a$, followed by prep TLC (silica, 12:1:0.1 CH$_2$Cl$_2$/MeOH/conc. NH$_4$OH) gave 92 mg (74%) of $31e$ as a brown-white solid. $^1$H NMR (CDCl$_3$, 400 MHz) $\delta$ 0.81 (3H, t, $J = 7.2$ Hz), 1.04-2.05 (45H, m), 2.22 (3H, s), 2.19-2.82 (m, 7H), 3.00 (3H, s), 3.08 (3H, s), 2.91-3.80 (6H, m), 3.95 (3H, m), 4.38 (3H, m), 4.88 (1H, d, $J = 4.4$ Hz), 5.04 (1H, dd, $J = 10.8$, 2.0 Hz), 7.33 (2H, d, $J = 7.6$ Hz), 7.71 (1H, s), 7.77 (2H, d, $J = 8.0$ Hz); $^{13}$C NMR (CDCl$_3$, 100MHz) $\delta$ 8.8, 9.4, 10.8, 12.5, 16.2, 18.2, 18.8, 20.0, 21.2, 21.5, 21.6, 25.1, 26.0, 26.7, 28.2, 28.6, 28.9, 29.9, 30.1, 35.0, 36.9, 37.4, 39.2, 39.4, 45.2, 45.4, 46.1, 49.6, 50.4, 50.8, 51.6, 58.1, 63.6, 65.9, 68.4, 69.5, 70.9, 72.8, 74.4, 78.0, 78.5, 81.2, 96.1, 120.1, 102.6, 126.1, 130.2, 147.4, 176.0; HRMS (ESI) calc for [C$_{54}$H$_{90}$N$_5$O$_{15}$ + H]$^+$ 1048.6486, found 1048.6427; M.P. = 87.0-97.0 $^\circ$C.

**Descladinose clarithromycin-N-benzyltriazolyloctahydroxamic acid (31f).** Reaction of descladinose-4'-ethynylbenzyl clarithromycin $29$ (0.10 g, 0.144 mmol) and 8-azidooctahydroxamic acid $24c$ (0.049 g, 0.246 mmol) within 2.5 h, according to the protocols of **Method A** described for the synthesis of compound $31a$, followed by prep TLC (silica, 10:1:0.1 CH$_2$Cl$_2$/MeOH/conc. NH$_4$OH) gave 117 mg (90 %) of $31f$ as a brown-white solid. $^1$H NMR (CDCl$_3$, 400 MHz) $\delta$ 0.81 (3H, t, $J = 7.2$ Hz), 1.10-2.09 (37H, m), 2.18 (3H, s), 2.19-2.68 (7H, m), 2.98-3.73 (4H, m), 3.83 (2H, s), 3.93 (1H, m), 4.36 (3H, m), 5.16 (1H, d, $J = 8.0$ Hz), 7.31 (2H, d, $J = 8.0$ Hz), 7.77 (2H, d, $J = 8.0$ Hz), 7.79 (1H, s); $^{13}$C NMR (CDCl$_3$, 100MHz) $\delta$ 8.5, 10.6, 12.8, 15.4, 16.4, 17.9, 18.9, 21.4,
21.6, 29.4, 36.1, 36.7, 37.7, 38.9, 44.7, 45.7, 49.8, 58.0, 65.2, 70.0, 70.4, 70.8, 74.4, 76.8, 78.2, 79.2, 83.6, 88.8, 106.9, 121.3, 128.7, 132.5, 139.6, 175.2; HRMS (FAB, thioglycerol) calc for [C_{46}H_{76}N_{5}O_{12} + H]^+ 890.5490, found 890.5562; M.P. = 90.0-95.0 °C.

**Clarithromycin-N-benzyltriazolylnonahydroxamic acid (31g).** Reaction of 4’-ethynylbenzyl clarithromycin 28 (0.100 g, 0.120 mmol) and 9-azidononahydroxamic acid 24d (0.043 g, 0.20 mmol) within 2.5 h, according to the protocols of **Method A** described for the synthesis of compound 31a, followed by prep TLC (silica, 12:1:0.1 CH₂Cl₂/MeOH/conc. NH₂OH) gave 54 mg (42%) of 31g as a brown-white solid. ¹H NMR (CDCl₃, 400 MHz) δ 0.81 (3H, t, J = 7.2 Hz), 1.04-2.02 (47H, m), 2.24 (3H, s), 2.10-2.97 (7H, m), 3.00, 3.09 (6H, s), 3.20-3.82 (6H, m), 3.88 (3H, m), 4.39 (3H, m), 4.88 (1H, d, J = 4.0 Hz), 5.05 (1H, d, J = 10.0 Hz), 7.35 (2H, d, J = 8.0 Hz), 7.77 (3H, d, J = 4.0 Hz); ¹³C NMR (CDCl₃, 100MHz) δ 9.0, 9.3, 10.8, 12.5, 16.1, 18.2, 18.8, 21.2, 21.5, 21.6, 21.7, 21.8, 26.3, 26.8, 28.7, 28.9, 29.1, 29.9, 30.3, 35.0, 37.0, 37.4, 39.3, 39.4, 45.2, 45.4, 49.6, 50.5, 50.8, 51.6, 57.8, 63.8, 65.8, 68.8, 69.2, 70.9, 72.7, 74.5, 76.8, 78.1, 78.4, 78.5, 81.1, 96.1, 102.9, 119.7, 125.9, 129.6, 129.9, 138.7, 147.6, 176.1; HRMS (ESI) calc for [C_{55}H_{91}N_{5}O_{15} + H]^+ 1062.6584, found 1062.6586; M.P. = 92.0-105.0 °C.

**Clarithromycin-N-benzyltriazolyldecahydroxamic acid (31h).** Reaction of 4’-ethynylbenzyl clarithromycin 28 (0.10 g, 0.120 mmol) and 10-azidodecahydroxamic acid 24e (0.045 g, 0.197 mmol) within 2.5 h, according to the protocols of **Method A** described for the synthesis of compound 31a, followed by prep TLC (silica, 12:1:0.1
CH₂Cl₂/MeOH/conc. NH₄OH) gave 68 mg (53 %) of 31h as a brown-white solid. ¹H NMR (CDCl₃, 400 MHz) 0.82 (3H, t, J = 7.2 Hz), 1.05-2.12 (49H, m), 2.24 (3H, s), 2.26-2.97 (7H, m), 3.01, 3.10 (6H, s), 3.19-3.80 (6H, m), 3.95 (3H, m), 4.39 (3H, m), 4.89 (1H, d, J = 4.0 Hz), 5.04 (1H, d, J = 8.0 Hz), 7.35 (2H, d, J = 8.0 Hz), 7.76 (1H, s), 7.79 (2H, d, J = 8.0 Hz); ¹³C NMR (CDCl₃, 100MHz) δ 9.3, 10.8, 12.5, 16.1, 18.2, 18.8, 20.0, 21.2, 21.5, 21.7, 25.4, 26.2, 28.9, 29.0, 29.3, 29.6, 29.9, 30.2, 35.0, 37.0, 37.4, 39.3, 39.4, 45.2, 45.4, 49.6, 50.5, 50.8, 51.6, 57.8, 63.8, 65.8, 68.8, 69.2, 70.9, 72.7, 74.5, 76.8, 78.1, 78.5, 81.1, 96.1, 102.9, 119.7, 125.9, 129.6, 129.8, 138.9, 147.6, 176.1 ; HRMS (ESI) calc for [C₅₆H₉₃N₅O₁₅+ H]⁺ 1076.6740, found 1076.6667; M.P. = 87.0-105.0 °C.

Azithromycin-N-benzyl-¹⁵N-triazolylhexahydroxamic acid (32a).

Reaction of 4’-ethynylbenzyl azithromycin 19 (0.080 g, 0.094 mmol) and 6-¹⁵N-azidohexahydroxamic acid 24f (0.032 g, 0.185 mmol) within 4 h, according to the protocols of Method B described for the synthesis of compound 23a, followed by prep TLC (silica, 12:1:0.1 CH₂Cl₂/MeOH/conc. NH₄OH) gave 50 mg (53%) of 32a as a yellowish-green solid. ¹H-NMR (CDCl₃, 400MHz) δ 0.82-1.51 (28H, m), 1.60-1.77 (6H, m), 1.86-2.08 (9H, m), 2.24-2.36 (9H, m), 2.46-2.56 (2H, m), 2.62-2.70 (2H, m), 3.00 (3H, s), 3.32 (2H, t, J = 8.8 Hz), 3.38-3.54 (6H, m), 3.60-3.78 (2H, m), 3.98-4.08 (2H, m), 4.36-4.50 (3H, m), 5.06-5.12 (2H, m), 7.33 (2H, d, J = 7.6 Hz), 7.77 (2H, d, J = 7.6 Hz); HRMS (ESI) calcd for [C₅₂H₈₈N₅O₁₄¹⁵N + H]⁺ 1022.6401, found 1021.6365
Azithromycin-N-benzyl-$^{15}$N-triazolylheptahydroxamic acid (32b).

Reaction of 4'-ethynylbenzyl azithromycin 19 (0.080 g, 0.094 mmol) and 7-$^{15}$N-azidoheptahydroxamic acid 24g (0.050 g, 0.267 mmol) within 4 h, according to the protocols of Method B described for the synthesis of compound 23a, followed by prep TLC (silica, 12:1:0.1 CH$_2$Cl$_2$/MeOH/conc. NH$_4$OH) gave 72 mg (74%) of 32b as a yellowish-green solid. $^1$H-NMR (CDCl$_3$, 400MHz) δ 0.82-1.53 (30H, m), 1.54-1.65 (6H, m), 1.70-2.14 (9H, m), 2.20-2.38 (9H, m), 2.46-2.56 (2H, m), 2.60-2.70 (2H, m), 3.00 (3H, s), 3.31 (2H, t, $J$ = 8.8 Hz), 3.38-3.54 (6H, m), 3.59 (1H, s), 3.79 (1H, d, $J$ = 12.8 Hz), 3.98-4.10 (2H, m), 4.35 (3H, d, $J$ = 7.2 Hz), 4.49 (1H, d, $J$ = 7.2 Hz), 5.11 (1H, d, $J$ = 4.0 Hz), 7.32 (2H, d, $J$ = 7.6 Hz), 7.73 (1H, s), 7.75 (2H, d, $J$ = 7.6 Hz); HRMS (ESI) calcd for [C$_{53}$H$_{90}$N$_5$O$_{14}$$^{15}$N + H]$^+$ 1036.6558, found 1036.6580

O-3’-Acetylated-4’-ethynylbenzyl azithromycin (33a). To a solution of 4’-ethynylbenzyl azithromycin 19 (0.140g, 0.164 mmol) in anhydrous CH$_2$Cl$_2$ (3 mL) was added acetic anhydride (0.02 mL, 0.196 mmol). The reaction mixture is heated to 40°C in a pressure tube and stirring continued for 48 h. The reaction mixture was cooled and diluted with CH$_2$Cl$_2$ (5 mL) and washed with saturated NaHCO$_3$ (2 x 10 mL), and saturated brine (10 mL). The organic layer was dried over Na$_2$SO$_4$. Solvent was evaporated off to give 140 mg (95%) of compound 33a as a yellowish solid (>95 % purity, TLC: CH$_2$Cl$_2$/MeOH 12:1). The crude 33a was used without further purification. $^1$H-NMR (CDCl$_3$, 400MHz) δ 0.11 (3H, s), 0.81-0.86 (6H, m), 0.92-0.97 (10H, m), 1.10-1.25 (19H, m), 1.36-1.56 (2H, m), 1.70-1.76 (2H, m), 1.94-2.17 (4H, m), 2.25-2.28 (7H, m), 2.51-2.78 (2H, m), 2.92-3.01 (3H, m), 3.05 (1H, s), 3.18 (3H, s), 3.37-3.47(4H, m),
3.60 (1H, br s), 3.65 (1H, s), 3.92-3.98 (1H, m), 4.11 (1H, br s), 4.43 (2H, d, \( J = 7.2 \) Hz), 4.77-5.02 (2H, m), 7.15 (2H, d, \( J = 8.0 \) Hz), 7.35 (2H, d, \( J = 8.4 \) Hz); \(^{13}\)C-NMR (CDCl\(_3\), 100MHz) \( \delta \) 0.9, 7.8, 11.1, 11.7, 14.5, 17.9, 20.7, 20.9, 21.1, 21.2, 21.5, 21.6, 24.0, 24.6, 27.0, 29.5, 31.1, 35.0, 36.6, 40.9, 42.9, 45.5, 49.2, 58.2, 60.8, 62.2, 65.6, 69.0, 71.1, 72.7, 73.7, 75.6, 76.1, 77.8, 83.6, 84.8, 96.9, 101.5, 120.4, 128.2, 131.8, 140.7, 169.8, 170.2, 176.3; HRMS (MALDI, CHCA) calc for \([C_{48}H_{78}N_{13}O_{13} + H]^+\) 891.5577, found 891.5374.

**O-3’-\(^{14}\)C-Acetylated-4’-ethynylbenzyl azithromycin (33b).** Reaction of 4’-ethynylbenzyl azithromycin 19 (0.140g, 0.164 mmol), acetic anhydride-carbonyl-\(^{14}\)C (0.08 µL, 0.008 mmol, 0.25 mCi), and acetic anhydride (0.02 mL, 0.174 mmol) within 48 h, according to the protocols described for the synthesis of 33a, gave 140 mg (95%) of 33b as a yellowish solid. The crude 33b was used without further purification. [>95 % purity, TLC: CHCl\(_2\)/MeOH 12:1]

**O-3’-Acetylated-azithromycin-N-benzyltriazylo-hexahydroxamic acid (34a).** O-3’-Acetylated-4’-ethynylbenzyl azithromycin 33a (0.040 g, 0.045 mmol) and 6-azidohexahydroxamic acid 24a (0.020 g, 0.115 mmol) were dissolved in anhydrous THF (5 mL) and stirred under argon at room temperature. Copper (I) iodide (0.011 g, 0.070 mmol), TBTA (0.038 g, 0.00 mmol), and Hunig’s base (0.2 mL) were then added to the reaction mixture, and stirring continued for 24 h. The reaction mixture was diluted with CH\(_2\)Cl\(_2\) (20 mL) and washed with 1:4 NH\(_4\)OH/saturated NH\(_4\)Cl (3 x 30 mL) and saturated NH\(_4\)Cl (30 mL). The organic layer was dried over Na\(_2\)SO\(_4\) and concentrated in vacuo. The crude product was purified by prep TLC (silica, 12:1:0.1 CH\(_2\)Cl\(_2\)/MeOH/conc.)
OH) to give 30 mg (63%) of 34a as a yellowish solid. \(^1\)H-NMR (CDCl\(_3\), 400MHz) \(\delta\)

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0.82-2.39 (52H, m), 2.56-2.72 (4H, m), 2.93-3.01 (3H, m), 3.18 (3H, s), 3.40-3.69 (10H, m), 3.96-4.08 (2H, m), 4.33-4.44 (4H, m), 4.79-4.94 (1H, m), 7.26 (2H, d, \(J = 7.6\) Hz), 7.70 (2H, d, \(J = 7.6\) Hz), 7.79 (1H, s); \(^{13}\)C-NMR (CDCl\(_3\), 100MHz) \(\delta\) 11.3, 11.9, 14.4, 17.0, 18.3, 21.1, 21.6, 21.7, 22.0, 22.9, 24.6, 25.1, 25.8, 29.9, 37.0, 58.6, 61.7, 64.1, 65.8, 69.3, 71.4, 72.9, 74.3, 75.7, 76.2, 78.1, 94.4, 97.1, 101.6, 120.0, 125.8, 129.2, 147.9, 170.3, 176.3; HRMS (MALDI, CHCA) calc for \([C_{54}H_{90}N_6O_{15} + H]^+\) 1063.6536, found 1063.6329.

**O-3’-Acetylated-azithromycin-N-benzyltriazolylheptahydroxamic acid (34b).**

Reaction of O-3’-Acetylated-4’-ethynylbenzyl azithromycin 33a (0.040 g, 0.045 mmol) and 7-azidoheptahydroxamic acid 24b (0.020 g, 0.114 mmol) within 24 h, as described for the synthesis of compound 34a, followed by prep TLC (silica, 12:1:0.1 CH\(_2\)Cl\(_2\)/MeOH/conc. NH\(_4\)OH) gave 20 mg (42%) of 34b as a yellowish solid. \(^1\)H-NMR (CDCl\(_3\), 400MHz) \(\delta\)

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0.84-2.36 (54H, m), 2.62-2.74 (4H, m), 2.95-3.04 (3H, m), 3.21 (3H, s), 3.40-3.71 (10H, m), 3.97-4.12 (2H, m), 4.36-4.45 (4H, m), 4.79 (1H, m), 7.29 (2H, d, \(J = 8.0\) Hz), 7.72 (2H, d, \(J = 7.6\) Hz), 7.78 (1H, s); \(^{13}\)C-NMR (CDCl\(_3\), 100MHz) \(\delta\) 8.0, 11.1, 11.7, 14.1, 18.0, 20.8, 20.9, 21.3, 21.5, 23.9, 24.9, 25.6, 27.9, 29.6, 29.8, 30.7, 31.8, 32.4, 35.0, 36.7, 45.4, 49.3, 50.0, 58.3, 61.0, 61.5, 65.5, 69.1, 71.1, 72.7, 74.0, 75.6, 76.1, 77.9, 96.9, 101.6, 119.6, 121.5, 128.9, 129.0, 139.8, 147.7, 170.1, 176.3; HRMS (MALDI, CHCA) calc for \([C_{55}H_{92}N_6O_{15} + H]^+\) 1077.6693, found 1077.6488.
O-3′-14C-Acetylated-azithromycin-N-benzyltriazolylhexahydroxamic acid (34c).

Reaction of O-3′-14C-acetylated-4′-ethynylbenzyl azithromycin 33b (0.070 g, 0.078 mmol) and 6-azidohexahydroxamic acid 24a (0.040 g, 0.231 mmol) within 24 h, as described for the synthesis of compound 34a, followed by prep TLC (silica, 12:1:0.1 CH2Cl2/MeOH/conc. NH4OH) gave 22 mg (27%) of 34c as a yellowish solid. [>95% purity, TLC: CHC2Cl2/MeOH/ NH4OH 12:1:0.1]

O-3′-14C-Acetylated-azithromycin-N-benzyltriazolylheptahydroxamic acid (34d).

Reaction of O-3′-14C-acetylated-4′-ethynylbenzyl azithromycin 33b (0.070 g, 0.078 mmol) and 7-azidoheptahydroxamic acid 24b (0.040 g, 0.230 mmol) within 24 h, as described for the synthesis of compound 34a, followed by prep TLC (silica, 12:1:0.1 CH2Cl2/MeOH/conc. NH4OH) gave 16 mg (19%) of 34d as a yellowish solid. [>95% purity, TLC: CHC2Cl2/MeOH/ NH4OH 12:1:0.1]

HDAC Activity Assay. In Vitro HDAC inhibition was assayed using the HDAC Fluorimetric Assay/Drug Discovery Kit as previously described.17,19 Briefly, 15 μl HeLa Nuclear extract was mixed with 5μl 10X compound and 5μl Assay Buffer. Fluorogenic substrate was added and reaction was allowed to proceed for 15 min at room temperature and then stopped by addition of a developer containing TSA. Fluorescence was monitored after 15 min at excitation and emission wavelengths of 360 and 460 nm, respectively. IC50 values were determined using logit plots.
Cell Culture and Viability. SK-MES-1 and NCI-H69 lung cancer cell lines and DU-145 prostate cancer cell line were obtained from ATCC (Manassas, VA) and were maintained in the recommended complete growth mediums. MCF-7 cell line (a generous gift from Dr. Al Merill) was maintained in EMEM containing 10% fetal bovine serum. Human mammary epithelial cells (HMEC) (Lonza Biosciences) were maintained in complete MEGM per product instructions. Human normal lung fibroblasts (a generous gift from Dr. Barker) were maintained in EMEM containing 10 percent FBS. All cell lines were maintained in a 37 °C environment containing 5 % CO₂. All compounds to be tested were dissolved to a concentration of 10 mM in DMSO and stored at -80 °C. For cell viability experiments, cells were passaged 24 h prior to dosing. All compounds were diluted to appropriate concentrations in DMSO and fresh medium such that the final concentration of DMSO was 0.1%. To control wells, only fresh medium was added. Viability was assessed after 72 h by Trypan Blue staining.

2.5.4 Nuclear Targeting HDACi Experimental

Ethyl 6-(4-anilyl)triazolylhexanoate (35a). Ethyl 6-azidohexanoate 2c (0.711 g, 3.84 mmol) and 4-ethynylaniline (0.300 g, 2.56 mmol) were dissolved in anhydrous THF (7 mL) and stirred under argon at room temperature. Copper (I) iodide (0.011 g, 0.07 mmol) and Hunig’s base (0.3 mL) were then added to the reaction mixture, and stirring continued for 24 h. The reaction mixture was diluted with CH₂Cl₂ (10 mL) and washed with 1:4 NH₄OH/saturated NH₄Cl (3 x 30 mL) and saturated NH₄Cl (30 mL). The organic layer was dried over Na₂SO₄ and concentrated in vacuo. The crude product was purified by flash chromatography (silica, gradient 1:1; 1:2; 1:3 Hexane/EtOAc) to give
696 mg (90%) of 35a as yellowish solid. $^1$H NMR (CDCl$_3$, 400 MHz) δ 1.19 (3H, t, $J = 7.2$ Hz), 1.29-1.37 (2H, m), 1.59-1.67 (2H, m), 1.86-1.94 (2H, m), 2.25 (2H, t, $J = 7.6$ Hz), 4.07 (2H, q, $J = 14.0$, 6.8 Hz), 4.32 (2H, t, $J = 7.2$ Hz), 6.68 (2H, d, $J = 8.0$ Hz), 7.58 (3H, t, $J = 3.6$ Hz); $^{13}$C NMR (CDCl$_3$, 100MHz) δ 14.1, 21.4, 25.8, 29.9, 33.8, 49.9, 60.2, 115.1, 118.1, 121.0, 126.7, 146.4, 148.0, 173.3

**Ethyl 7-(4-anilyl)triazolylheptanoate (35b).** Reaction of ethyl 7-azidoheptanoate 2d (0.711 g, 3.57 mmol) and 4-ethynylaniline (0. 300g, 2.56 mmol) within 24 h as described for the synthesis of 35a, followed by flash chromatography (silica, gradient 1:1; 1:2; 1:3 Hexane/EtOAc) gave 700 mg (87%) of 35b as yellowish solid. $^1$H NMR (CDCl$_3$, 400 MHz) δ 1.15 (3H, t, $J = 7.2$ Hz), 1.20-1.30 (4H, m), 1.48-1.54 (2H, m), 1.78-1.82 (2H, m), 2.18 (2H, t, $J = 7.2$ Hz), 4.02 (2H, q, $J = 14.4$, 6.8 Hz), 4.22 (2H, t, $J = 7.2$ Hz), 6.62 (2H, d, $J = 8.4$ Hz), 7.51 (1H, s), 7.53 (2H, d, $J = 4.8$ Hz); $^{13}$C NMR (CDCl$_3$, 100MHz) δ 13.9, 24.3, 25.8, 28.1, 29.8, 33.8, 49.8, 59.9, 114.9, 118.0, 120.6, 126.5, 146.4, 147.7, 173.3

**Methyl 8-(4-anilyl)triazolyloctanoate (35c).** Reaction of methyl 8-azidooctanoate 2e (1.200 g, 6.03 mmol) and 4-ethynylaniline (0. 500g, 4.27 mmol) within 24 h as described for the synthesis of 35a, followed by flash chromatography (silica, gradient 1:1; 1:2; 1:3 Hexane/EtOAc) gave 923 mg (69%) of 35c as yellowish solid. $^1$H NMR (CDCl$_3$, 400 MHz) δ 1.24-1.32 (6H, m), 1.54-1.58 (2H, m), 1.84-1.87 (2H, m), 2.24 (2H, t, $J = 7.6$ Hz), 3.61 (3H, s), 4.29 (2H, t, $J = 7.2$ Hz), 6.67 (2H, d, $J = 8.4$ Hz), 7.56 (2H, d, $J = 2.0$ Hz).
Hz), 7.58 (1H, s); 13C NMR (CDCl3, 100MHz) δ 24.6, 26.1, 28.5, 28.7, 30.1, 33.8, 50.1, 51.3, 115.0, 118.0, 121.0, 126.7, 146.4, 147.9, 174.0

Ethyl 6-(4-azidophenyl)triazolylhexanoate (36a). To a solution of ethyl 6-(4-anilino)triazolylhexanoate 35a (0.676 g, 2.238 mmol) in 17.2% aqueous HCl (15 mL) at 0 °C was added NaNO2 (0.140 g, 2.027 mmol) and stirring continued at 0 °C for 1 h. Sodium azide (0.270 g, 4.153 mmol) was then added and stirring continued at 0 °C for additional 3 h. The reaction was poured into cold water (30 mL) and extracted with CH2Cl2 (3 x 30 mL). The combined organic layer was washed with saturated brine (30 mL) and dried over dried over Na2SO4. Solvent was evaporated off to give 400 mg of compound 36a as a yellow solid. The crude 36a was used in the next step without further purification.

Ethyl 7-(4-azidophenyl)triazolylheptanoate (36b). To a solution of ethyl 7-(4-anilino)triazolylheptanoate 35b (0.300 g, 0.948 mmol) in 17.2% aqueous HCl (5 mL) at 0 °C was added NaNO2 (0.062 g, 0.898 mmol) and stirring continued at 0 °C for 1 h. Sodium azide (0.120 g, 1.846 mmol) was then added and stirring continued at 0 °C for additional 3 h. The reaction was poured into cold water (20 mL) and extracted with CH2Cl2 (3 x 20 mL). The combined organic layer was washed with saturated brine (20 mL) and dried over dried over Na2SO4. Solvent was evaporated off to give 132 mg of compound 36b as a yellow solid. The crude 36b was used in the next step without further purification.
Methyl 8-(4-azidophenyl)triazolyloctanoate (36c). To a solution of methyl 8-(4-anil)triazolyloctanoate 35c (0.519 g, 1.642 mmol) in 17.2% aqueous HCl (10 mL) at 0 °C was added NaNO₂ (0.107 g, 1.550 mmol) and stirring continued at 0 °C for 1 h. Sodium azide (0.207 g, 3.185 mmol) was then added and stirring continued at 0 °C for additional 3 h. The reaction was poured into cold water (20 mL) and extracted with CH₂Cl₂ (3 x 20 mL). The combined organic layer was washed with saturated brine (20 mL) and dried over dried over Na₂SO₄. Solvent was evaporated off to give 332 mg of compound 36c as a yellow solid. The crude 36c was used in the next step without further purification.

6-(4-azidophenyl)hexanoic acid (37a). To a solution of ethyl 6-(4-azidophenyl)hexanoate 36a (0.180 g, 0.549 mmol) in 3:1 THF (6 mL) and distilled water (2 mL) was added lithium hydroxide monohydrate (0.066 g, 1.573 mmol) and the stirring continued for 24 h. The reaction was partitioned between distilled water (10 mL) and EtOAc (20 mL). The two layers were separated, and the aqueous layer was extracted with EtOAc (2 x 20 mL). The aqueous layer was acidified to pH 2 in an ice bath with 1N HCl and extracted with EtOAc (3 x 20 mL). The combined organic layer was dried over Na₂SO₄ and concentrated in vacuo to give 164 mg (100%) of 37a as a yellowish solid.

\[^1\text{H}\text{-NMR (DMSO-d}_6, 400MHz) \delta 1.23-1.29 (2H, m), 1.48-1.55 (2H, m), 1.82-1.87 (2H, m), 2.19 (2H, t, J = 7.2 Hz), 4.36 (2H, t, J = 6.8 Hz), 7.18 (2H, d, J = 8.4 Hz), 7.86 (2H, d, J = 7.2 Hz), 8.57 (1H, s), 12.0 (1H, s); \[^{13}\text{C}\text{-NMR (DMSO-d}_6, 100MHz) \delta 23.8, 25.4, 29.3, 33.4, 49.4, 119.6, 121.1, 126.6, 127.8, 138.6, 145.5, 174.3
7-(4-azidophenyl)triazolyleptanoic acid (37b). Reaction of ethyl 7-(azidophenyl)triazolyleptanoate 36b (0.120 g, 0.350 mmol) and lithium hydroxide monohydrate (0.044 g, 1.049 mmol) within 24 h as described for the synthesis of 37a gave 108 mg (100%) of 37b as pale yellowish solid. $^1$H NMR (DMSO-d$_6$, 400 MHz) $\delta$ 1.23-1.32 (4H, m), 1.43-1.50 (2H, m), 1.79-1.87 (2H, m), 2.17 (2H, t, J = 7.2 Hz), 4.36 (2H, t, J = 7.6 Hz), 7.17 (2H, d, J = 8.8 Hz), 7.86 (2H, d, J = 8.4 Hz), 8.57 (1H, s), 11.9 (1H, br s); $^{13}$C NMR (DMSO-d$_6$, 100MHz) $\delta$ 24.2, 25.5, 27.8, 29.4, 33.5, 49.4, 119.6, 121.1, 126.6, 127.8, 138.6, 145.4, 174.4

8-(4-azidophenyl)triazolyloctanoic acid (37c). Reaction of methyl 8-(azidophenyl)triazolyloctanoate 36c (0.332 g, 0.971 mmol) and lithium hydroxide monohydrate (0.122 g, 2.909 mmol) within 24 h as described for the synthesis of 37a gave 317 mg (100%) of 37c as pale yellowish solid. $^1$H NMR (DMSO-d$_6$, 400 MHz) $\delta$ 1.26 (6H, br s), 1.40-1.51 (2H, m), 1.79-1.87 (2H, m), 2.16 (2H, t, J = 7.2 Hz), 4.36 (2H, t, J = 7.6 Hz), 7.17 (2H, d, J = 8.8 Hz), 7.86 (2H, d, J = 8.4 Hz), 8.57 (1H, s), 11.9 (1H, br s); $^{13}$C NMR (DMSO-d$_6$, 100MHz) $\delta$ 24.3, 25.7, 28.0, 28.3, 29.5, 33.5, 49.5, 119.6, 121.1, 126.6, 127.8, 138.6, 145.5, 174.4

6-(azidophenyl)triazolyl-0-trityl hexahydroxamate (38a). To a solution of 6-(4-azidophenyl)triazolylhexanoic acid 37a (0.100 g, 0.333 mmol) in anhydrous DMF (3 mL) was added EDC (0.083 g, 0.433 mmol), HOBT (0.067 g, 0.496 mmol), and NMM (0.05 mL, 0.455 mmol) in succession. Stirring continued under Ar for 20 mins. O-trityl hydroxamine (0.120 g, 0.435 mmol) was then added to the reaction mixture, and stirring
continued under Ar for 24 h. The reaction was diluted with CH$_2$Cl$_2$ (10 mL) and wash with ½ saturated NaHCO$_3$ (2 x 15 mL), distilled water (15 mL), and saturated brine (15 mL). The organic layer was dried over Na$_2$SO$_4$ and concentrated in vacuo. The crude product was purified by flash chromatography (silica, 1:1 Hexane/EtOAc) to give to give 135 mg (73%) of 38a as a yellowish solid. $^1$H-NMR (CDCl$_3$, 400MHz) δ 0.98-1.08 (2H, m), 1.23-1.32 (2H, m), 1.54-1.62 (2H, br s), 1.72-1.84 (2H, m), 4.29 (2H, t, J = 7.2 Hz), 7.05 (2H, d, J = 8.4 Hz), 7.29 (15H, br s), 7.43 (1H, br s), 7.68 (1H, s), 7.80 (2H, d, J = 8.8 Hz); $^{13}$C NMR (CDCl$_3$, 100MHz) δ 22.4, 25.7, 29.9, 30.7, 50.0, 93.3, 119.4, 127.0, 127.5, 128.1, 128.9, 139.6, 140.9, 141.7, 146.8, 176.6; HRMS (ESI) calcd for [C$_{33}$H$_{31}$N$_7$O$^+ + Na$]$^+$ 580.2437, found 580.2383

7-(azidophenyl)triazolyl-O-trityl heptahydroxamate (38b). Reaction of 7-(azidophenyl)triazolylheptanoic acid 37b (0.105 g, 0.334 mmol) and O-trityl hydroxylamine (0.120g, 0.435 mmol) within 24 h as described for the synthesis of 38a followed by flash chromatography (silica, 1:1 Hexane/EtOAc), gave 140 mg (74%) of 38b as yellowish solid. $^1$H NMR (CDCl$_3$, 400 MHz) δ 1.06-1.23 (6H, m), 1.56-1.61 (2H, m), 1.81-1.87 (2H, m), 4.31 (2H, t, J = 6.4 Hz), 7.05 (2H, d, J = 8.4 Hz), 7.30 (15H, br s), 7.44 (1H, br s), 7.67 (1H, s), 7.79 (2H, d, J = 8.4 Hz); $^{13}$C NMR (CDCl$_3$, 100MHz) δ 23.0, 26.0, 28.2, 29.6, 30.1, 31.0, 50.2, 119.2, 119.4, 127.0, 127.5, 128.1, 129.0, 139.6, 141.0, 141.8, 146.9, 176.8; HRMS (ESI) calcd for [C$_{34}$H$_{33}$N$_7$O$_2$ + Na$]$$^+$ 594.2593, found 594.2575
8-(azidophenyl)triazolyl-O-trityl octahydroxamate (38c). Reaction of 8-(azidophenyl)triazolyloctanoic acid 37c (0.100 g, 0.309 mmol) and O-trityl hydroxylamine (0.120g, 0.435 mmol) within 24 h as described for the synthesis of 38a followed by flash chromatography (silica, 1:1 Hexane/EtOAc), gave 133 mg (74%) of 38c as brown solid. $^1$H NMR (CDCl$_3$, 400 MHz) $\delta$ 0.95-1.10 (2H, m), 1.25 (6H, br s), 1.50-1.70 (2H, m), 1.86-1.90 (2H, m), 4.35 (2H, t, $J = 6.8$ Hz), 7.07 (2H, d, $J = 8.0$ Hz), 7.32 (15H, br s), 7.45 (1H, br s), 7.71 (1H, s), 7.81 (2H, d, $J = 8.0$ Hz); $^{13}$C NMR (CDCl$_3$, 100MHz) $\delta$ 23.2, 26.1, 28.5, 29.6, 30.1, 31.0, 50.3, 119.1, 119.4, 127.0, 127.5, 128.1, 129.0, 139.6, 141.0, 141.8, 146.9, 176.8; HRMS (ESI) calcd for [C$_{35}$H$_{35}$N$_7$O$_2$ + H]$^+$ 586.2925, found 586.2944

$N$-Boc NLS triazole-linked phenyltriazolyl-O-trityl hexahydroxamates (39a). 6-(azidophenyl)triazolyl-O-trityl hexahydroxamate 38a (0.010 g, 0.018 mmol) and alkyne-modified NLS peptides PCS-37689-PI (0.020 g, 0.010 mmol) were dissolved in anhydrous THF (4 mL) and anhydrous DMF (1 mL) stirred under argon at room temperature. Copper (I) iodide (0.001 g, 0.006 mmol), TBTA (0.006 g, 0.011 mmol), and Hunig’s base (0.01 mL) were then added in succession to the reaction mixture, and stirring continued for 24 h. The reaction mixture was diluted with CH$_2$Cl$_2$ (10 mL) and washed with 1:4 NH$_2$OH/saturated NH$_4$Cl (3 x 30 mL) and saturated NH$_4$Cl (30 mL). The organic layer was dried over Na$_2$SO$_4$ and concentrated in vacuo. The crude product was purified by prep TLC (silica, 12:1:0.1 CH$_2$Cl$_2$/MeOH/NH$_4$OH) to give 23 mg (99%) of 39a as brown solid. $^1$H-NMR (DMSO-d$_6$, 400MHz) $\delta$ 0.81-1.98 (110H, m), 2.69-3.00 (15H, m). 3.15 (3H, d, $J = 5.2$ Hz), 3.61 (2H, d, $J = 6.0$ Hz), 3.72 (6H, br s), 4.08-4.32
(8H, m), 6.71 (4H, br s), 7.30 (10H, br s), 7.78-8.21 (13H, m), 8.63 (2H, d, $J = 5.2$ Hz), 10.1 (1H, s); HRMS (ESI) calc for [C$_{122}$H$_{179}$N$_{27}$O$_{26}$S + Na + H]$^{2+}$ 2494.3200, found 2494.2948

**$N$-Boc NLS triazole-linked phenyltriazolyl-O-trityl heptahydroxamates (39b).**

Reaction of 7-(azidophenyl)triazolyl-O-trityl heptahydroxamate 38b (0.010 g, 0.018 mmol) and alkyne-modified NLS peptides PCS-37689-PI (0.020g, 0.010 mmol) within 24 h as described for the synthesis of 39a, followed by prep TLC (silica, 12:1:0.1 CH$_2$Cl$_2$/MeOH/NH$_4$OH), gave 23 mg (99%) of 39b as yellowish solid. $^1$H-NMR (DMSO-d$_6$, 400MHz) $\delta$ 0.81-1.98 (112H, m), 2.61-2.98 (15H, m), 3.49 (3H, s), 3.61 (2H, d, $J = 5.6$ Hz), 3.68-3.78 (6H, m), 4.08-4.28 (4H, m), 4.32-4.39 (2H, m), 4.46 (2H, d, $J = 4.8$ Hz), 6.72 (4H, br s), 7.30 (15H, br s), 7.57-8.45 (13H, m), 8.63 (1H, s), 8.67 (1H, s), 10.1 (1H, s); HRMS (ESI) calc for [C$_{123}$H$_{181}$N$_{27}$O$_{26}$S + 2H]$^{2+}$ 2486.3310, found 2486.3338

**$N$-Boc NLS triazole-linked phenyltriazolyl-O-trityl octahydroxamates (39c).**

Reaction of $N$-Boc 8-(azidophenyl)triazolyl-O-trityl octahydroxamate 38c (0.015 g, 0.026 mmol) and alkyne-modified NLS peptides PCS-37689-PI (0.020g, 0.010 mmol) within 24 h as described for the synthesis of 39a, followed by prep TLC (silica, 12:1:0.1 CH$_2$Cl$_2$/MeOH/NH$_4$OH), gave 24 mg (100%) of 39c as yellowish solid. $^1$H-NMR (DMSO-d$_6$, 400MHz) $\delta$ 0.81-1.98 (114H, m), 2.65-2.98 (15H, m), 3.49 (3H, s), 3.61 (2H, d, $J = 6.0$ Hz), 3.68-3.78 (6H, m), 4.09-4.30 (4H, m), 4.39 (2H, t, $J = 5.6$ Hz), 4.47 (2H, d, $J = 4.8$ Hz), 6.73 (4H, br s), 7.29 (15H, br s), 7.58-8.25 (13H, m), 8.63 (1H, s), 8.68
(1H, s), 10.1 (1H, s); HRMS (ESI) calc for [C_{123}H_{183}N_{27}O_{26}S + Na]^+ 2523.3155, found 2523.3111

**NLS triazole-linked phenyltriazozylyhexahydroxamate (40a).** N-Boc NLS O-trityl hexahydroxamates 39a (0.010 g, 0.004 mmol) was dissolved in 90:5:5 TFA/TIPS/phenol (1 mL) and stirring continued for 2 h. The reaction mixture was divided into two equal halves by volume and pipette into two 1.5 mL eppendorf tubes. Anhydrous diethyl ether (1 mL) was added into each eppendorf tubes, and white precipitates were observed upon the addition of solvent. The eppendorf tubes were then vortex for 1 min, and further centrifuge down at 5000 rpm for 5 mins. The supernatant were removed and additional anhydrous diethyl ether (1 mL) was added into each eppendorf tube, followed by vortex and centrifugation. This process was repeated one more time, and the residual precipitates were air-dried for 20 mins and concentrate in vacuo. The residues were redissolved in distilled water, freezed in an acetone-dry ice bath, and lyophilized overnight to give 6 mg (100%) of 40a as pale yellowish solid. Partial ¹H-NMR (DMSO-d₆, 400MHz) δ 0.82 (8H, t, J = 6.4 Hz), 1.07 (8H, t, J = 8.4 Hz), 1.27-1.62 (30H, m), 1.85-1.95 (14H, m), 2.23-2.72 (10H, m), 3.38-4.39 (14H, m), 6.72 (1H, d, J = 8.4 Hz), 7.08-7.15 (3H, m), 7.31 (3H, s), 7.60-7.78 (11H, br s), 7.94-8.08 (8H, m), 8.18-8.24 (3H, m), 8.62 (1H, s), 8.68 (1H, s), 10.38 (1H, s); HRMS (ESI) calcd for [C_{70}H_{117}N_{27}O_{15} + H]^+ 1576.9300, found 1576.9420

**NLS triazole-linked phenyltriazozylyheptahydroxamate (40b).** Reaction of N-Boc NLS O-trityl heptahydroxamates 39b (0.010 g, 0.004 mmol) within 2 h as described for the synthesis of 40a gave 6 mg (100%) of 40b as yellowish solid. Partial ¹H-NMR
(DMSO-d$_6$, 400MHz) $\delta$ 0.83 (8H, t, $J$ = 6.0 Hz), 1.03-1.62 (41H, m), 1.85-1.97 (14H, m), 2.22-2.73 (10H, m) 3.36-4.39 (14H, m), 6.71 (1H, d, $J$ = 8.4 Hz), 7.10 (3H, s), 7.32 (3H, s), 7.60-7.85 (11H, br s), 7.95-8.10 (8H, m), 8.20-8.25 (3H, m), 8.64 (1H, s), 8.69 (1H, s), 10.34 (1H, s); HRMS (MALDI) calc for [C$_{71}$H$_{119}$N$_{27}$O$_{15}$ + H]$^+$ 1590.9451, found 1590.9450

NLS triazole-linked phenyltriazoxyoctahydroxamate (40c). Reaction of N-Boc NLS O-trityl octahydroxamates 39c (0.010 g, 0.004 mmol) within 2 h as described for the synthesis of 40a gave 6 mg (100%) of 40c as pale yellowish solid. Partial $^1$H-NMR (DMSO-d$_6$, 400MHz) $\delta$ 0.83 (8H, t, $J$ = 4.4 Hz), 1.03-1.63 (43H, m), 1.85-2.02 (14H, m), 2.22-2.74 (10H, m), 3.34-4.42 (14H, m), 6.71 (1H, d, $J$ = 8.4 Hz), 7.10-7.18 (3H, m), 7.32 (1H, s), 7.60-7.82 (11H, m), 7.95-8.10 (8H, m), 8.20-8.24 (3H, m), 8.64 (1H, s), 8.70 (1H, s), 10.33 (1H, s); HRMS (MALDI) calc for [C$_{72}$H$_{121}$N$_{27}$O$_{15}$ + H]$^+$ 1604.9699, found 1604.9608

Alkyne BODIPY (42). Alkyne aldehyde 41 (0.450 g, 2.22 mmol) and 2,4-dimethylpyrrole (0.47 mL, 4.50 mmol) were dissolves in anhydrous CH$_2$Cl$_2$ (40 mL). Two drops of trifluoroacetic acid were added, and the solution was stirred at room temperature (covered with tin foil) overnight. A solution of 2,3-dichloro-5,6-dicyano-p-benzoquinone (0.514 g, 2.22 mmol) in CH$_2$Cl$_2$ (30 mL) was added and stirring continued at room temperature for 4 h. Triethylamine (12 mL) was added to the reaction followed by a dropwise addition of BF$_3$:OEt$_2$ (12 mL) within 30 mins at 0°C. The reaction was gradually warmed to room temperature, and stirring continued overnight. The reaction
was washed with dilute NaHCO$_3$ (60 mL). The organic layer was passed through a celite plug, which the celite was washed with more CH$_2$Cl$_2$ (30 mL). The combined organic layer was washed with distilled water, dried over Na$_2$SO$_4$, and concentrated \textit{in vacuo}. The crude product was purified by flash chromatography (silica, gradient 10:1; 8:1 Hexane/EtOAc) to give 340 mg (54\%) of 42 as reddish solid. $^1$H NMR (CDCl$_3$, 400 MHz) $\delta$ 1.40 (6H, s), 1.72-1.76 (2H, m), 1.91-1.97 (3H, m), 2.26-2.30 (2H, m), 2.52 (6H, s), 4.01 (2H, t, $J = 6.0$ Hz), 5.95 (2H, s), 6.96 (2H, d, $J = 8.0$ Hz), 7.12 (2H, d, $J = 8.0$ Hz); $^{13}$C NMR (CDCl$_3$, 100MHz) $\delta$ 14.5, 18.1, 25.0, 28.2, 67.3, 68.7, 83.9, 114.9, 121.0, 126.9, 129.1, 131.8, 141.8, 143.1, 155.1, 159.5

**BODIPY-4-triazolylbenzyl alcohol (43).** Reaction of alkyne BODIPY 39 (0.050g, 0.119 mmol) and 4-azidobenzyl alcohol (0.035g, 0.238 mmol) within 24 h as described for the synthesis of 35a, followed by flash chromatography (silica, 1:2 Hexane/EtOAc) gave 66 mg (99\%) of 43 as reddish solid. $^1$H NMR (CDCl$_3$, 400 MHz) $\delta$ 1.22 (2H, t, $J = 7.2$ Hz), 1.38 (6H, s), 1.91-1.93 (2H, m), 2.50 (6H, s), 2.86 (2H, t, $J = 7.6$ Hz) 4.01-4.10 (2H, m), 4.73 (2H, s), 5.93 (2H, s), 6.95 (2H, d, $J = 8.4$ Hz), 7.09 (2H, d, $J = 8.4$ Hz), 7.46 (2H, d, $J = 8.0$ Hz), 7.66 (2H, d, $J = 8.4$ Hz), 7.75 (1H, s); $^{13}$C NMR (CDCl$_3$, 100MHz) $\delta$ 14.5, 20.9, 25.2, 25.9, 28.7, 29.6, 60.3, 64.1, 67.5, 114.9, 118.9, 120.3, 121.0, 126.8, 127.9, 129.0, 131.7, 136.1, 141.6, 141.8, 143.1, 148.4, 155.1, 159.4

**BODIPY-4-triazolylbenzyl mesylate (44).** Triethylamine (0.06 mL, 0.400 mmol) was added to a solution of BODIPY-4-triazolylbenzyl alcohol 43 (0.076 g, 0.134 mmol) in anhydrous CH$_2$Cl$_2$ (5 mL). The reaction mixture was stirred for 15 minutes at 0\(^\circ\)C, and
then methanesulfonyl chloride (0.03 mL, 0.400 mmol) was added. The reaction mixture is allowed to warm up to room temperature and stirring continued for 5 h. The reaction mixture was quenched with ice cold distilled water (10 mL) and extracted with anhydrous diethyl ether (2 x 10 mL). The combined organic layer was washed in succession with 1 N HCl, distilled water, saturated NaHCO$_3$, and then again with distilled water. The organic layer was dried over Na$_2$SO$_4$, and concentrated in vacuo to give 85 mg (99 %) of crude 44 as reddish solid. $^1$H-NMR (CDCl$_3$, 400MHz) $\delta$ 1.21 (2H, br s), 1.37 (6H, s), 1.91 (2H, br s), 2.48 (6H, s), 2.84 (2H, br s), 3.63 (3H, s), 4.02 (2H, br s), 5.23 (2H, s), 5.92 (2H, s), 6.94 (2H, d, $J = 8.4$ Hz), 7.07 (2H, d, $J = 8.4$ Hz), 7.52 (2H, d, $J = 8.4$ Hz), 7.54 (2H, d, $J = 8.4$ Hz), 7.8 (1H, br s); $^{13}$C NMR (CDCl$_3$, 100MHz) $\delta$ 14.4, 25.1, 25.8, 29.5, 31.4, 38.0, 52.4, 67.4, 70.1, 114.8, 120.3, 120.9, 126.6, 128.9, 129.9, 131.6, 133.7, 137.5, 141.8, 143.0 154.9, 159.4

**BODIPY-4-triazolylbenzyl azide (45).** Sodium azide (0.038 g, 0.593 mmol) was added to a solution of BODIPY-4-triazolylbenzyl mesylate 44 (0.096 g, 0.148 mmol) in anhydrous DMF (5 mL). The reaction mixture was heated to 60°C and stirring continued for 3 h. The reaction mixture was then cooled to room temperature and quenched with distill water (10 mL). The cooled reaction mixture was extracted with dichloromethane (3 x 10 mL), and the combined organic layers were washed with distilled water (3 x 10 mL), saturated brine (2 x 10 mL), and dried over Na$_2$SO$_4$. The solvent was removed and concentrated in vacuo. The crude product was purified by prep TLC (silica, 1:1 Hexane/EtOAc) to give 56 mg (64%) of 45 as reddish solid. $^1$H-NMR (CDCl$_3$, 400MHz) $\delta$ 1.20-1.23 (2H, m), 1.38 (6H, s), 1.91-1.93 (2H, m), 2.50 (6H, m), 2.87 (2H, t, $J = 6.8$
Hz), 4.01-4.10 (2H, m), 4.38 (2H, s), 5.93 (2H, s), 6.95 (2H, d, J = 8.4 Hz), 7.09 (2H, d, J = 8.4 Hz), 7.43 (2H, d, J = 8.4 Hz), 7.72 (2H, d, J = 8.4 Hz), 7.77 (1H, s); 13C NMR (CDCl₃, 100MHz) δ 14.4, 25.2, 25.8, 28.6, 29.5, 53.9, 60.3, 67.5, 114.9, 118.8, 120.5, 121.0, 126.7, 129.0, 129.3, 131.7, 135.8, 136.8, 141.8, 143.0, 148.5, 155.0, 159.4; HRMS (FAB, thioglycerol) calcd for [C₃₂H₃₃BF₂N₈O] 594.2838, found 594.1867

N-Boc NLS triazole-linked benzyltriazolyl-BODIPY (46). Reaction of BODIPY-4-triazolylbenzyl azide 45 (0.010 g, 0.018 mmol) and alkyne-modified N-Boc NLS peptides PCS-37689-PI (0.015g, 0.008 mmol) within 24 h as described for the synthesis of 39a, followed by prep TLC (silica, 12:1:0.1 CH₂Cl₂/MeOH/NH₄OH), gave 16 mg (94%) of 46 as orange solid. Partial ¹H-NMR (DMSO-d₆, 400MHz) δ 0.81 (13H, br s), 1.08 (12H, t, J = 6.8 Hz), 1.19-1.46 (51H, m), 1.59 (7H, br s), 1.78-1.84 (11H, m), 1.99 (7H, s), 2.16-2.20 (8H, m), 2.58-2.65 (7H, m), 2.78-2.85 (12H, m), 2.94-2.99 (7H, m), 3.62 (2H, d, J = 5.6 Hz), 3.69-3.73 (6H, m), 4.07-4.46 (10H, m), 5.61 (2H, s), 6.15 (2H, s), 6.71 (3H, br s), 7.08 (3H, t, J = 8.4 Hz), 7.23 (3H, d, J = 6.8 Hz), 7.47 (2H, d, J = 8.0 Hz), 7.86-8.20 (12H, m), 8.59 (1H, s); HRMS (ESI) calcd for [C₁₂₁H₁₸₁BN₂₈O₂₅F₂S + 2H]²⁺ 2505.3680, found 2505.3240

NLS triazole-linked benzyltriazolyl-BODIPY (47). Reaction N-Boc NLS triazole-linked benzyltriazolyl-BODIPY dye 46 (0.016 g, 0.006 mmol) within 2 h as described for the synthesis of 40a gave 11 mg (100%) of 47 as reddish solid. ¹H-NMR (DMSO-d₆, 400MHz) δ 0.82 (6H, t, J = 6.0 Hz), 1.02 (2H, s), 1.28-1.93 (67H, m), 2.18 (5H, t, J = 8.4 Hz), 3.04 (2H, br s) 3.62 (3H, d, J = 6.0 Hz), 3.70-3.74 (8H, m), 3.09 (5H, br s), 4.01-
BODIPY triazole-linked phenyltriazolyl-O-triyl hexahydroxamates (48). Reaction of 6-(azidophenyl)triazolyl-O-trityl hexahydroxamate 38a (0.095 g, 0.171 mmol) and alkyne BODIPY 42 (0.048g, 0.114 mmol) within 24 h as described for the synthesis of 39a, followed by prep TLC (silica, 1:3 Hexane/EtOAc), gave 86 mg (77%) of 48 as reddish solid. ¹H-NMR (CDCl₃, 400MHz) δ 1.23 (6H, t, J = 6.8 Hz), 1.401 (6H, s), 1.52-1.62 (2H, m), 1.70-2.01 (4H, m), 2.51 (6H, m), 2.90 (2H, t, J = 6.8 Hz), 4.03-4.12 (2H, m), 4.31 (2H, t, J = 6.8 Hz), 5.94 (2H, s), 6.97 (2H, d, J = 8.8 Hz), 7.12 (2H, d, J = 8.4 Hz), 7.29 (15H, br s), 7.40 (1H, br s), 7.75-7.80 (4H, m), 7.96 (2H, d, J = 8.4 Hz); HRMS (ESI) calcd for [C₅₈H₅₈N₉O₃F₂B + H]⁺ 978.4797, found 978.4785

BODIPY triazole-linked phenyltriazolyl hexahydroxamates (49). Reaction BODIPY O-trityl hexahydroxamates 48 (0.036 g, 0.036 mmol) within 2 h as described for the synthesis of 40a gave 11 mg (42%) of 49 as reddish solid. ¹H-NMR (CD₃OD, 400MHz) δ 1.16 (6H, t, J = 6.8 Hz), 1.30-1.41 (2H, m), 1.66-1.80 (2H, m), 1.81-2.00 (6H, br s), 2.05-2.15 (2H, m), 2.43 (6H, s) 2.90 (2H, t, J = 6.8 Hz), 4.20 (2H, t, J = 5.2 Hz), 4.48 (2H, t, J = 7.2 Hz), 6.42 (2H, s), 7.16 (2H, d, J = 8.8 Hz), 7.36 (2H, d, J = 8.8 Hz), 7.92 (2H, d, J = 8.8 Hz), 8.02 (2H, d, J = 8.8 Hz), 8.41 (1H, s), 8.44 (1H, s); HRMS (FAB, thioglycerol) calcd for [C₃₉H₄₄N₉O₃F₂B + 2H-BF₂]²⁺ 688.3723, 688.3736
2.6 References


CHAPTER 3
TARGETED NUCLEAR DELIVERY OF GOLD NANOPARTICLES

3.1 Gold Nanoparticles in Medicinal Application

Nanobiotechnology and nanomedicine are emerging fields engendered by convergence of multiple scientific fields for biologic applications and recently discovered novel properties of materials at the nanoscale. Nanoparticles, because of their small sizes and unique properties, have been widely used in drug delivery,\(^1\)-\(^4\) cellular imaging,\(^5\)-\(^9\) and biomedical diagnostics and therapeutics.\(^10\)-\(^13\) Plasmonic nanoparticles are especially useful for these applications because of their enhanced resonant absorption and scattering properties as well as strong Raman scattering, which are essential properties for their applications in photothermal therapy,\(^14\)-\(^25\) optical imaging,\(^26\)-\(^31\) and Raman probe design.\(^32\)-\(^38\)

Nuclear targeting of nanoparticles in live cells is generating widespread interest because of the prospect of developing novel diagnostic and therapeutic strategies such as gene therapy. However, nuclear delivery of the nanoparticles requires bypassing the formidable barriers of the cellular membrane and the nuclear membrane. The cellular membrane endocytoses non-specific particles on the cell surface, enveloping them with a phospholipid membrane and restricting interaction with the general cell cytoplasm. The nuclear membrane is a double-layer membrane marked with nuclear pores that allow diffusion of molecules and selective uptake into the nucleus. One common approach to targeted nuclear delivery is the conjugation of drug molecules and nanoparticles to nuclear membrane-penetrating peptides.
Gold nanoparticles (AuNPs) are examples of extremely attractive candidate for attachment to such carrier peptides due to their small sizes, ease of preparation and bioconjugation,\textsuperscript{39,40} strong absorbing and scattering properties,\textsuperscript{41-44} as well as their well-known biocompatibility.\textsuperscript{45} Tkachenko et al.,\textsuperscript{46,47} using video-enhanced color differential interference contrast microscopy, have demonstrated nuclear entry of 20 nm gold nanospheres indirectly conjugated to various nuclear localization signaling (NLS) peptides through a shell of bovine serum albumin (BSA) protein. However, these gold nanospheres only absorb in the visible region, thus limiting their use in \textit{in vivo} applications such as photothermal therapy, which requires near-infrared region where the light penetration is optimal.

Unlike gold (Au) nanospheres, Au nanorods possess unique optical properties ideal for \textit{in vivo} applications. They have two surface Plasmon absorption bands, a strong long-wavelength band due to longitudinal oscillation of their electrons and a weak short wavelength band around 520 nm due to the transverse electronic oscillation.\textsuperscript{42,43,48-51} The longitudinal absorption band is very sensitive to the size of the nanorods. By increasing the aspect ratio (length divide by width), the longitudinal absorption maximum red-shifts with an increase in the absorption intensity. This provides the opportunity for their applications as near-infrared photoabsorbers and scatterers.\textsuperscript{23}

Au nanorods are provide a scaffold for observing surface-enhanced Raman scattering (SERS).\textsuperscript{52-59} The well-developed synthesis of nanorods with different aspect ratios provides the opportunity to tune the surface Plasmon band to the excitation laser to obtain the largest enhancement. It has been shown that the enhancement factors on the order of $10^4$-$10^5$ could be observed for the adsorbed molecules on Au nanorods, which no
such enhancement was observed on nanospheres. Biological species are known to have small Raman scattering cross section and sensitivity toward photochemical damage and interference from fluorescence when UV or visible laser excitation is used. In order to overcome these difficulties, the size of Au nanorods can be adjusted to have its enhancement in the near-infrared region away from the biomolecular excitation transition that could lead to fluorescence or photo-chemical decomposition. Moreover, the surface chemistry of Au nanorods allows for multiple functionalization so that the capping molecules, such as cetyltrimethylammonium bromide (CTAB), could be replaced or conjugated with many functional groups.

In the present work, we describe the preparation of Au nanorods directly conjugated to a simian virus (SV40) NLS peptide through a thioalkyl-triazole linker. Using a simple conventional microscope in the dark field, we are able to image the intracellular localization of these nanorods. Furthermore, cellular components are observed by surface-enhanced Raman scattering of these Au nanorod-peptide conjugates. Our results demonstrate that these nanorods are useful for cellular delivery applications, especially nuclear targeting and cellular component sensing.

3.2 Peptide Conjugation to Gold Nanorods

The use of NLS for targeting of proteins, peptides, nucleic acids, and small organic molecules to the cell nucleus has been extensively investigated. One of the most studied is the NLS of SV40 Large T antigen, which has been shown to act as a “Trojan horse”, efficiently distributing appended payloads into the cell nucleus. However, the chemical syntheses of NLS-conjugated payloads are very difficult, often
Scheme 3-1. Synthesis of thiolalkyl-triazole linked NLS peptide. Conditions: (a) NaN₃, DMF, 110°C; (b) MsCl, Et₃N, CH₂Cl₂, rt; (c) potassium thioacetate, THF, reflux; (d) conc. HCl, methanol, reflux; (e) alkyne-modified NLS (PC-37044-PI), TBTA, CuI, DMF, rt.

requiring technically challenging compound purification and characterization protocols.

In the case of nanoparticle-peptide conjugates, an alternative synthetic approach has been
to indirectly couple these nanoparticles to carrier peptides through a secondary protein, such as bovine serum albumin (BSA) protein.\textsuperscript{46,47} The resulting conjugates are large and possess highly branched polypeptides that are very difficult to characterize.

We observed in this study that thiol-terminated NLS peptides incorporating appropriate spacer groups could be easily accessed by Cu(I)-catalyzed cycloaddition reaction (click chemistry)\textsuperscript{71} between an alkyne-terminated NLS, such as PC-37044-PI, and an appropriate azidothiols (scheme 3-1). Subsequent reaction of the resulting thioalkyl-triazole linked NLS\textsuperscript{54} with Au nanorods which are stabilized with CTAB molecules yields peptide-conjugated Au nanorods by forming Au-S bonds between the peptide and the Au surface. There are some other previous reports of bioconjugation to Au nanoparticles through Au-S bonds. For example, HS-mPEG has been reported to rapidly assemble onto Au nanoshells through Au-S bonds within 1 h.\textsuperscript{14} In this case, there are no capping molecules on the surface of the Au layer. In comparison to a bare Au surface, it takes a longer time to assemble thiolated molecules to the surface of Au nanorods. The adsorption of HS-mPEG onto Au nanorods requires stirring for 24 h,\textsuperscript{61} while thiolated DNA is bound to Au nanorods after 72 h of incubation.\textsuperscript{73} Au nanorods are capped with CTAB surfactants, which form a bilayer structure around the Au surface.\textsuperscript{74} The inner layer of the surfactant is bound to the Au surface via the surfactant head groups. In our work, the thiolated peptide and Au nanorod solution are left for up to 72 h. The absorption spectra in Figure 3-1 show that there is no change in the peak position after CTAB replacement, but the peak intensity decreases slightly. This intensity damping is due to the increase of the positive charge on the capping molecules when positive CTAB is replace by more positively charged peptide molecules
3.3 SERS of Peptide-Conjugated Gold Nanorods

Figure 3-2 shows a comparison of the Raman spectrum of the peptide-conjugated Au nanorods with that of the pure peptide and CTAB-capped Au nanorods. It can be seen that the Raman spectrum of the peptide-conjugated Au nanorods show some signals from CTAB molecules, which indicated that a small proportion of the CTAB molecules remained on the rod surface. It is very interesting to note that the amide I C=O stretching vibration of the peptide at 1665 cm\(^{-1}\) is not enhanced when it is bound to the nanorod surface. The CH bending at 1432 cm\(^{-1}\) for the peptide is shifted to 1440 cm\(^{-1}\) on the rod surface. The amide III C—N stretching at 1240 cm\(^{-1}\) is the same for peptide and peptide bound to Au nanorods. The amide II N—H vibrations (1500-1570 cm\(^{-1}\) region) are not observed in both the peptide powder and the peptide bound onto Au nanorods. The thioalkyl-triazole linker shows a strong CH\(_2\) rocking vibration\(^{75}\) at 760 cm\(^{-1}\) and C—C stretch\(^{75, 76}\) at 1092 cm\(^{-1}\). The CH bending is found to overlap with the CH bending in the peptide at 1440 cm\(^{-1}\).\(^{52}\) The C—N stretch in CTAB molecules overlaps with C-N stretch
Figure 3.2. SERS of CTAB capping molecules on Au nanorod, thioalkyl—triazole—peptide on Au nanorod and Raman spectrum of peptide. (A): 300-1400 cm⁻¹ region; (B): 1400-3000 cm⁻¹ region. Experiment performed by Dr. Xiaohua Huang.

in the thioalkyl-triazole to give a strong peak at 1002 cm⁻¹. The C-H stretching vibrations of the peptide, the thioalkyl-triazole, and the CTAB are at 2836 cm⁻¹ and 2930 cm⁻¹. The peptide residues show their strong COO⁻ deformation at 722 cm⁻¹.
signal at 831 cm$^{-1}$ is assigned to the proline and valine C—C stretch, and the 1031 cm$^{-1}$ signal is assigned to C—N stretch vibrations from glycine and proline.$^{77}$

The CTAB capping molecules show strong Raman bands at 1137 cm$^{-1}$ for the C—C stretch vibrations, 1267 cm$^{-1}$ for $\delta$ (CH) of the CH$_2$—N+(CH$_3$)$_3$ group and 1450 cm$^{-1}$ and 1490 cm$^{-1}$ for CH$_2$ bending vibration.$^{52}$ From Figure 3-2, it can be seen that these bands are still observed in the Raman spectrum of the peptide-conjugated Au nanorods due to the incomplete replacement of the CTAB molecules. In comparison to the Raman spectrum of the antibody-conjugated Au nanorods in which CTAB molecules are replaced,$^{78}$ the intensity of these bands in the peptide-conjugated Au nanorods are much weaker due to the replacement of the capping molecules with the thiolated peptides.

### 3.4 Light Scattering Images of Peptide-Conjugated Gold Nanorods Inside Cells

Figure 3-3 shows that dark field light scattering images of cells after incubation with 1 nM CTAB capped nanorods and 1 nM thioalkyl—triazole—peptide conjugated Au nanorods for 2 h. It can be seen clearly that, while the CTAB-capped Au nanorods are not efficiently absorbed by the bells, the thiolalkyl—triazole—peptide conjugated Au nanorods enter both cell lines with much higher efficiency. This result indicated that peptide conjugate enhances the cellular uptake of the Au nanorods. Importantly, it is worth noting that the observed enhancement of Au nanorod uptake took place in medium containing a very low concentration of peptide-conjugated nanorods (∼ 1 nM) within a very short incubation time (2 h). This enhanced Au nanorod uptake may be due to the receptor-mediated endocytosis. Moreover, we observed that the thioalkyl—triazole—
Figure 3-3. Dark field images of cells after incubation with Au nanorods and peptide-conjugated Au nanorods for 2 h. (A) HaCaT normal cells incubated with Au nanorods, (B) HSC cancer cells incubated with Au nanorods, (C) HaCaT normal cells incubated with peptide conjugated Au nanorods, (D) HSC cancer cells incubated with peptide conjugated Au nanorods. Peptide conjugation promotes the cellular uptake of Au nanorods. Scale bar: 10μm. Experiment performed by Dr. Xiaohua Huang

peptide conjugated nanorods are distributed into both nucleus and cytoplasm in either normal or cancer cells. However, the nanorods are more concentrated in the nucleus of the cancer cells. Reasons for increased nuclear distribution particles in the malignant cells could reflect increased specific and active uptake of the NLS peptide or decreased regulation of passive uptake of cytoplasmic components due to the disruption of the normal cellular process. It is quite possible that the nuclear pores of the nuclear
membrane which routinely restrict entrance into the nucleus may be faulty and thereby facilitate the penetration of the peptide-conjugated nanorods into the nucleus. Identification of alterations in cellular pathways, such as nuclear pore function, by this multimodal method suggest further avenues of investigation that may yield novel methods of exploiting cellular defects of carcinogenesis for therapeutic applications.

3.5 SERS of Peptide-Conjugated Gold Nanorods Inside Cells.

The Raman spectra of cells incubated with the peptide-conjugated Au nanorods are also measured (Figure 3-4). The Raman laser spot covers the major part of a single cell. Statistically, over twenty cells for each cell line are measured. They gave similar spectra with slight differences in some peak intensities. Shown in Figure 3-4A, B are five typical spectra for both normal and cancer cells. Figure 3-4C compares a typical spectrum of normal and cancer cell as well as the spectrum when the peptide-conjugated nanorods are outside the cells. Spectra of the peptide-conjugated Au nanorods reveal a difference pre- and post-cellular uptake. New bands at 620, 655, and 1158 cm\(^{-1}\) are observed after cellular translocation of the conjugates. The 620 cm\(^{-1}\) band is due to protein COO\(^{-}\) wag vibration and tyrosine \(\delta\) (ring) vibration.\(^{79}\) The 655 cm\(^{-1}\) band is due to an overlapping of a strong tyrosine vibration and the guanine bands.\(^{36,80}\) The DNA backbone vibrations are reflected most likely at bands in the region between 1030 and 1137 cm\(^{-1}\), such as 1058 cm\(^{-1}\) for the DNA C—O stretch\(^{76}\) and 1094 cm\(^{-1}\) for O—P—O vibrations.\(^{81}\) The 1151 cm\(^{-1}\) band could be assigned to chromatin and histone octamer
Figure 3-4. Raman spectra of peptide conjugated Au nanorods inside HaCat normal cells (A) and HSC cancer cells (B). The Raman spectrum of the peptide conjugated Au nanorods outside cells is shown in (C) as the bottom curve for comparison. Raman spectra from five cells for each cell line are shown. Experiment performed by Dr. Xiaohua Huang
Raman signals. The intensities around 820 and 850 cm\(^{-1}\) bands are greatly increased inside the cells. This indicates that such residues as phenylalanine (830 cm\(^{-1}\), CH\(_2\) rock) and tyrosine (850 cm\(^{-1}\), ring breath) in cell proteins are probably enhanced. The lysine Raman signal at 900 cm\(^{-1}\) in the peptide is shifted to 914 cm\(^{-1}\) when it is present inside the cells, suggesting interaction of this residue with the cell components. The Raman intensity of the thioalkyl-triazole C—N stretch band at 760 cm\(^{-1}\) inside the cell is greatly decreased in comparison to that outside the cells. This suggests a change in linker conformation upon nanorod translocation into the cells.

The normal and cancer cells show some differences in their Raman signals as well. The 731 cm\(^{-1}\) line which is assigned to adenine is stronger in the cancer cells than that in the normal cells. In addition, a peak at 398 cm\(^{-1}\) is observed only in cancer cells. All these spectral differences might be useful for cancer diagnostics.

Feld, Kneipp, and some other groups have demonstrated that individual and aggregated Au nanospheres could be useful for single cell micro-Raman microscopy. However, the inability of controlled delivery of the particles within the cell has limited their applications. The data in this project suggest that the intracellular distribution of the particles inside the cell can be controlled and simultaneously provides information regarding the local biochemical environment. Identification of spectral differences, such as an increased adenine signal within the cancer cell nucleus, provides detailed biochemical information at the molecular level with concurrent imaging of the particles in subcellular compartments. This multimodal and ultrasensitive probe has a significant potential for a range of investigative and medical diagnostic applications.
3.6 Targeted Nuclear Delivery of Gold Nanoparticles into Malignant Cells

Cells-selectivity is necessary to further explore the diagnostic and therapeutic applications of NLS-AuNPs conjugates. To ensure cells-selectivity, we incorporate other delivery peptides, such as RGD peptides, acting as a ligand that selectively and potently bind to an unique cell surface receptors on tumor cells called \( \alpha_\nu\beta_3 \) integrins. It is heterodimeric proteins that mediate cell-cell attachment and cellular adhesion to the extracellular matrix. They are overexpressed in both invasive tumor cells and the endothelium of the tumor vasculature. It is however important that the RGD peptide be linked through an appropriate linking moiety as improper conjugation of RGD to the

![Diagram of modified RGD/NLS-conjugated Au nanosphere](image)

**Figure 3-5.** Representative schematic of modified RGD/NLS-conjugated Au nanosphere.
AuNPs has been observed to result in loss of RGD-mediated RME into the tumor cells. To eliminate the linker issue, we designed a 15mers (RGDRGDRGDRGDPGC) that contained repeated sequence of RGD tripeptides with terminal cysteine residue.

![Figure 3-6](image_url)

**Figure 3-6.** Dark field images of cells after incubation with RGD/NLS-conjugated Au nanospheres for 2 h. (A) HaCat normal cells incubated with only RGD-conjugated Au nanosphere, (B) HaCat normal cells incubated with only NLS-conjugated Au nanosphere, (C) HaCat normal cells incubated with RGD/NLS-conjugated Au nanospheres, (D) HSC cancer cells incubated with only RGD-conjugated Au nanosphere, (E) HSC cancer cells incubated with only NLS-conjugated Au nanospheres, (F) HSC cancer cells incubated with RGD/NLS-conjugated Au nanospheres. Combination of RGD and NLS promote selective nuclear delivery of Au nanosphere into HSC cancer cells. Experiment performed by Dr. Xiaohua Huang.

for conjugation on the Au surface (Figure 3-5). Preliminary whole cell studies have indicated that 40 nm Au nanosphere conjugated with both thioalkyl-triazole linked NLS and cysteine-terminated RGD peptides exhibit both cellular selectivity and nuclear delivery toward HSC cancer cells within 2 h. As controls, stand alone modified RGD-Au nanosphere conjugates displayed selective delivery into HSC cancer cells, and NLS-conjugated Au nanosphere confirmed early results with NLS-Au nanorod conjugates.
Further studies are currently ongoing for the RGD/NLS-Au nanosphere conjugates to be utilized in diagnostic application and experimental therapeutic techniques such as photothermal therapy.

3.7 Conclusions

We have demonstrated in this work that Au nanorods as well as nanosphere directly conjugated to a SV40 virus NLs peptide through a thioalkyl-triazole linker and/or modified RGD peptides could be efficiently and selectively delivered into cells within a very short time. The RGD/NLS-conjugated Au nanospheres are selectively translocated into both the cytoplasm and the nucleus portion. Raman spectra reveal enhanced signal of the NLS peptides as well as molecules from both the cytoplasm and the nucleus. In addition, the Raman signal distinguishes the malignant from nonmalignant cells. The study demonstrated that Au nanorods can be used for nuclear targeting as a novel type of imaging-based contrast agent and an ultrasensitive Raman probe for intracellular molecular sensing as well.

However, the successful implementations of the promised applications of AuNPs are still limited in part by the formidable barriers imposed by the complexity of a whole organism in contrast to simple cell based studies that formed the bed rock of most of the proof-of-principle investigations. The recent results from the phase I clinical trial on Aurimune™, indicating a safe and targeted delivery of Aurimune™ in and around tumor sites, are particularly intriguing and encouraging. This has provided a very important evidence that AuNPs-based therapeutic agents could overcome the barriers presented by the human immune and circulatory systems to achieve delivery at diseased sites without
uptake by healthy tissues. In principle, such improved targeted delivery could make other 
AuNPs-based experimental therapeutic techniques, such as photothermal therapy, 
practicable. With the “right” combination of delivery agents and particle size, AuNPs-
based therapeutics could effectively kill the diseased cells while eliminating the 
horrrendous side effects of the conventional chemotherapeutic agents. Nevertheless, more 
still needs to be done regarding our understanding of the pharmacokinetics and toxicity 
profiles of AuNPs.

3.8 General Procedure and Experimental

11-Bromoundecan-1-ol and all chemicals used for the synthesis of gold nanorods 
are purchased from Sigma Aldrich. Anhydrous solvents and other reagents are purchased 
and used without purification. Analtech silica gel plate (60 F_{254}) is used for analytical 
TLC, and UV light is used to examine the spots. Tris-(benzyltriazolylmethyl)amine 
(TBTA) is prepared by using the procedure described by Chan et al. Alkyne-modified 
NLS (PCS-37044-PI) and cysteine-terminated RGD peptides (PCS-37420-PI) are 
synthesized by the solid-phase method at Peptide International, Louisville, Kentucky. 
NMR spectra are recorded on a Varian-Germini 400 magnetic resonance spectrometer. 
$^1$H NMR spectra are recorded in parts per million (ppm) relative to the peak of CDCl$_3$ 
(7.24 ppm). Mass spectra are recorded at the Georgia Institute of Technology mass 
spectrometry facility in Atlanta.

1-Azidoundecan-11-ol (50). This compound was synthesized by adapting the method of 
Shon et al. Briefly, sodium azide (1.2 g, 18.5 mmol) is added to a solution of 1-

126
bromoundecan-11-ol (2.0 g, 7.96 mmol) in anhydrous DMF (20 mL). The reaction mixture is heated to 110°C in a pressure tube and stirring continued for 24 h. The reaction mixture is then cooled to room temperature and quenched with distill water (30 mL). The cooled reaction mixture was extracted with anhydrous diethyl ether (3 x 20 mL), and the combined organic layers were washed with distilled water (3 x 20 mL), and dried over Na₂SO₄. The solvent is removed and concentrated in vacuo to give 1.275 g (75 %) of 50 as pale yellowish oil. ¹H-NMR (CDCl₃, 400MHz) δ1.25-1.38 (14H, m), δ 1.50-1.60 (4H, m), δ3.22 (2H, t, J = 7.2 Hz), δ 3.61 (2H, t, J = 7.2 Hz).

1-Azidoundecan-11-methylsulfonate (51). Triethylamine (4.35 mL, 31.2 mmol) is added to a solution of 1-azidoundecan-11-ol 50 (2.42 g, 11.3 mmol) in anhydrous CH₂Cl₂ (100 mL). The reaction mixture is stirred for 15 minutes at 0°C, and then methanesulfonyl chloride (2.41 mL, 31.2 mmol) is added. The reaction mixture is allowed to warm up to room temperature and stirring continued for 2 h. The reaction mixture was quenched with ice cold distilled water (35 mL) and extracted with anhydrous diethyl ether (2 x 35 mL). The combined organic layer was washed in succession with 1 N HCl, distilled water, saturated NaHCO₃, and then again with distilled water. The organic layer was dried over Na₂SO₄, and concentrated in vacuo to give 2.934 g (89 %) of 51 as pale yellowish oil. ¹H-NMR (CDCl₃, 400MHz) δ 1.25-1.40 (14H, m), δ 1.53-1.60 (2H, m), δ 1.68-1.75 (2H, m), δ 2.97 (3H, s), δ 3.22 (2H, t, J = 7.2 Hz), δ 4.19 (2H, t, J = 6.8 Hz).
1-Azidoundecan-11-thioacetate (52). Potassium thioacetate (2.02 g, 6.79 mmol) is added to a solution of 1-azidoundecan-11-sulfonate 51 (1.00 g, 3.43 mmol) in anhydrous THF (10 mL). The reaction mixture is degassed, refilled with argon and heated under reflux for 3 h. The reaction mixture was quenched with ice cold distilled water (30 mL) and extracted with anhydrous diethyl ether (3 x 30 mL). The combined organic layer was dried over Na$_2$SO$_4$ and concentrated in vacuo to give 856 mg (87 %) of 52 as yellowish oil. $^1$H-NMR (CDCl$_3$, 400MHz) δ 1.23-1.38 (14H, m), δ 1.50-1.60 (4H, m), δ 2.29 (3H, s), δ 2.83 (2H, t, $J$ = 7.6 Hz), δ 3.22 (3H, t, $J$ = 7.6 Hz).

1-Azidoundecane-11-thiol (53). A solution of 1-azidoundecane-11-thiolacetate 52 (0.1 g, 0.369 mmol) in anhydrous methanol (7.0 mL) was first degassed and refilled with argon. Concentrated HCl (0.4 mL) was added to the solution and the resulting mixture refluxed for 3 h. The reaction mixture was quenched with distilled water (10 mL) and extracted with anhydrous diethyl ether (2 x 10 mL). The organic layer was dried over Na$_2$SO$_4$ and concentrated in vacuo to give 85 mg (100%) of 53 as yellowish oil. $^1$H-NMR (CDCl$_3$, 400MHz) δ 1.25-1.38 (14H, m), δ 1.53-1.62 (4H, m), δ 2.50 (2H, q, $J$ = 15.2 Hz, 7.2 Hz), δ 3.23 (2H, t, $J$ = 5.6 Hz).

Thiolalkyl-triazole linked NLS peptide (54). Anhydrous CuI (0.003 g, 0.011 mmol) and TBTA (0.007 g, 0.011 mmol) is added to a solution of 1-azidoundecane-11-thiol 53 (0.02 g, 0.079 mmol) and alkyne-modified NLS peptides PC-37044-PI (0.05 g, 0.039 mmol) in anhydrous DMF (2 mL). The solution is stirred at room temperature under argon for 48 h. Excess DMF is removed in vacuo, and the residue is quenched with
distill water (5 mL). The resulting mixture is extracted with dichloromethane (2 x 10 mL) to remove unreacted 1-azidoundecane-11-thiol 53. The remaining aqueous layer was frozen in acetone-dry ice bath and concentrated in vacuo to give 50 mg (43%) of 54 as brown solid. ¹H NMR (DMSO-d₆, 400 MHz) δ 0.83 (2H, t, J = 6.0 Hz), δ 1.23-1.36 (6H, m), δ 1.42-1.54 (3H, m), δ 1.56-1.68 (1H, m), δ 2.13-2.24 (2H, m), δ 2.40-2.50 (5H, m), δ 2.66-2.80 (2H, m), δ 2.87 (1H, s), δ 3.29 (1H, t, J = 8.0 Hz), δ 3.34-3.54 (1H, m), δ 3.62-3.78 (1H, m), δ 4.10-4.30 (1H, m), δ 7.26-7.36 (1H, m), δ 7.64-7.88 (3H, m), δ 7.94 (1H, s), δ 8.10-8.32 (2H, m); MS (MALDI, CHCA) calc for [C₆₇H₁₂₃N₂₃O₁₃S + H]⁺ 1490.9, found 1491.0.

**Synthesis of gold nanorods and peptide conjugation.** The gold nanorods are synthesized according to the seed-mediated growth method. Briefly, 600 μL 0.01 M ice-cold sodium borohydride is quickly added into a 10 mL 0.5 mM stirring auric acid, which is dissolved in 0.2 M CTAB solution. 2 to 5 nM gold nanoparticles are formed after 2 min as a seed solution. In a separate flask, 1 mL of 4 mM silver citrate is added to 50 mL growth solution, which contains 0.2 M CTAB, 0.15 M benzyldimethylammonium chloride hydrate (BDAC), and 1 mM auric acid. 0.07 mL of 0.0788 M ascorbic acid is added to the growth solution to reduce the auric acid to form HAuCl₂ solution. 0.08 mL seed solution is injected into the growth solution to initiate the rod formation and growth. In this procedure, gold nanorods with absorption maximum at 650 nm (aspect ratio of 2.4) are obtained overnight. For the conjugation of peptide 5 to gold nanorods, 100 μL 1 mM peptide 5 is added to 10 mL gold nanorod solution (OD₆₅₀ = 1.0), and the mixture is
left to react for 72 h. The unbound peptide is separated by centrifugation at 5000 rpm for 10 min.

**Cell culture and cellular delivery of peptide conjugated gold nanorod.** One nonmalignant epithelial cell line, HaCaT (human keratinocytes) and one malignant epithelial cell lines, HSC 3 (human oral squamous cell carcinoma), were cultured on coverslips in DMEM (Dulbecco’s Modification of Eagle’s Medicum, Cellgro) plus 10% FBS at 37°C under 5% CO₂ for 24 h. The DMEM medium is then taken out and rinsed with PBS buffer. Fresh DMEM medium containing 1 nM thioalkyl-triazole peptide/Au nanorod conjugates is added, and the cells were put back in incubator for 2 h. After the nanorods incubation, the cells are rinsed with PBS buffer, fixed with paraformaldehyde, coated with glycerol, and sealed with another cover slip.

**Dark field imaging.** The dark field images are taken under an inverted Olympus IX70 microscope. A dark field condenser (U-DCW) with a numerical aperture between 0.9-1.2 is used to deliver a very narrow beam of white light from a tungsten lamp to the sample. A 100x/1.35 oil Iris objective (UPLANAPO) is used to collect only the scattered light from samples. In this mode, the samples with highly scattering properties are shown as a bright object in a dark background.

**Raman measurements.** The Raman spectra are obtained with a Holoprobe series 5000 Micro-spectrometer (Kaiser optical systems, Inc, Ann Arbor, MI) in a 180° reflective configuration with a 50x objective. The excitation wavelength is 785 nm from a diode
laser. The spectral resolution in Raman experiments was 5 cm$^{-1}$ and the laser power is 25 mW. Each spectrum are obtained in 10s collection time with six accumulations.

3.9 References


35. Morjani, H.; Riou, J. F.; Nabiev, I.; Lavelle, F.; Manfait, M. Molecular and cellular interactions between intoxilivine, DNA, and Topoisomerase II studied by


Numerous examples of Cu (I) catalyzed Huigsen Cycloaddition reaction have appeared in the literature (A comprehensive list is available at http://www.scripps.edu/chem/sharpless/click.html). Cited here are two pioneering examples: (a) Rostovtsev, V. V.; Green, L. G.; Fokin, V. V.; Sharpless, K. B. A Stepwise Huigsen Cycloaddition Process : Copper(I)-Catalyzed Regioselective ‘Ligation’ of Azides and Terminal Alkynes. Angew. Chem. Int. Ed. 2002, 41, 2596-2599. (b) Tornoe, C. W.; Christensen, C.; Meldal, M. Peptidotriazoles on Solid Phase: [1,2,3]-Triazoles by Regiospecific Copper(I)-Catalyzed 1,3-Dipolar Cycloadditions of Terminal Alkynes to Azides. J. Org. Chem. 2002, 67, 3057-3064.


4.1 \(\beta\)-lactam Antibiotics

\(\beta\)-lactam antibiotics such as penicillin and cephalosporins are the most commonly prescribed antibacterial agents. Their antibacterial efficacy derives from ability to acylate a serine residue at the penicillin binding proteins (PBPs) active site.\(^1,2\) Serine acylation inhibits the enzymatic activities of PBPs, thus interfering with the essential polymerization and cross-linking of the peptidoglycan components of the bacterial cell wall.\(^1\) However, the emergence of multi-drug resistant bacteria strains such as \textit{Staphylococcus aureus} is threatening the clinical effectiveness of \(\beta\)-lactam antibiotics.\(^3\) The common resistance mechanism in gram-negative bacteria is the cellular expression of \(\beta\)-lactamases (or penicillinase), which hydrolyze the \(\beta\)-lactam ring and thus inactivate the antibiotics.\(^4-7\) Therapeutic agents targeting \(\beta\)-lactamase resistance include clavulanate, sulbactam, and tazobactam. These are generally administered in combination with \(\beta\)-lactamase susceptible \(\beta\)-lactams.\(^8,9\) However, the emergence of multi-strains \(\beta\)-lactamase has reduced the efficacy of some of these \(\beta\)-lactamase inhibitors.\(^10,11\)

There are also other intracellular factors that attenuate the antibacterial activities of \(\beta\)-lactams. For example, the N-5 amide moiety in penicillins is highly reactive and has been proposed to facilitate the hydrolysis of the \(\beta\)-lactam ring in acidic media (Figure 4-1).\(^12\) Incorporation of electron-withdrawing groups at the N-acyl side chain has been shown to reduce the acid sensitivity of penicillins. Another approach to reduction of \(\beta\)-
lactam acid instability is to introduce steric bulk, commonly tolerated by PBPs, into the N-acyl side chains. Examples of such penicillins which can withstand an acidic environment similar to human gastric juice include ampicillin, phenethicillin and propicillin (Figure 4-2).13

![Diagram of penicillin hydrolysis](image_url)

Figure 4-1. Self-induced acid hydrolysis of penicillin.

Similarly, structure activity relationship (SAR) studies have revealed that β-lactamases are intolerant of bulky substituents on the N-acyl side chain of β-lactams. This has led to the development of several useful penicillinase resistant β-lactams such as methicillin, oxacillin, and cloxacillin (Figure 4-2).14

Another shortcoming to the effectiveness of β-lactamase antibiotics is the bacterial outer membrane, which is a formidable barrier in gram negative organisms. In
addition, the bacterial membrane could acquire efflux pumps that facilitate active elimination of drug from the bacterial cytosol.\textsuperscript{15,16} All these changes could prevent the intracellular accumulation of pharmacologically relevant concentrations of the $\beta$-lactam antibiotics. One clever strategy to facilitate $\beta$-lactams diffusion across membrane is to covalently link them to membrane penetrating groups such as sideophores and peptides.\textsuperscript{1-3,17} However, the synthesis of such $\beta$-lactam conjugates often involve long synthetic schemes and complicated compound purifications protocols.\textsuperscript{2b-e}

![Chemical structures](image)

**Figure 4-2.** The structure of some acid- and $\beta$-lactamase-resistant penicillins.

One of the most fruitful $\beta$-lactam SAR studies has been the modification of the N-acyl groups. These studies have led to the discovery of $\beta$-lactam analogs with superior antibacterial activity.\textsuperscript{16,18-20} Much recent studies have shown that attachment of N-acyl
groups incorporating the structural feature of peptidoglycan result in PBP specific β-lactams and cephalosporins.\textsuperscript{3,17,21} PBPs can also tolerate an assorted variety of non-amide groups at the C-6 and C-7 positions of penicillins and cephalosporins, respectively.\textsuperscript{20} Some of these non-amide β-lactam analogs, in addition to possessing potent antibacterial activity, have differential affinity for PBP isoforms (Figure 4-3). For example, amidocillin, a β-lactam analog with an unusual 6-formylimido moiety, derives its antibacterial activity from its specific interaction with PBP-2.\textsuperscript{22} Very few β-lactams with traditional amide bond isosteres have been reported to date. One such example is the sulfonamide derivatives. A subset of these compounds have been synthesized and tested. However these compounds are still prone to chemical degradation and hydrolysis by β-lactamases.\textsuperscript{23}

![Figure 4-3. Structure of amide isosteric β-lactams.](image)

We proposed that 1,4-disubstituted-1,2,3-triazoles could serve as an effective isostere for the N-acyl group of penicillins and cephalosporins. Due to bond characteristics similar to an amide bond, the triazole group is commonly used as an amide bond isostere.\textsuperscript{24,25} Unlike amide bond, the triazole moiety is not susceptible to
Replacement of the reactive β-lactam acyl bond with a triazole ring could lead to β-lactams that are more stable in acidic environment. Because β-lactamases are relatively intolerant of side chain steric hindrance, introduction of the triazole ring could lead to β-lactams with improved activity against β-lactamase producing organisms. In addition, the triazole ring will facilitate facile conjugation of β-lactams to assorted cell permeable peptides and peptoids for promoted uptake of β-lactams into bacterial cell. Here, we report our efforts on the design, synthesis, and preliminary biological activities of 6-triazolylpenicillanic acid.

4.2 Molecular design of 6-triazolylpenicillanic acid

The key intermediate in the synthesis of the proposed 6-triazolylpenicillanic acids 56 is the 6-azidopenicillanates 57. It is anticipated that Cu(I)-catalyzed cycloaddition between 57 and appropriate alkynes (Sharpless Click Chemistry)26 followed by deprotection of the ester protection group will furnish the desired triazolylpenicillanic acid 56 (scheme 4-1). The synthesis of protected 6-azidopenicillanates similar to 57a-c has been described in the literature.27-30 These compounds are generally synthesized, in moderate yields, using multi-step reactions. For example, Barrett and Sakadarat described the synthesis of benzyl protected 6-azidopenicillanate, the final intermediate in their total synthesis of 6-aminopenicillanic acid (6-APA), in a six-step reaction scheme with a total
yield of about 15%. For the synthesis of 6-triazolylpenicillanates, we desired a more
general and mild synthetic strategy to 6-azidopenicillanates.

As a new general alternative, we envisioned that azidopenicillanic acid could be
obtained from commercially available 6-aminopenicillanic acids through a diazo-transfer
reaction using an appropriately carboxyl protected ester. The diazo-transfer reaction is a
mild, high yielding reaction that has been used to effect direct conversion of assorted
amines to azides. In addition, a recent observation from our lab has extended the scope
of this versatile reaction to include azide functionalization of amine-coated solid supports
under heterogeneous reaction conditions. The requisite carboxyl protected
aminopenicillanates 58a-c were synthesized adapting literature protocols. Using
the PNB protected compound 58a, we initiated diazo-transfer reaction with freshly
prepared triflyl azide under basic conditions in the classic CH₂Cl₂/MeOH/H₂O solvent
mixture. These conditions, however, gave a complex mixture from which we only
isolated a minute amount of the ring-opened methyl ester azide 59. This product was
presumably obtained from Et₃N promoted methanolysis of compound 58a in addition to
the desired diazo-transfer reaction. The reaction was repeated in anhydrous CH₂Cl₂ and

**Scheme 4-1.** Synthetic approach to 6-triazolylpenicillanic acids.
Et₃N. Gratifyingly, we obtained the desired azide 60a in yields of 50-68% within 2 to 2.5 h of reaction, though other uncharacterized degradation products persisted. Because of the potential hazard of handling triflyl azide in halogenated solvent, we investigated the compatibility of this reaction with non-halogenated solvents. The reaction worked equally well when CH₂Cl₂ was replaced with toluene, yielding azide 60a in 70% yields. Similarly, azides 60b and 60c were obtained from amines 57b and 57c in moderate to good yields (scheme 4-2). The β orientation of the azide group in 60a-c was authenticated by ¹H NMR (J₅,₆ coupling constant = 4 Hz), thereby confirming that the reaction occurred with the characteristic retention of configuration.

![Scheme 4-2. Synthesis of protected 6-azidopenicillanic acid. Conditions: (a) TfN₃, Et₃N, CH₂Cl₂/MeOH/H₂O, rt, (b) TfN₃, Et₃N, DMAP, rt, 2-2.5 hrs, (c) TfN₃, Et₃N, toluene, rt, 1.5-2.5 hrs.]

To identify optimum conditions for Cu(I)-catalyzed cycloaddition reaction, we investigated the reaction of azides 60b and 60c with 3-phenyl-1-propyne 61 and 2-ethynyl-1,3-dimethoxybenzene 62. Our choice of terminal alkynes 61 and 62 is partly informed by the possibility that cycloaddition between these alkynes and azides 60 will
respectively furnish triazolyl isosteres of penicillin G and methicillin, two historically useful penicillin derivatives. Cu(I)-catalyzed reaction of azide 60b or 60c with alkyne 61 resulted in triazoles 63a and 63b in excellent yield. Subsequent TFA deprotection of the PMB and the BzD group furnished the desired penicillanic acid 64a in excellent yield. Similarly, the reaction of azide 60c with alkyne 62 followed by deprotection of the BzD group gave penicillanic acid 64 in 72% overall yield (scheme 4-3). Because of the rapidity of deprotection of the BzD group and the product quality, we focused much of our attention on the reactivity of azide 60c.

Scheme 4-3. Cu(I)-catalyzed cycloadditions reaction between 6-azidopenicillanates and representative terminal alkynes. Conditions: (a) CuI, Hunig’s base, alkynes 61 or 62, THF, rt, (b) TFA, anisole, CH₂Cl₂, -5°C.

4.3 Structural Activity-Relationship Studies of 6-Triazolylpenicillanic Acids

We turned to investigate the reactivity of azide 60c with assorted terminal alkynes in order to probe the scope of this reaction. We selected a subset of terminal alkynes whose closely related carboxylic acid analogs have been shown to support the antimicrobial activities of β-lactams. Alkynes such as 62, 65h, 65i, and 65k that we could not obtain from commercial sources were synthesized from the corresponding
Scheme 4-4. Scope of Cu(I)-catalyzed cycloaddition between 6-azidopenicillanate and terminal alkynes. Conditions: a) CuI, Hunig’s base, alkynes 65, THF, rt, b) TFA, anisole CH2Cl2, -5°C.

carboxylic acid, through the intermediacy of aldehyde, using the Bestmann-Ohira reagent.41-43 Hydroxyl alkyne 65f and 65l were obtained by a direct Grignard reaction of ethynylmagnesium bromide with the appropriate aldehydes. Detailed protocols for the synthesis of these alkynes are reported in the experimental section.

Cu(I)-catalyzed reaction of alkynes 65a-l with azidopenicillanate 60b or 60c proceeded smoothly at ambient temperature, leading to uneventful formation of triazole 66a-l in good to excellent yields. Subsequent carbonyl group deprotection furnished the desired 6-triazolylpenicillanic acid 67a-l (scheme 4-4).

4.4 Preliminary Antibacterial Activity Studies of 6-Triazolylpenicillanic Acids

The synthesized 6-triazolylpenicillanic acids were screened at the National Institute of Allergy and Infectious Disease (NIAID) through the In vitro and Animal
Models for Emerging Infectious Diseases and Biodefense Screening Program.

Compounds were screened following the procedures recommended by the Clinical Laboratory Standard Institute (CLSI). Compound 64a, 67e, 67g, and 67l exhibited some antibacterial activity against the Gram-positive bacteria strain \textit{S. pneumoniae} with MIC value of 4 to 8 µg/mL. Similarly, compound 67 showed a moderate activity (MIC ≈ 8 µg/mL) against \textit{B. anthracis}. Other compounds listed in Table 4-1 displayed comparatively poor antibacterial activity (MIC > 8µg/mL).

\textbf{Table 4-1.} Minimal Inhibitory Concentration (MIC) of 6-triazolylepenicillanic acids in µg/mL: Each MIC values were obtained from an average of three independent experiments.

<table>
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<th>Compound</th>
<th>( R_2 )</th>
<th>\textit{S. aureus} ATCC 29213 (µg/mL)</th>
<th>\textit{E. coli} ATCC 25922 (µg/mL)</th>
<th>\textit{S. pneumoniae} ATCC 49619 (µg/mL)</th>
<th>\textit{B. anthracis} AMES (µg/mL)</th>
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* X = Na
** Denotes that one of the 3 replicates had MIC value of 8 µg/mL.
*** No Data
4.5 Conclusion

We have reported a convenient route for synthesis of 6-triazolylpenicillanic acid. Preliminary biological evaluations have demonstrated that some of these compounds possess moderate antibacterial activity. Currently, we are investigating the SAR of the lead compounds in order to identify 6-triazolylpenicillanic acid with improved antibacterial activity against drug resistant bacterial strains. More importantly, we have identified a facile, high yielding synthetic scheme of 6-azidopenicillanes enabling of conjugation to cell permeable peptides or peptoids for potential bacterial cell-selectivity.

4.6 General Procedure and Experimental

6-aminopenicillanoic acid (6-APA) was purchased from Sigma Aldrich. Anhydrous solvents and other reagents were purchased and used without further purification. Analtech silica gel plates (60 F\textsubscript{254}) were used for analytical TLC, and uv light was used to examine the spots. 200-400 Mesh silica gel was used in column chromatography. NMR spectra were recorded on a Varian-Gemini 400 magnetic resonance spectrometer. \textsuperscript{1}H NMR spectra are recorded in parts per million (ppm) relative to the peak of CDCl\textsubscript{3}, (7.24 ppm), acetone-d\textsubscript{6} (2.09 ppm), or DMSO-d\textsubscript{6} (2.49 ppm). \textsuperscript{13}C spectra were recorded relative to the central peak of the CDCl\textsubscript{3} triplet (77.0 ppm), acetone-d\textsubscript{6} (205.8 ppm), or the DMSO-d\textsubscript{6} septet (39.7 ppm), and were recorded with complete hetero-decoupling. High-resolution mass spectra were recorded at the Georgia Institute of Technology mass spectrometry facility in Atlanta. Triflyl azide was prepared as described before and used without storage.\textsuperscript{32} The Bestmann-Ohira reagent was prepared as described by Ghosh \textit{et al}\textsuperscript{42} while diphenyldiazomethane was prepared by
using the procedure described by Ko et al.\textsuperscript{37} 6-Aminopenicillanates 58a-c were synthesized adapting literature procedures.\textsuperscript{21, 33-37}

**Representative Procedure for the Alkyne Transformation Reaction.** 2-Ethynyl-1,3-dimethoxybenzene (62). 2,6-dimethoxybenzaldehyde (0.45 g, 2.71 mmol) was first dissolved in anhydrous MeOH (25 mL) and stirred under argon at room temperature. Anhydrous K$_2$CO$_3$ (1.12 g, 8.12 mmol) and Bestmann-Ohira reagent (1.03 g, 5.42 mmol) were added to the reaction mixture and stirring continued for 24 h at room temperature. Solvent was evaporated off, and the remaining residue was dissolved in CH$_2$Cl$_2$ (25 mL) and washed with saturated NH$_4$Cl (3 x 20 mL). The organic layer was dried over Na$_2$SO$_4$ and concentrated \textit{in vacuo}. The crude product was purified by flash column chromatography (silica gel, 5:1 Hexane/EtOAc) to give 220 mg (50%) of 62 as a white solid. $^1$H-NMR (CDCl$_3$, 400MHz) $\delta$ 3.55 (1H, s), 3.88 (6H, s), 6.53 (2H, d, $J = 8.4$ Hz), 7.24 (1H, t, 7.4 Hz); $^{13}$C-NMR (CDCl$_3$, 100MHz) $\delta$ 56.0, 76.2, 85.7, 100.0, 103.5, 130.3, 162.0.

4-(4-Ethynylphenyl)pyridine (65k). Reaction of 4-(4-formylphenyl)pyridine (0.3 g, 1.64 mmol) and Bestmann-Ohira reagent (0.627 g, 3.27 mmol) within 24 h as described for the synthesis of compound 7 followed by flash chromatography (silica gel, 2:1 Hexane/EtOAc) gave 261 mg (89%) of 65k as a white solid. $^1$H-NMR (CDCl$_3$, 400MHz) $\delta$ 3.16 (1H, s), 7.47 (2H, d, $J = 6.4$ Hz), 7.59 (4H, s), 8.66 (2H, d, $J = 6.4$Hz). HRMS (EI) calc for [C$_{13}$H$_9$N] 179.0735, found 179.0746.
2-Ethynlbiphenyl (65i). The required Biphenyl-2-carbaldehyde was synthesized from the corresponding carboxylic acid through NaBH₄/ BF₃:OEt₂ reduction to the primary alcohol.⁴³ Subsequent PDC oxidation of the primary alcohol furnished Biphenyl-2-carbaldehyde. Reaction of biphenyl-2-carbaldehyde (0.66 g, 3.63 mmol) and Bestmann-Ohira reagent (1.72 g, 7.27 mmol) within 24 h followed by flash chromatography (silica gel, 5:1 Hexane/EtOAc) yielded 523 mg (81%) of 65i as a colorless oil. ¹H-NMR (CDCl₃, 400MHz) δ 3.03 (1H, s), 7.28-7.32 (1H, m), 7.36-7.45 (5H, m), 7.57-7.63 (3H, m).

3-Ethynlbiphenyl (65h). The required Biphenyl-3-carbaldehyde was synthesized from the corresponding carboxylic acid through NaBH₄/ BF₃:OEt₂ reduction to the primary alcohol.⁴³ Subsequent PDC oxidation of the primary alcohol furnished Biphenyl-3-carbaldehyde. Reaction of biphenyl-3-carbaldehyde (0.87 g, 4.78 mmol) and Bestmann-Ohira reagent (1.72 g, 9.56 mmol) within 24 h followed by flash chromatography (silica gel, 5:1 Hexane/EtOAc) gave 850 mg (100%) of 65h as a reddish oil. ¹H-NMR (CDCl₃, 400MHz) δ 3.03 (1H, s), 7.33-7.47 (5H, m), 7.55-7.57 (3H, m), 7.72 (1H, s). HRMS(EI) calc for [C₁₄H₁₀] 178.0782, found 178.0797.

Representative Procedure for the Alkyne Transformation via Grignard Reaction.
1-phenylprop-2-yn-1-ol (65f). To a solution of benzaldehyde (0.5 g, 4.71 mmol) in anhydrous THF (2 mL) was added ethynylmagnesium bromide (14.0 mL, 0.5M in THF) at room temperature under argon. The reaction mixture was stirred for 1 h and quenched with distilled water. The reaction mixture was partitioned between CH₂Cl₂ (10 mL) and
water (15 mL), and the two layers separated. The organic layer was washed in succession with distilled water (2 x 10 mL) and saturated brine (2 x 10 mL), and dried over Na$_2$SO$_4$. Solvent was evaporated off to give 0.626 g (100%) of 65f as a brownish oil. $^1$H-NMR (CDCl$_3$, 400MHz) $\delta$ 2.66 (1H, d, $J = 2.4$ Hz), 5.45 (1H, d, $J = 4.0$ Hz), 7.30-7.40 (3H, m), 7.54 (2H, d, $J = 7.2$ Hz); HRMS (FAB, thioglycerol) calc for [C$_9$H$_8$O] $132.0575$, found 132.0566.

1-(4-(pyrdin-4-yl)prop-2-yn-1-ol (65l). To a solution of benzaldehyde (0.5 g, 2.73 mmol) in anhydrous THF (2 mL) was added ethynylmagnesium bromide (8.2 mL, 0.5M in THF) at room temperature under argon. The reaction mixture was stirred for 1 h and quenched with distilled water. The reaction mixture was partitioned between CH$_2$Cl$_2$ (10 mL) and water (15 mL), and the two layers separated. The organic layer was washed in succession with distilled water (2 x 10 mL) and saturated brine (2 x 10 mL), and dried over Na$_2$SO$_4$. Solvent was evaporated off to give 0.491 (86%) of 65l as a brownish solid. $^1$H-NMR (CD$_3$OD, 400MHz) $\delta$ 3.05 (1H, s), 5.46 (1H, s), 7.66-7.78 (6H, m), 8.56 (2H, d, $J = 9.2$ Hz); HRMS (FAB, thioglycerol) calc for [C$_{14}$H$_{11}$NO + H]$^+$ 210.0918, found 210.0909.

Representative Procedures for Diazo-transfer Reaction. Method A: $p$-Nitrobenzyl 6-azidopenicillanate (60a). 4-Nitrobenzyl 6-aminopenicillanate salt 58a (5.0 g, 9.5 mmol) was suspended in EtOAc (40 mL) and washed with saturated NaHCO$_3$ (2 x 30 mL). The organic layer was dried over Na$_2$SO$_4$ and concentrated in vacuo to give pure 4-nitrobenzyl 6-aminopenicillanate. To a solution of 4-nitrobenzyl 6-aminopenicillanate in
anhydrous CH$_2$Cl$_2$ (10 mL) was added triflyl azide solution (25 mmol) in CH$_2$Cl$_2$ (25 mL), and Et$_3$N (2.1 mL, 15.0 mmol). The reaction mixture was stirred at room temperature for 2 h. Solvent was evaporated off and the residue was directly purified by flash chromatography (silica, gradient 4:1; 3:1 Hexane/EtOAc) to give 2.35 g (68%) of 60a as a colorless gel. $^1$H-NMR (CDCl$_3$, 400MHz) $\delta$ 1.38 (3H, s), 1.61 (3H, s), 4.49 (1H, s), 4.92 (1H, d, $J$ = 4.0 Hz), 5.23 (2H, q, $J$=24.0, 12.0 Hz), 5.43 (1H, d, $J$ = 4.0 Hz), 7.50 (2H, d, $J$ = 8.8 Hz), 8.17 (2H, d, $J$ = 8.8 Hz); $^{13}$C-NMR (CDCl$_3$, 100MHz) $\delta$ 26.4, 31.5, 64.3, 65.7, 66.5, 67.6, 70.0, 123.7, 128.0, 141.5, 147.9, 167.0, 169.8; 

Method B: Benzhydryl 6-azidopenicillanate (60c). Benzhydryl 6-aminopenicillanate salt 58c (3.5 g, 6.32 mmol) was suspended in EtOAc (60 mL) and washed with saturated NaHCO$_3$ (3 x 40 mL). The organic layer was dried over Na$_2$SO$_4$ and concentrated in vacuo to give 2.4 g of pure benzhydryl 6-aminopenicillanate. To a solution of benzhydryl 6-aminopenicillanate in anhydrous CH$_2$Cl$_2$ (15 mL) was added triflyl azide solution (14 mmol) in CH$_2$Cl$_2$ (35 mL), DMAP (1.15 g, 9.24 mmol), and Et$_3$N (0.1 mL). The reaction mixture was stirred at room temperature for 1.5 h and solvent was evaporated off. The residue was directly purified by flash chromatography (silica, gradient 1:0; 5:1 Hexane/EtOAc) to give 1.38 g (54%) of 60c as a colorless gel. $^1$H-NMR (CDCl$_3$, 400MHz) $\delta$ 1.26 (3H, s), 1.64 (3H, s), 4.57 (1H, s), 4.89 (1H, d, $J$ = 4.0 Hz), 5.47 (1H, d, $J$ = 4.0 Hz), 6.93 (1H, s), 7.28-7.35 (10H, m); $^{13}$C-NMR (CDCl$_3$, 100MHz) $\delta$ 26.2, 31.9, 64.7, 66.6, 67.8, 70.2, 78.4, 126.8, 127.5, 128.1, 128.4, 128.5, 138.8, 166.5, 169.8; HRMS (FAB, mnba) calc for [C$_{21}$H$_{20}$N$_4$O$_3$S + H]$^+$ 409.1334, found 409.1357.
Method C:  \( p \)-Nitrobenzyl 6-azidopenicillanate (60a). \( p \)-Nitrobenzyl 6-aminopenicillanate salt 58a (0.75 g, 1.4 mmol) was suspended in EtOAc (30 mL) and washed with saturated NaHCO\(_3\) (2 x 20 mL). The organic layer was dried over Na\(_2\)SO\(_4\) and concentrated \textit{in vacuo} to give pure 4-nitrobenzyl 6-aminopenicillanate. To a solution of pure 4-nitrobenzyl 6-aminopenicillanate in anhydrous toluene (5 mL) was added triflyl azide solution (2.97 mmol) in toluene (25 mL), and Et\(_3\)N (0.3 mL, 2.1 mmol). The reaction mixture was stirred at room temperature for 2 h. The reaction was diluted with EtOAc (10 mL) and washed with 1N HCl (20 mL), distilled water (2 x 20 mL), and saturated brine (20 mL). The organic layer was dried over Na\(_2\)SO\(_4\) and concentrated \textit{in vacuo} to give 361 mg (70%) of 60a as a yellowish solid. \(^1\)H-NMR (CDCl\(_3\), 400MHz) \( \delta \) 1.42 (3H, s), 1.66 (3H, s), 4.52 (1H, s), 4.92 (1H, d, \( J = 4.0 \) Hz), 5.26 (2H, q, \( J = 24.0, 12.0 \) Hz), 5.46 (1H, d, \( J = 4.0 \) Hz), 7.53 (2H, d, \( J = 8.8 \) Hz), 8.24 (2H, d, \( J = 8.8 \) Hz).

\( p \)-Methoxybenzyl 6-azidopenicillanate (60b). 6-azidopenicillanate 60b was prepared from \( p \)-Methoxybenzyl 6-aminopenicillanate 58b (4.0 g, 7.9 mmol) and triflyl azide (25 mmol) in CH\(_2\)Cl\(_2\) (total volume = 25 mL), and Et\(_3\)N (1.44 mL, 10.3 mmol) using \textbf{method A}. The reaction mixture was stirred at room temperature for 2 h. Purification of the crude product by flash chromatography (silica, gradient 4:1; 3:1 Hexane/EtOAc) afforded 1.40 g (49%) of 60b as a colorless gel. \(^1\)H-NMR (CDCl\(_3\), 100MHz) \( \delta \) 1.36 (3H, s), 1.61 (3H, s), 3.79 (3H, s), 4.45 (1H, s), 4.88 (1H, d, \( J = 4.0 \) Hz), 5.10 (2H, app. q, \( J = 24.0, 12.0 \) Hz), 5.44 (1H, d, \( J = 4.0 \) Hz), 6.87 (2H, d, \( J = 8.8 \) Hz), 7.27 (2H, d, \( J = 8.8 \) Hz); \(^{13}\)C-NMR (CDCl\(_3\), 400MHz) \( \delta \) 26.6, 31.7, 55.3, 64.6, 66.5, 67.4, 67.7, 70.2, 113.9, 126.5,
Representative Procedure for Cu(I)-Catalyzed Cycloaddition Reaction. *p*-Methoxybenzyl benzyl-6-triazolylpenicillanate (63a). *p*-Methoxybenzyl 6-azidopenicillanate 60b (0.27g, 0.635 mmol) and 3-phenyl-1-propyne 61 (0.17 mL, 1.39 mmol) were dissolved in anhydrous THF (8 mL) and stirred under argon at room temperature. Copper (I) iodide (0.011 g, 0.06 mmol), and Hunig’s base (0.1 mL) were then added to the reaction mixture, and stirring was continued for 2 h. The reaction mixture was diluted with CH$_2$Cl$_2$ (40 mL) and washed with 1:4 NH$_4$OH/saturated NH$_4$Cl (3 x 30 mL) and again with saturated NH$_4$Cl (30 mL). The organic layer was dried over Na$_2$SO$_4$ and concentrated *in vacuo*. The crude product was purified by flash chromatography (silica, gradient 3:1; 2:1; 3:2 Hexane/EtOAc) to give 246 mg (71%) of 63a as a white solid. $^1$H-NMR (CDCl$_3$, 400MHz) δ 1.35 (3H, s), 1.57 (3H, s), 3.79 (3H, s), 4.08 (2H, s), 4.47 (1H, s), 5.12 (2H, app. q, $J = 24.0$, 12.0 Hz), 5.69 (1H, d, $J = 4.0$ Hz), 6.27 (1H, d, $J = 4.4$ Hz), 6.88 (2H, d, $J = 8.8$ Hz), 7.20-7.30 (7H, m), 7.42 (1H, s); $^{13}$C-NMR (CDCl$_3$, 100MHz) δ 26.9, 30.9, 32.0, 55.2, 65.3, 66.2, 67.3, 67.5, 70.5, 114.0, 122.4, 126.5, 128.5, 128.6, 130.6, 138.5, 147.2, 160.0, 167.1, 168.1; HRMS (FAB, thioglycerol) calc for [C$_{25}$H$_{26}$N$_4$O$_4$S + H]$^+$ 479.1753, found 479.1756.

Benzhydryl benzyl-6-triazolylpenicillanate (63b). Reaction of benzhydryl 6-azidopenicillanate 60c (0.270 g, 0.661 mmol) and 3-phenyl-1-propyne 61 (0.17 mL, 1.39 mmol) within 2.5 h followed by flash chromatography (silica, gradient 3:1; 2:1; 3:2...
Hexane/EtOAc) gave 246 mg (71%) of \textbf{63b} as a white solid. $^1$H-NMR (CDCl$_3$, 400MHz) δ 1.61 (3H, s), 1.57 (3H, s), 4.09 (2H, s), 4.59 (1H, s), 5.73 (1H, d, $J = 4.0$ Hz), 6.29 (1H, d, $J = 4.4$ Hz), 6.94 (1H, s), 7.21-7.35 (15H, m), 7.43 (1H, s); $^{13}$C-NMR (CDCl$_3$, 100MHz) δ 26.7, 31.2, 32.0, 65.4, 66.3, 67.4, 70.6, 78.6, 122.4, 126.5, 126.9, 127.6, 128.3, 128.5, 128.6, 128.7, 138.5, 138.7, 138.8, 147.3, 166.3, 168.1; HRMS (FAB, mnba) calcd for [C$_{30}$H$_{28}$N$_4$O$_3$S + H]$^+$ 525.1956, found 525.1960.

**Benzhydryl 2,6-dimethoxyphenyl-6-triazolylpenicillanate (63c).** Reaction of benzhydryl 6-azidopenicillanate \textbf{60c} (0.227 g, 0.555 mmol) and 2-Ethynyl-1,3-dimethoxybenzene \textbf{62} (0.06 g, 0.37 mmol) within 4 h followed by flash chromatography (silica gel, 1:1 Hexane/EtOAc) gave 120 mg (57%) of \textbf{63c} as a white solid. $^1$H-NMR (CDCl$_3$, 400MHz) δ 1.26 (3H, s), 1.69 (3H, s), 3.78 (6H, s), 4.64 (1H, s), 5.80 (1H, d, $J = 4.8$ Hz), 6.42 (1H, d, $J = 4.4$ Hz), 6.62 (2H, d, $J = 8.4$ Hz), 6.96 (1H, s), 7.26-7.35 (11H, m), 7.95 (1H, s); $^{13}$C-NMR (CDCl$_3$, 100MHz) δ 26.7, 31.4, 56.0, 65.2, 66.4, 67.7, 70.6, 78.6, 104.1, 125.2, 126.9, 127.6, 128.3, 128.5, 128.6, 128.7, 129.9 138.9, 139.9, 158.3, 166.4, 168.6; HRMS (FAB, thioglycerol) calcd for [C$_{31}$H$_{30}$N$_4$O$_5$S + H]$^+$ 571.2015, found 571.2049.

**Benzhydryl 4-pyridyl-6-triazolylpenicillanate (66a).** 4-Ethynlypyridyl hydrochloride (0.06 g, 0.414 mmol) was suspended in EtOAc (30 mL) and washed with saturated NaHCO$_3$ (1 x 30 mL). The organic layer was dried over Na$_2$SO$_4$ and concentrated \textit{in vacuo} to give 4-ethynylpyridine \textbf{65a} as white brown solid. The reaction of benzhydryl 6-azidopenicillanate \textbf{60c} (0.15 g, 0.367 mmol) and \textbf{65a} (0.076 g, 0.734 mmol) within 3 h
followed by flash chromatography (silica gel, gradient 1:1; 1:4 Hexane/EtOAc) gave 166 mg (74%) of 66a as a white solid. $^1$H-NMR (CDCl$_3$, 400MHz) $\delta$ 1.26 (3H, s), 1.69 (3H, s), 4.65 (1H, s), 5.80 (1H, d, $J = 4.0$ Hz), 6.39 (1H, d, $J = 4.0$ Hz), 6.96 (1H, s), 7.32-7.36 (10H, m), 7.77 (2H, d, $J = 5.20$ Hz ), 8.19 (1H, s), 9.03(2H, d, $J = 5.20$ Hz); $^{13}$C-NMR (CDCl$_3$, 100MHz) $\delta$ 26.8, 31.1, 65.7, 66.2, 67.2, 70.8, 78.8, 120.1, 122.1, 126.7, 127.5, 128.2, 128.4, 128.5, 138.6, 144.5, 149.2, 165.9, 167.5; HRMS (FAB, thioglycerol) calc for [C$_{28}$H$_{25}$N$_5$O$_3$S + H]$^+$ 512.1756, found 512.1763.

**Benzhydryl 3-pyridyl-6-triazolylpenicillanate (66b).** The reaction of benzhydryl 6-azidopenicillanate 60c (0.15 g, 0.441 mmol) and 3-ethynylpyridine 65b (0.068 g, 0.661 mmol) within 3 h followed by flash chromatography (silica gel, gradient 1:1; 1:3 Hexane/EtOAc) gave 170 mg (76%) of 66b as a white solid. $^1$H-NMR (CDCl$_3$, 400MHz) $\delta$ 1.26 (3H, s), 1.70 (3H, s), 4.66 (1H, s), 5.80 (1H, d, $J = 4.0$ Hz), 6.40 (1H, d, $J = 4.0$ Hz), 6.96 (1H, s), 7.32-7.36 (10H, m), 8.13 (1H, s), 8.26 (1H, d, $J = 8.24$ Hz ), 8.58 (1H, br s), 9.03(1H, br s); $^{13}$C-NMR (CDCl$_3$, 100MHz) $\delta$ 26.9, 31.1, 65.7, 66.3, 67.3, 70.7, 78.7, 120.8, 126.7, 127.5, 128.2, 128.4, 128.5, 133.8, 138.5, 138.6, 138.6, 144.0, 146.1, 148.3, 166.0, 167.6; HRMS (FAB, thioglycerol) calc for [C$_{28}$H$_{25}$N$_5$O$_3$S + H]$^+$ 512.1756, found 512.1773.

**Benzhydryl 2-pyridyl-6-triazolylpenicillanate (66c).** The reaction of benzhyldryl 6-azidopenicillanate 60c (0.15 g, 0.367 mmol) and 2-ethynylpyridine 65c (0.06 mL, 0.55 mmol) within 6 h followed by flash chromatography (silica gel, gradient 4:1; 2:1; 1:2; Hexane/EtOAc) gave 145 mg (64%) of 66c as a white solid. $^1$H-NMR (CDCl$_3$, 400MHz)
\[ \delta 1.25 \text{ (3H, s), 1.71 (3H, s), 4.64 (1H, s), 5.78 (1H, d, } J = 4.0 \text{ Hz), 6.39 (1H, d, } J = 4.0 \text{ Hz), 6.96 (1H, s), 7.32-7.36 \text{ (10H, m), 7.78 (1H, m), 8.18 (1H, d, } J = 7.69 \text{ Hz), 8.45 (1H, s), 8.58 (1H, d, } J = 4.39 \text{ Hz); } ^{13}\text{C-NMR (CDCl}_3, 100MHz) \delta \text{ 26.9, 31.3, 65.7, 66.5, 67.4, 70.8, 78.7, 120.5, 123.0, 126.8, 127.5, 128.2, 128.4, 128.5, 128.6, 138.6, 138.7, 166.1, 167.8; HRMS (FAB, thioglycerol) calc for [C}_{28}\text{H}_{25}\text{N}_{5}\text{O}_{3}\text{S + H}]^+ \text{ 512.1756, found 512.1765.} \]

**Benzhydryl 2-thiopyl-6-triazolylpenicillanate (66d).** Reaction of benzhydryl 6-azidopenicillanate 60c (0.15 g, 0.367 mmol) and 3-ethynylthiopene 65d (0.06 g, 0.55 mmol) within 5 h followed by flash chromatography (silica gel, gradient 1:0; 5:1; 3:1 Hexane/EtOAc) gave 150 mg (79%) of 66d as a white solid. \(^1\text{H-NMR (CDCl}_3, 400MHz) \delta 1.25 \text{ (3H, s), 1.69 (3H, s), 4.64 (1H, s), 5.78 (1H, d, } J = 4.4 \text{ Hz), 6.36 (1H, d, } J = 4.0 \text{ Hz), 6.96 (1H, s), 7.30-7.38 \text{ (11H, m), 7.44 (1H, dd, } J = 4.0, 1.2 \text{ Hz), 7.70 (1H, dd, } J = 2.0, 1.2 \text{ Hz), 7.90 (1H, s); } ^{13}\text{C-NMR (CDCl}_3, 100MHz) \delta 26.7, 30.9, 66.1, 67.3, 70.8, 78.7, 120.1, 121.6, 125.7, 126.4, 126.9, 127.6, 128.3, 128.6, 128.7, 131.1, 138.7, 138.8, 143.8, 166.3, 168.2; HRMS (FAB, thioglycerol) calc for [C}_{27}\text{H}_{24}\text{N}_{4}\text{O}_{3}\text{S}_2\text{ + H}]^+ \text{ 517.1368, found 517.1374.} \]

**p-Methoxybenzyl phenyl-6-triazolylpenicillanate (66e).** Reaction of p-methoxybenzyl-6-azidopenicillanate 60b (0.15 g, 0.414 mmol) and phenylacetylene 65e (0.14 mL, 1.24 mmol) within 2 h followed by flash chromatography (silica gel, gradient 3:1; 3:2 Hexane/EtOAc) gave 165 mg (86%) of 66e as a white foam. \(^1\text{H-NMR (CDCl}_3, 400MHz) \delta 1.38 \text{ (3H, s), 1.66 (3H, s), 3.80 (3H, s), 4.54 (1H, s), 5.14 (2H, app. q, } J = \)
24.0, 12.0 Hz), 5.75 (1H, d, J = 4.0 Hz), 6.37 (1H, d, J = 4.0 Hz), 6.89 (2H, d, J = 8.8 Hz), 7.29-7.35 (3H, m), 7.39-7.42 (2H, m), 7.82 (2H, m), 7.99 (1H, s); $^{13}$C-NMR (CDCl$_3$, 100MHz) δ 27.0, 30.8, 55.2, 65.4, 66.1, 67.2, 67.6, 70.7, 114.0, 120.3, 125.8, 126.5, 128.4, 128.8, 129.9, 130.6, 147.5, 160.0, 167.1, 168.2; HRMS (FAB, thioglycerol) calc for [C$_{24}$H$_{24}$N$_4$O$_4$S + H]$^+$ 465.1596, found 465.1571.

**Benzhydryl α-hydroxylbenzyl-6-triazolylpenicillanate (66f).** Reaction of benzhydryl 6-azidopenicillanate 60c (0.2 g, 0.49 mmol) and 1-phenylprop-2-yn-1-ol 65f (0.13 g, 0.985 mmol) within 2 h followed by flash chromatography (silica gel, 2:1 Hexane/EtOAc) gave 191 mg (72%) of 66f as a white solid. $^1$H-NMR (CDCl$_3$, 400MHz) δ 1.23 (3H, d, J = 12 Hz), 1.59 (3H, d, J = 8.0 Hz), 4.61 (1H, s), 5.71 (1H, d, J = 4.4 Hz), 5.99 (1H, d, J = 5.2 Hz), 6.23 (1H, dd, J = 4.0, 1.6 Hz), 6.95 (1H, s), 7.24-7.42 (15H, m), 7.57 (1H, s). $^{13}$C-NMR (CDCl$_3$, 100MHz) δ 26.6, 31.2, 31.3, 65.3, 65.3, 66.3, 66.4, 67.3, 67.4, 68.7, 68.9, 70.4, 70.5, 78.5, 122.0, 122.1, 126.1, 126.2, 126.3, 126.4, 126.6, 126.7, 127.1, 127.3, 127.6, 127.7, 128.0, 128.2, 128.3, 128.4, 138.5, 138.6, 141.5, 150.9, 166.0, 167.4, 167.5; HRMS (FAB, thioglycerol) calc for [C$_{30}$H$_{28}$N$_4$O$_4$S + H]$^+$ 541.1909, found 541.1936.

**Benzhydryl 4-biphenyl-6-triazolylpenicillanate (66g).** Reaction of benzhydryl 6-azidopenicillanate 60c (0.15 g, 0.367 mmol) and 4-ethynylbiphenyl 65g (0.098 g, 0.55 mmol) within 5 h followed by flash chromatography (silica gel, gradient 1:0; 5:1; 3:1 Hexane/EtOAc) gave 158 mg (73%) of 66g as a white solid. $^1$H-NMR (DMSO-d$_6$, 400MHz) δ 1.26 (3H, s), 1.69 (3H, s), 4.92 (1H, s), 5.88 (1H, d, J = 2.0 Hz), 6.81 (1H, d,
\( J = 2.0 \text{ Hz}, 6.96 (1\text{H}, \text{s}), 7.32 (2\text{H}, \text{d}, J = 8.0 \text{ Hz}), 7.38 (5\text{H}, \text{t}, J = 7.2 \text{ Hz}), 7.47 (5\text{H}, \text{t}, J = 6.8 \text{ Hz}), 7.52 (2\text{H}, \text{d}, J = 8.0 \text{ Hz}), 7.77 (2\text{H}, \text{d}, J = 8.0 \text{ Hz}), 7.90 (2\text{H}, \text{d}, J = 8.0 \text{ Hz}), 8.66 (1\text{H}, \text{s}) \)

**Benzhydryl 3-biphenyl-6-triazolylpenicillanate (66h).** Reaction of benzhydryl 6-azidopenicillanate \( 60c \) (0.15 g, 0.367 mmol) and 3-ethynylbiphenyl \( 65h \) (0.098 g, 0.55 mmol) within 5 h followed by flash chromatography (silica gel, gradient 1:0; 5:1; 3:1 Hexane/EtOAc) gave 215 mg (100%) of \( 66h \) as a white solid. \(^1\text{H-NMR (CDCl}_3\text{,} 400\text{MHz}) \) \( \delta 1.14 (3\text{H}, \text{s}), 1.58 (3\text{H}, \text{s}), 4.54 (1\text{H}, \text{s}), 5.68 (1\text{H}, \text{d}, J = 4.4 \text{ Hz}), 6.28 (1\text{H}, \text{d}, J = 4.0 \text{ Hz}), 6.86 (1\text{H}, \text{s}), 7.12 (1\text{H}, \text{s}), 7.19-7.24 (10\text{H}, \text{m}), 7.31-7.39 (3\text{H}, \text{m}), 7.45 (1\text{H}, \text{d}, J = 8.0 \text{ Hz}), 7.52 (2\text{H}, \text{d}, J = 8.0 \text{ Hz}), 7.69 (2\text{H}, \text{d}, J = 8.0 \text{ Hz}), 7.95 (1\text{H}, \text{s}); \(^{13}\text{C-NMR (CDCl}_3\text{, 100MHz}) \) \( \delta 26.7, 30.9, 65.5, 66.2, 67.3, 70.7, 78.7, 120.5, 124.6, 124.7, 126.9, 127.2, 127.5, 127.6, 128.3, 128.5, 128.6, 128.7, 129.3, 130.4, 138.7, 138.8, 140.6, 141.9, 147.5, 166.3, 168.2; \) HRMS (FAB, thioglycerol) calc for \([\text{C}_{35}\text{H}_{30}\text{N}_4\text{O}_3\text{S} + \text{H}]^+\) 587.2116, found 587.2143.

**Benzhydryl 2-biphenyl-6-triazolylpenicillanate (66i).** Reaction of benzhydryl 6-azidopenicillanate \( 60c \) (0.15 g, 0.367 mmol) and 2-ethynylbiphenyl \( 65i \) (0.098 g, 0.55 mmol) within 5 h followed by flash chromatography (silica gel, gradient 1:0; 5:1; 3:1 Hexane/EtOAc) gave 150 mg (70%) of \( 66i \) as a white solid. \(^1\text{H-NMR (CDCl}_3\text{, 400MHz}) \) \( \delta 1.20 (3\text{H}, \text{s}), 1.47 (3\text{H}, \text{s}), 4.47 (1\text{H}, \text{s}), 5.63 (1\text{H}, \text{d}, J = 4.0 \text{ Hz}), 6.26 (1\text{H}, \text{d}, J = 4.4 \text{ Hz}), 6.80 (1\text{H}, \text{s}), 6.93 (1\text{H}, \text{s}), 7.22-7.45 (18\text{H}, \text{m}), 8.15 (1\text{H}, \text{d}, J = 8.0 \text{ Hz}); \(^{13}\text{C-NMR (CDCl}_3\text{, 100MHz}) \) \( \delta 26.7, 31.3, 65.4, 66.3, 67.4, 70.4, 78.6, 123.0, 126.9, 127.2, 127.5,
127.8, 128.1, 128.2, 128.5, 128.6, 128.7, 128.8, 129.2, 130.3, 138.8, 140.2, 141.5, 166.3, 167.0; HRMS (FAB, thioglycerol) calc for $[\text{C}_{35}\text{H}_{30}\text{N}_{4}\text{O}_{5}\text{S} + \text{H}]^+$ 587.2116, found 587.2143.

**Benzhydryl 6-methoxynapthaly-6-triazolylpenicillanate (66j).** Reaction of benzhydryl 6-azidopenicillanate 60c (0.15 g, 0.367 mmol) and 2-ethynyl-6-methoxynaphthalene (0.067 g, 0.37 mmol) within 5 h followed by flash chromatography (silica gel, gradient 1:0; 5:1; 3:1 Hexane/EtOAc) gave 175 mg (81%) of 66j as a white solid. $^1$H-NMR (CDCl$_3$, 400MHz) $\delta$ 1.27 (3H, s), 1.72 (3H, s), 3.91 (3H, s), 4.67 (1H, s), 5.80 (1H, d, $J = 4.4$ Hz), 6.41 (1H, d, $J = 4.4$ Hz), 6.97 (1H, s), 7.13-7.16 (2H, m), 7.31-7.35 (10H, m), 7.77 (2H, dd, $J = 6.4$, 2.8 Hz), 7.88 (1H, dd, $J = 6.4$, 1.6 Hz), 8.08 (1H, s), 8.26 (1H, s); $^{13}$C-NMR (CDCl$_3$, 100MHz) $\delta$ 26.7, 31.0, 55.3, 65.5, 66.2, 67.3, 70.8, 78.7, 119.3, 120.2, 124.3, 124.6, 125.1, 126.9, 127.4, 127.6, 128.3, 128.6, 128.7, 128.9, 129.7, 134.4, 138.7, 138.8, 147.7, 158.0, 166.3, 168.2; HRMS (FAB, mna) calc for $[\text{C}_{34}\text{H}_{30}\text{N}_{4}\text{O}_{4}\text{S} + \text{H}]^+$ 591.2066, found 591.2061.

**Benzhydryl 4-pyridylphenyl-6-triazolylpenicillanate (66k).** Reaction of benzhydryl 6-azidopenicillanate 60c (0.15 g, 0.367 mmol) and 4-(4-ethynylphenyl)pyridine 65k (0.098 g, 0.55 mmol) within 5 h followed by flash chromatography (silica gel, gradient 1:1; 1:2; 1:4 Hexane/EtOAc) gave 202 mg (94%) of 66k as a white solid. $^1$H-NMR (CDCl$_3$, 400MHz) $\delta$ 1.27 (3H, s), 1.71 (3H, s), 4.66 (1H, s), 5.80 (1H, d, $J = 4.0$ Hz), 6.40 (1H, d, $J = 4.0$ Hz), 6.97 (1H, s), 7.31-7.35 (12H, m), 7.61 (2H, br s), 7.72 (2H, d, $J = 8.0$ Hz), 7.97 (2H, d, $J = 8.0$ Hz), 8.09 (1H, s); $^{13}$C-NMR (CDCl$_3$, 100MHz) $\delta$ 26.7, 30.9, 65.6,
Benzhydryl 4-pyridylbenzylhydroxyl-6-triazolylpenicillanate (66l). Reaction of benzhydryl 6-azidopenicillanate 60c (0.15 g, 0.367 mmol) and 1-(4-(pyridin-4-yl)prop-2-yn-1-ol 65l (0.115 g, 0.55 mmol) within 2 h followed by flash chromatography (silica gel, gradient 1:2; 1:3 Hexane/EtOAc) gave 190 mg (84%) of 66l as a white solid. $^1$H-NMR (CDCl$_3$, 400MHz) δ 1.22 (3H, d, $J = 12$ Hz), 1.60 (3H, d, $J = 5.6$ Hz), 4.58 (1H, s), 5.72 (1H, d, $J = 4.4$ Hz), 6.08 (1H, s), 6.28 (1H, d, $J = 4.4$ Hz), 6.92 (1H, s), 7.24-7.32 (10H, m), 7.44 (2H, dd, $J = 6.0$, 1.6 Hz ) 7.52-7.58 (4H, m), 7.67 (1H, s), 8.53 (2H, dd, $J = 6.4$, 1.6 Hz). $^{13}$C-NMR (CDCl$_3$, 100MHz) δ 26.7, 30.6, 31.1, 31.2, 64.3, 65.4, 66.2, 66.3, 67.3, 68.4, 68.5, 70.5, 78.6, 121.4, 122.0, 126.6, 126.9, 127.1, 127.4, 128.1, 128.3, 128.4, 128.5, 137.1, 137.2, 138.5, 142.9, 147.8, 149.6, 150.8, 150.9, 166.0, 167.5, 167.6; HRMS (FAB, thioglycerol) calc for [C$_{35}$H$_{31}$N$_5$O$_4$S + H]$^+$ 618.2175, found 618.2180

Representative Procedure for Deprotection of Carboxyl Protecting Group. Benzyl-6-triazolylpenicillanic acid (64a). $p$-Methoxybenzyl benzyl-6-triazolylpenicillanate 63a (0.13 g, 0.26 mmol) was dissolved and stirred in anhydrous CH$_2$Cl$_2$ (1 mL) at -5°C. Anhydrous anisole (0.2 mL, 1.83 mmol) and trifluoroacetic acid (0.5 mL, 6.49 mmol) were added, and the reaction mixture stirred for 2 h. The reaction mixture was diluted with cold Et$_2$O (10 mL), and the solvent was evaporated off in an ice bath. The residue was concentrated in vacuo on an ice bath for 15 minutes, and redissolved in THF (5 mL)
and ½ saturated NaHCO$_3$ (15 mL) at 0°C. The resulting mixture was stirred at 0°C for 15 minutes and partitioned between deionized water (5 mL) and EtOAc (20 mL). The two layers were separated and the aqueous layer was extracted with EtOAc (2 x 20 mL). The aqueous layer was acidified to pH 3 in an ice bath with 1N HCl and extracted with EtOAc (3 x 20 mL). The combined organic layer was dried over Na$_2$SO$_4$ and concentrated in vacuo to give 89 mg (100%) of 64a as a white solid. $^1$H-NMR (acetone-d$_6$, 400MHz) δ 1.55 (3H, s), 1.70 (3H, s), 4.07 (2H, s), 4.55 (1H, s), 5.85 (1H, d, $J = 4.4$ Hz), 6.58 (1H, d, $J = 4.4$ Hz), 7.26-7.28 (5H, m), 7.79 (1H, s); $^{13}$C-NMR (acetone-d$_6$, 100MHz) δ 26.8, 31.0, 31.8, 65.1, 67.0, 67.8, 70.7, 123.2, 126.7, 128.8, 128.9, 129.0, 139.9, 168.4, 168.5; HRMS (FAB, mnba) calc for [C$_{17}$H$_{18}$N$_4$O$_3$S + H]$^+$ 359.1177, found 359.1177.

2,6-Dimethoxyphenyl-6-triazolylpenicillanic acid (64b). The reaction of a CH$_2$Cl$_2$ solution of 63c with anisole and TFA within 2 h, as described for the synthesis of 64a from 63a, afforded 85 mg (100%) of 64b as a white solid. $^1$H-NMR (acetone-d$_6$, 400MHz) δ 1.59 (3H, s), 1.77 (3H, s), 3.77 (6H, s), 4.62 (1H, s), 5.93 (1H, d, $J = 4.4$ Hz), 6.73 (1H, d, $J = 4.4$ Hz), 6.74 (2H, d, $J = 8.4$ Hz), 7.35 (1H, t, $J = 8.4$ Hz), 8.15 (1H, s); $^{13}$C-NMR (acetone-d$_6$, 400MHz) δ 26.9, 31.2, 55.9, 65.2, 67.2, 68.0, 70.7, 104.6, 126.2, 131.0, 158.7, 168.6; HRMS (FAB, mnba) calc for [C$_{18}$H$_{20}$N$_4$O$_5$S + H]$^+$ 405.1232, found 405.1214.

4-pyridyl-6-triazolylpenicillanic acid (67a). The reaction of a CH$_2$Cl$_2$ solution of 66a with anisole and TFA within 0.5 h, as described for the synthesis of 64a from 63a, afforded 80 mg (100%) of 67a as a white solid. $^1$H-NMR (acetone-d$_6$, 400MHz) δ 1.56
(3H, s), 1.73 (3H, s), 4.60 (1H, s), 5.95 (1H, d, J = 4.0 Hz), 6.78 (1H, d, J = 4.0 Hz), 8.57
(2H, br s), 9.01 (2H, br s), 9.07 (1H, s); $\textsuperscript{13}$C-NMR (acetone-d$_6$, 100MHz) $\delta$ 27.1, 31.6,
65.7, 67.7, 68.0, 71.4, 122.8, 126.8, 142.8, 143.6, 146.6, 167.5, 170.7; HRMS (FAB, mnba) calc for C$_{15}$H$_{15}$N$_5$O$_3$S + H]$^+$ 346.0973, found 346.0972.

3-pyridyl-6-triazolylpenicillanic acid (67b). The reaction of a CH$_2$Cl$_2$ solution of 66b
with anisole and TFA within 0.5 h, as described for the synthesis of 64a from 63a,
afforded 80 mg (100%) of 67b as a reddish white solid. $^1$H-NMR (acetone-d$_6$, 400MHz) $\delta$ 1.57 (3H, s), 1.74 (3H, s), 4.60 (1H, s), 5.95 (1H, d, J = 4.0 Hz), 6.76 (1H, d, J = 4.0 Hz), 8.15 (1H, br s), 8.84 (1H, s), 8.92 (2H, m), 9.41 (1H, br s); HRMS (FAB, mnba) calc
for [C$_{15}$H$_{15}$N$_5$O$_3$S + H]$^+$ 346.0973, found 346.0972.

2-pyridyl-6-triazolylpenicillanic acid (67c). The reaction of a CH$_2$Cl$_2$ solution of 66c
with anisole and TFA within 0.5 h, as described for the synthesis of 64a from 63a,
afforded 80 mg (100%) of 67c as a white solid. $^1$H-NMR (acetone-d$_6$, 400MHz) $\delta$ 1.57
(3H, s), 1.76 (3H, s), 4.63 (1H, s), 5.95 (1H, d, J = 4.0 Hz), 6.79 (1H, d, J = 4.0 Hz), 7.79
(1H, m), 8.39 (1H, m), 8.48 (1H, m), 8.90 (1H, d, J = 5.1 Hz), 8.95 (1H, s); $\textsuperscript{13}$C-NMR
(acetone-d$_6$, 100MHz) $\delta$ 27.1, 31.5, 65.7, 67.6, 68.0, 71.2, 122.8, 125.3, 125.7, 143.2,
145.9, 146.7, 160.2, 167.8, 168.6; HRMS (FAB, mnba) calc for [C$_{15}$H$_{15}$N$_5$O$_3$S + H]$^+$
346.0973, found 346.0974.

2-Thiopyl-6-triazolylpenicillanic acid (67d). The reaction of a CH$_2$Cl$_2$ solution of 66d
with anisole and TFA within 1 h, as described for the synthesis of 64a from 63a, afforded
81 mg (100%) of 67d as a yellowish solid. $^1$H-NMR (acetone-d$_6$, 400MHz) δ 1.57 (3H, s), 1.74 (3H, s), 4.59 (1H, s), 5.90 (1H, d, $J = 4.0$ Hz), 6.65 (1H, d, $J = 4.0$ Hz), 7.54 (1H, m), 7.57 (1H, dd, $J = 3.0, 1.5$ Hz), 7.87 (1H, dd, $J = 3.0, 1.5$ Hz), 8.34 (1H, s); $^{13}$C-NMR (acetone-d$_6$, 100MHz) δ 27.1, 31.3, 65.4, 67.3, 68.0, 71.2, 121.4, 121.6, 126.3, 127.1, 132.4, 143.7, 168.3, 168.9; HRMS (FAB, mnba) calc for [C$_{14}$H$_{14}$N$_4$O$_3$S$_2$ + H]$^+$ 351.0585, found 351.0577.

**Phenyl-6-triazolylpenicillanic acid (67e).** The reaction of a CH$_2$Cl$_2$ solution of 66e with anisole and TFA within 2 h, as described for the synthesis of 64a from 63a, afforded 68.5 mg (93%) of 67e as a white solid. $^1$H-NMR (acetone-d$_6$, 400MHz) δ 1.47 (3H, s), 1.67 (3H, s), 4.54 (1H, s), 5.80 (1H, d, $J = 4.0$ Hz), 6.76 (1H, d, $J = 4.4$ Hz), 7.34 (1H, m), 7.44 (2H, m), 7.89 (2H, m), 8.60 (1H, s); $^{13}$C-NMR (acetone-d$_6$, 100MHz) δ 27.2, 31.2, 65.4, 67.3, 68.0, 71.2, 121.6, 126.1, 126.1, 128.6, 129.4, 131.3, 147.3, 168.4; HRMS (FAB, mnba) calc for [C$_{16}$H$_{16}$N$_4$O$_3$S + H]$^+$ 345.1021, found 345.1015.

**α-Hydroxybenzyyl-6-triazolylpenicillanic acid (67f).** The reaction of a CH$_2$Cl$_2$ solution of 66f with thioanisole and TFA within 0.5 h, as described for the synthesis of 64a from 63a, afforded 76 mg (100%) of 67f as a pale yellowish solid. $^1$H-NMR (acetone-d$_6$, 400MHz) δ 1.53 (3H, s), 1.67 (3H, s), 3.37 (1H, s), 4.55 (1H, s), 5.84 (1H, bs), 5.97 (1H, s), 6.55 (1H, bs), 7.22-7.43 (4H, m), 7.80 (1H, s); HRMS (FAB, thioglycerol) calc for [C$_{17}$H$_{18}$N$_4$O$_4$S + H]$^+$ 375.1127, found 375.1192.
4-Biphenyl-6-triazolylpenicillanic acid (67g). The reaction of a CH$_2$Cl$_2$ solution of 66g with anisole and TFA within 2 h, as described for the synthesis of 64a from 63a, afforded 65 mg (100%) of 67g as a yellowish solid. $^1$H-NMR (DMSO-d$_6$, 400MHz) δ 1.48 (3H, s), 1.65 (3H, s), 4.38 (1H, s), 5.79 (1H, d, $J = 4.0$ Hz), 6.72 (1H, d, $J = 4.0$ Hz), 7.35-7.40 (1H, m), 7.47 (2H, t, $J = 8.0$ Hz), 7.74 (4H, dd, $J = 11.2$, 8.0 Hz), 7.99 (2H, d, $J = 8.0$ Hz), 8.63 (1H, s); HRMS (FAB, mnba) calc for [C$_{22}$H$_{20}$N$_4$O$_3$S + H]$^+$ 421.1334, found 421.1333.

3-Biphenyl-6-triazolylpenicillanic acid (67h). The reaction of a CH$_2$Cl$_2$ solution of 66h with anisole and TFA within 2 h, as described for the synthesis of 64a from 63a, afforded 54 mg (83%) of 67h as a white solid. $^1$H-NMR (acetone-d$_6$, 400MHz) δ 1.59 (3H, s), 1.77 (3H, s), 4.61 (1H, s), 5.93 (1H, d, $J = 4.0$ Hz), 6.71 (1H, d, $J = 4.0$ Hz), 7.38 (1H, m), δ 7.48 (2H, m), 7.54 (1H, t, $J = 8.0$ Hz), 7.64 (1H, m), 7.72 (2H, m), 7.96 (1H, dt, $J = 8.0$, 1.5 Hz), 8.23 (1H, t, 1.5 Hz), 8.58 (1H, s); $^{13}$C-NMR (acetone-d$_6$, 100MHz) δ 27.2, 31.2, 65.5, 67.3, 68.0, 71.3, 122.0, 124.6, 125.1, 127.2, 127.5, 128.1, 129.4, 130.0, 131.9, 141.1, 142.2, 147.2, 168.4, 168.9; HRMS (ESI, mnba) calc for [C$_{22}$H$_{20}$N$_4$O$_3$S + H]$^+$ 421.1329, found 421.1312.

2-Biphenyl-6-triazolylpenicillanic acid (67i). The reaction of a CH$_2$Cl$_2$ solution of 66i with anisole and TFA within 1 h, as described for the synthesis of 64a from 63a, afforded 65 mg (100%) of 67i as a white solid. H-NMR (acetone-d$_6$, 400MHz) δ 1.53 (3H, s), 1.59 (3H, s), 4.41 (1H, s), 5.78 (1H, d, $J = 4.0$ Hz), 6.55 (1H, d, $J = 4.0$ Hz), 7.00 (1H, s),
7.22-7.25 (2H, m), 7.32-7.52 (6H, m), 8.07 (1H, dd, \( J = 5.8, 1.5 \) Hz); HRMS (ESI, mnba) calc for [C\(_{22}\)H\(_{20}\)N\(_4\)O\(_3\)S + H\(^+\)] 421.1329, found 421.1359.

6-Methoxynaphthaly-6-triazolylpenicillanic acid (67j).  The reaction of a CH\(_2\)Cl\(_2\) solution of 66j with anisole and TFA within 1 h, as described for the synthesis of 64a from 63a, afforded 56 mg (89%) of 67j as a white foam. \(^1\)H-NMR (DMSO-d\(_6\), 400MHz) \( \delta \) 1.58 (3H, s), 1.78 (3H, s), 3.91 (3H, s), 4.17 (1H, s), 5.80 (1H, d, \( J = 4.0 \) Hz), 6.62 (1H, d, \( J = 4.0 \) Hz), 7.16 (1H, dd, \( J = 6.5, 2.2 \) Hz), 7.30 (1H, m), 7.85 (2H, dd, \( J = 3.0, 5.0 \) Hz), 7.98 (1H, m), 8.39 (1H, m), 8.55 (1H, s); \(^{13}\)C-NMR (acetone-d\(_6\), 100MHz) \( \delta \) 26.7, 30.7, 55.1, 65.0, 67.0, 67.6, 70.8, 106.1, 119.5, 121.3, 124.4, 124.5, 126.2, 127.7, 129.3, 129.9, 134.9, 147.4, 158.4, 168.3, 168.4; HRMS (FAB, thioglycerol) calc for [C\(_{21}\)H\(_{20}\)N\(_4\)O\(_4\)S + H\(^+\)] 425.1283, found 425.1264.

4-Pyridylphenyl-6-triazolylpenicillanic acid (67k).  The reaction of a CH\(_2\)Cl\(_2\) solution of 66k with anisole and TFA within 1 h, as described for the synthesis of 64a from 63a, afforded 37 mg (65%) of 67k as a white foam. \(^1\)H-NMR (acetone-d\(_6\), 400MHz) \( \delta \) 1.58 (3H, s), 1.77 (3H, s), 4.61 (1H, s), 5.94 (1H, d, \( J = 4.0 \) Hz), 6.74 (1H, d, \( J = 4.0 \) Hz), 8.17 (4H, m), 8.47 (2H, br s), 8.64 (1H, s), 9.04 (2H, br s); HRMS (FAB, mnba) calc for [C\(_{21}\)H\(_{19}\)N\(_5\)O\(_3\)S + H\(^+\)] 422.1287, found 422.1301.

4-Pyridylbenzylhydroxyl-6-triazolylpenicillanic acid salt (67l). Benzhydryl 4-pyridylphenyl-6-triazolylpenicillanate 66l (0.09 g, 0.14 mmol) was dissolved and stirred in anhydrous CH\(_2\)Cl\(_2\) (1 mL) at -5°C. Anhydrous thioanisole (0.2 mL, 1.83 mmol) and
TFA (0.5 mL, 6.49 mmol) were added, and the reaction mixture stirred for 0.5 h. The reaction mixture was diluted with cold Et₂O (10 mL), and the solvent was evaporated off in an ice bath. The residue was concentrated in vacuo on an ice bath for 15 minutes, and redissolved in aqueous NaHCO₃ (0.018 g, 0.21 mmol) at 0°C. The resulting mixture was stirred at 0°C for 15 minutes and partitioned between deionized water (5 mL) and EtOAc (20 mL). The two layers were separated and the aqueous layer was extracted with EtOAc (2 x 20 mL). The aqueous layer was frozen in acetone-dry ice bath and concentrated in vacuo to give 35 mg (52%) of 67l as a white solid. ¹H-NMR (DMSO-d₆, 400MHz) δ 1.46 (3H, s), 1.61 (3H, m), 4.51 (1H, s), 5.75 (1H, d, J = 4.4 Hz), 5.94 (1H, s), 6.67 (1H, d, J = 4.0 Hz), 7.57 (2H, d, J = 8.0 Hz), 7.86 (4H, m), 8.75 (2H, m); HRMS (FAB, mnba) calc for [C₁₂₂H₂₀N₅NaO₄S + H]⁺ 474.1212, found 474.1234.

4.7 References


26. Numerous examples of click chemistry have appeared in the literature. Cited here are two pioneering examples: (a) Rostovtsev, V. V.; Green, L. G.; Fokin, V. V.; Sharpless, K. B. A stepwise Huisgen Cycloaddition Process: Copper(I)-Catalyzed Regioselective “ligation” of Azide and Terminal Alkynes. Angew. Chem., Int. Ed. 2002, 41, 2596-2599. (b) Tornoe, C. W.; Christensen, C.; Meldal, M. Peptidotriazoles on solid phase: [1,2,3]-triazoles by regiospecific copper(I)-catalyzed 1,3-dipolar cycloadditions of terminal alkynes to azides. J. Org. Chem. 2002, 67, 3057-3064.


5.1 Solid Phase Synthesis

Solid-phase synthesis (SPS) has become a routine and unmatched method for the synthesis of biomacromolecules such as peptide, DNA and RNA. Vital to the success of SPS is an optimal covalent attachment of the leader monomer onto insoluble supports. Majority of the protocols for the immobilization of leader monomer onto solid supports are based on alkylation and acylation reactions. However, these protocols usually require special procedures, which in untrained hands, are often difficult and time consuming. For example, attachment of a substrate nucleoside onto a commercially available insoluble solid support such as 3-aminopropylated-CPG could take days to accomplish, under rigorous exclusion of moisture. Also, partial support-loading could result in unwanted side-reactions which could compromise product quality. To address these problems and expand the scope of functionalities which can serve as handle for SPS, a large collection of linkers have been investigated and developed. However, the vast majority of these linkers still rely on the traditional coupling protocols for the monomer support attachment. It is therefore of prime interest to have flexible synthetic methodologies for rapid loading of leader monomers onto the supports. This realization has continued to spawn immense efforts in the literature.

The utility of organic azides in bioconjugations and synthetic organic chemistry applications is enjoying a renaissance. They are extensively used in photoaffinity labeling
of biomacromolecules, the Staudinger ligation reaction, and protection strategy for amines. Moreover, with the recent discovery of Cu(I) catalysis, termed click chemistry by Sharpless and co-workers, Huigsen cycloaddition reaction between azides and terminal alkynes has become the premier conjugation technique in chemistry, biology and material science applications. Click chemistry has allowed rapid construction of complex macromolecules, whole cell and organism modification, and small molecules with diverse biological properties.

There are several methods for the synthesis of organic azides. Among these are substitution reactions between various electrophiles and azide nucleophiles to generate aliphatic azides. Aromatic azides can be easily prepared by diazotization of the corresponding amines, and reaction of aryl Grignard or lithium reagents and aryl amide salts with para-tosylazide. Solid-supported azides have been principally generated by direct nucleophilic displacement of appropriately activated supports. This reaction usually proceeds rather sluggishly under harsh conditions. More conveniently, a direct conversion of organic amines to azides (diazo-transfer) is commonly achieved by the reaction of the corresponding amine with triflyl azide [Caution! Triflyl azide may be explosive and it should be handled with care. However, we have not experienced any difficulties in handling triflyl azide]. Diazo-transfer is a high-yielding reaction that proceeds under mild conditions, and it is especially useful for the synthesis of tertiary azides where steric hindrance precludes the traditional synthesis through a direct nucleophilic displacement of leaving groups such as halides and sulfates. Commercially available are numerous amine-attached insoluble solid supports, whose surfaces offer a challenging steric maze that may be well-suited for diazo-transfer
reaction. Inspired by this possibility, we investigated the feasibility of heterogeneous diazo-transfer reaction. We report that triflyl azide rapidly reacts with a series of amine functionalized solid-supports to generate azide-coated supports. The “azide-coat” was further shown to allow a facile loading of alkyne-functionalized leader nucleoside-monomers and subsequent assembly of oligonucleotides by conventional SPS methods. These results extend the scope of amine-azide conversion reaction and also demonstrate the suitability of click-chemistry in the support-attachment of leader nucleoside-monomers for solid phase oligonucleotide synthetic applications.

5.2 Heterogeneous Diazo-transfer Reaction

Firstly, we investigated the possibility of reaction of triflyl azide with ArgoPore-NH₂ resin. Diazo-transfer reaction was initiated with seven-mole excess of freshly prepared triflyl azide (Figure 5-1a). The reaction progress was monitored by quantitative ninhydrin analysis and FTIR. We noticed a clear distinction between solution-phase and heterogeneous diazo-transfer reaction; namely, the heterogeneous reaction proceeded rapidly in the presence of Cu (II) catalyst. The reaction reached its maxima within 4h as judged by quantitative ninhydrin analysis (Table 5-1) and the presence of azide moiety was confirmed by FTIR (2097 cm⁻¹) (Figure 5-1b). In the absence of Cu (II) catalyst, the reaction proceeded at a much slower rate, reaching maximal conversion at 21h (Table 5-1).
Figure 5-1: Analysis of heterogeneous diazo-transfer Reactions. (a) Conditions for heterogeneous diazo-transfer reaction (the blue circle represents the part of the resin unaffected by the reaction). (b) FTIR spectra (KBr) of Cu (II) catalyzed reaction: (i) unmodified resin, (ii) 20 min reaction time, and (iii) 21 h reaction time.
Table 5-1: Quantification of the extent of heterogeneous diazo-transfer reaction of ArgoPore-NH$_2$ resin in the presence (a) and absence (b) of Cu (II) catalyst (Quantitative ninhydrin analysis).

(a)  

<table>
<thead>
<tr>
<th>Reaction Time (min)</th>
<th>mmol/g NH$_2$</th>
<th>mmol/g N$_3$ $^a$</th>
<th>% NH$_2$ Conversion</th>
</tr>
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<tbody>
<tr>
<td>0</td>
<td>1.174 $^b$</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>20</td>
<td>0.126</td>
<td>1.048</td>
<td>89.3</td>
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<td>0.084</td>
<td>1.090</td>
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<tr>
<td>240</td>
<td>0.048</td>
<td>1.126</td>
<td>95.9</td>
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<tr>
<td>1260</td>
<td>0.057</td>
<td>1.117</td>
<td>95.1 $^c$</td>
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</table>

(b)  

<table>
<thead>
<tr>
<th>Reaction Time (min)</th>
<th>mmol/g NH$_2$</th>
<th>mmol/g N$_3$ $^a$</th>
<th>% NH$_2$ Conversion</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>1.174 $^b$</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>20</td>
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<td>0.491</td>
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<td>0.914</td>
<td>77.9</td>
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<tr>
<td>240</td>
<td>0.142</td>
<td>1.032</td>
<td>87.9</td>
</tr>
<tr>
<td>1260</td>
<td>0.039</td>
<td>1.135</td>
<td>96.7</td>
</tr>
</tbody>
</table>

$^a$ mmol/g N$_3$ was calculated from the difference between mmol/g NH$_2$ of unmodified resin and mmol/g NH$_2$ at a given time during the course of the reaction. $^b$ standard loading ArgoPore-NH$_2$ resin, recommended manufacturer loading capacity is 0.90mmol/g, c. within the limit of assay error obtained from standard deviation from two experiments.

Based on this interesting result, we sought to probe the generality of this reaction. We turned to investigate the reactivity of four additional commercial amine-terminated resins, namely: aminopropylated-CPG, Tentagel S-NH$_2$, polystyrene A-NH$_2$ and (aminomethyl) polystyrene. These resins each offer different arrays of polymeric backbones and conceivably varied steric environments. Tentagel S-NH$_2$, polystyrene A-NH$_2$ and (aminomethyl) polystyrene reacted with triflyl azide in similar fashion to the ArgoPore resin yielding azide-coated resins in excellent yields (Table 2). However, aminopropylated-CPG failed to react with triflyl azide under identical conditions used for ArgoPore resin. We then tested various solvents and temperature conditions and found that the reaction occurred at slightly elevated temperature, between 37°C to 40°C, to give azide-coated aminopropylated-CPG in 50% yield within 24h (Table 5-2). The
introduction of azide onto solid supports by the conventional nucleophilic displacement
reactions required the synthesis of activated supports or the use of limited commercial
activated supports such as Merrifield resin. These reactions proceed slowly under
extreme conditions. Operationally however, the current heterogeneous diazo-transfer
reaction proceeds rapidly and smoothly in conventional reaction flask at ambient
conditions without any need for an elaborate technical handling.

Table 5-2. Heterogeneous diazo-transfer reaction explored on amine-terminated resins.

<table>
<thead>
<tr>
<th>Type of Resins</th>
<th>Reaction Temperature (°C)</th>
<th>Azide Conversion* (%)</th>
<th>Reaction Time (h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ArgoPore</td>
<td>25</td>
<td>90</td>
<td>4</td>
</tr>
<tr>
<td>Polystyrene A</td>
<td>25</td>
<td>90</td>
<td>4</td>
</tr>
<tr>
<td>(Aminoethyl) Polystyrene</td>
<td>25</td>
<td>90</td>
<td>4</td>
</tr>
<tr>
<td>Tentagel</td>
<td>25</td>
<td>90</td>
<td>4</td>
</tr>
<tr>
<td>CPG</td>
<td>37</td>
<td>50</td>
<td>24</td>
</tr>
</tbody>
</table>

*Calculated from quantitative ninhydrin analysis.

5.3 Synthesis of Alkyne Functionalized Nucleosides

Among the bioconjugations protocols utilizing organic azides, Cu (I) catalyzed
alkyne-azide cycloaddition reaction (Sharpless click chemistry) has attracted the most
attention in macromolecular fabrication. The application of click chemistry in the
immobilization of oligonucleotides probes on self-assembled monolayers on thiol-coated
silicon wafers has recently appeared in the literature. Similarly, click-chemistry has
been used to directly functionalize resins for the solid phase synthesis of a library of
dopaminergic arylcarbamides,\textsuperscript{24} tertiary amines,\textsuperscript{25} and peptidotriazole.\textsuperscript{16b,30} As an
extension of the application of this versatile chemistry, we explored the suitability of two
of the synthesized azido-resins in the loading of nucleoside leader monomers for solid
phase oligonucleotide synthesis. Toward this end we have prepared new alkyne
functionalized nucleosides \textbf{70a}, \textbf{70b} and \textbf{72a}. Acylation of 5’-\textit{O}-
dimethoxytritylthymidine \textbf{68} with acid fluorides \textbf{69a-b} adapting the procedure described
by Oliver and Oyelere furnished 3’-alkyne esters \textbf{70a} and \textbf{70b} in good yields.\textsuperscript{31} Similarly, 2’-deoxyadenosine-3’-alkyne ester \textbf{72a} was obtained from acylation of commercially
available protected 2’-deoxyadenosine \textbf{71} and acid fluoride \textbf{69b} (Scheme 5-1). These
alkyne functionalized nucleosides were evaluated as substrates for attachment to azide-
coated supports by click chemistry.
Scheme 5-1. Synthesis of Alkyne Functionalized Nucleosides

5.4 Attachment of Leader Alkyne-monomers to Solid Supports and Solid Phase Oligonucleotide Synthesis.

The azide-coated resins were first treated with acetic anhydride to cap any traces of unreacted amines using standard protocol. To identify the optimum reaction conditions for the attachment of the alkyne nucleosides onto azide-coated supports, we tested resin derivatization of alkynes 70a and 70b by copper catalyzed Huisgen reaction on ArgoPore-N3 resin (Scheme 5-2).
In a typical reaction, 70a or 70b was incubated with ArgoPore-N₃ resin and CuI in THF and Hunig’s base mixture in the presence of tris-(benzyltriazolylmethyl)-amine (TBTA).³² The reaction proceeded smoothly at ambient conditions resulting in an uneventful resin loading. Quantitative trityl group³³ analysis revealed that as little as 0.5 equivalents of the alkyne 70a or 70b is sufficient for a near quantitative nucleoside resin loading within 2h. Because such a maximal resin loading may not be ideal for an SPS application, a series of loading reactions were then investigated to obtain nucleoside resin loadings at typical concentrations for solid phase oligonucleotide synthesis. We found that the reaction of about 0.1 equivalents of 70a or 70b resulted in ArgoPore-N₃ resin loading of about 7-10 μmol/g within 30 min while a prolonged reaction time resulted in increase nucleoside attachment. For example, within 2h, a loading of about 30-46 μmol/g was obtained. We saw no significant difference in the loading capacity of compounds 70a and 70b. Interestingly, about ten times as much of compound 71a is necessary to obtain nucleoside loadings comparable to that of 70a or 70b; we could however not ascertain the source of this coupling disparity. Due to the wide application of aminopropylated-CPG
resin in solid phase oligonucleotide synthesis, we turned our attention to study the coupling behavior of CPG-N₃ resin. We found that using 2 mole equivalents of compound 70b under similar conditions described for ArgoPore-N₃ resin resulted in coupling yields of \(40 \pm 5 \, \mu\text{mol/g}\) within 2h (Scheme 5-2).

Encouraged by these results, we then decided to investigate the suitability of this solid-support coupling protocol in solid phase oligonucleotide synthesis. Thymidine- and deoxyadenosine-linked ArgoPore resins 73 (a or b) and 74 respectively and thymidine-linked CPG resin 75 prepared above were employed in automated oligonucleotide synthesis in Expedite⁴⁴ and Applied Biosystems DNA synthesizers using phosphoramidite chemistry.¹c, ³⁵ In these experiments, we synthesized on 1 \(\mu\text{mol}\) scale a 15mer (AGC CAG ATT TGA GCT) and a 30mer (AGC CAG ATT TGA GCT TGG GGC TCT CTG GCT) on thymidine immobilized supports; and a 30mer (TGC CAG ATT TGA GCT TGG GGC TCT CTG GCA) on 2’-deoxyadenosine immobilized support. Oligonucleotide syntheses, and the subsequent deprotection and cleavage of the support-bound products followed standard procedures.⁵ Figure 5-2 shows the reverse-phase HPLC elution pattern of a representative crude product. This HPLC profile compared favorably well with that of similar oligo made from commercial resins. The identities of the HPLC-purified oligos were further confirmed by UV-vis absorption and MALDI-TOF MS analysis (see Chapter 6. Experimental and Spectra Data for details).
Figure 5-2. Reverse-phase HPLC elution pattern of a crude oligonucleotide. Deprotected crude 15mer (AGC CAG ATT TGA GCT), HPLC conditions: Phenomenex RP C-18 column; solvent A 0.1 M NH₄OAc; solvent B, acetonitrile, gradient 6-28 % of solvent B in 45 min; flow rate 1 mL/min.

5.5 Conclusion

In summary, we have demonstrated the feasibility of heterogeneous diazo-transfer reaction on commercial, amine-terminated resins. We also showed that the so-formed azide-coat can be used to load leader nucleoside-monomers on solid supports using click chemistry under benign experimental conditions. This approach avoids expensive, toxic and moisture sensitive additives, that are required for traditional monomer support-attachment by esterification reactions. Furthermore, we demonstrated the potential utility of the support coupling protocol, herein described, in oligonucleotide solid phase synthetic applications. Part of our ongoing work is the syntheses of nuclear targeting oligopeptides and second generation-linkers that will allow facile cleavage of SPS assembled macromolecules from various insoluble supports.
5.6 General Procedure and Experimental

Tentagel S-NH₂, polystyrene A-NH₂ and (aminomethyl) polystyrene resins were procured from Fluka. ArgoPore-NH₂ resin was obtained from Argonaut Technologies Inc (www.biotage.com) while aminopropylated-CPG was purchased from Proligo Reagents. Cyanuric fluoride was from Alfa and was used without further purification. Anhydrous solvents and other reagents were purchased from Aldrich and used without purification. Analtech silica gel plates (60 F₂₅₄) were used for analytical TLC, and the spots were examined with UV light. Column chromatography was carried out on 200-400 mesh silica gel. Triflyl azide was prepared as described by Titz et al, and Liu and Tor.²⁶h-i Acid fluorides were made according to Oliver and Oyelere³¹ while 5’-O-Dimethoxytritylthymidine 68 was prepared following the procedure of Meier et al.³⁶ Oligonucleotides HPLC purification was performed using Phenomenex RP C-18 column, eluting with a gradient of 6-28 % acetonitrile in 0.1 M triethylammonium acetate (pH = 7.1).

5’-O-(4,4’-Dimethoxytrityl)-3’-O-pent-4-ynoate)thymidine (70a). 5’-O-Dimethoxytritylthymidine 68 (0.750 g, 1.38 mmol) was added to an oven-dried round bottom flask (10 mL) under argon and coevaporated with anhydrous pyridine (2 × 5 mL). Dry dichloromethane (4 mL), dry pyridine (0.4 mL) and dry triethylamine (0.5 mL) were added under argon. A solution of pent-4-ynoyl fluoride 69a (0.200 g, 2.00 mmol) in dry dichloromethane (1 mL) was added to the solution and the mixture stirred at room temperature under argon for 24 h. The reaction was partitioned between dichloromethane (40 mL) and saturated sodium bicarbonate (30 mL). The two layers were separated and
the organic layer was washed with saturated sodium bicarbonate (30 mL), saturated brine (30 mL) and dried over Na₂SO₄. Solvent was concentrated and the crude material purified by flash chromatography (silica gel, step gradient 8:1; 6:1 CH₂Cl₂/acetone) to give 0.560 g (64%) of 70a. ¹H NMR (CDCl₃, 400 MHz) δ 1.35 (3H, s), 2.01 (1H, t, J = 2.4 Hz), 2.51 (6H, m), 3.46 (2H, m), 3.77 (6H, s), 4.13 (1H, br s), 5.47 (1H, d, J = 5.6 Hz), 6.44 (1H, m), 6.82 (4H, d, J = 8.8 Hz), 7.22-7.38 (9H, m), 7.59 (1H, s), 9.10 (1H, s); ¹³C NMR (CDCl₃, 100 MHz). δ 11.6,14.3, 33.1, 37.9, 55.2, 63.6, 69.6, 75.8, 81.9, 83.9, 84.2, 87.1, 111.7, 113.2, 127.2, 128.0, 128.1, 130.0, 130.3, 135.0, 135.1, 135.3, 144.1, 150.6, 158.7, 158.7, 163.8, 171.3. HRMS (FAB, thioglycerol) calcd for [C₃₆H₃₆N₂O₈ + H]⁺ 625.2550, found 625.2544.

5'-O-(4,4'-Dimethoxytrityl)-3'-O-hex-5-ynoate)thymidine (70b). Reaction of 5'-O-Dimethoxytritylthymidine 68 (0.750 g, 1.38 mmol) and hex-5-ynoyl fluoride 69b (0.229 g, 2.00 mmol) for 24 h as described for compound 70a followed by flash chromatography (silica gel, step gradient 8:1; 6:1 CH₂Cl₂/acetone) gave 0.571 g (65%) of chromatographically pure 70b. ¹H NMR (CDCl₃, 400 MHz) δ 1.35 (3H, s), 1.82 (2H, m), 1.97 (1H, t, J = 2.6 Hz), 2.25 (2H, m), 2.46 (4H, m), 3.45 (2H, m), 3.77 (6H, s), 4.11 (1H, br. s), 5.44 (1H, d, J = 4.8 Hz), 6.43 (1H, app. t), 6.82 (4H, d, J = 8.8 Hz), 7.22-7.38 (9H, m), 7.59 (1H, s), 9.20 (1H, s); ¹³C NMR (CDCl₃, 100 MHz). δ 11.6,17.7, 23.2, 32.7, 37.9, 55.2, 63.6, 69.5, 75.3, 82.9, 83.9, 84.2, 87.1, 111.7, 113.2, 127.1, 128.0, 128.0, 130.0, 130.0, 135.0, 135.1, 135.3, 144.1, 150.6, 158.7, 158.7, 163.8, 172.5. HRMS (FAB, thioglycerol) calcd for [C₃₇H₃₈N₂O₈ + H]⁺ 638.2628, found 638.2606.
5'-O-(4,4'-Dimethoxytrityl)-6-N-(benzoyl)-2'-deoxy-3'-O-hex-5-ynoate)adenosine (72a). Reaction of protected nucleoside 67 (0.200 g, 0.30 mmol), hex-5-ynoyl fluoride 69b (0.114 g, 1.00 mmol) and catalytic amount of DMAP in dichloromethane (4 mL), dry pyridine (0.4 mL) and dry triethylamine (0.5 mL) for 2 h followed by flash chromatography (silica gel, step gradient 12:1; 10:1 CH$_2$Cl$_2$/acetone) gave 0.184 g (82%) of chromatographically pure 72a. $^1$H NMR (CDCl$_3$, 400 MHz) $\delta$ 1.82 (2H, m), 1.99 (1H, t, $J$ = 2.6 Hz), 2.25 (2H, m), 2.50 (2H, app. t), 2.66 (1H, m), 2.98 (1H, m), 3.43 (2H, d, $J$ = 4.0 Hz), 3.74 (6H, s), 4.28 (1H, m), 5.53 (1H, d, $J$ = 6.0 Hz), 6.50 (1H, m), 6.77 (4H, d, $J$ = 8.8 Hz), 7.17-7.29 (9H, m), 7.36 (2H, d, $J$ = 7.2 Hz), 7.47 (2H, app. t), 7.56 (1H, app. t), 8.00 (2H, d, $J$ = 8.4 Hz), 8.17 (1H, s), 8.69 (1H, s), 9.14 (1H, br. s); $^{13}$C NMR (CDCl$_3$, 100 MHz). $\delta$ 17.7, 23.3, 32.7, 38.2, 55.2, 63.5, 69.5, 75.2, 82.9, 84.4, 84.5, 86.7, 113.1, 127.0, 127.8, 127.9, 128.0, 128.8, 129.9, 130.0, 132.7, 133.5, 135.4, 144.1, 144.3, 149.3, 151.6, 152.6, 158.5, 164.7, 172.3. HRMS (FAB, thioglycerol) calcd for [C$_{44}$H$_{41}$N$_5$O$_7$ + H]$^+$ 752.3084, found 752.3110.

Representative Procedure for Heterogeneous Diazo-transfer Reaction. ArgoPore-N$_3$ Resin. ArgoPore-NH$_2$ (1.0 g, 0.90 mmol/g) was swelled in a homogeneous mixture of CH$_2$Cl$_2$ (5.6 mL), MeOH (5.2 mL), distilled H$_2$O (4.9 mL), and Et$_3$N (0.21 mL) for 2 hours. After 2 hours, the swelling solution was decanted. Triflyl azide (7.0 mmol) in CH$_2$Cl$_2$ (12 mL), and CuSO$_4$ (0.011 g, 0.04 mmol) in distilled H$_2$O (0.13 mL) were added to the swelled resin and gently stirred (or gently rocked) at 25$^\circ$C. Small portions of the resin were taken out at 10 min, 20 min, 40 min, 1 h, 2 h, 4 h, and 21 h for quantitative ninhydrin analysis. The resin was washed in succession with swelling solvent, conc.
NH₄OH (2 x 10 mL), swelling solvent; and dried in vacuo to give brownish solid. IR 2097 cm⁻¹. [Note: the reaction worked equally well when CH₂Cl₂ is replaced with toluene. Because of its better safety profile, we recommend toluene as a solvent of choice]

**Polystyrene A-N₃ Resin.** This resin was synthesized from polystyrene A-NH₂ as described for the synthesis of ArgoPore-N₃ from ArgoPore-NH₂ to give brown-greenish solid. IR 2090 cm⁻¹.

**(Aminomethyl) Polystyrene-N₃ Resin.** This resin was synthesized from (aminomethyl) polystyrene as described for the synthesis of ArgoPore-N₃ from ArgoPore-NH₂ to give light yellow solid. IR 2092 cm⁻¹.

**Tentagel-N₃ Resin.** This resin was synthesized from Tentagel S-NH₂ as described for the synthesis of ArgoPore-N₃ from ArgoPore-NH₂ to give greenish solid. IR 2106 cm⁻¹.

**CPG-N₃ Resin.** This resin was synthesized from CPG-NH₂ as described for the synthesis of ArgoPore-N₃ from ArgoPore-NH₂ except that the reaction was stirred at 37°C for 24 h to give white-blue solid. IR 2359, 2341 cm⁻¹.

**Representative protocol for nucleoside loading.** In a typical reaction, a mixture of 66a (0.019g, 0.03 mmol), ArgoPore-N₃ resin (0.065g, approx 0.06 mmol), CuI (0.003g, 0.02 mmol) and TBTA (0.009g, 0.02 mmol) in THF (1.2 mL) and Hunig’s base (12 μL) was
shaken at rt for 2h. The resin was filtered off, washed with THF, CH₂Cl₂ and dried in vacuo. Quantitative DMT group analysis gave a nucleoside loading of approximately 0.5 mmol/g.

5.7 References


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APPENDIX A

$^1$H and $^{13}$C NMR characterization for both SAHA-like Triazole-linked HDACi, Nonpeptide Macrocyclic HDACi, NLS-HDACi, Fluorescent Tagged NLS and HDACi and their intermediates
$^1$H NMR of 3a:
$^{13}$C NMR of 3a:
$^1$H NMR of 3b:
$^1$H NMR of 3c:
$^{13}$C NMR of 3c:
H NMR of 6a:
$^{13}$C NMR of 6a:
$^1$H NMR of 6m:
$^{13}$C NMR of 6m:
$^1$H NMR of 6n:
$^{13}$C NMR of 6n:
H NMR of 60:
$^{13}$C NMR of 6o:
$^1$H NMR of $6p$: 

[Diagram of NMR spectrum]

[Structural formula of $6p$]
$^{13}$C NMR of 6p:
$^{13}$C NMR of 6r:
H NMR of 6u:
$^{13}$C NMR of 6u:
$^1$H NMR of 4a:
$^{13}$C NMR of 4a:
$^1$H NMR of 4b:
$^{13}$C NMR of 4b:
\(^1\)H NMR of 4c:
$^{13}$C NMR of 4c:
$^1$H NMR of 7a:
$^{13}$C NMR of 7a:
$^1$H NMR of 7m:
C NMR of 7m:

\[ \text{HO} - \text{N} - \text{O} - \text{N} = \text{N} - \text{O} \]
$^1$H NMR of 7n:
$^{13}$C NMR of 7n:
$^1$H NMR of 7o:
H NMR of 7p:
$^1$H NMR of 7r:
$^{13}$C NMR of 7r:
$^1$H NMR of 7u:
$^{13}$C NMR of 7u:
$^1$H NMR of 10:
$^{13}$C NMR of 10:
$^1$H NMR of 14:
$^{13}$C NMR of 14:
$^1$H NMR of 15:
$^{13}$C NMR of 15:
$^1$H NMR of 17:
$^1$H NMR of 19:
$^{13}$C NMR of 19:
$^1$H NMR of 20:
$^{13}$C NMR of 20:
$^1$H NMR of 22:
$^{13}$C NMR of 22:
$^1$H NMR of 23a:
$^{13}$C NMR of 23a:
\( ^1H \) NMR of 23b:
$^{13}$C NMR of 23b:
$^1$H NMR of 23c:
$^{13}$C NMR of 23c:
$^1$H NMR of 23d: (in CDCl$_3$)
$^1$H NMR of 23d: (in acetone-d$_6$)
$^{13}$C NMR of 23d:
$^1$H NMR of 23e:
\(^{13}\)C NMR of 23e:
$^1$H NMR of 23f:
$^{13}$C NMR of 23f:
$^1$H NMR of 23g:
$^{13}\text{C NMR of 23g:}$
$^1$H NMR of 23h:
$^{13}\text{C NMR of } 23\text{h:}$
$^1$H NMR of 24a:
$^{13}$C NMR of 24a:
$^1$H NMR of 24b:
$^{13}$C NMR of 24b:
$^{1}$H NMR of 24c:
$^{13}$C NMR of 24c:
$^1$H NMR of 2,44.
C NMR of 24d
$^1\text{H NMR of 24e}$
$^{13}$C NMR of 24e:
$^1$H NMR of 26a:
$^{13}$C NMR of 26a:
$^1$H NMR of 26b:
$^{13}$C NMR of 26b:
H NMR of 28:
$^{13}$C NMR of 28:
$^1$H NMR of 29:
$^{13}$C NMR of 29:
$^1$H NMR of 30:
$^{13}$C NMR of 30:
H NMR of 31a:
$^{13}$C NMR of 31a:
$^1$H NMR of 31b: (in CDCl$_3$)
$^1$H NMR of 31b: (in acetone-d$_6$)
$^{13}$C NMR of 31b:
$^1$H NMR of 31c:
$^{13}$C NMR of 31c:
$^1$H NMR of 31d:
$^{13}$C NMR of 31d:
$^1$H NMR of 31e:
$^{13}$C NMR of 31e:
$^1$H NMR of 31f:
$^{13}$C NMR of 31f:
$^1$H NMR of 31g:
$^{13}$C NMR of 31g:
$^1$H NMR of 31h:
$^{13}$C NMR of 31h:
$^1$H NMR of 33a:
$^{13}$C NMR of 33a:
$^1$H NMR of 34a:
$^{13}$C NMR of 34a:
^1H NMR of 34b:
$^{13}$C NMR of 34b:
\( ^1H \) NMR of 35a:
$^{13}$C NMR of 35a:
$^1$H NMR of 35b:
\(^{13}\text{C}\) NMR of 35b:
$^1$H NMR of 35c:
$^{13}$C NMR of 35c:
$^1$H NMR of 37a:
$^{13}$C NMR of 37a:
$^1$H NMR of 37b:
$^{13}$C NMR of 37b:
$^1$H NMR of 37c:
$^{13}\text{C NMR}$ of 37c:
$^1$H NMR of 38a:
$^{13}$C NMR of 38a:
$^1$H NMR of 38b:
$^{13}$C NMR of 38b:
$^1$H NMR of 38c:
$^{13}$C NMR of 38c:
$^1$H NMR of 39a:
$^1$H NMR of 39b:
$^1$H NMR of 39c:
$^1$H NMR of 40a:
$^1$H NMR of 40b:
$^1$H NMR of 40c:
$^{1}H$ NMR of 42:
$^{13}$C NMR of 42:
$^1$H NMR of 43:
$^{13}$C NMR of 43:
$^1$H NMR of 44:
$^{13}$C NMR of 44:
$^1$H NMR of $^{45}$:
$^{13}$C NMR of 45:
$^1$H NMR of 46:
$^1$H NMR of 47:
$^1$H NMR of 48:
$^{13}$C NMR of 48:
$^1$H NMR of 49:
APPENDIX B

$^1$H NMR characterization for thiolalkyl-triazole-linked NLS peptide and its intermediates
$^1$H NMR of 50:
$^1$H NMR of 51:
$^1$H NMR of 52:
$^1$H NMR of 53:
APPENDIX C

$^1$H and $^{13}$C NMR characterization for 6-triazolylpenicillanic acids and their intermediates
$^1$H NMR of 58a:
H NMR of 58b:
$^1$H NMR of 58c:
$^1$H NMR of 60a:
$^{13}$C NMR of 60a:
H NMR of 60b:
$^{13}$C NMR of 60b:
$^1$H NMR of 60c:
$^{13}$C NMR of 60c:
$^1$H NMR of 62:
$^{13}$C NMR of 62:
$^1$H NMR of 65f:
$^1$H NMR of 65h:
$^1$H NMR of 65i:
$^1$H NMR of 65k:
$^1$H NMR of 65I:
$^1$H NMR of 63a:
$^{13}$C NMR of 63a:
$^1$H NMR of 63b:
$^{13}$C NMR of 63b:
$^1$H NMR of 63c:
$^{13}$C NMR of 63c:
$^1$H NMR of 64a:
$^{13}$C NMR of 64a:
$^1$H NMR of 64b:
$^{13}$C NMR of 64b:
H NMR of 66a:
$^{13}$C NMR of 66a:
$^1$H NMR of 66b:
$^{13}$C NMR of 66b:
$^1$H NMR of 66c:
$^{13}$C NMR of 66c:
$^1$H NMR of 66d:
$^{13}$C NMR of 66d:
H NMR of 66e:
$^{13}$C NMR of 66e:
$^1$H NMR of 66f:
$^{13}$C NMR of 66f:
\textsuperscript{1}H NMR of 66g:
$^{1}$H NMR of 66h:
\[^{13}\text{C}\] NMR of 66h:
$^1$H NMR of 66i:
$^{13}$C NMR of 66i:
$^1$H NMR of 66j:
$^{13}$C NMR of $66j$: 

[Graph of $^{13}$C NMR spectrum for $66j$]
$^1$H NMR of 66k:
$^{13}$C NMR of 66k:
H NMR of 66l:
$^{13}$C NMR of 66l:
$^1$H NMR of 67a:
$^{13}$C NMR of 67a:
$^1$H NMR of 67b:
$^{13}$C NMR of 67b:
H NMR of 67c:
$^{13}$C NMR of 67c:
$^1$H NMR of 67d:
$^{13}$C NMR of 67d:
$^1$H NMR of 67e:
$^{13}$C NMR of 67e:
$^1$H NMR of 67f:
$^1$H NMR of 67g:
$^1$H NMR of 67h:
$^{13}$C NMR of 67h:
$^1$H NMR of 67i:
$^1$H NMR of 67j:
$^{13}$C NMR of 67j:
$^1$H NMR of 67k:
$^1$H NMR of 67I:
Appendix D

Additional Spectra for Chapter 5
Heterogeneous Diazo-Transfer Reaction With Cu(II)

Abs

nm.

500.00
600.00
700.00

1 = Unmodified AgarPore-NH2 Resin (Dilution Factor of 4)
2 = 20 mins
3 = 1 hr
4 = 21 hrs
5 = 4 hrs
6 = 21 hrs (2x Triflic Azide)
Figure D1. (a) and (b) are UV absorbance spectra, after quantitative ninhydrin assay, in the presence and absence of CuSO$_4$ respectively, of 5mg portion of ArgoPore-NH$_2$ resin at specified time. Note that the dilution factors of unmodified resin and reactions without Cu (II) at 20min are six times that of the reaction with Cu (II) in order to bring the absorbance value to the linear range of the Beer-Lambert law.
Figure D2. MALDI-TOF MS analysis and HLPC profile of purified 15mer (AGC CAG ATT TGA GCT).
Figure D3. MALDI-TOF MS analysis and HLPC profile of purified 30mer (AGC CAG ATT TGA GCT TGG GGC TCT CTG GCT).
$^1$H NMR of 70a:
$^{13}$C NMR of 70a:
H NMR of 70b:

[Image of NMR spectrum and chemical structure]
$^{13}$C NMR of 70b:
H NMR of 72a:
$^{13}C$ NMR of 72a:
**ArgoPore-NH$_2$ Resin:**

![IR Spectrum of ArgoPore-NH$_2$ Resin]

**ArgoPore-N$_3$ Resin:**

![IR Spectrum of ArgoPore-N$_3$ Resin]

**Figure D4.** IR spectra of ArgoPore-NH$_2$ and ArgoPore-N$_3$ resins.
**Figure D5.** IR spectra of CPG-NH$_2$ resin and CPG-N$_3$ resins.
(Aminomethyl) Polystyrene-NH₂ Resin:

Figure D6. IR spectra of (Aminomethyl) Polystyrene-NH₂ and (Aminomethyl) Polystyrene-N₃ resins.
Figure D7. IR spectra of Polystyrene A-NH$_2$ and Polystyrene A-N$_3$ resins.
Figure D8. IR spectra of Tentagel-NH$_2$ and Tentagel-N$_3$ resins.
ArgoPore-N$_3$ Resin prepared with triflyl-azide dissolved in toluene:

Figure D9. IR spectra of ArgoPore-N$_3$ resins prepared in toluene solution of triflyl azide.
Po Chen was born in Taipei, Taiwan on October 10, 1980 to Frank and Ann Chen. He has a younger brother named Wei. Po obtained his nickname, Bob, from his English teacher in Taiwan. Bob spent his first eleven years in Taipei, Taiwan. Bob came over to the United State when he was twelve years old. He grew up in Florence, South Carolina and graduated from South Florence High School in May 1999. In May of 2003, Bob graduated with B. S. in Chemistry from Vanderbilt University, where he also worked as an undergraduate research for Dr. Piotr Kaszynski. After graduation, he attended graduate school at Georgia Institute of Technology. Throughout his graduate career, he published six papers in respected chemistry journals such as Journal of Medicinal Chemistry, Journal of Organic Chemistry, Bioorganic & Medicinal Chemistry, and Bioconjugate Chemistry. In 2006 and 2008, he was a recipient of the GAANN predoctoral fellowship from Georgia Tech Center for Drug Design, Development, and Delivery. He received his Ph.D. in organic chemistry in May 2009 from Georgia Institute of Technology under the guidance of Dr. Adegboyega (Yomi) Oyelere.