SYNTHESIS OF FLUOROGENIC PROBES FOR STUDYING BIOMASS DEGRADATION AND SYNTHESIS OF NEW ANTIFUNGAL AMINOGLYCOSIDES

by

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This dissertation is composed of two research projects. The first research project is aimed at using synthetic fluorogenic probes to study the possible or dominant linkages in biomass. These probes that mimic the linkages found in lignin-cellulosic biomass are designed to select the optimal fungi from direct evaluation process or could be tested against other microbials to screen candidates which can break ligno-hemicellulose bonds. For the first stage, these probes would be tested against white rot fungi extract. The white rot fungi are used for the first stage to see if releasing or degrading carbohydrates while keeping lignin largely intact is possible or not.

These probes can help to answer fundamental questions, such as what could be the dominant linkages between lignin and hemicellulose, and what are the possible mechanisms for the cleavage of carbohydrates in biomasses. Understanding the linkages in these biomass will enable high efficient
degradation or release of carbohydrates, primarily hemicelluloses, from biomass.

The second project is focused on synthesizing new aminoglycoside analogs and exploring the potential to revive traditional antibacterial kanamycin as new types of antifungal agents. Aminoglycosides are widely used broad spectrum antibiotics. Although mainly used as antibacterial agents, there have been studies to show amphiphilic aminoglycoside derivatives could be possibly employed as antifungal agents.

A concise and novel method for site-selective alkylation of tetra-azidokanamycin has been developed that leads to the divergent synthesis of three classes of kanamycin derivatives. These new amphiphilic kanamycin derivatives bearing alkyl chains length of 4, 6, 7, 8, 9, 10, 12, 14, 16 have been synthesized and tested against bacteria and fungi. Surprisingly, the antibacterial effect of the synthesized kanamycin derivatives decline or disappear compared with the original kanamycin A, but some of the compounds show very strong activity as antifungal agents.

(312 pages)
PUBLIC ABSTRACT

Synthesis of Fluorogenic Probes for Studying Biomass Degradation and Synthesis of New Antifungal Aminoglycosides

by

Qian Zhang, Doctor of Philosophy
Utah State University, 2015

For the first project, a library of fifteen commercially purchased and synthetic fluorogenic probes were employed for the investigation of biomass degradation using extract of white-rot fungi. These discoveries prove that it is possible to employ fungi for selective degradation or release of hemicelluloses from biomass. This work is supported by Sun Grant Western Regional Center/DOT “Bioprospecting for Enzymes to Break Lignin-Hemicellulose Bond” ($300,000,09/01/2011-08/31/2013)

The second project is focused on synthesizing new aminoglycoside analogs and exploring the potential to revive traditional antibacterial kanamycin as new type of antifungal agents. Although aminoglycosides antibiotics are mainly used as antibacterial agents, this project prove that it is possible to convert kanamycin to amphiphilic antifungal agents through simple chemical modification.
DEDICATION

I would like to dedicate this work to my loving parents, my father Genyuan Zhang and my mother Tuqing Tang.
ACKNOWLEDGMENTS

First of all, I would like to express my sincere gratitude to my supervisor, Dr. Cheng-Wei Tom Chang, for all that I have learned from him throughout my academic program at Utah State University. He helped me greatly to develop my skills in the research of chemical reactions, to improve my understanding of the field of synthetic organic chemistry and medicinal chemistry, and to gain a deep appreciation for the perseverance required to perform such research. The joy and enthusiasm he has for his research was contagious and motivational for me during the entire time of my Ph.D. pursuit.

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A special thanks to Dr. Jon Y. Takemoto, I have learned a lot biological techniques from him and conducted many research in his laboratory. I will never forget that he helped unlock the door at 11:00 pm to let me into his lab for collecting data for my research. He is a nice, smart and really easy-going professor.
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I have to thank all my friends here in Logan. Research plays a big part of PhD life, but not all of it. We have shared many great moments including go hiking and fishing together. I express my deep and sincere appreciation to my wife, Yexiang Wu, for her love and support during all the years we have gone through together. I also thank my parents and my parents-in-law for their support and encouragement.

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Qian Zhang, 2015
CONTENTS

ABSTRACT ........................................................................................................ iii
PUBLIC ABSTRACT ..................................................................................... V
DEDICATION ................................................................................................. VI
ACKNOWLEDGMENTS ................................................................................ VII
LIST OF TABLES ........................................................................................... XI
LIST OF FIGURES ........................................................................................ XII
LIST OF SCHEMES ........................................................................................ XIII
LIST OF ABBREVIATIONS ......................................................................... XIV
LIST OF SPECTRA ..................................................................................... XVI

CHAPTER

1. GENERAL INTRODUCTION ..................................................................... 1
1-1. Overview of Project 1: Biomass, a Promising Source of Renewable Energy ........................................................................................................ 1
  1-1.1. Rationale for the Planned Work ..................................................... 1
  1-1.2. Aims and Objectives ..................................................................... 7
1-2. Overview of Project 2: Traditional Aminoglycosides and their New Application .............................................................................................................................. 8
  1-2.1. Introduction to Aminoglycosides .................................................... 8
  1-2.2. Investigation of Antibacterial to Antifungal Conversion of Kanamycin ......................................................................................................................... 10
  1-2.3. Research Summary for Previous Work......................................... 16
1-3. REFERENCES .................................................................................... 17
2. USING FLUOROGENIC PROBES FOR INVESTIGATION OF BIOMASS DEGRADATION BY FUNGI ................................................................. 23
  2-1. Abstract .................................................................................................. 23
  2-2. Introduction .............................................................................................. 24
  2-3. Design andSynthesis of Fluorogenic Probes which Mimic Lignin-Hemicellulose Ether Bonds ............................................................ 27
  2-4. Results and Discussion ........................................................................... 34
  2-5. Experimental Section ............................................................................ 41
  2-6. References ................................................................................................ 53

3. SYNTHESIS OF ANTIFUNGAL KANAMYCIN A ANALOGS .................. 57
  3-1. Abstract .................................................................................................... 57
  3-2. Introduction ............................................................................................... 58
  3-3. Synthesis of Kanamycin Derivatives for Converting Traditional
       Antibacterial Agents to New Antifungal Agents ..................................... 61
  3-4. Conclusions and Significance ................................................................ 79
  3-5. Experimental Section ............................................................................. 80
  3-6. References ................................................................................................ 99

APPENDICES .................................................................................................. 101
  Appendix A ...................................................................................................... 102
  Appendix B ...................................................................................................... 262
  CURRICULUM VITAE .................................................................................. 277
<table>
<thead>
<tr>
<th>Table</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-1.</td>
<td>MIC Values of <strong>FG01, FG02, and FG08</strong> (Against <em>F. graminearum</em> Strains)</td>
</tr>
<tr>
<td>1-2.</td>
<td>MIC Values of <strong>FG (03-18)</strong> Compounds</td>
</tr>
<tr>
<td>3-1.</td>
<td>Example of Naming System for Octylated Kanamycin</td>
</tr>
<tr>
<td>3-2.</td>
<td>MIC Comparison between K4608, K608, K408 and FG17, FG06, FG03</td>
</tr>
<tr>
<td>3-3.</td>
<td>Alkylation of Tetraazidokanamycin with Various Chain Length</td>
</tr>
<tr>
<td>3-4.</td>
<td>Yield of Mono-armed and Di-armed Amphiphilic Aminoglycosides after Staudinger Reaction</td>
</tr>
<tr>
<td>3-5.</td>
<td>MIC of Kanamycin A Analogs</td>
</tr>
</tbody>
</table>
# LIST OF FIGURES

<table>
<thead>
<tr>
<th>Figure</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-1.</td>
<td>6</td>
</tr>
<tr>
<td>1-2.</td>
<td>8</td>
</tr>
<tr>
<td>1-3.</td>
<td>9</td>
</tr>
<tr>
<td>1-4.</td>
<td>11</td>
</tr>
<tr>
<td>1-5.</td>
<td>12</td>
</tr>
<tr>
<td>2-1.</td>
<td>26</td>
</tr>
<tr>
<td>2-2.</td>
<td>28</td>
</tr>
<tr>
<td>2-3.</td>
<td>29</td>
</tr>
<tr>
<td>2-4.</td>
<td>35</td>
</tr>
<tr>
<td>2-5.</td>
<td>35</td>
</tr>
<tr>
<td>2-6.</td>
<td>36</td>
</tr>
<tr>
<td>2-7.</td>
<td>36</td>
</tr>
<tr>
<td>2-8.</td>
<td>37</td>
</tr>
<tr>
<td>2-9.</td>
<td>39</td>
</tr>
<tr>
<td>3-1.</td>
<td>70</td>
</tr>
</tbody>
</table>

1-1. Representative Structure of Cellulose, Hemicellulose and Lignin
1-2. Streptomycin
1-3. Structure of Kanamycin Class
1-4. Structure of FG08 and K20
1-5. Structures of Amphiphilic Kanamycin B Derivatives in the SAR Investigation
2-1. Possible Linkages between Hemicelluloses and Lignin
2-2. Types of Fluorescent Chromophores
2-3. Structures of Employed Probes
2-4. RFI Values of Class I Probes
2-5. RFI Values of Class Ila Probes
2-6. RFI Values of Class Iib Probes
2-7. RFI Values of Class III Probes
2-8. Possible Degradation of Probes which will not Generate Fluorescence
2-9. Incubation of 4MU with Aromatic Compounds
3-1. Structure Comparison between K4608, K608, K408 and FG17, FG06, FG03
LIST OF SCHEMES

Scheme

2-1. Synthesis of Probe 14, Probe 16 and Probe 17...............................30
2-2. Synthesis of Probe 1 and Probe 3..............................................31
2-3. Synthesis of Probe 4 and Probe 5..............................................32
2-4. Synthesis of Probe 10 and Probe 11............................................33
3-1. Synthesis of FG08........................................................................60
3-2. Synthesis of Glycosyl Donors for FG14-18.................................62
3-3. Synthesis of FG14-18.................................................................64
3-4. Synthesis of 1,3,6′,3″-Tetraazidokanamycin...............................65
3-5. Octylation of Tetraazidokanamycin.............................................66
3-6. Acetylation of Octylated Tetraazidokanamycin.............................68
LIST OF ABBREVIATIONS

Ac: acetyl
APCI: Atmospheric pressure chemical ionization
Bn: benzyl
Boc: tert-butyloxycarbonyl
Bz: benzoyle
COSY: correlation spectroscopy
DMAP: 4-(dimethylamino)pyridine
DMF: dimethylformamide
DMSO: dimethyl sulfoxide
ESI: electrospray ionization
Et₂O: ethyl ether
FAB: fast atom bombardment
HOAc: acetic acid
MALDI: Matrix-assisted laser desorption/ionization
NIS: N-iodosuccinimide
Py: pyridine
PMe₃: trimethyl phosphine
r.t.: room temperature
TBAI: tetrabutylammonium iodide
TEA: triethylamine
Tf₂O: trifluoromethanesulfonyl acid anhydride
TFA: trifluoroacetic acid
THF: tetrahydrofuran
TMSOTf: trimethylsilyl trifluoromethanesulfonate
Trityl: triphenylmethyl
Ts: tosyl
TsOH: \( p \)-toluenesulfonic acid
TsCl: toluenesulfonyl chloride
<table>
<thead>
<tr>
<th>Spectrum</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spectrum 1</td>
<td>$^1$H NMR of Methyl-6-O-(6-Methoxy-2-aphthenylmethyl)-mannopyronoside (probe 1)</td>
<td>103</td>
</tr>
<tr>
<td>Spectrum 2</td>
<td>$^{13}$C NMR of Methyl-6-O-(6-Methoxy-2-aphthenylmethyl)-mannopyronoside (probe 1)</td>
<td>104</td>
</tr>
<tr>
<td>Spectrum 3</td>
<td>$^1$H NMR of Methyl-6-(4-methyl-umbelliferyl)-O-$\alpha$-D-mannopyranoside (probe 3)</td>
<td>105</td>
</tr>
<tr>
<td>Spectrum 4</td>
<td>$^{13}$C NMR of Methyl-6-(4-methyl-umbelliferyl)-O-$\alpha$-D-mannopyranoside (probe 3)</td>
<td>106</td>
</tr>
<tr>
<td>Spectrum 5</td>
<td>$^1$H NMR of 6-(4-Methyl-umbelliferyl)-O-D-xylofuranose (probe 4)</td>
<td>107</td>
</tr>
<tr>
<td>Spectrum 6</td>
<td>$^{13}$C NMR of 6-(4-Methyl-umbelliferyl)-O-D-xylofuranose (probe 4)</td>
<td>108</td>
</tr>
<tr>
<td>Spectrum 7</td>
<td>$^1$H NMR of 3-(6-Methoxy-2-naphthalenylmethyl)-O-D-xylofumose (probe 5)</td>
<td>109</td>
</tr>
<tr>
<td>Spectrum 8</td>
<td>$^{13}$C NMR of 3-(6-Methoxy-2-naphthalenylmethyl)-O-D-xylofumose (probe 5)</td>
<td>110</td>
</tr>
<tr>
<td>Spectrum 9</td>
<td>$^1$H NMR of 2-((1-(3,4-dimethoxyphenyl)ethoxy)methyl)-6-methoxynaphthalene (probe 10)</td>
<td>111</td>
</tr>
</tbody>
</table>
Spectrum 10  $^{13}$C NMR of 2-((1-(3,4-dimethoxyphenyl)ethoxy)methyl)-6-methoxynaphthalene (probe 10) ........................................ 112

Spectrum 11  $^1$H NMR of 1-(3,4-dimethoxyphenyl)-2-(4-methyl-umbelliferoxy)-ethanone (probe 11) ............................... 113

Spectrum 12  $^{13}$C NMR of 1-(3,4-dimethoxyphenyl)-2-(4-methyl-umbelliferoxy)-ethanone (probe 11) ............................... 114

Spectrum 13  $^1$H NMR of (6-Methoxy-2-naphthalenylmethyl)-β-D-glucopyranoside (probe 14) ................................................ 115

Spectrum 14  $^{13}$C NMR of (6-Methoxy-2-naphthalenylmethyl)-β-D-glucopyranoside (probe 14) ................................................ 116

Spectrum 15  $^1$H NMR of (6-Methoxy-2-naphthalenylmethyl)-β-D-xylopyranoside (probe 16) ................................................ 117

Spectrum 16  $^{13}$C NMR of (6-Methoxy-2-naphthalenylmethyl)-β-D-xylopyranoside (probe 16) ................................................ 118

Spectrum 17  $^1$H NMR of (6-Methoxy-2-naphthalenylmethyl)-β-D-cellubioside (probe 17) ................................................ 119

Spectrum 18  $^{13}$C NMR of (6-Methoxy-2-naphthalenylmethyl)-β-D-cellubioside (probe 17) ................................................ 120

Spectrum 19  $^1$H NMR of (6-Methoxy-2-naphthalenylmethyl)-2,3,4,6-tetra-O-acetyl-β-D-glucopyranoside (8) ......................... 121

Spectrum 20  $^{13}$C NMR of (6-Methoxy-2-naphthalenylmethyl)-2,3,4,6-tetra-O-acetyl-β-D-glucopyranoside (8) ......................... 122
| Spectrum 21 | $^1$H NMR of (6-Methoxy-2-naphthalenylmethyl)-2,3,4,-tri-O-acetyl-D-xylopyranoside (10) ........................................... 123 |
| Spectrum 22 | $^{13}$C NMR of (6-Methoxy-2-naphthalenylmethyl)-2,3,4,-tri-O-acetyl-D-xylopyranoside (10) ........................................... 124 |
| Spectrum 23 | $^1$H NMR of Methyl-2,3,4-tri-benzoyl-O-6-(6-Methoxy-2-naphthalenylmethyl)-α-D-mannopyranoside (13) ..................... 125 |
| Spectrum 24 | $^{13}$C NMR of Methyl-2,3,4-tri-benzoyl-O-6-(6-Methoxy-2-naphthalenylmethyl)-α-D-mannopyranoside (13) ..................... 126 |
| Spectrum 25 | $^1$H NMR of 1,2-O-isopropylidene-6-(4-methyl-umbelliferyl)-O-α-D-xylofuranose (16) .................................................. 127 |
| Spectrum 26 | $^{13}$C NMR of 1,2-O-isopropylidene-6-(4-methyl-umbelliferyl)-O-α-D-xylofuranose (16) .................................................. 128 |
| Spectrum 27 | $^1$H NMR of 4″,6″-Di-O-butyl-1,3,6′,3″-tetraazidokanamycin (K4604N3) ................................................................. 129 |
| Spectrum 28 | $^{13}$C NMR of 4″,6″-Di-O-butyl-1,3,6′,3″-tetraazidokanamycin (K4604N3) ................................................................. 130 |
| Spectrum 29 | $^1$H NMR of 4″-O-Butyl-1,3,6′,3″-tetraazidokanamycin (K404N3) .......................................................... 131 |
| Spectrum 30 | $^{13}$C NMR of 4″-O-Butyl-1,3,6′,3″-tetraazidokanamycin (K404N3) .......................................................... 132 |
| Spectrum 31 | $^1$H NMR of 4″,6″-Di-O-hexyl-1,3,6′,3″-tetraazidokanamycin (K4606N3) .......................................................... 133 |
Spectrum 32  $^{13}$C NMR of 4",6"-Di-O-hexyl-1,3,6',3"-tetraazidokanamycin (K4606N3) ........................................... 134

Spectrum 33  $^1$H NMR of 4"-O-Hexyl-1,3,6',3"-tetraazidokanamycin (K406N3) ............................................................... 135

Spectrum 34  $^{13}$C NMR of 4"-O-Hexyl-1,3,6',3"-tetraazidokanamycin (K406N3) ............................................................... 136

Spectrum 35  $^1$H NMR of 4",6"-Di-O-heptyl-1,3,6',3"-tetraazidokanamycin (K4607N3) ........................................................ 137

Spectrum 36  $^{13}$C NMR of 4",6"-Di-O-heptyl-1,3,6',3"-tetraazidokanamycin (K4607N3) ........................................................ 138

Spectrum 37  $^1$H NMR of 4"-O-Heptyl-1,3,6',3"-tetraazidokanamycin (K407N3) ................................................................. 139

Spectrum 38  $^{13}$C NMR of 4"-O-Heptyl-1,3,6',3"-tetraazidokanamycin (K407N3) ................................................................. 140

Spectrum 39  $^1$H NMR of 4",6"-Di-O-octyl-1,3,6',3"-tetraazidokanamycin (K4608N3) ......................................................... 141

Spectrum 40  $^{13}$C NMR of 4",6"-Di-O-octyl-1,3,6',3"-tetraazidokanamycin (K4608N3) ......................................................... 142

Spectrum 41  $^1$H NMR of 6"-O-Octyl-1,3,6',3"-tetraazidokanamycin (K608N3) ................................................................. 143

Spectrum 42  $^{13}$C NMR of 6"-O-Octyl-1,3,6',3"-tetraazidokanamycin (K608N3) ................................................................. 144
Spectrum 43

$^1$H NMR of 4"-O-Octyl-1,3,6',3"-tetraazidokanamycin (K408N3) ......................................................... 145

Spectrum 44

$^{13}$C NMR of 4"-O-Octyl-1,3,6',3"-tetraazidokanamycin (K408N3) ......................................................... 146

Spectrum 45

$^1$H NMR of 4",6"-Di-O-nonyl-1,3,6',3"-tetraazidokanamycin (K4609N3) ..................................................... 147

Spectrum 46

$^{13}$C NMR of 4",6"-Di-O-nonyl-1,3,6',3"-tetraazidokanamycin (K4609N3) ..................................................... 148

Spectrum 47

$^1$H NMR of 6"-O-Nonyl-1,3,6',3"-tetraazidokanamycin (K609N3) ................................................................. 149

Spectrum 48

$^{13}$C NMR of 6"-O-Nonyl-1,3,6',3"-tetraazidokanamycin (K609N3) ................................................................. 150

Spectrum 49

$^1$H NMR of 4"-O-Nonyl-1,3,6',3"-tetraazidokanamycin (K409N3) ................................................................. 151

Spectrum 50

$^{13}$C NMR of 4"-O-Nonyl-1,3,6',3"-tetraazidokanamycin (K409N3) ................................................................. 152

Spectrum 51

$^1$H NMR of 4",6"-Di-O-decyl-1,3,6',3"-tetraazidokanamycin (K4610N3) ......................................................... 153

Spectrum 52

$^{13}$C NMR of 4",6"-Di-O-decyl-1,3,6',3"-tetraazidokanamycin (K4610N3) ......................................................... 154

Spectrum 53

$^1$H NMR of 6"-O-Decyl-1,3,6',3"-tetraazidokanamycin (K610N3) ................................................................. 155
Spectrum 54
$^{13}$C NMR of 6″-O-Decyl-1,3,6′,3″-tetraazidokanamycin (K610N3) ................................................................. 156
Spectrum 55
$^1$H NMR of 4″-O-Decyl-1,3,6′,3″-tetraazidokanamycin (K410N3) ................................................................. 157
Spectrum 56
$^{13}$C NMR of 4″-O-Decyl-1,3,6′,3″-tetraazidokanamycin (K410N3) ................................................................. 158
Spectrum 57
$^1$H NMR of 4″,6″-Di-O-dodecyl-1,3,6′,3″-tetraazidokanamycin (K4612N3) ......................................................... 159
Spectrum 58
$^{13}$C NMR of 4″,6″-Di-O-dodecyl-1,3,6′,3″-tetraazidokanamycin (K4612N3) ......................................................... 160
Spectrum 59
$^1$H NMR of 6″-O-Dodecyl-1,3,6′,3″-tetraazidokanamycin (K612N3) ................................................................. 161
Spectrum 60
$^{13}$C NMR of 6″-O-Dodecyl-1,3,6′,3″-tetraazidokanamycin (K612N3) ................................................................. 162
Spectrum 61
$^1$H NMR of 4″-O-Dodecyl-1,3,6′,3″-tetraazidokanamycin (K412N3) ................................................................. 163
Spectrum 62
$^{13}$C NMR of 4″-O-Dodecyl-1,3,6′,3″-tetraazidokanamycin (K412N3) ................................................................. 164
Spectrum 63
$^1$H NMR of 4″,6″-Di-O-tetradecyl-1,3,6′,3″-tetraazidokanamycin (K4614N3) ...................................................... 165
Spectrum 64
$^{13}$C NMR of 4″,6″-Di-O-tetradecyl-1,3,6′,3″-tetraazidokanamycin (K4614N3) ...................................................... 166
Spectrum 65  $^1$H NMR of 4"-O-Tetradecyl-1,3,6',3"-tetraazidokanamycin (K414N3) ................................................................. 167

Spectrum 66  $^{13}$C NMR of 4"-O-Tetradecyl-1,3,6',3"-tetraazidokanamycin (K414N3) ................................................................. 168

Spectrum 67  $^1$H NMR of 4",6"-Di-O-hexadecyl-1,3,6',3"-tetraazidokanamycin (K4616N3) ......................................................... 169

Spectrum 68  $^{13}$C NMR of 4",6"-Di-O-hexadecyl-1,3,6',3"-tetraazidokanamycin (K4616N3) ................................................................. 170

Spectrum 69  $^1$H NMR of 4"-O-Hexadecyl-1,3,6',3"-tetraazidokanamycin (K416N3) ................................................................. 171

Spectrum 70  $^{13}$C NMR of 4"-O-Hexadecyl-1,3,6',3"-tetraazidokanamycin (K416N3) ................................................................. 172

Spectrum 71  $^1$H NMR of 2',3',4',2"-Tetra-O-acetyl-4",6"-di-O-butyl-1,3,6',3"-tetraazidokanamycin (K4604Ac) ......................... 173

Spectrum 72  $^1$H-$^1$H COSY of 2',3',4',2"-Tetra-O-acetyl-4",6"-di-O-butyl-1,3,6',3"-tetraazidokanamycin (K4604Ac) ......................... 174

Spectrum 73  $^1$H NMR of 2',3',4',2",6"-Pent-O-acetyl-4"-O-butyl-1,3,6',3"-tetraazidokanamycin (K404Ac) ..................................... 175

Spectrum 74  $^1$H-$^1$H COSY of 2',3',4',2",6"-Pent-O-acetyl-4"-O-butyl-1,3,6',3"-tetraazidokanamycin (K404Ac) ......................... 176

Spectrum 75  $^1$H NMR of 2',3',4',2"-Tetra-O-acetyl-4",6"-di-O-hexyl-1,3,6',3"-tetraazidokanamycin (K4606Ac) ......................... 177
Spectrum 76  \(^1\)H-\(^1\)H COSY NMR of 2',3',4',2"-Tetra-O-acetyl-4",6"-di-O-hexyl-1,3,6',3"-tetraazidokanamycin (K4606Ac) ......................... 178

Spectrum 77  \(^1\)H NMR of 2',3',4',2",6"-Pent-O-acetyl-4"-O-hexyl-1,3,6',3"-tetraazidokanamycin (K406Ac) .................................. 179

Spectrum 78  \(^1\)H-\(^1\)H COSY NMR of 2',3',4',2",6"-Pent-O-acetyl-4"-O-hexyl-1,3,6',3"-tetraazidokanamycin (K406Ac) ......................... 180

Spectrum 79  \(^1\)H NMR of 2',3',4',2",6"-Pent-O-acetyl-4",6"-di-O-heptyl-1,3,6',3"-tetraazidokanamycin (K4607Ac) ................................. 181

Spectrum 80  \(^1\)H-\(^1\)H COSY NMR of 2',3',4',2",6"-Pent-O-acetyl-4",6"-di-O-heptyl-1,3,6',3"-tetraazidokanamycin (K4607Ac) ......................... 182

Spectrum 81  \(^1\)H NMR of 2',3',4',2",6"-Pent-O-acetyl-4"-O-heptyl-1,3,6',3"-tetraazidokanamycin (K407Ac) .................................. 183

Spectrum 82  \(^1\)H-\(^1\)H COSY NMR of 2',3',4',2",6"-Pent-O-acetyl-4"-O-heptyl-1,3,6',3"-tetraazidokanamycin (K407Ac) ......................... 184

Spectrum 83  \(^1\)H NMR of 2',3',4',2"-Tetra-O-acetyl-4",6"-di-O-octyl-1,3,6',3"-tetraazidokanamycin (K4608Ac) .................................. 185

Spectrum 84  \(^1\)H-\(^1\)H COSY NMR of 2',3',4',2"-Tetra-O-acetyl-4",6"-di-O-octyl-1,3,6',3"-tetraazidokanamycin (K4608Ac) ......................... 186

Spectrum 85  \(^1\)H NMR of 2',3',4',2",4"-Pent-O-acetyl-6"-O-octyl-1,3,6',3"-tetraazidokanamycin (K608Ac) .................................. 187

Spectrum 86  \(^1\)H-\(^1\)H COSY NMR of 2',3',4',2",4"-Pent-O-acetyl-6"-O-octyl-1,3,6',3"-tetraazidokanamycin (K608Ac) ......................... 188
Spectrum 87  \(^1\)H NMR of 2′,3′,4′,2″,6″-Pent-O-acetyl-4″-O-octyl-1,3,6′,3″-tetraazidokanamycin (K408Ac) .......................... 189

Spectrum 88  \(^1\)H-\(^1\)H COSY NMR of 2′,3′,4′,2″,6″-Pent-O-acetyl-4″-O-octyl-1,3,6′,3″-tetraazidokanamycin (K408Ac) .................. 190

Spectrum 89  \(^1\)H NMR of 2′,3′,4′,2″-Tetra-O-acetyl-4″,6″-di-O-nonyl-1,3,6′,3″-tetraazidokanamycin (K4609Ac) .......................... 191

Spectrum 90  \(^1\)H-\(^1\)H COSY NMR of 2′,3′,4′,2″-Tetra-O-acetyl-4″,6″-di-O-nonyl-1,3,6′,3″-tetraazidokanamycin (K4609Ac) ............. 192

Spectrum 91  \(^1\)H NMR of 2′,3′,4′,2″,4″-Pent-O-acetyl-6″-O-nonyl-1,3,6′,3″-tetraazidokanamycin (K609Ac) .......................... 193

Spectrum 92  \(^1\)H-\(^1\)H COSY NMR of 2′,3′,4′,2″,4″-Pent-O-acetyl-6″-O-nonyl-1,3,6′,3″-tetraazidokanamycin (K609Ac) ............. 194

Spectrum 93  \(^1\)H NMR of 2′,3′,4′,2″,6″-Pent-O-acetyl-4″-O-nonyl-1,3,6′,3″-tetraazidokanamycin (K409Ac) .......................... 195

Spectrum 94  \(^1\)H-\(^1\)H COSY NMR of 2′,3′,4′,2″,6″-Pent-O-acetyl-4″-O-nonyl-1,3,6′,3″-tetraazidokanamycin (K409Ac) ............. 196

Spectrum 95  \(^1\)H NMR of 2′,3′,4′,2″-Tetra-O-acetyl-4″,6″-di-O-decyl-1,3,6′,3″-tetraazidokanamycin (K4610Ac) .......................... 197

Spectrum 96  \(^1\)H-\(^1\)H COSY NMR of 2′,3′,4′,2″-Tetra-O-acetyl-4″,6″-di-O-decyl-1,3,6′,3″-tetraazidokanamycin (K4610Ac) ............. 198

Spectrum 97  \(^1\)H NMR of 2′,3′,4′,2″,4″-Pent-O-acetyl-6″-O-decyl-1,3,6′,3″-tetraazidokanamycin (K610Ac) .......................... 199
Spectrum 98  $^1$H-$^1$H COSY NMR of 2′,3′,4′,2″,4″-Pent-O-acetyl-6″-O-decyl-1,3,6′,3″-tetraazidokanamycin (K610Ac)……………  200

Spectrum 99  $^1$H NMR of 2′,3′,4′,2″,6″-Pent-O-acetyl-4″-O-decyl-1,3,6′,3″-tetraazidokanamycin (K410Ac)……………  201

Spectrum 100  $^1$H-$^1$H COSY NMR of 2′,3′,4′,2″,6″-Pent-O-acetyl-4″-O-decyl-1,3,6′,3″-tetraazidokanamycin (K410Ac)……………  202

Spectrum 101  $^1$H NMR of 2′,3′,4′,2″-Tetra-O-acetyl-4″,6″-di-O-dodecyl-1,3,6′,3″-tetraazidokanamycin (K4612Ac)……………  203

Spectrum 102  $^1$H-$^1$H COSY NMR of 2′,3′,4′,2″-Tetra-O-acetyl-4″,6″-di-O-dodecyl-1,3,6′,3″-tetraazidokanamycin (K4612Ac)……………  204

Spectrum 103  $^1$H NMR of 2′,3′,4′,2″,4″-Pent-O-acetyl-6″-O-dodecyl-1,3,6′,3″-tetraazidokanamycin (K612Ac)……………  205

Spectrum 104  $^1$H-$^1$H COSY NMR of 2′,3′,4′,2″,4″-Pent-O-acetyl-6″-O-dodecyl-1,3,6′,3″-tetraazidokanamycin (K612Ac)……………  206

Spectrum 105  $^1$H NMR of 2′,3′,4′,2″,6″-Pent-O-acetyl-4″-O-dodecyl-1,3,6′,3″-tetraazidokanamycin (K412Ac)……………  207

Spectrum 106  $^1$H-$^1$H COSY NMR of 2′,3′,4′,2″,6″-Pent-O-acetyl-4″-O-dodecyl-1,3,6′,3″-tetraazidokanamycin (K412Ac)……………  208

Spectrum 107  $^1$H NMR of 2′,3′,4′,2″-Tetra-O-acetyl-4″,6″-di-O-tetradecyl-1,3,6′,3″-tetraazidokanamycin (K4614Ac)……………  209

Spectrum 108  $^1$H-$^1$H COSY NMR of 2′,3′,4′,2″-Tetra-O-acetyl-4″,6″-di-O-tetradecyl-1,3,6′,3″-tetraazidokanamycin (K4614Ac)……  210
Spectrum 109  \( ^1\text{H} \) NMR of 2′,3′,4′,2″,4″-Pent-O-acetyl-6″-O-tetradecyl-1,3,6′,3″-tetraazidokanamycin (K614Ac)…………………  211

Spectrum 110  \( ^1\text{H}-^1\text{H} \) COSY NMR of 2′,3′,4′,2″,4″-Pent-O-acetyl-6″-O-tetradecyl-1,3,6′,3″-tetraazidokanamycin (K614Ac)……  212

Spectrum 111  \( ^1\text{H} \) NMR of 2′,3′,4′,2″,6″-Pent-O-acetyl-4″-O-tetradecyl-1,3,6′,3″-tetraazidokanamycin (K414Ac)…………………  213

Spectrum 112  \( ^1\text{H}-^1\text{H} \) COSY NMR of 2′,3′,4′,2″,6″-Pent-O-acetyl-4″-O-tetradecyl-1,3,6′,3″-tetraazidokanamycin (K414Ac)……  214

Spectrum 113  \( ^1\text{H} \) NMR of 2′,3′,4′,2″-Tetra-O-acetyl-4″,6″-di-O-hexadecyl-1,3,6′,3″-tetraazidokanamycin (K4616Ac)…………………  215

Spectrum 114  \( ^1\text{H}-^1\text{H} \) COSY NMR of 2′,3′,4′,2″-Tetra-O-acetyl-4″,6″-di-O-hexadecyl-1,3,6′,3″-tetraazidokanamycin (K4616Ac)……  216

Spectrum 115  \( ^1\text{H} \) NMR of 2′,3′,4′,2″,6″-Pent-O-acetyl-4″-O-hexadecyl-1,3,6′,3″-tetraazidokanamycin (K416Ac)……………………………………  217

Spectrum 116  \( ^1\text{H}-^1\text{H} \) COSY NMR of 2′,3′,4′,2″,6″-Pent-O-acetyl-4″-O-hexadecyl-1,3,6′,3″-tetraazidokanamycin (K416Ac)……  218

Spectrum 117  \( ^1\text{H} \) NMR of 4″,6″-Di-O-butyl kanamycin (K4604)…………………  219

Spectrum 118  \( ^13\text{C} \) NMR of 4″,6″-Di-O-butyl kanamycin (K4604)…………………  220

Spectrum 119  \( ^1\text{H} \) NMR of 4″-O-Butyl kanamycin (K404)…………………………  221
| Spectrum 120 | $^{13}$C NMR of 4\"-O-Butyl kanamycin (K404) | 222 |
| Spectrum 121 | $^1$H NMR of 4\"-,6\"-Di-O-hexyl kanamycin (K4606) | 223 |
| Spectrum 122 | $^{13}$C NMR of 4\"-,6\"-Di-O-hexyl kanamycin (K4606) | 224 |
| Spectrum 123 | $^1$H NMR of 4\"-,O-Hexyl-1,3,6\',3\" kanamycin (K406) | 225 |
| Spectrum 124 | $^1$H NMR of 4\"-,6\"-Di-O-heptyl kanamycin (K4607) | 226 |
| Spectrum 125 | $^{13}$C NMR of 4\"-,6\"-Di-O-heptyl kanamycin (K4607) | 227 |
| Spectrum 126 | $^1$H NMR of 4\"-,O-Heptyl kanamycin (K407) | 228 |
| Spectrum 127 | $^{13}$C NMR of 4\"-,O-Heptyl kanamycin (K407) | 229 |
| Spectrum 128 | $^1$H NMR of 4\"-,6\"-Di-O-octyl kanamycin (K4608) | 230 |
| Spectrum 129 | $^{13}$C NMR of 4\"-,6\"-Di-O-octyl kanamycin (K4608) | 231 |
| Spectrum 130 | $^1$H NMR of 6\"-,O-Octyl kanamycin (K608) | 232 |
Spectrum 131  $^{13}$C NMR of 6″-O-Octyl kanamycin (K608) .......................... 233

Spectrum 132  $^1$H NMR of 4″-O-Octyl kanamycin (K408) .......................... 234

Spectrum 133  $^{13}$C NMR of 4″-O-Octyl kanamycin (K408) .......................... 235

Spectrum 134  $^1$H NMR of 4″,6″-Di-O-nonyl kanamycin (K4609) .............. 236

Spectrum 135  $^{13}$C NMR of 4″,6″-Di-O-nonyl kanamycin (K4609) .......... 237

Spectrum 136  $^1$H NMR of 6″-O-Nonyl kanamycin (K609) ....................... 238

Spectrum 137  $^{13}$C NMR of 6″-O-Nonyl kanamycin (K609) ..................... 239

Spectrum 138  $^1$H NMR of 4″-O-Nonyl kanamycin (K409) ....................... 240

Spectrum 139  $^{13}$C NMR of 4″-O-Nonyl kanamycin (K409) ..................... 241

Spectrum 140  $^1$H NMR of 4″,6″-Di-O-decyl kanamycin (K4610) ............ 242

Spectrum 141  $^{13}$C NMR of 4″,6″-Di-O-decyl kanamycin (K4610) .......... 243
Spectrum 142  $^1$H NMR of 6"-O-Decyl kanamycin (K610)…………………  244

Spectrum 143  $^{13}$C NMR of 6"-O-Decyl kanamycin (K610) ……………..  245

Spectrum 144  $^1$H NMR of 4"-O-Decyl kanamycin (K410) …………………  246

Spectrum 145  $^{13}$C NMR of 4"-O-Decyl kanamycin (K410) ……………….  247

Spectrum 146  $^1$H NMR of 4",6"-Di-O-dodecyl kanamycin (K4612)………..  248

Spectrum 147  $^{13}$C NMR of 4",6"-Di-O-dodecyl kanamycin (K4612)………..  249

Spectrum 148  $^1$H NMR of 6"-O-Dodecyl kanamycin (K612)………………..  250

Spectrum 149  $^{13}$C NMR of 6"-O-Dodecyl kanamycin (K612)……………….  251

Spectrum 150  $^1$H NMR of 4"-O-Dodecyl kanamycin (K412)………………..  252

Spectrum 151  $^{13}$C NMR of 4"-O-Dodecyl kanamycin (K412)……………….  253

Spectrum 152  $^1$H NMR of 4",6"-Di-O-tetradecyl kanamycin (K4614)……..  254
| Spectrum 153 | $^{13}$C NMR of 4″,6″-Di-O-tetradecyl kanamycin (K4614) | 255 |
| Spectrum 154 | $^1$H NMR of 4″-O-Tetradecyl kanamycin (K414) | 256 |
| Spectrum 155 | $^{13}$C NMR of 4″-O-Tetradecyl kanamycin (K414) | 257 |
| Spectrum 156 | $^1$H NMR of 4″,6″-Di-O-hexadecyl kanamycin (K4616) | 258 |
| Spectrum 157 | $^{13}$C NMR of 4″,6″-Di-O-hexadecyl kanamycin (K4616) | 259 |
| Spectrum 158 | $^1$H NMR of 4″-O-Hexadecyl kanamycin (K416) | 260 |
| Spectrum 159 | $^{13}$C NMR of 4″-O-Hexadecyl kanamycin (K416) | 261 |
1-1. Overview of Project 1: Biomass, a Promising Source of Renewable Energy

1-1.1. Rationale for the Planned Work

Nowadays, the United States imports large amounts of petroleum to help satisfy energy and chemical needs. Alternatively, both fuels and chemicals can be generated from biomass in bio-refineries. Corn starch was used as feedstock for the first generation bio-refineries. Unfortunately, cultivating agricultural crops as a source of biomass is energy intensive and escalates competition between the use of land for food or for fuel. Thus, second and third generation bio-refineries have focused on ligno-cellulosic sources of biomass including agricultural waste, energy crops such as switchgrass, and wood waste.

At the same time, with the increasing price of fossil fuel and the serious problem of global warming, discovery of sustainable and renewable energy and reduction of fossil fuel consumption are the imminent challenge for mankind to ensure continuing prosperity. Many national government agencies, including NSF and DOE, have missions and emphasis on the development of technologies to produce energy (or alternative energy) from wind, solar, biomass, and geothermal sources. While there are many reasons to embrace alternative energy, common problems like cost and reliability, are often associated with each form and, thus, hinder the application of these technologies. For instance, problems with wind energy are its noise, availability and capacity: wind droughts
Biomass is a very promising renewable energy source, bio-energy has been now accepted as major part of the projected renewable energy provisions of the future as biofuels in the form of gas, liquid or solid fuels or electricity and heat. The most widely used method to obtain biofuels is to ferment the sugar components from starch crops to produce ethanol, which can be used as gasoline (the 1st generation of biofuel production).

We believe that non-edible biomass is a superior source for alternative energy in the category of biofuel form which is known as the 2nd generation of biofuels. Unlike solar or wind energy, the supply of feedstock biomass is immense and low cost. For many years, the main issues of utilizing non-edible biomass is the difficulties in developing a low-cost and continuous separation methods for extracting fermentable carbohydrates (cellulose or hemicellulose) from the biomass.

Transformation of biomass into energy, commonly in the form of ethanol, has been intensively studied. And procedures for industrial scale production has already been established. Cellulose, lignin and hemicellulose are the three major biomaterials in biomass. Despite decades of intensive investigations, progress on utilizing cellulose in biomass as the energy source is still not satisfactory. Cellulose contains sturdy and complex structures, which make it difficult to be broken down using chemical, mechanical or biological methods. The similar problem also presents in degradation of lignin. Therefore, there is a growing trend of employing hemicellulose (or lignocellulose) as the feedstock for
ethanol production. The common practices for removing hemicellulose from biomass, especially those employed by paper-making industry during the wood pulping, involve the uses of caustic chemicals (acids, alkaline, ammonia, ozone) and/or harsh mechanical conditions (pyrolysis, steam explosion).\textsuperscript{19-21} These practices have the disadvantages of producing chemical wastes that requires further treatments or immense energy investment during the operations. As a result, there is an ongoing effort in searching for "green" process for hemicellulose isolation, and microbes for cost-effective biofuel production.\textsuperscript{10}

Due to the capability of fast rotting of wood, fungi have been the focus for biodegradation of biomass. This process can be free of hazardous chemicals and requires little energy investment.\textsuperscript{8,22} Extensive investigations have been devoted into the search for fungi, the studies of fungal enzymes and the genetic modification of microbes.\textsuperscript{24} However, little progress has been noted. The major challenges are the complexity of enzymes employed by various fungi: not all the targeted fungi can degrade wood in a satisfactory efficiency. To make matters worse, the composition of enzymes may change upon alteration of growing environments and factors, such as, temperature, humidity and nutrition. Consequently, inconsistency in the outcomes was often observed. Additionally, a main obstacle is that the degradation of lignin will generate phenols or quinones, which are toxic to the microbes (commonly yeasts) that will be used for the fermentation of carbohydrates produced from biomass. Over degradation of lignin will also lead to the formation of excess tar during the gasification of biomass.\textsuperscript{26-28} Therefore, chemical treatments are required to remove these
phenols or quinones which further complicate the process of ethanol or biofuel production. All of the above-mentioned problems make the biofuel production from hemicellulose or cellulose using biomass not effective in cost and energy-consumption. In summary, an ideal method for converting ligno-cellulosic biomass that can be used as the substitutes of fossil fuel and coal has not been completely developed, yet. Thus, we believe that understanding the linkages in biomass is pivotal in developing a practical method of utilizing carbohydrates in biomass.

White rot fungi have long been investigated for their capability to break down the biomass in wood. Among the many species, *Phanerochaete chrysosporium* (ATCC24725) has been studied by Prof. Steve Aust in the Department of Chemistry and Biochemistry for many years at Utah State University. White rot fungi use cellulases and peroxidases to degrade cellulose, lignin and hemicellulose.\(^{29-30}\) However, there is no selectivity observed between cellulose/hemicellulose and lignin. This is common among most of the wood decaying fungi.\(^ {25}\)

The dominant structures of cellulose, hemicellulose and lignin are shown in Figure 1-1. Cellulose contains polysaccharide chains predominately consisting of glucose in \(\beta-1,4\) linkages (Figure 1-a). There are two major types of hemicellulose: xylan and mannan (Figure 1-b).\(^ {31}\) Xylan has polysaccharide chains consisting of \(\beta-1,4\) linked xylose. The polysaccharide chains are decorated with small molecules and sugars. Mannan has polysaccharide chains
consisting β-1,4 linked mannose as the major repeating unit and little α-1,6 linked mannose. It has been proposed that hemi-celluloses are covalently linked to lignin via ether or alkyl types of linkages. However, the details of these linkages have not been fully revealed. Lignin is a rather complex biopolymer that contains methoxy or hydroxyphenylpropanediol or propanetriol as the major units (Figure 1-c).32-33
a) Representative Structure of Cellulose

\[
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\text{\includegraphics[width=\textwidth]{cellulose_structure.png}}
\end{array}}
\]

b) Representative Structure of Hemicellulose

**Xylan**

\[
\text{\begin{array}{c}
\text{\includegraphics[width=\textwidth]{xylan_structure.png}}
\end{array}}
\]

**Mannan**

\[
\text{\begin{array}{c}
\text{\includegraphics[width=\textwidth]{mannan_structure.png}}
\end{array}}
\]

c) Representative Structure of Lignin

\[
\text{\begin{array}{c}
\text{\includegraphics[width=\textwidth]{lignin_structure.png}}
\end{array}}
\]

**Figure 1-1**: Representative Structure of Cellulose, Hemicellulose and Lignin
1-1.2. Aims and Objectives

The proposed research is aimed at using synthetic fluorogenic probes to study the possible or dominant linkages in biomass. These probes that mimic the linkage found in lingo-cellulosic biomass are designed to select the optimal fungi from direct evaluation process. The selected fungi are expected to release or degrade carbohydrates while keeping lignin largely intact.

These probes can help to answer the fundamental questions, such as what could be the dominant linkages between lignin and hemicellulose, and what are the possible mechanisms for the cleavage of carbohydrates in biomass. Understanding the linkages in this source of biomass will enable highly efficient degradation or release of carbohydrates, primarily hemicelluloses, from biomass. The resulting carbohydrates can be used for biofuel production via fermentation, or thermochemical or thermo-catalytic conversions. The successful manipulation and selection of new fungi will help to address the problems of biofuel production from biomass, and consequently problems associated with alternative energy production from other sources. Overall, it may allow a convenient and economical utilization of these carbohydrates as the feedstock for biofuel production, and alleviate the dependence of fossil fuel of modern civilization.
1-2. Overview of Project 2: Traditional Aminoglycosides and Their New Application

1-2.1. Introduction to Aminoglycosides

Aminoglycoside antibiotics are one of the most important broad spectrum antimicrobials that have been used in clinic for more than 60 years. Even though aminoglycosides have a problem of potential nephrotoxicity and ototoxicity, they remain valuable and sometimes play an irreplaceable role for the treatment of infectious diseases.\textsuperscript{34} Streptomycin (\textbf{Figure 1-2}) was discovered as the first anti-tuberculosis agent in 1944 from the bacterial culture supernatants.\textsuperscript{35} Since then, more and more aminoglycosides were isolated from nature including kanamycin, gentamicin, and tobramycin, which were used to treat bacterial infectious diseases and have saved millions of lives.

\textbf{Figure 1-2:} Streptomycin
Naturally occurring aminoglycosides are produced by *Streptomyces* and *Micromonospora* soil bacteria,\(^{36}\) which proactively methylate their ribosomes to survive the bactericidal action of their secondary metabolites.\(^{37}\) Among the commonly used aminoglycoside antibiotics, the kanamycin class (Figure 1-3) show prominent antibacterial activity for both Gram-positive and Gram-negative bacterial. Nevertheless, the prevalence of aminoglycosides-resistant bacteria has significantly reduced the effectiveness of kanamycin. In order to solve the problem of drug resistance, chemical modification of kanamycin is the most popular and effective method to revive its activity against resistant bacterial.\(^{38-39}\) The discovery of amphiphilic aminoglycosides that can exert unexpected antimicrobial activities has led to a new strategy of broadening the applications of both amphiphilic neomycin and kanamycin.\(^{40-42}\)

![Figure 1-3. Structure of Kanamycin Class Aminoglycosides](image-url)

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AHB: (S)-4-amino-2-hydroxybutyryl
1-2.2. Investigation of Antibacterial to Antifungal Conversion of Kanamycin

Compared to the investment in the development of antibacterial, antiviral and anti-cancer therapeutics, antifungal drug discovery is relatively neglected in medical research. Pathogenic fungi, such as *Aspergillus fumigatus*, *Candida albicans*, *Cryptococcus neoformans*, and *Histoplasma capsulatum* pose serious threats to human health.\(^{43}\) Only a few new fungicides have been introduced since the mid-1980s.\(^{44}\) Resistance to existing antifungal drugs is another serious challenge. Many fungicides used in agriculture are heterocyclic compounds which are chemically synthesized, such as triazoles and pyrimidines. These heterocyclic compounds highly resemble the antifungal drugs used for treatment of fungus infections in humans. As a result, resistant fungi found in agriculture and the environment have counterparts that have evolved among pathogenic fungi found in humans.\(^{45}\) Thus, there is an urgent need for the development of novel fungicides.

Our group has previously reported the synthesis and antifungal investigation of a novel broad-spectrum fungicide **FG08** and **K20** (Figure 1-4).\(^{46,47}\) Plant leaf infection assays and greenhouse studies showed that **FG08** is capable of suppressing wheat fungal infections by *Fusarium graminearum* which is the causative agent of Fusarium Head Blight. **FG08** can be viewed as a kanamycin B derivative with three distinct structural modifications, all on ring III: a linear octyl group at O-4" position, deoxygenation at O-6" position and the replacement of the 3"-NH\(_2\) with an OH. Among these modifications, the attachment of O-4" octyl
group is essential for converting the antibacterial kanamycin into an antifungal agent. Further investigations have confirmed that **FG08** exerts its antifungal activity by specifically disrupting the fungal plasma membrane, a mechanism of action that differs from the antibacterial action of kanamycin of binding ribosomal RNA and interfering with protein synthesis.

![Figure 1-4: Structure of FG08 and K20](image_url)

**Antifungal, but not antibacterial**

To elucidate the structure activity relationship (SAR) for this phenomenon, a library of kanamycin analogs, alkylated with several different chain lengths and at various hydroxyl groups of kanamycin were synthesized (Figure 1-5). The synthesized kanamycin B analogs were again tested for growth inhibitory activities against the filamentous fungus *F. graminearum*, the G- bacterium *E. coli* (ATCC25922) and the G+ bacterium *S. aureus* (ATCC25923, G+) (Table 1-1 and Table 1-2).
Figure 1-5: Structures of Amphiphilic Kanamycin B Derivatives in the SAR Investigation

FG08, R = C₆H₁₃
FG01, R = C₄H₉
FG02, R = C₁₂H₂₅

FG05, R = C₃H₁₃
FG06, R = C₈H₁₇

FG09
FG10, R = OH
FG11, R = NH₂

FG12, R = C₆H₁₇, R' = OH
FG13, R = OH, R' = C₆H₁₇

FG14, R¹ = C₆H₁₇, R² = H, R³ = H
FG15, R¹ = H, R² = C₆H₁₇, R³ = H
FG16, R¹ = H, R² = H, R³ = C₆H₁₇
FG17, R¹ = H, R² = C₆H₁₇, R³ = C₆H₁₇
FG18, R¹ = C₆H₁₇, R² = C₆H₁₇, R³ = H
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**Table 1-1**: MIC Values of FG01, FG02, and FG08 (Against *F. graminearum* Strains)
<table>
<thead>
<tr>
<th>Entry</th>
<th>Alkylation site</th>
<th>Compound</th>
<th>MIC (µg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td><em>F. graminearum</em></td>
</tr>
<tr>
<td>1</td>
<td>O-2''</td>
<td>FG09</td>
<td>20</td>
</tr>
<tr>
<td>2</td>
<td>O-3''</td>
<td>FG07</td>
<td>62.5</td>
</tr>
<tr>
<td>3</td>
<td>O-4''</td>
<td>FG03 (6''-OH)</td>
<td>7.8</td>
</tr>
<tr>
<td>4</td>
<td>O-4''</td>
<td>FG08 (6''-H)</td>
<td>7.8</td>
</tr>
<tr>
<td>5</td>
<td>O-6'' (C₆H₁₃)</td>
<td>FG05</td>
<td>125</td>
</tr>
<tr>
<td>6</td>
<td>O-6'' (C₈H₁₇)</td>
<td>FG06</td>
<td>31.3</td>
</tr>
<tr>
<td>7</td>
<td>O-5</td>
<td>FG10 (3''-OH)</td>
<td>≥500</td>
</tr>
<tr>
<td>8</td>
<td>O-5</td>
<td>FG11 (3''-NH₂)</td>
<td>31.3</td>
</tr>
<tr>
<td>9</td>
<td>O-3' &amp; O-4'</td>
<td>FG12&amp;FG13</td>
<td>≥500</td>
</tr>
<tr>
<td>10</td>
<td>O-2''</td>
<td>FG14</td>
<td>62.5</td>
</tr>
<tr>
<td>11</td>
<td>O-4''</td>
<td>FG15</td>
<td>15.6</td>
</tr>
<tr>
<td>12</td>
<td>O-6''</td>
<td>FG16</td>
<td>31.3</td>
</tr>
<tr>
<td>13</td>
<td>O-4'' and O-6''</td>
<td>FG17</td>
<td>31.3</td>
</tr>
<tr>
<td>14</td>
<td>O-2'' and O-4''</td>
<td>FG18</td>
<td>31.3</td>
</tr>
<tr>
<td>15</td>
<td>-</td>
<td>kanamycin</td>
<td>≥500</td>
</tr>
</tbody>
</table>

**Table 1-2**: MIC Values of FG (03-18) Compounds
From our study, we have shown that O-4” position is the optimal site for attaching a linear alkyl chain that will enable the conversion of antibacterial kanamycin into an antifungal agent (Table 1-2, Entry 3, 4 and 11). Octylation at the hydroxyl groups on ring I or II causes the loss of antibacterial activity and no gain in antifungal activity. We have showed that the 3”-NH₂ group has no or even negative role in generating antifungal activity. We have also revealed that the structure of the core molecule and the site where the linear alkyl chain is essential for the selective antifungal activity. The fact that FG03 has the same level of antifungal activity as FG08 indicates deoxygenation at 6”-OH is not necessary. These findings provides a more accessible synthetic route for scale up production of future lead compound(s). There is a growing interest in the antibacterial activities of amphiphilic aminoglycosides. Our work here not only offers a new application of amphiphilic aminoglycosides, but also details in the structure activity relationship that will maximize the antifungal activity. These SAR investigations could possibly lead to the development of new antifungal kanamycin derivative that can be produced in large quantity.²⁰
1-2.3. Research Summary for Previous Work

FG compounds show very promising potential for becoming antifungal amphiphilic aminoglycosides. Unfortunately, the syntheses of these FG compounds usually involves multistep chemistry including protection/de-protection strategies and glycosylation reactions that limit the synthesis and application of the FG compounds. The quantity of final FG compounds we can make is usually around 0.1 g. All the FG compounds from our library are derivatives of kanamycin B, which is not naturally abundant among kanamycin family. On the other hand, kanamycin A is much more economical and accessible in large quantity compared to kanamycin B, however it has not been investigated a lot for its potential to become new type of antifungal agent. So the aim of the follow up research is to perform chemical modification of kanamycin A to answer the following questions:

1. Will kanamycin A show antifungal property after being chemical modified to amphiphilic type of aminoglycosides?
2. Is there a simplified synthetic strategy which could reduce the synthetic steps and improve the yield of the modified aminoglycosides?
3. How will the alkyl chain length affect the antifungal activity and is 8 carbon chain really the best choice?
1-3. REFERENCES


44. Leonard P. Gianessi, L. P.; Reigner, N. The Value of Fungicides in U.S. Crop Production. Review article from Crop Protection Research Institute, CropLife Foundation, **2005**.


CHAPTER 2

USING FLUOROGENIC PROBES FOR THE INVESTIGATION OF SELECTIVE BIOMASS DEGRADATION BY FUNGI

2-1. Abstract

A library of fifteen commercially purchased and synthetic fluorogenic probes was employed for the investigation of biomass degradation using extracts of white-rot fungi. These probes were selected or designed to mimic the dominant linkages in celluloses, hemicelluloses, and lignin, the three most abundant polymers found in biomass. The results show that white-rot fungi display a high preference for cleaving mannose- and glucose-based probes, which mimic hemicelluloses. Low degrees of cleavages were noted for xylose- and cellobiose-based probes. No cleavages were observed for probes that mimic the linkages in lignin. Overall, these discoveries prove that it is possible to employ fungi for selective degradation or release of hemicelluloses from biomass.

2-2. Introduction

\[^{1}\text{Adapted with permission from [Qian Zhang, Xinrui Peng, Michelle Grilley, Jon Takemoto, Cheng-Wei Tom Chang, Green Chem. 2015, 17, 1918-1925.]. Copyright 2015 The Royal Society of Chemistry.}\]
Celluloses, hemicelluloses (commonly present in forms, such as xylan, arabinoxylan, mannan and glucomannan) and lignin are the three most abundant polymers found in biomass. Non-edible biomass is a superior source for alternative energy in the category of 2nd generation biofuels. Unlike solar or wind energy, the supply of feedstock biomass is immense and low cost. Using fungi for biodegradation of biomass and release of carbohydrates is an effective and environmentally friendly way of providing feedstock for biofuel production. However, for many years the main issue for utilizing non-edible biomass has been the difficulties in developing low-cost and continuous separation methods for extracting fermentable carbohydrates (cellulose or hemicellulose) from the biomass. The common practices for removing hemicellulose from biomass, especially those employed by the paper-making industry during wood pulping, involve the uses of caustic chemicals (acids, alkaline, ammonia, ozone) and/or harsh mechanical conditions (pyrolysis, steam explosion). These practices have the shortcoming of producing chemical wastes that require further treatments or immense energy investments. As a result, there is an ongoing effort in searching for "green" processes for hemicellulose isolation, and microbes for cost-effective cellulose-based biofuel production.

Due to the capability of fast rotting of wood, fungi have been the focus for biodegradation of biomass. This process can be free of hazardous chemicals and requires little energy investment. Extensive investigations have been devoted to the search for fungi, studies of fungal enzymes and the genetic modification of microbes. Nevertheless, little progress has been noted. The major challenges
are the complexity of enzymes employed by various fungi: not all the targeted fungi can degrade wood with satisfactory efficiency. Another obstacle is that the degradation of lignin will generate phenols or quinones, which are toxic to the microbes, commonly yeasts, that will be used for the fermentation of the carbohydrates produced from biomass. Over degradation of lignin will also lead to the formation of excess tar during the gasification of biomass. Therefore, chemical treatments are required to remove these phenols or quinones which further complicates the process of ethanol or biofuel production. We believe that an ideal solution is to identify strains of fungi that can selectively degrade or release hemicellulose from biomass without significant degradation of lignin.

Hemicelluloses are thought to link to lignin via covalent bonds. Although the exact linkages have not been fully characterized, they most likely involve in the form of glycosidic bond to anomeric carbon or to hydroxyl groups in the form of ether bond (Figure 2-1). For achieving the goal of selective degradation or releasing hemicellulose, a library of synthetic and commercially available fluorogenic probes were screened to show their usefulness in identifying and characterizing such linkages. These probes were designed to contain linkages that mimic those found in biomass, including:

A. The first type (class I) with a chromophore attached to the anomeric position of cellobiose to mimic cellulose;
B. The second type with chromophores attached to the anomeric positions of xylose, mannose, and glucose (class IIa) to mimic the anomeric linkages between hemicelluloses, or with chromophores attached to the primary
hydroxy groups of mannopyranose and xylofuranose to mimic the ether
linkages between hemicelluloses and lignin (class IIb);

C. The third type (class III) are designed to mimic various linkages found in
lignin.

Figure 2-1: Possible linkages between hemicelluloses and lignin

2-3. Design and Synthesis of Fluorogenic Probes which Mimic Lignin-
Hemicellulose Ether Bonds
Two types of chromophores were employed. 4-Methylumbelliferone (4MU), 1 will be used in the designs that mimic the phenolic link. 6-methoxynaphthalencarbonyl (6MN) motif, 2 was used in the designs that mimic linkages between hemicellulose and lignin at phenolic and benzylic positions (Figure 2-2). These molecules show no fluorescence when linked to carbohydrates or other structural moieties. However, upon degradation or breaking of the designed chemical bonds, fluorescent molecules are released, and are thus termed fluorogenic. In addition, 4MU undergoes hydrolytic cleavage whereas 6MN fluorescence only occurs after oxidative cleavage. Thus, these two probes can also differentiate the possible mechanisms involved in the breakage of polymers in biomass. All the examined probes are shown in Figure 2-3.
Figure 2-2: Types of Fluorescent Chromophores
Figure 2-3: Structures of Employed Probes
All the probes used with 4MU at the anomeric position are commercially available. The synthesis of probe 17 began with converting the per-acetylated cellobiose into the glycosyl bromide followed by glycosylation using (6-methoxy-2-naphthyl)methanol, 7 as the glycosyl acceptor. Deacetylation of 6 afforded the desired probe 17. Similar processes were used for the syntheses of probes 14 and 16 (Scheme 2-1).

Scheme 2-1: Synthesis of Probe 14, Probe 16 and Probe 17
Probe 1 was synthesized with alkylation of methyl 2,3,4,-tri-O-benzoyl-α-D-mannoside, 11 using a 6MN derivative, (6-methoxynaphthalen-2-yl)methyl-2,2,2-trichloroacetimidate, 12 followed by the removal of the benzoyl groups (Scheme 2-2). Probe 3 was prepared from methyl mannopyranoside, 14 via a Mitsunobu reaction using 4MU as the nucleophile (Scheme 2-2).

Scheme 2-2: Synthesis of Probe 1 and Probe 3
Similar to the synthesis of probe 3, probe 4 was prepared from 1,2-O-isopropylidene-α-D-xylofuranose via a Mitsunobu reaction followed by the removal of the isopropylidene group (Scheme 2-3). In a separate route, the 3-OH of compound 15 was alkylated with 6MN. Following the removal of both trityl and isopropylidene groups, probe 5 is isolated which could exist as an equilibrium of furanose and pyranose forms (Scheme 2-3).

Scheme 2-3: Synthesis of Probe 4 and Probe 5

Probe 10 can be synthesized by alkylation of the hydroxyl group of 17 with 19 (Scheme 2-4). Probe 11 can be prepared by bromination of the α-carbon of 20 followed by nucleophilic substitution of Br using 1 as the nucleophile. Probe 12
wassynthesized via a Mitsunobu reaction, and it has also been synthesized and used as a lignin linkage mimic.⁹

Scheme 2-4: Synthesis of Probe 10, Probe 11 and Probe 12
2-4. Results and Discussion

White rot fungus, *Phanerochaete chrysosporium* (ATCC24725), which uses cellulases and peroxidases to degrade cellulose, lignin and hemicellulose, has been studied for many years.⁴ᵈ,⁸ All the probes were tested using crude extract of *P. chrysosporium*. The fluorescence intensities of the inoculated solutions were observed every ten minutes using excitation at 360 nm and emission at 460 nm, and the data was collected up to five and half hours. Relative fluorescence intensity (RFI) values were calculated as the difference in fluorescence intensity between the inoculated solutions and controls (no crude extract of *P. chrysosporium* added). The RFI value for each probes was determined 6-9 times and the averaged RFI values for all probes were summarized in Figures 2-4 to Figures 2-7.
Figure 2-4: RFI Values of Class I Probes

Figure 2-5: RFI Values of Class IIa Probes
Figure 2-6: RFI Values of Class IIb Probes

Figure 2-7: RFI Values of Class III Probes
III.2 Discussion and Conclusion

The data show that only two of the class IIa probes (probes 7 and 8) displayed a significant fluorescence increase, showing a time-dependent increase in RFI. Probe 9 also manifested a time-dependent but rather small increase in RFI. All three probes have 4MU attached at the anomeric position, whereas similar probes with 6MN attached at the anomeric position (probes 14 and 16) showed no obvious fluorescence increase. Since 6MN can be fluorescent only via oxidative cleavage, this result suggests that the activity of glycosidases and not peroxidases are responsible for the degradation. However, it is worth mentioning that the lack of fluorescence from probes 14 and 16 cannot be considered as the lack of fungal enzyme activity. In fact, we believe that fungal glycosidases can degrade these two probes. Nevertheless, the possible adducts 6-methoxy-2-naphthlenemethanol from such degradation were not fluorescent (Figure 2-8).

![Figure 2-8: Possible Degradation of Probes which will not Generate Fluorescence](image-url)
The lack of fluorescence increase from class IIb probes attached with either 4MU or 6MN also implies that peroxidases or etherases cannot degrade xylose- or mannose-based hemicelluloses, which are connected to lignin via ether linkages. Therefore, it is possible to release or degrade glucose, xylose or mannose-based hemicelluloses only when these are connected to lignin via glycosidic bonds.

The lack of fluorescence increase from class III probes and very little fluorescence increase from class I probes supports the idea of using fungi to selectively degrade or release hemicelluloses without breaking down celluloses or lignin from biomass. To confirm that the aromatic compounds generated from the degradation of class III probes (probes 10, 11, and 12) will not cause fluorescence quenching, we have measured the fluorescence of pre-mixed aromatic compounds and 4MU. Three aromatic adducts from the possible degradation of class III probes were used for this investigation: 3,4-dimethoxyacetophene (A), 3,4-dimethoxybenzyl alcohol (B) and 2-(3,4-dimethoxylphenol)-ethanol (C). The fluorescence measure showed no quenching of 4MU from these aromatic compounds. There was only a slight decrease in fluorescence when 4MU was mixed with 3,4-dimethoxyacetophene and no changes on the other two combinations. The results confirm that no fluorescence quenching if the degradation of class III probes occurs (Figure 2-9).
Nevertheless, there is a slight and time-dependent fluorescence increase for probe 18. It is possible that celluloses may still be degraded but at a much slower rate than that of the hemicelluloses. In contrast to probe 8 (mannose with β-linked 4MU), probe 19 (mannose with α-linked 4MU) did not exhibit an increase in RFI. Plant mannans are mannose-based polysaccharides with mannose linked with β(1–4) linkages while yeasts and mammalian glycoproteins have mannose-based oligosaccharides carrying mannose linked with α(1–6) as the backbone, and α(1–2) and α(1–3) linked at the branches. The lack of RFI increase from
probe 19 strongly indicates that the fungal enzymatic degradation is very selective toward the plant mannans.

Finally, based on the time-dependent but small increase in RFI of probe 18, the possibility that the rate of enzymatic degradation/release on hemicelluloses may be slower for polymers consisting of xylose, mannose or glucose cannot be ruled out. It is difficult to design or envision a fluorogenic probe that can mimic the glycosidic bond between two carbohydrate units. However, since the cellobiose-based probe (probe 18) and, likely, probe 17 were degraded by the fungal enzymes, it is possible that the glycosidic bonds between xylose, mannose, or glucose-containing hemicelluloses and lignin can also be broken by fungal enzymes albeit, probably, at a slower rate when compared to the monosaccharide-based probes.
2-6. Experimental Section

Proton magnetic resonance spectra were recorded using JEOL ECX-300, or Bruker ARX-400 spectrometer. Chemical shifts were reported as parts per million (ppm) downfield from tetramethylsilane in δ unit and coupling constants were given in cycles per second (Hz). Signal multiplicities were indicated by s (singlet), d (doublet), t (triplet), and q (quartet), m (multiplet). $^{13}$C NMR spectra were obtained using JEOL ECX-300 at 75 MHz, or Bruker ARX-400 at 100 MHz. Routine $^{13}$C NMR spectra were fully decoupled by broad-broad WALTZ decoupling. All NMR spectra were recorded at ambient temperature. Low-resolution fast-atom bombardment (LRFAB), high-resolution fast-atom bombardment (HRFAB), high-resolution MALDI, chemical ionization (CI), atmospheric pressure chemical ionization (APCI) or electrospray ionization (ESI) were provided by the Mass Spectrometry Facility, University of California, Riverside.

Chemical reagents and chromatography solvents were purchased from Aldrich Chemical Co. or Acros Chemical Co. and were used without purification unless otherwise noted. Dichloromethane was freshly distilled from calcium hydride under nitrogen atmosphere. Pyridine and triethylamine were distilled and stored over 4Å molecular sieve. Column chromatographic purifications were carried out on silica gel 230x450 mesh, Sorbent Tech. Analytical TLC was performed on Sorbent Technologies silica gel glass TLC plates. Visualization was accomplished with UV light (254 or 366nm). TLC staining were performed by
treated with diluted sulfuric acid (5% in methanol) solution or treating in iodine chamber, TLC staining for compounds bearing amine were done by treating with ninhydrin (in ethanol) and heating.

**General procedure for assay of fluorogenic probes.** White rot basidiomycete, *Phanerochaete chrysosporium*, ATCC24725 was grown on potato dextrose agar (PDA) plates and incubated around 30°C for one week before the fungus was transferred to the liquid culture media. The culture medium contained, per liter: 2 g KH$_2$PO$_4$ (40 × stock), 0.5 g MgSO$_4$·7H$_2$O (40 × stock), 0.1 g CaCl$_2$·2H$_2$O (40 × stock), 1% glucose (10 × stock), 10 mM pH 4.2 2,2-dimethyl succinate (10 × stock), 1 mg thiamin-HCl (1000 × stock), 0.2 g ammonium tartrate (40 × stock), and 10 mL of trace element solution (see below). The thiamin and KH$_2$PO$_4$ stock solutions are filter sterilized; the others are autoclaved as separate solutions. The stock solution of trace elements is made by first dissolving 1.5 g of nitrilotriacetic acid in 800 mL water and the pH of the solution was adjusted to approximately 6.5 with KOH. The following were then added with dissolution: 0.5 g MnSO$_4$·H$_2$O, 1.0 g NaCl, 0.1 g FeSO$_4$·7H$_2$O, 0.1 g CoSO$_4$, 0.1 g ZnSO$_4$·7H$_2$O, 10 mg CuSO$_4$·5H$_2$O, 10 mg AlK(SO$_4$)$_2$·12H$_2$O, 10 mg H$_3$BO$_3$, and 10 mg Na$_2$MoO$_4$·2H$_2$O. Sterile distilled water was added to make the total volume of one liter. The medium was inoculated with a 100 mL suspension of fungal culture containing spores (absorbance of 0.5 at 650 nm).

For fungal growth, 10 mL portions of liquid cultures in the growth medium were incubated in 125 mL Erlenmeyer flasks capped with rubber-stopper at 30-
39°C. Enzyme production was stimulated by including 0.4 mM veratryl alcohol final concentration and trace elements (7 times the above concentration) at the time of inoculation. After one week growth, the 10 mL culture aliquots were centrifuged at 1500 RPM, 5°C for 15 minutes, and the supernatant was collected and directly used for enzymatic assays. In a 96 well microtiter plate, each well contained 100 uL 125 mM sodium tartrate (pH 3), 50 uL 10 mM probes, 50 uL 2 mM H2O2, 50 uL culture supernatant. For the control, each well contains 100 uL 125 mM sodium tartrate (pH 3), 50 uL 10 mM probes, 50 uL 2 mM H2O2 (freshly prepared), 50 uL H2O. Fluorescence was measured by using excitation wavelength at 360/40 and emission wavelength 460/40 at 37°C (40 stands for the filter bandwidth). Two duplicated experiments were conducted in parallel. Each probes was assayed at least 6-9 times.

**General procedure for the Mitsunobu reaction using primary alcohol and 4-methylumbelliferone (probe 3 and 16).** To a solution of starting material (0.5 g) and 4-methylumbelliferone (1 equiv.) in 20 mL of anhydrous THF, triphenylphosphine (1.5 equiv.) and DIAD (1.5 equiv.) was added. The reaction was heated at 50 °C overnight. After completion of the reaction (confirmed by TLC, eluted withEtOAc), the solvent was removed and the product was purified by column chromatography (hexane/EtOAc from 80/20 to 0/100). The obtained product was further purified with recrystallization in a mixture of EtOAc/Methanol.

**General Procedure for the Methanolsysis of Acetyl Groups by NaOMe in MeOH.** To a solution of acetylated sugars (0.1 g) in anhydrous MeOH (10 mL), a catalytic amount of NaOMe (ca. 1 M in MeOH) was added. The solution was
stirred at room temperature for 6 hours. After completion of the reaction (confirmed by TLC, eluted with EtOAc/MeOH = 9/1), the reaction was quenched by adding Amberlite 120 (H⁺) resin. The reaction mixture was filtered through a short column packed with Celite, and the filtrate was collected and concentrated. The product usually would be in good quality but can be purified by a gradient column chromatography (eluted with CH₂Cl₂/MeOH from 100/0 to 4/1). The yields for deacetylation were usually between 90 – 100%.

**Methyl-6-O-(6-Methoxy-2-naphthalenylmethyl)-mannopyronoside (probe 1).** \(^1\)H NMR (300 MHz, METHANOL-D4) δ 7.7 (m, 3H), 7.43 (dd, J = 9.42, 1.74 Hz, 1H), 7.19 (d, J = 2.43 Hz, 1H), 7.1 (dd, J = 8.91, 2.73 Hz), 4.7 (s, 3H), 4.62 (d, J = 1.74, 1H, H1), 3.87 (s, 3H), 3.81 (t, J = 10.32 Hz, 1H), 3.63-3.76 (m, 5H), 3.36 (s, 3H); \(^1^3\)C NMR (100 MHz, METHANOL-D4) δ 158.3, 134.7, 133.7, 129.3, 129.2, 127.1, 126.7, 126.6, 118.8, 105.7, 101.8, 73.6, 72.4, 71.6, 71.0, 70.1, 67.7, 54.7, 54.3; ESI/APCI calcd for C₁₉H₂₄O₇⁺ ([M]⁺) 364.1522; measured 364.1499.

**Methyl-6-(4-methyl-umbelliferyl)-O-α-D-mannopyranoside (probe 3).** \(^1\)H NMR (300 MHz, METHANOL-D4) δ 7.66 (d, J = 8.94Hz, 1H), 7.0 (dd, J = 8.94, 2.4 Hz, 1H), 6.94 (d, J = 2.4 Hz, 1H), 6.14 (d, J = 1.02 Hz, 1H), 4.63 (d, J = 1.38 Hz, 1H), 4.37 (dd, J = 10.65, 1.71 Hz, 1H), 4.26 (dd, J = 10.65, 5.85 Hz, 1H), 3.8 (m, 2H), 3.73 (t, J = 8.91 Hz, 1H), 3.69 (m, 1H), 3.36 (s, 3H), 2.42 (d, J = 1.35Hz, 3H). \(^1^3\)C NMR (100 MHz, METHANOL-D4) δ 162.6, 162.3, 155.2, 154.5, 126.1, 113.7, 112.9, 110.9, 101.7, 101.3, 71.4, 71.3, 70.7, 68.3, 62.3, 54.0, 17.4. ESI/APCI calcd for C₁₇H₂₀O₈⁺ ([M]⁺) 352.1158; measured 352.1152.
1,2-O-isopropylidene-6-(4-methyl-umbelliferyl)-O-α-D-xylofuranose (14). ¹H NMR (300 MHz, CDCl₃) δ 7.45 (d, J = 8.9 Hz, 1H), 6.88 (dd, J = 8.9, 2.4 Hz, 1H), 6.84 (d, J = 2.4 Hz, 1H), 6.1 (d, 1H), 6.01 (d, J = 3.5 Hz, 1H, H-1), 4.59 (d, J = 3.8 Hz, 1H, H-2), 4.55 (ddd, J = 11.3, 5.5, 2.7 Hz, 1H, H-4), 4.40 (d, J = 2.7 Hz, 1H, H-3), 4.32 (ddd, J = 16.5, 10.3, 6.2 Hz, 2H, H-6), 2.77 (s, 1H, OH), 2.37 (d, 3H), 1.52 (s, 3H), 1.33 (s, 3H); ¹³C NMR (100 MHz, CDCl₃) 161.7, 155.2, 152.9, 125.8, 114.1, 112.9, 112.3, 105.2, 101.8, 85.5, 78.4, 75.3, 66.4, 29.9, 27.0, 26.4, 22.1, 18.9; ESI/APCI calcd for C₁₈H₂₀O₇⁺ ([M + H]⁺) 349.1287; measured 349.1295.

6-(4-Methyl-umbelliferyl)-O-D-xylofuranose (probe 4). 0.20 g (0.57 mmol) 1,2-O-isopropylidene-6-(4-methyl-umbelliferyl)-O-α-D-xylofuranose was desovled into a 5mL solution (HOAc:H₂O:TFA = 80:20:1) in a 10mL round bottom flask. The the solution was heated at 60 °C under reduced pressure (rotavapor system) for 2 hours. The completion of the reaction was confirmed by TLC (eluted with EtOAc/hexane = 3/7). Then the solvent was removed under reduced pressure. 5 mL dichloromethane was added and the solvent was removed under reduced pressure, this was repeated 3 times in order to remove AcOH. Then the product was pumped dry by oil pump for 24 hours. Pure product was obtained after chromatography (Hexane/EtOAc = 100/10 to 0/100, yield 110 mg, 0.35 mmol, 62%). ¹H NMR (300 MHz, CDCl₃) δ 7.66 (dd, J = 8.9, 1.7 Hz), 7.0 - 6.9 (m, 1H), 6.9 – 6.8 (m, 1H), 6.14 (d, J =1.1 Hz), 5.39 (d, J = 4.1 Hz, 0.4 H), 5.13 (s, 0.4H, this compound is a 1:1 mixture of α and β), 4.5 – 4.4 (m, 1H),
4.4 – 4.3 (m, 1H), 4.3 – 4.2 (m, 2H), 4.2 – 4.1 (m, 1H), 4.1 – 4.0 (m, 1H), 2.42 (d, J = 1.1 Hz, 1H); \(^{13}\)C NMR (100 MHz, CDCl\textsubscript{3}) for the sugar ring there are two sets of peaks since it is a 1:1 mixture of \(\alpha\) and \(\beta\). 162.5, 162.3, 155.1, 154.1, 126.1, 113.7, 112.8, 110.9, 103.3, 101.3, 96.8, 81.1, 80.2, 77.0, 76.9, 75.9, 75.8, 68.6, 67.9, 17.4; ESI/APCI calcd for C\(_{15}\)H\(_{17}\)O\(_{7}\)\(^+\) ([M + H]\(^+\)) 309.0947; measured 309.0959.

3-(6-Methoxy-2-naphthalenylmethyl)-O-D-xylofurnose (probe 5). This compound was prepared using the similar procedure for preparing probe 4 with the isolated yield of 55 %. \(^1\)H NMR (300 MHz, MeOH) \(\delta\) 7.79 (s, 1H), 7.72 (d, J = 1.7 Hz, 1H), 7.69 (d, J = 2.4 Hz), 7.52 (dd, J = 8.6, 1.7 Hz, 1H), 7.18 (d, J = 2.7 Hz, 1H), 7.08 (dd, J = 8.9, 1.7 Hz, 1H), 5.01 (d, J = 3.4 Hz, 0.6 Hz, \(\alpha\)), 4.96 (d, 2H), 4.42 (d, J = 7.2 Hz, \(\beta\)), 3.87 (s, 3H), 3.8 - 3.5 (m, 5H), 3.3 – 3.2 (m, 1H); \(^{13}\)C NMR (100 MHz, MeOH) for the sugar ring there are two sets of peaks since it is a mixture of \(\alpha\) and \(\beta\). 157.9, 134.5, 134.4, 129.1, 128.9, 126.8, 126.6, 126.4, 125.3, 118.5, 105.5, 97.8, 93.1, 84.8, 81.9, 74.9, 74.9, 74.8, 72.5, 70.1, 70.0, 65.9, 61.9, 54.5; ESI/APCI calcd for C\(_{17}\)H\(_{20}\)O\(_{6}\)\(^+\) ([M]\(^+\)) 320.1260; measured 320.1263.

2-((1-(3,4-dimethoxyphenyl)ethoxy)methyl)-6-methoxynaphthalene (probe 10). 2-(3,4-dimethoxyphenyl)-ethanol was prepared by reducing 3,4-dimethoxyacetophenone using NaBH\(_4\) in MeOH. 2-bromomethyl-6-methoxynaphthalene was prepared by treating 6-methoxynaphthyl-methanol with PBr\(_3\) in Et\(_2\)O for 5 hours. The reaction was quenched by adding sat. NaHCO\(_3\) to the
reaction solution until no bubbling. Then the organic layer was washed by Sat. NaHCO₃ for two times and Brine for one time. The organic layer was dried over Na₂SO₄ and the solvent was removed under reduced pressure. The crude product was use directly for next step. (0.5 g, 2.7 mmol) 2-(3,4-dimethoxyphenyl)-ethanol and 1eq. 2-bromomethyl-6-methoxy-naphthalene was dissolved in THF, then 10 equiv. of NaH was added. After 6 hours, the reaction solution was poured to a flask with ice to quench the reaction. 300 mL of EtOAc was added to extract the product. The organic layer was washed by 100 mL distilled water, 100 mL 1N HCl and Brine, then dried over Na₂SO₄. Filtration and concentration of the solvent gave the crude solid product, the solid crude product was subjected to purification with gradient silica gel column chromatography (Hexane/EtOAc = 100:0 to 40/60) to afford the desired product (Yield 0.69g, 1.97 mmol, 73%). ¹H NMR (300 MHz, CDCl₃) δ 7.7 – 7.6 (m, 3H), 7.40 (dd, J = 8.4, 1.8 Hz, 1H), 7.1 – 7.0 (m, 2H), 6.9 – 6.8 (m, 3H), 4.58 – 4.38 (dd, J = 46, 11.67 Hz, 2H), 4.47 (q, J = 6.54, 1H), 3.92 (s, 3H), 3.90 (s, 3H), 3.89 (s, 3H), 1.49 (d, J = 6.5 Hz, 3H); ¹³C NMR (100 MHz, CDCl₃) δ 157.8, 149.4, 148.6, 136.5, 134.3, 133.9, 129.5, 128.9, 127.2, 126.8, 126.7, 119.0, 111.1, 109.3, 105.9, 70.5, 56.1, 56.1, 55.5, 29.9, 24.4; ESI/APCI calcd for C₂₂H₂₄O₇⁺ ([M]⁺) 352.1673; measured 352.1675.

1-(3,4-dimethoxyphenyl)-2-(4-methyl-umbelliferoxy)-ethanone (probe 11). To a cooled (0°C) solution of 3,4-dimethoxyacetophenone (0.50 g, 2.71 mmol) in diethyl ether (50 mL), bromine (0.26 g, 3.32 mmol) dissolved in ether (20 mL) was added dropwise. After 10 min, the reaction mixture was worked up,
and the organic layer was wash with water and brine. Then organic layer was
dried over sodium sulfate. Filtration and evaporation of the solvent gave a crude
product which was used directly for the next step. The crude product from last
step, potassium carbonate (0.56 g, 4.05 mmol) and 4-methylumbelliferone (0.47
g, 2.73 mmol) was dissolved into 50 mL of acetone. The solution was heated
under reflux for overnight. Upon completion of the reaction (check by TLC, eluted
with EtOAc/hexane = 1/9), the solvent was removed by compress air. 100 ml
EtOAc was added to the crude yellowish solid. After being stirred for half an hour
in EtOAC, there was still some insoluble yellowish solid which was collected by
filtration. The insoluble yellowish solid was confirmed as the right product by H
NMR (Yield 0.78 g, 2.21 mmol, 82%). \(^1\)H NMR (300 MHz, CDCl\(_3\)) \(\delta\) 7.62 (dd, \(J = \)
8.25, 2.04 Hz, 1H), 7.5 - 7.4 (m, 2H), 7.0 - 6.9 (m, 2H), 6.79 (d, \(J = 2.76\) Hz, 1H),
6.14 (d, \(J = 1.38\) Hz, 1H), 5.33 (s, 2H), 3.97 (s, 3H), 3.93 (s, 3H), 2.4 (d, 1.38 Hz);
\(^{13}\)C NMR (100 MHz, CDCl\(_3\)) \(\delta\) 191.9, 161.3, 161.2, 155.2, 154.5, 152.7, 149.7,
127.5, 125.9, 122.8, 114.5, 112.9, 112.6, 110.4, 110.4, 102.1, 70.7, 56.4, 56.3,
18.7; ESI/APCI calcd for C\(_{20}\)H\(_{18}\)O\(_6\)\(^+\) ([M]\(^+\)) 354.1103; measured 354.1091.

(6-Methoxy-2-naphthalenylmethyl)-\(\beta\)-D-glucopyranoside (probe 14).
\(^1\)H NMR (300 MHz, METHANOL-D4) \(\delta\) 7.8 – 7.7 (m, 3H), 7.48 (dd, \(J = 8.5, 1.7\)
Hz, 1H), 7.20 (d, \(J = 2.7\) Hz, 1H ), 7.10 (dd, \(J = 8.9, 2.4\) Hz), 5.0 – 4.7 (dd, \(J =
75.9, 11.7\) Hz), 4.32 (d, \(J = 7.5\) Hz, H-1), 3.9 – 3.8 (m, 1H), 3.88 (s, 3H), 3.7 – 3.6
(m, 1H), 3.4 – 3.2 (m, 3H); \(^{13}\)C NMR (100 MHz, METHANOL-D4) \(\delta\) 158.1, 134.6,
132.9, 129.2, 128.9, 126.7, 126.6, 118.6, 105.5, 102.0, 76.9, 76.85 (2C), 73.9,
70.7, 70.5, 61.6, 54.5; ESI/APCI calcd for C_{18}H_{22}O_{7}Na⁺ ([M + Na]⁺) 373.1263; measured 373.1273.

(6-Methoxy-2-naphthenylmethyl)-β-D-xylopyranoside (probe 16). \(^1\)H NMR (300 MHz, METHANOL-D4) δ 7.8 – 7.7 (m, 3H), 7.46 (dd, J = 8.6, 1.7 Hz, 1H), 7.19 (d, J = 2.4 Hz, 1H), 7.09 (dd, J = 8.9, 2.4 Hz), 4.9 – 4.7 (dd, J = 67.3, 11.7 Hz), 4.32 (d, J = 7.2 Hz, H-1), 3.9 – 3.8 (m, 1H), 3.88 (s, 3H), 3.6 – 3.4 (m, 2H), 3.3 – 3.1 (m, 2H); \(^{13}\)C NMR (100 MHz, METHANOL-D4) 134.6, 132.9, 129.1, 128.9, 126.8, 126.6, 126.57, 118.6, 1015.5, 102.9, 76.6, 73.8, 70.8, 70.1, 65.8, 54.5; ESI/APCI calcd for C_{18}H_{22}O_{7}Na⁺ ([M + Na]⁺) 343.1158; measured 343.1167.

(6-Methoxy-2-naphthenylmethyl)-β-D-cellubioside (probe 17). \(^1\)H NMR (300 MHz, METHANOL-D4) δ 7.8 – 7.7 (m, 3H), 7.48 (dd, J = 8.5, 1.7 Hz, 1H), 7.20 (d, J = 2.7 Hz, 1H), 7.10 (dd, J = 8.9, 2.4 Hz), 5.0 – 4.7 (dd, J = 71.8, 11.7 Hz), 4. (s, 2H), 4.40 (dd, J = 7.5, 3.1 Hz, 2H), 3.9 – 3.8 (m, 3H), 3.88 (s, 3H), 3.7 – 3.4 (m, 4H), 3.4 – 3.3 (m, 2H), 3.20 (t, J = 8.6 Hz, 1H); \(^{13}\)C NMR (100 MHz, METHANOL-D4) δ 158.1, 134.6, 132.9, 129.2, 128.9, 126.7, 126.6, 118.6, 105.5, 103.4, 101.9, 79.5, 76.9, 76.6, 75.4, 75.2, 73.7, 70.8, 70.2, 61.2, 60.7, 54.5. ESI/APCI calcd for C_{24}H_{32}O_{12}Na⁺ ([M + Na]⁺) 535.1791; measured 535.1787.

(6-Methoxy-2-naphthenylmethyl)-2,3,4,6-tetra-O-acetyl-β-D-glucopyranoside (8). Bromo-2,3,4,6-acetyl-O-α-D-glucopyranoside was prepared by treating 1,2,3,4,6-penta-O-acetyl-D-glucose (0.5 g, 1.28 mmol) with HBr (3mL, 33% in AcOH) in a 20 mL flask with 3 mL anhydrous dichloromethane
as solvent for 2 hours. The reaction mixture was extracted by 100 mL EtOAc and
the organic layer was washed by distilled water, Sat. NaHCO₃ for three times and
Brine for one time. Then the organic layer was dried over anhydrous Na₂SO₄ and
filtered. The bromo-2,3,4,6-acetyl-O-α-D-glucopyranoside was obtained by
removing the solvent under reduced pressure and used directly for next step.
Bromo-2,3,4,6-acetyl-O-α-D-glucopyranoside (1.28 mmol) and (6-Methoxy-2-
naphthyl)methanol (0.34 g, 1.8 mmol) and 4 Å MS was stirred in 20 mL of
anhydrous dichloromethane for 15 minutes before adding in Ag₂CO₃ (0.49 g, 1.8
mmol ). The mixture was stirred for overnight before filtered through a syringe
packed with celite and silica gel. Then the solvent was removed and the crude
product was loaded to a column and purified with gradient (Hexane/EtOAc =
100/10 to 50/50). Pure product was obtained (0.41g, 0.79 mmol, 65 % yield over
two steps). ¹H NMR (300 MHz, CDCl₃) δ 7.8 – 7.7 (m, 3H), 7.37 (dd, J = 8.3, 1.4
Hz), 7.2 – 7.1 (m, 2H), 5.70 (d, J = 5.1 Hz, H-1 ),5.22 (t, J = 2.8 Hz, H-2), 4.91
(dd, J = 9.3, 2.1 Hz, H-3), 4.67 (s, 2H), 4.35 (dd, J = 4.5, 3.1 Hz, H-4), 4.2 – 4.1
(m, 2H), 3.97 (ddd, J = 9.6, 5.5, 3.5 Hz, H-5), 3.9 (s, 3H), 2.1 – 1.8 (m, 12H); ¹³C
NMR (100 MHz, CDCl₃) δ 170.9, 169.9, 169.4, 157.9, 134.3, 132.8, 129.6 128.9,
127.3, 126.6, 126.5, 121.7, 119.2, 105.9, 97.22, 73.4, 70.3, 68.4, 67.2, 66.3,
63.3, 55.5, 21.2, 21.0 (3C); ESI/APCI calcd for C₂₆H₃₉O₁₁Na⁺ ([M + Na]+) m/z
541.1686; measured m/z 541.1677.

(6-Methoxy-2-naphthalenylmethyl)-2,3,4,-tri-O-acetyl-D-
xylopyranoside (10). This compound was prepared using the similar procedure
for preparing compound 8 with the isolated yield of 45 %. ¹H NMR (300 MHz,
The compound was obtained as a mixture of alpha and beta conformation (α/β = 1.3/1) δ 7.8 – 7.7 (m, 4.6 H), 7.5 – 7.4 (m, 1.6 H), 7.2 – 7.1 (m, 3.1 H), 5.7 (d, J = 6.9 Hz, 1H, H α-1), 5.58 (d, J = 4.5 Hz, 1.3H, H β-1), 5.28 (t, J = 2.8 Hz, 1.3H), 5.20 (t, J = 8.3, 1H), 5.1 – 4.8 (m, 4H), 4.68 (s, 2H), 4.3 – 4.2 (m, 1.4H), 4.15 (dd, J = 12.0, 5.2 Hz, 1H), 4.0 – 3.9 (m, 1H), 3.90 (s, 5.1H), 3.71 (dd, J = 12.4, 6.9 Hz, 1.4H), 3.51 (dd, J = 12.0, 8.6 Hz, 1 H ), 2.1 – 2.0 (m, 21H); 13C NMR (100 MHz, CDCl₃) δ 170.1, 170.0, 169.5, 169.3, 169.2, 157.9, 134.3, 132.8, 129.6, 128.9, 127.3, 126.7, 126.6, 126.1, 125.7, 122.6, 119.1, 105.9, 96.8, 92.3, 74.5, 71.2, 69.7, 69.6, 68.8, 68.5, 67.6, 65.6, 63.0, 59.9, 55.5, 22.9 (2C), 21.0, 20.9, 20.87, 20.81; ESI/APCI calcd for C₂₃H₂₆O₉Na⁺ ([M + Na]⁺) m/z 469.1475; measured m/z 469.1468.

Methyl-2,3,4-Tri-benzoyl-O-6-(6-Methoxy-2-naphthalenylmethyl)-α-D-mannopyranoside (13). 6-methoxy-2-naphthalenylmethyl trichloroacetimidate (0.33 g, 1 mmol) and methyl 2,3,4-tri-O-benzoyl-α-D-mannopyranoside (0.35 g, 0.7 mmol), 4 Å molecular sieve in anhydrous dichloromethane (20 mL) was stirred for 30 minutes and then cooled to -78 °C. After addition of several drops of BF₃·Et₂O, the reaction mixture was stirred overnight. After completion of the reaction (check by TLC, eluted with EtOAc/hexane = 1/9), the reaction mixture was filtered through a short column packed with Celite and silica gel. After removal of most of the solvent, the crude product was purified with a gradient column chromatography (hexane/EtOAc = 100/0 to 75/25) to furnish the desired product (0.48 g, 68%). 1H NMR (CDCl₃, 300 MHz) δ 8.1-8.0 (m, 2H), 7.82-7.9 (m, 4H), 7.7-7.2 (m, 10H), 7.0-7.1 (m, 2H), 6.02 (t, J = 9.69 Hz, 1H, H4), 5.85 (dd, J =
9.96, 3.09 Hz, 1H, H3), 5.7 (dd, J = 3.45, 1.71 Hz, 1H, H2), 5.0 (d, J = 1.71 Hz, 1H, H1), 4.71 (dd, J = 26.1, 11.67 Hz, 2H), 4.27 (dt, J = 9.63, 3.45 Hz, 1H, H5), 3.91 (s, 3H), 3.79 (d, J = 3.75 Hz, 2H, H6), 3.54 (s, 3H); $^{13}$C NMR (CDCl$_3$, 75 MHz) δ 165.8, 165.7, 163.6, 157.8, 134.3, 133.6, 133.4, 133.3, 133.2(2C), 130.1(2C), 130.0(3C), 129.9, 129.6, 129.6, 129.44, 129.4, 128.7(2C), 128.5(2C), 128.5(2C), 127.1, 126.80, 126.7, 118.92, 105.9, 98.8, 74.1, 70.7, 70.5, 70.3, 69.1, 67.5, 55.7, 55.5. ESI/APCI calcd for C$_{40}$H$_{36}$O$_{10}$ $^{+}$ ([M]$^{+}$) 676.2308; measured 676.2300.
2-6. REFERENCES


CHAPTER 3
SYNTHESIS OF ANTIFUNGALKANAMYCIN A ANALOGS

3-1. Abstract

A concise and novel method for site-selective alkylation of tetraazidokanamycin has been developed that leads to the divergent synthesis of three classes of kanamycin derivatives. These new amphiphilic kanamycin derivatives bearing alkyl chains length of 4, 6, 8, 9, 10, 12, 16 have been synthesized and tested as antibiotics. Surprisingly, the antibacterial effect of the synthesized kanamycin derivatives decline or disappear compared to the original kanamycin A, but some of the compounds show very strong activity as antifungal agents.

\[\text{Coauthored by Qian Zhang, Madher Alfindee, Xinrui Peng, Yukie Kawasaki, Jon Y. Takemoto, and Cheng-Wei Tom Chang, \textit{Manuscript in preparation}.}\]
3-2. Introduction

Fungal diseases are one of the major threats to human health and food security.¹ Fungal crop diseases like wheat head blight or scab (caused by *Fusarium graminearum*) and stem rust (caused by *Puccinia graminis*) result in large economic losses and threats to the world’s food supplies.² Traditional and commonly used antifungals such as amphotericinB and azoles are still in use to treat invasive fungal infections, and fungicidal triazoles, pyrimidines and strobilurins continue to be used in massive quantities for wheat and other major crops.³ The effectiveness of the current antifungals keep decreasing due to fungal resistance, and traditional crop antifungals are causing huge disturbances to the natural ecosystem. In consequence, there is a growing and urgent need to develop novel and effective antifungal agents.

Although aminoglycosides are generally known as anti-bacterials, it has been reported that certain classical aminoglycosides could inhibit important crop pathogenic fungal oomycetes.⁴ A recent report revealed inhibition of plant pathogenic oomycetes *Phytophthora* and *Pythium sp.* by streptomycin, neomycin, ribostamycin and paramomycin, although they showed comparatively little activity against several other fungal genera. Also there are some structurally unusual aminoglycosides show inhibition activities against yeasts and protozoans.⁵
Five years ago, it was demonstrated in our lab that there is a new possibility of antifungal instead of traditional antibacterial capabilities of aminoglycosides. Compound **FG08** (Scheme 3-1) synthesis was started by alkylation of 22 using bromooctane and NaH as the base to afford glycosyl donor 23. Then stereo-selective glycosylation between pre-prepared glycosyl acceptor 24 and donor 23 gave compound 25. Deacetylation, Staudinger reduction of azido groups and hydrogenolysis of benzyl groups of 25 offered the final compound **FG08**. Leaf infection assays and greenhouse studies showed that **FG08** could suppress wheat fungal infections by *Fusarium graminearum*—the causative agent of Fusarium head blight—at concentrations that are minimally phytotoxic. Instead of typical aminoglycoside action of ribosomal protein translation miscoding, **FG08**'s antifungal action involves perturbation of the plasma membrane. This antibacterial to antifungal transformation paved the way for the development of a new class of aminoglycoside-based fungicides which could be useful in treating crop disease. In addition, this strategy is an example of reviving a clinically obsolete drug by chemical modification to offer a new application.
Scheme 3-1: Synthesis of FG08

FG08, with broad-spectrum antifungal properties but did not inhibit tested bacterial and mammalian cells, is great start for the study of antifungal aminoglycoside. But the long and complicated synthetic route put a huge limit for the application of this new chemically modified aminoglycoside. The shortcomings of FG08 prompted our search for similar amphiphilic aminoglycosides using alternative synthetic approaches.8
3-3. Synthesis Of Kanamycin Derivatives For Converting Antibacterial Agents To Antifungal Agents

In our recent study for investigation of structure-activity relationship for antibacterial to antifungal conversion of Kanamycin B, I have participated in the synthesis of compounds **FG14-18**. The syntheses of the needed glycosyl donors for these additional kanamycin analogs began with the preparation of phenyl 3-azido-3-deoxy-1-thio-β-D-glucopyranoside, **26** and its derivatives, **27** and **28** *(Scheme 3-2)*. Using a similar approach, compounds **29** and **30** were prepared with Bn and octyl group incorporated at O-4 position, respectively. Alkylation of **29** and acetylation of **30** led to the formation of glycosyl donors, **33c** and **33e**.

Using appropriate protecting groups and protection strategy, glycosyl donor, **33a** was synthesized from **27** whereas glycosyl donors **33d** were prepared from **28**.

Following the glycosylation and deacetylation, compounds were subjected to Staudinger reduction and hydrogenolysis, and the desired kanamycin analogs were purified by column chromatography using either CG50 (NH₄⁺) resin or silica gel *(Scheme 3-3)*. After ion-exchange, these analogs were prepared as chloride salts.
Scheme 3-2: Synthesis of Glycosyl Donors for FG14-18
Scheme 3-3: Synthesis of FG14-18

For the synthesis of FG14-18, in collaboration with my labmate, it took us almost one year to finish the syntheses of the 5 targeted amphiphilic aminoglycosides. The objectives of F14-F18 synthesis in the SAR investigation were to understand two key points a) importance of 3"-NH2, (Does it have to be replaced by OH?) and b) the effect of multiple octyl groups(Will di-alkylated kanamycin show better activity compared to mono-alkylated?) We found that replacing 3"-NH2 with a hydroxyl group has almost no effect in improving antifungal activity which means
we do not need to modify the 3″-NH₂. This is a promising result because both kanamycin A and Kanamycin B naturally have the 3″-NH₂. Compounds incorporated with two octyl groups (FG17 and FG18), showed no improvement in antifungal activities, but they had enhanced activities against S. aureus (ATCC25923). Perhaps, as the lipophilicity increases, these amphiphilic aminoglycosides are less fungal-specific and behave like other amphiphilic aminoglycosides that contain longer alkyl chain (ex. hexadecyl) and that show strong antibacterial activities. All these lessons we learned from the SAR study suggest us: For the synthesis of amphiphilic aminoglycosides, we should have a chance to modify kanamycin directly while skipping complicated protection steps and glycosylation reaction which usually put the limit for the scale of the reaction. Also, we would like to start from kanamycin A which is economical and available in large quantity.

First, we synthesized 1,3,6′,3″-tetraazidokanamycin (will be abbreviated as tetraazidokanamycin in the following text) from commercial available kanamycin sulfate according to literature report (Scheme 3-4)⁹. Azides serve as a good masking group for amines because of its stability in both acid and basic conditions. Also the azide could be turned back into amine easily by Staudinger reaction.¹⁰ This reaction has been tested to be relative high yield (~ 75%) and doable at over 10 gram scale, which makes the tetraazidokanamycin very accessible.
Scheme 3-4: Synthesis of 1,3,6′,3″-Tetraazidokanamycin

In an effort to enable the production of amphiphilic aminoglycosides in an easy and efficient process, we decided to investigate the feasibility of direct alkylation of tetra-azido kanamycin\textsuperscript{35} (Scheme 3-5). For the test reaction, we use tetraazidokanamycin as the substrate, 20 equivalents of NaH as the base and 1.5 equivalent of bromooctane for octylation. This design is aimed at exploring if mono-alkylation of the primary 6″-OH is possible or not. To our delight and surprise, we actually could separate three different products from the test reaction. The major product is the \textbf{36a}, 4″, 6″-O-octyl tetra-azido kanamycin. Also \textbf{36b}, 6″-O-octyl tetra-azido kanamycin and \textbf{36c}, 4″-O-octyl tetra-azido kanamycin was isolated. Acetylation of the \textbf{36a}, \textbf{36b} and \textbf{36c} offered \textbf{37a}, \textbf{37b}, and \textbf{37c}, respectively (Scheme 3-6). The octylation site was confirmed after performing H-H correlation spectroscopy (COSY) spectrum and the spectrum was provided in the appendix. Then reducing the azide of \textbf{36a}, \textbf{36b} and \textbf{36c} by Staudinger
reaction afford the final amphiphilic aminoglycosides 38a, 38b and 38c (Scheme 3-7).

For our own convenience for further research, we have developed a naming system for alkylated tetraazidokanamycin (Table 3-1):

Scheme 3-5: Octylation of Tetraazidokanamycin
<table>
<thead>
<tr>
<th>Compound Code</th>
<th>Compound Name</th>
</tr>
</thead>
<tbody>
<tr>
<td>K4608N3</td>
<td>4″,6″-Di-O-octyl-1,3,6’,3″-tetraazidokanamycin</td>
</tr>
<tr>
<td>K608N3</td>
<td>6″-O-Octyl-1,3,6’,3″-tetraazidokanamycin</td>
</tr>
<tr>
<td>K408N3</td>
<td>4″-O-Octyl-1,3,6’,3″-tetraazidokanamycin</td>
</tr>
<tr>
<td>K4608Ac</td>
<td>2’,3’,4’,2″-Tetra-O-acetyl-4″,6″-di-O-octyl-1,3,6’,3″-tetraazidokanamycin</td>
</tr>
<tr>
<td>K608Ac</td>
<td>2’,3’,4’,2″,4″-Pent-O-acetyl-6″-O-octyl-1,3,6’,3″-tetraazidokanamycin</td>
</tr>
<tr>
<td>K408Ac</td>
<td>2’,3’,4’,2″,6″-Pent-O-acetyl-4″-O-octyl-1,3,6’,3″-tetraazidokanamycin</td>
</tr>
<tr>
<td>K4608</td>
<td>4″,6″-Di-O-octyl kanamycin</td>
</tr>
<tr>
<td>K608</td>
<td>6″-O-Octyl kanamycin</td>
</tr>
<tr>
<td>K408</td>
<td>4″-O-Octyl kanamycin</td>
</tr>
</tbody>
</table>

Table 3-1: Example of Naming System for Octylated Kanamycin

Note: 4 and 6 indicate the site of alkylation (4″ and 6″ are the two alkylation sites), 08 stands for the carbon chain length, N3 stands for 1,3,6’,3″-tetraazidokanamycin and Ac stands for acetylation.
Scheme 3-6: Acetylation of Octylated Tetraazidokanamycin

Scheme 3-7: Amphiphilic Aminoglycosides after Staudinger Reaction
Using a three step synthesis, we have successfully obtained three different types of amphiphilic aminoglycosides. K4608, K608, and K408 are structurally very close to our previously synthesized FG17, FG06 and FG03. The only difference is: K4608, K608 and K408 are derivatives from kanamycin A, at 2′ position there is OH group; FG17, FG06 and FG03 are derivatives from kanamycin B, at 2′ position there is NH₂ group (Figure 3-1). We then immediately submitted K4608, K608 and K408 for bioactivity testing against F. graminearum and bacteria. The minimum inhibitory concentrations (MICs) value are listed in Table 3-2 and the results were compared with FG17, FG06 and FG03. From the result of MIC values, K4608, K608 and K408 did not show very good antibacterial activity, which is consistent with our previous findings that alkylated aminoglycosides are not good antibacterial agents. To our delight, K4608, K608 and K408 showed even better activity against F. graminearum compared with FG compounds. We are extremely happy about the results, because not only did we successfully improved the antifungal activity, we have also cut down the synthetic steps to only three steps. No complicated protection strategy is needed, and no glycosylation step is needed. The most important advantage compared with FG compounds synthesis is that we could scale up the reaction to offer large quantity of product.
Figure 3-1: Structure Comparison between K4608, K608, K408 and FG17, FG06, FG03

Table 3-2: MIC Comparison between K4608, K608, K408 and FG17, FG06, FG03
For the FG project, we have found that shortening the octyl chain to a butyl group (FG01) or extending the chain length to a dodecyl group (FG02) diminished the antifungal activity. We are wondering if this would happen again if butyl group and dodecyl group were attached to kanamycin A. Due to the complexity of synthesizing FG compounds, we did not have time to explore more alkyl chain length in our previous study. And our results from FG project always face the question: Is 8 carbon chain really the best chain length? With this promising result from the K4608 compound, we now have the ability to synthesize more amphiphilic aminoglycosides with various chain lengths following the new synthetic route (Table 3-3). Using the established protocol, apart from the alkylation with bromooctane (8 carbon), we also did the alkylation of tetraazidokanamycin using 1-bromobutane (4 carbon), 1-bromohexane (6 carbon), 1-bromoheptane (7 carbon), 1-bromononane (9 carbon), 1-bromodecane (10 carbon), 1-bromododecane (12 carbon), 1-bromotetradecane (14 carbon) and 1-bromohexyldecane (16 carbon), respectively.


**Table 3-3:** Alkylation of Tetraazidokanamycin with Various Chain Length

All alkylations with various alkyl bromides were done following this general procedure: to a solution of tetraazidokanamycin (0.5 g, 0.85 mmol) 35, in 20 mL of anhydrous DMF, NaH (20 equiv.) was added. The reaction mixture was stirred for 10 minutes before adding the corresponding alkyl bromide (1.5 equiv.). A catalytic amount of TBAI was added as catalyst and the reaction mixture was
stirred at room temperature overnight. Completion of the reaction was confirmed using TLC (eluted with EtOAc). Then the reaction was quenched by adding MeOH (5 mL). The mixture was concentrated and purified with a gradient silica gel column chromatography (eluted from EtOAc/Hexane 30:70 to EtOAc/MeOH 90:10) to obtain three products. The observed polarity of three products was as follows: 4"-momo-armed tetraazidokanamycin>6"-mono-armed tetraazidokanamycin>> 4", 6"-di-armed tetraazidokanamycin. The big polarity difference between mono-armed and di-armed kanamycin makes it very easy to separate the di-armed product from the mono-armed product. There is a very small polarity difference between 4"-momo-armed tetraazidokanamycin and 6"-mono-armed tetraazidokanamycin. The silica gel column chromatography purification was successful for almost half of the reactions we tried (Table 3-3, Entry 4, 5 and 6), but in other cases it is challenging to separate the pure 6"-mono-armed tetraazidokanamycin (Table 3-3, Entry 1, 2, 3, 7 and 8) since this type of product is the minor product compared to the other two types. We do observe the formation of the 6"'-mono-armed tetraazidokanamycin for all the cases, an estimated of compound 39 yield is given according to H NMR if 6"-mono-armed tetraazidokanamycin is not isolated to good purity.

The purified 4"-mono-armed tetraazidokanamycin 40, 6"-mono-armed tetraazidokanamycin 39 and 4", 6"-di-armed tetraazidokanamycin 38 were submitted for Staudinger reaction to reduce azide back to amine to afford the amphiphilic aminoglycosides (Table 3-4). All the purified aminoglycosides were tested for both antibacterial and antifungal activity.
\[ \begin{align*}
\text{R} &= \text{alkyl or H} \\
\text{1) PMe}_3 (5 \text{ eq.}, \text{THF}) \\
\text{2) Dowex 1X8-200 (Cl-)}
\end{align*} \]

<table>
<thead>
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<th>Compound</th>
<th>Yields (%)</th>
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</tr>
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<td>K610</td>
<td>66%</td>
</tr>
<tr>
<td>K410</td>
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The growth inhibition activities of these amphiphilic kanamycin A analogs were examined against *F. graminearum*, *E. coli* (ATCC25922) and *S. aureus* (ATCC25923) and the results are summarized in Table 3-5. It was clear that the presence of 2''-NH₂ does not increase the antifungal activity (*FG17* vs. *K4608*, *FG06* vs. *K608*, and *FG03* vs. *K408*). For the amphiphilic aminoglycosides alkylated with various chain length, 8 carbon chain was proved to be the best chain length even though 6, 7, 9 and 10 carbon alkylated kanamycin did show moderate to good antifungal activity. Again, placement of the octyl group at the O-4'' position offered optimal antifungal activity as compared to 6''-alkylated or

<table>
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**Table 3-4:** Yield of Mono-armed and Di-armed Amphiphilic Aminoglycosides after Staudinger Reaction
4",6"-di-alkylated kanamycin. Compounds incorporated with two octyl groups (K4608, K4609, and K4610) do not show improvement in antifungal activities compared to the corresponding mono-alkylated aminoglycosides, but slight better activities against S. aureus (ATCC25923) and E. Coli (ATCC25922). It is also shown again that when alkylation chain become too short (shorter than 6) or too long (longer than 10), the antifungal activity diminishes or disappears. To convert the traditional antibacterial kanamycin A to new types of antifungal agents, alkylation with chain length of 6 to 10 would be good choices. Again, 8 carbon chain length proved to be the best choice.
<table>
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<tr>
<th>Entry</th>
<th>Alkylation site (Alkylation length)</th>
<th>Compound</th>
<th>MIC (µg/mL)</th>
<th>F. graminearum</th>
<th>E. coli</th>
<th>S. aureus</th>
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Table 3-5: MIC of Kanamycin A Analogs

In collaboration with Dr. Takemoto’s lab of the Biology Department, Utah State University, the mechanism of FG08’s antifungal property has been studied. FG08’s growth inhibitory specificity for fungi is suggested to lie in its ability to increase plasma membrane permeability by mechanisms that are influenced by the lipid composition of the fungal plasma membrane. We propose that our Kanamycin A analogs probably follow the same mechanism of action as FG08 (Kanamycin B analog) due to the structure similarity. These active new kanamycin A analogs have broad-spectrum antifungal activities, almost no antibacterial activity that would promote antibiotic resistance. They appear to be promising candidates for novel antifungal agents.
3-4. Conclusions and Significance

Inspired by the leading compounds FG08 from our previous study, we have demonstrated that the amphiphilic aminoglycosides have general potential to be converted to antifungal agents. This new type of broad spectrum antifungal property is completely different from the original antibacterial activity. Importantly, we have shown that our new developed approach, involving a three step reaction route, could offer simple and efficient synthesis of amphiphilic aminoglycosides. Through this new synthetic route, we have synthesized alkylated aminoglycosides with various chain length. Interestingly, we have shown that the secondary 4"-OH of the Kanamycin A is more reactive than the primary 6"-OH. The 4",6" di-armed kanamycin is not more active compared with the 4" mono-armed kanamycin which is consistent with our previous study, 8 carbon chain length is the best chain length for yielding antifungal activity. Kanamycin alkylated with 6, 7, 9 and 10 carbon chain length also show antifungal activity, but the antifungal activity diminish or disappear when the alkyl chain becomes too short (shorter than 6) or too long (longer than 10). In conclusion, we have provided a possible large scale synthesis route for the preparation of antifungal aminoglycosides. These new findings may expedite further research for amphiphilic aminoglycosides, whether to use amphiphilic aminoglycosides to fight against bacterial resistance or revive traditional amphiphilic aminoglycosides as a new type of antifungal agents.
3-5. Experimental Section

Proton magnetic resonance spectra were recorded using JEOL ECX-300, or Bruker ARX-400 spectrometer. Chemical shifts were reported as parts per million (ppm) downfield from tetramethylsilane in δ unit and coupling constants were given in cycles per second (Hz). Signal multiplicities were indicated by s (singlet), d (doublet), t (triplet), and q (quartet), m(multiplet). $^{13}$C NMR spectra were obtained using JEOL ECX-300 at 75 MHz, or Bruker ARX-400 at 100 MHz. Routine $^{13}$C NMR spectra were fully decoupled by broad-broad WALTZ decoupling. All NMR spectra were recorded at ambient temperature. Low-resolution fast-atom bombardment (LRFAB), high-resolution fast-atom bombardment (HRFAB), high-resolution MALDI, chemical ionization (CI), atmospheric pressure chemical ionization (APCI) or electrospray ionization (ESI) were provided by the Mass Spectrometry Facility, University of California, Riverside.

Chemical reagents and chromatography solvents were purchased from Aldrich Chemical Co. or Acros Chemcial Co. and were used without purification unless otherwise noted. Dichloromethane was freshly distilled from calcium hydride under nitrogen atmosphere. Pyridine and triethylamine were distilled and stored over 4Å molecular sieve. Column chromatographic purifications were carried out on silica gel 230x450 mesh, Sorbent Tech. Analytical TLC was performed on Sorbent Technologies silica gel glass TLC plates. Visualization was accomplished with UV light (254 or 366nm). TLC staining were performed by
treat with dilute sulfuric acid (5% in methanol) solution or treat in iodine chamber, TLC staining for compounds bearing amine were done by treat with ninhydrin (in ethanol) and heating.

**Procedure for Synthesis of 1,3,6′,3″-Tetraazidokanamycin.** To a solution of NaN₃ (25.4 g, 388 mmol) in a mixture of H₂O/CH₂Cl₂ (200 mL, 1:1 v/v) at 0°C, Tf₂O (33 mL, 199 mmol) was added. The reaction mixture was stirred at room temperature for 2 h. After been quenched with saturated aqueous NaHCO₃, the layers were separated, and the aqueous layer was extracted with CH₂Cl₂ (100 mL). The organic layers were combined to afford 200 mL of TfN₃ solution, which was added to a solution of kanamycin sulfate (9.45 g, 16.2 mmol) in H₂O (200 mL), MeOH (600 mL), and CuSO₄ (250 mg) prepared as a stock solution of MeOH/H₂O/NaHCO₃ (50 mL, 3/3/4 v/v/v). The reaction mixture was stirred at room temperature for 2 h. Then solid NaHCO₃ (20 g) was added carefully. The mixture was filtered through buchner funnel, and the residue organic solvents were concentrated by rotavapor. The crude product was purified by column chromatography with gradient (EtOAc/MeOH 100:0 to 50:50) to afford azidokanamycin, yield 60 - 85%. The synthesized 1,3,6′,3″-tetraazidokanamycin was characterized by H NMR and confirmed to be the right compound.

**General Procedure for O-Alkylation of 1,3,6′,3″-Tetraazidokanamycin.** To a solution of azidokanamycin (0.5 g, 0.85 mmol) in 20 mL of anhydrous DMF, NaH (20 equiv.) was added. Catalytic amount of TBAI was added as catalyst and the reaction mixture was stirred for 10 minutes before
adding the corresponding alkyl bromide (1.5 equiv.). The reaction mixture was stirred at room temperature overnight. Completion of the reaction was confirmed by TLC (Eluted with EtOAc), and the reaction was quenched by adding MeOH (5 mL). The mixture was concentrated and purified with a gradient column chromatography (eluted from EtOAc/Hexane 30:70 to EtOAc/MeOH 90:10) to afford three products. All alkylation products were characterized by H NMR, $^{13}$C NMR and mass spectrum.

**General Procedure for Staudinger Reaction.** The O-alkylated tetraazidokanamycin (0.2 g) was dissolved into 20 mL THF in a 50 mL round bottom flask. Several drops of water and 5 equiv. of PMe$_3$ (1 M solution in THF) was added to the solution. The reaction mixture was heated at 60°C for 2 hours. The reaction mixture was concentrated and then diluted with water. After been filtered through Celite, the crude product was loaded to a CG50 column (NH$_4^+$ form) and purified by a mixture of water and ammonium hydroxide (H$_2$O/NH$_2$OH 100:0 to 50:50) to afford the purified product. The product was concentrated and acidified with 5% HOAc in water to afford the acetate salt in water first. Then the aqueous solution was removed and re-dissolved into water. Eluting the aqueous solution containing acetate salt through ion exchange column (Dowex 1X8 chloride form) afforded the final product as chloride salt.

**4″-O-Butyl-1,3,6′,3″-tetraazidokanamycin (K404N3).**

$^1$H NMR (300 MHz, METHANOL-D4) δ 5.24 (d, $J = 3.78$ Hz, 1H), 5.18 (d, $J = 3.42$ Hz, 1H), 4.0-4.2 (m, 2H), 3.3 - 3.8, (m, 16H), 3.17 (t, $J = 9.9$ Hz, 1H), 2.3 - 2.4 (m, 1H), 2.0 - 2.1 (m, 1H), 1.5 - 1.6 (m, 3H), 1.2 - 1.4 (m, 2H), 0.89 (t, $J = 7.23$ Hz, 3H); $^{13}$C
NMR (100 MHz, METHANOL-D4) δ 101.17, 98.51, 83.82, 80.35, 76.35, 74.46, 73.54, 72.65, 72.24(2C), 71.39, 71.10, 70.85, 66.88, 61.02, 60.27, 59.63, 51.52, 32.331, 29.22, 19.10, 13.01. ESI/APCI calcd for C_{22}H_{36}N_{12}O_{11}Na ([M + Na]^+) m/z 667.2532; measured m/z 667.2516.

4″,6″-Di-O-hexyl-1,3,6′,3″-tetraazidokanamycin (K4606N3). $^1$H NMR (300 MHz, METHANOL-D4) δ 5.24 (d, J = 3.78 Hz, 1H), 5.16 (d, J = 3.78 Hz, 1H), 4.1-4.2 (m, 1H), 4.0-4.1 (m, 1H), 3.3 - 3.7, (m, 18H), 3.18 (t, J = 9.8 Hz, 1H), 2.3 - 2.4 (m, 1H), 1.5 - 1.6 (m, 5H), 1.2 - 1.4 (m, 12H), 0.89 (t, J = 6.4 Hz, 6H). $^{13}$C NMR (100 MHz, METHANOL-D4) δ 101.16, 98.44, 83.83, 80.22, 76.65, 74.40, 73.56, 72.65, 72.56, 72.24, 71.40, 71.12, 70.85, 70.49, 68.90, 66.97, 61.05, 59.63, 51.52, 32.20, 31.63(2C), 30.11, 29.47, 25.87(2C), 22.50(2C), 13.18(2C). ESI/APCI calcd for C_{30}H_{52}N_{12}O_{11}Na ([M + Na]^+) m/z 779.3784; measured m/z 779.3785.

4″-O-Hexyl-1,3,6′,3″-tetraazidokanamycin (K406N3). $^1$H NMR (300 MHz, METHANOL-D4) δ 5.23 (d, J = 3.78 Hz, 1H), 5.18 (d, J = 3.78 Hz, 1H), 4.1-4.0 (m, 2H), 3.8 - 3.3, (m, 16H), 3.17 (t, J = 9.8 Hz, 1H), 2.4 - 2.3 (m, 1H), 1.6 - 1.5 (m, 3H), 1.4 - 1.2 (m, 6H), 0.89 (t, J = 6.4 Hz, 3H); $^{13}$C NMR (100 MHz, METHANOL-D4) δ 101.18, 98.51, 83.80, 80.37, 76.36, 74.49, 73.55, 72.65, 72.54, 72.25, 71.42, 71.14, 70.86, 66.91, 61.03, 60.30, 59.64, 51.53, 31.63, 30.16, 29.52, 29.22, 25.71, 22.48, 13.17. ESI/APCI calcd for C_{24}H_{40}N_{12}O_{11}Na ([M + Na]^+) m/z 695.2856; measured m/z 695.2857.
4″,6″-Di-O-heptyl-1,3,6′,3″-tetraazidokanamycin (K4607N3). \(^1\)H NMR (300 MHz, METHANOL-D4) \(\delta\) 5.24 (d, \(J = 3.8\) Hz, 1H), 5.16 (d, \(J = 3.8\) Hz, 1H), 4.2 – 4.1 (m, 1H), 4.1 – 4.0 (m, 1H), 3.8 – 3.3 (m, 18H), 3.18 (t, \(J = 10.0\) Hz, 1H), 2.34 (ddd, \(J = 18.9, 9.4, 4.5\) Hz, 1H)), 1.6 – 1.5 (m, 5H), 1.4 – 1.2 (m, 16H), 0.89 (t, \(J = 6.5\) Hz, 6H); \(^{13}\)C NMR (100 MHz, METHANOL-D4) \(\delta\) 101.2, 98.5, 83.8, 80.2, 76.7, 74.4, 73.6, 72.7, 72.7, 72.2, 71.4, 71.1, 70.9, 70.5, 68.9, 66.9, 61.1, 59.6, 51.5, 31.8(2C), 30.2, 29.5(2C), 29.1(2C), 26.2, 26.0, 22.6(2C), 13.2(2C). ESI/APCI calcd for C\(_{32}\)H\(_{56}\)N\(_{12}\)O\(_{11}\)Na ([M + Na\(^+\)] \(m/z\) 807.4084; measured \(m/z \) 807.4101.

4″-O-Heptyl-1,3,6′,3″-tetraazidokanamycin (K407N3). \(^1\)H NMR (300 MHz, METHANOL-D4) \(\delta\) 5.24 (d, \(J = 3.8\) Hz, 1H), 5.18 (d, \(J = 3.8\) Hz, 1H), 4.2 – 4.1 (m, 1H), 4.1 – 4.0 (m, 1H), 3.8 – 3.3 (m, 16H), 3.18 (t, \(J = 9.6\) Hz, 1H), 2.34 (ddd, \(J = 12.7, 9.4, 4.5\) Hz, 1H)), 1.6 – 1.5 (m, 3H), 1.4 – 1.2 (m, 8H), 0.86 (t, \(J = 6.5\) Hz, 3H); \(^{13}\)C NMR (100 MHz, METHANOL-D4) \(\delta\) 101.2, 98.5, 83.8, 80.3, 76.4, 74.5, 73.6, 72.7, 72.5, 72.3, 71.4, 71.1, 70.9, 66.9, 61.1, 60.3, 59.7, 51.5, 32.2, 31.8, 29.5, 29.1, 25.9, 22.4, 13.2. ESI/APCI calcd for C\(_{25}\)H\(_{42}\)N\(_{12}\)O\(_{11}\)Na ([M + Na\(^+\)] \(m/z\) 709.2988; measured \(m/z \) 709.3000.

4″,6″-Di-O-octyl-1,3,6′,3″-tetraazidokanamycin(K4608N3). \(^1\)H NMR (300 MHz, METHANOL-D4) \(\delta\) 5.24 (d, \(J = 3.8\) Hz, 1H), 5.17 (d, \(J = 3.8\) Hz, 1H), 4.2 – 4.1 (m, 1H), 4.1 – 4.0 (m, 1H), 3.8 – 3.3 (m, 18H), 3.18 (t, \(J = 10.0\) Hz, 1H), 2.34 (ddd, \(J = 18.9, 9.4, 4.5\) Hz, 1H)), 1.6 – 1.5 (m, 5H), 1.4 – 1.2 (m, 20H), 0.88 (t, \(J = 6.8\) Hz, 6H); \(^{13}\)C NMR (100 MHz, METHANOL-D4) \(\delta\) 101.2, 98.4, 83.8, 80.2, 76.7, 74.4, 73.6, 72.7, 72.6, 72.3, 71.4, 71.1, 70.9, 70.5, 68.9, 66.9, 61.0,
59.6, 51.5, 32.2, 31.8(2C), 30.2, 29.5, 29.4(2C), 29.3(2C), 26.2, 26.1, 22.6(2C), 13.3(2C). ESI/APCI calcd for C_{34}H_{60}N_{12}O_{11}Na ([M + Na]^+) m/z 835.4397; measured m/z 835.4397.

6”-O-Octyl-1,3,6′,3″-tetraazidokanamycin (K608N3). \(^1\)H NMR (300 MHz, METHANOL-D4) δ 5.26 (d, J = 4.1 Hz, 1H), 5.19 (d, J = 3.4 Hz, 1H), 4.2 – 4.1 (m, 1H), 4.1 – 4.0 (m, 1H), 3.8 – 3.3 (m, 17H), 2.34 (ddd, J = 16.8, 8.6, 4.1 Hz, 1H), 1.6 – 1.5 (m, 3H), 1.4 – 1.2 (m, 10H), 0.86 (t, J = 6.2 Hz, 3H); \(^{13}\)C NMR (100 MHz, METHANOL-D4) δ 101.1, 98.4, 83.7, 80.3, 76.4, 74.5, 73.6, 72.6, 72.2, 71.6, 71.1, 70.9, 69.3, 68.8, 67.3, 60.9, 59.6, 51.5, 32.2, 31.8, 29.5, 29.4, 29.3, 26.0, 22.5, 13.3; ESI/APCI calcd for C_{26}H_{44}N_{12}O_{11}Na ([M + Na]^+) m/z 723.3145; measured m/z 723.3165.

4”-O-Octyl-1,3,6′,3″-tetraazidokanamycin (K408N3). \(^1\)H NMR (300 MHz, METHANOL-D4) δ 5.24 (d, J = 3.8 Hz, 1H), 5.19 (d, J = 3.8 Hz, 1H), 4.2 – 4.1 (m, 1H), 4.1 – 4.0 (m, 1H), 3.8 – 3.3 (m, 16H), 3.17 (t, J = 9.8 Hz, 1H), 2.34 (ddd, J = 16.8, 8.6, 4.1 Hz, 1H), 1.6 – 1.5 (m, 3H), 1.4 – 1.2 (m, 10H), 0.86 (t, J = 6.8 Hz, 3H); \(^{13}\)C NMR (100 MHz, METHANOL-D4) δ 101.2, 98.5, 83.8, 80.4, 76.4, 74.5, 73.6, 72.7, 72.6, 72.2, 71.4, 71.1, 70.8, 66.9, 61.0, 59.7, 51.5, 32.2, 31.8, 30.2, 29.5, 29.4, 29.2, 26.1, 22.5, 13.2; ESI/APCI calcd for C_{26}H_{44}N_{12}O_{11}Na ([M + Na]^+) m/z 723.3145; measured m/z 723.3157.

4″,6″-Di-O-nonyl-1,3,6′,3″-tetraazidokanamycin (K4609N3). \(^1\)H NMR (300 MHz, METHANOL-D4) δ 5.25 (d, J = 3.8 Hz, 1H), 5.17 (d, J = 3.8 Hz, 1H), 4.2 – 4.1 (m, 1H), 4.1 – 4.0 (m, 1H), 3.8 – 3.3 (m, 18H), 3.19 (t, J = 9.8 Hz, 1H), 2.4 – 2.3 (m, 1H), 1.6 – 1.5 (m, 5H), 1.4 – 1.2 (m, 24H), 0.88 (t, J = 6.5 Hz,
$\delta$ 101.2, 98.4, 83.8, 80.2, 76.7, 74.4, 73.6, 72.7, 72.6, 72.2, 71.4, 71.1, 70.9, 70.5, 68.9, 66.9, 61.0, 59.6, 51.5, 32.3, 31.9(2C), 30.2, 29.6(2C), 29.5(2C), 29.4(2C), 29.3, 26.2, 26.1, 22.6(2C), 13.3(2C); ESI/APCI calcd for C$_{36}$H$_{64}$N$_{12}$O$_{11}$Na ([M + Na]$^+$) $m/z$ 863.4710; measured $m/z$ 863.4710.

6″-O-Nonyl-1,3,6′,3″-tetraazidokanamycin (K609N3). $^1$H NMR (300 MHz, METHANOL-D4) δ 5.26 (d, $J = 3.8$ Hz, 1H), 5.18 (d, $J = 3.8$ Hz, 1H), 4.2 – 4.1 (m, 1H), 4.1 – 4.0 (m, 1H), 3.8 – 3.3 (m, 17H), 2.4 – 2.3 (m, 1H), 1.6 – 1.5 (m, 3H), 1.4 – 1.2 (m, 12H), 0.88 (t, $J = 7.2$ Hz, 3H); $^{13}$C NMR (100 MHz, METHANOL-D4) δ 101.1, 98.4, 83.7, 80.4, 74.5, 73.6, 72.7, 72.2, 71.6, 71.2, 71.1, 70.9, 69.3, 68.9, 67.3, 60.9, 59.6, 51.5, 32.2, 31.9, 29.5, 29.45, 29.2, 26.0, 25.4, 22.5, 13.2; ESI/APCI calcd for C$_{27}$H$_{46}$N$_{12}$O$_{11}$Na ([M + Na]$^+$) $m/z$ 737.3301; measured $m/z$ 737.3316.

4″-O-Nonyl-1,3,6′,3″-tetraazidokanamycin (K409N3). $^1$H NMR (300 MHz, METHANOL-D4) δ 5.24 (d, $J = 4.1$ Hz, 1H), 5.18 (d, $J = 3.4$ Hz, 1H), 4.2 – 4.1 (m, 1H), 4.1 – 4.0 (m, 1H), 3.8 – 3.3 (m, 16H), 3.17 (t, $J = 9.6$ Hz, 1H), 2.4 – 2.3 (m, 1H), 1.6 – 1.5 (m, 3H), 1.4 – 1.2 (m, 12H), 0.88 (t, $J = 7.2$ Hz, 3H); $^{13}$C NMR (100 MHz, METHANOL-D4) δ 101.2, 98.5, 83.8, 80.4, 76.4, 74.5, 73.6, 72.7, 72.5(2C), 72.2, 71.4, 71.2, 70.9, 66.9, 61.0, 59.7, 51.5, 32.2, 31.8, 30.2, 29.5, 29.4, 29.2, 26.0, 22.5, 13.2; ESI/APCI calcd for C$_{27}$H$_{46}$N$_{12}$O$_{11}$Na ([M + Na]$^+$) $m/z$ 737.3301; measured $m/z$ 737.3312.
4″,6″-Di-O-decyl-1,3,6′,3″-tetraazidokanamycin(K4610N3). $^1$H NMR (300 MHz, METHANOL-D4) δ 5.24 (d, J = 3.8 Hz, 1H), 5.16 (d, J = 3.8 Hz, 1H), 4.2 – 4.1 (m, 1H), 4.1 – 4.0 (m, 1H), 3.8 – 3.3 (m, 18H), 3.19 (t, J = 10.0 Hz, 1H), 2.4 – 2.3 (m, 1H), 1.6 – 1.5 (m, 5H), 1.4 – 1.2 (m, 28H), 0.88 (t, J = 6.5 Hz, 6H); $^{13}$C NMR (100 MHz, METHANOL-D4) δ 101.2, 98.4, 83.8, 80.2, 76.7, 74.4, 73.6, 72.7, 72.6, 72.3, 71.4, 71.1, 70.9, 70.5, 68.9, 66.9, 61.0, 59.6, 51.5, 32.2, 31.9(2C), 30.1, 29.6(5C), 29.4(2C), 29.3(2C), 26.2, 26.1, 22.6(2C), 13.3(2C); ESI/APCI calcd for C$_{38}$H$_{68}$N$_{12}$O$_{11}$Na ([M + Na]$^+$) 891.5023; measured m/z 891.4995.

6″-O-Decyl-1,3,6′,3″-tetraazidokanamycin (K610N3). $^1$H NMR (300 MHz, METHANOL-D4) δ 5.26 (d, J = 3.8 Hz, 1H), 5.19 (d, J = 3.8 Hz, 1H), 4.2 – 4.1 (m, 1H), 4.1 – 4.0 (m, 1H), 3.8 – 3.3 (m, 17H), 2.4 – 2.3 (m, 1H), 1.6 – 1.5 (m, 3H), 1.4 – 1.2 (m, 14H), 0.88 (t, J = 7.2 Hz, 3H); $^{13}$C NMR (100 MHz, METHANOL-D4) δ 101.2, 98.4, 83.8, 80.2, 76.7, 74.4, 73.6, 72.7, 72.6, 71.4, 71.1, 70.9, 70.5, 68.9, 67.0, 61.0, 59.6, 51.5, 32.2, 31.9, 29.6(2C), 29.4, 29.3, 26.2, 26.1, 22.5, 13.3; ESI/APCI calcd for C$_{28}$H$_{48}$N$_{12}$O$_{11}$Na ([M + Na]$^+$) 751.3466; measured m/z 751.3466.

4″-O-Decyl-1,3,6′,3″-tetraazidokanamycin(K410N3). $^1$H NMR (300 MHz, METHANOL-D4) δ 5.24 (d, J = 4.1 Hz, 1H), 5.18 (d, J = 3.5 Hz, 1H), 4.2 – 4.1 (m, 1H), 4.1 – 4.0 (m, 1H), 3.8 – 3.3 (m, 16H), 3.17 (t, J = 9.8 Hz, 1H), 2.4 – 2.3 (m, 1H), 1.6 – 1.5 (m, 3H), 1.4 – 1.2 (m, 18H), 0.88 (t, J = 6.5 Hz, 3H); $^{13}$C NMR (100 MHz, METHANOL-D4) δ 101.1, 98.5, 83.8, 80.4, 76.4, 74.5, 73.6,
72.7, 72.5, 72.2, 71.4, 71.1, 70.9, 66.9, 61.0, 60.3, 59.6, 51.5, 32.2, 31.8, 29.6,
29.5, 29.4, 29.2, 26.0, 25.4, 22.5, 13.2; ESI/APCI calcd for C_{28}H_{48}N_{12}O_{11}Na ([M + Na]^+) 751.3466; measured \textit{m/z} 751.3477.

4″,6″-Di-O-dodecyl-1,3,6′,3″-tetraazidokanamycin (K4612N3). $^1$H NMR (300 MHz, METHANOL-D4) $\delta$ 5.24 (d, $J = 3.8$ Hz, 1H), 5.16 (d, $J = 3.8$ Hz, 1H), 4.2 – 4.1 (m, 1H), 4.1 – 4.0 (m, 1H), 3.8 – 3.3 (m, 17H), 3.19 (t, $J = 10.0$ Hz, 1H), 2.4 – 2.3 (m, 1H), 1.6 – 1.5 (m, 5H), 1.4 – 1.2 (m, 36H), 0.88 (t, $J = 7.2$ Hz, 6H); $^{13}$C NMR (100 MHz, METHANOL-D4) $\delta$ 101.2, 98.4, 83.8, 80.2, 76.7, 74.4, 73.6, 72.7, 72.6, 72.2, 71.4, 71.1, 70.9, 70.5, 68.9, 66.9, 61.0, 59.6, 54.5, 32.2, 31.9(2C), 30.1, 29.6(9C), 29.4(2C), 29.3(2C), 26.2, 26.1, 22.6(2C), 13.3(2C); ESI/APCI calcd for C_{42}H_{75}N_{12}O_{11}Na ([M + Na]^+) 947.5649; measured \textit{m/z} 947.5681.

6″-O-Dodecyl-1,3,6′,3″-tetraazidokanamycin (K612N3). $^1$H NMR (300 MHz, METHANOL-D4) $\delta$ 5.26 (d, $J = 3.8$ Hz, 1H), 5.16 (d, $J = 3.8$ Hz, 1H), 4.2 – 4.1 (m, 1H), 4.1 – 4.0 (m, 1H), 3.8 – 3.3 (m, 17H), 2.4 – 2.3 (m, 1H), 2.1 – 2.0 (m, 1H), 1.6 – 1.5 (m, 2H), 1.4 – 1.2 (m, 18H), 0.88 (t, $J = 6.2$ Hz, 3H); $^{13}$C NMR (100 MHz, METHANOL-D4) $\delta$ 101.1, 98.4, 83.7, 80.3, 74.5, 73.6, 72.7, 72.2, 71.6, 71.2, 71.1, 70.9, 69.3, 68.9, 67.3, 60.9, 59.6, 51.5, 32.2, 31.9, 29.6(6C), 29.3, 26.0, 22.5, 13.2; ESI/APCI calcd for C_{30}H_{52}N_{12}O_{11}Na ([M + Na]^+) 779.3711; measured \textit{m/z} 779.3797.

4″-O-Dodecyl-1,3,6′,3″-tetraazidokanamycin (K412N3). $^1$H NMR (300 MHz, METHANOL-D4) $\delta$ 5.24 (d, $J = 4.1$ Hz, 1H), 5.18 (d, $J = 3.5$ Hz, 1H), 4.2 –
4.1 (m, 1H), 4.1 - 4.0 (m, 1H), 3.8 - 3.3 (m, 16H), 3.17 (t, J = 9.8 Hz, 1H), 2.4 - 2.3 (m, 1H), 2.1 - 2.0 (m, 1H), 1.6 - 1.5 (m, 2H), 1.4 - 1.2 (m, 18H), 0.88 (t, J = 6.5 Hz, 3H); 13C NMR (100 MHz, METHANOL-D4) δ 101.1, 98.5, 83.8, 80.3, 76.4, 74.5, 73.6, 72.7, 72.5, 72.2, 71.4, 71.2, 70.9, 66.9, 61.0, 60.3, 59.7, 51.5, 32.2, 31.9, 30.2, 29.6(4C), 29.4, 29.2, 26.0, 22.5, 13.2; ESI/APCI calcd for C30H52N12O11Na ([M + Na]+) 779.3711; measured m/z 779.3791.

4″,6″-Di-O-tetradecyl-1,3,6′,3″-tetraazidokanamycin (K4614N3). 1H NMR (300 MHz, METHANOL-D4) δ 5.25 (d, J = 3.78 Hz, 1H), 5.18 (d, J = 3.78 Hz, 1H), 4.1-4.2 (m, 1H), 4.0- 4.1 (m, 1H), 3.3 - 3.7, (m, 18H), 3.20 (t, J = 9.6 Hz, 1H), 2.3 - 2.4 (m, 1H), 1.5 - 1.6 (m, 5H), 1.2 - 1.4 (m, 44H), 0.89 (t, J = 6.6 Hz, 6H); 13C NMR (100 MHz, METHANOL-D4) δ 101.13, 98.39, 83.79, 80.17, 78.27, 76.65, 74.36, 73.56, 72.64, 72.24, 71.37, 71.12, 70.84, 70.48, 68.90, 66.97, 61.02, 59.62, 51.62, 32.27, 31.25(2C), 30.15(2C), 29.66(10C), 29.55(2C), 29.43(2C), 29.35(2C), 26.28, 26.07, 22.59(2C), 13.71(2C); ESI/APCI calcd for C46H84N12O11Na ([M + Na]+) 1003.6275; measured m/z 1003.6308.

4″-O-Tetradecyl-1,3,6′,3″-tetraazidokanamycin (K414N3). 1H NMR (300 MHz, METHANOL-D4) δ 5.25 (d, J = 3.78 Hz, 1H), 5.18 (d, J = 3.78 Hz, 1H), 4.0- 4.1 (m, 2H), 3.3 - 3.8, (m, 16H), 3.18 (t, J = 9.9 Hz, 1H), 2.3 - 2.4 (m, 1H), 1.5 - 1.6 (m, 3H), 1.2 - 1.4 (m, 22H), 0.89 (t, J = 6.5 Hz, 3H); 13C NMR (100 MHz, METHANOL-D4) δ 101.14, 98.43, 83.82, 80.17, 76.64, 74.38, 73.56,72.66, 72.24, 71.34, 71.10, 70.84, 70.46, 68.89, 66.96, 61.05, 59.62, 51.51, 31.89,
30.10, 29.60(6C), 29.43, 29.29, 26.23, 26.03, 22.54, 13.71; ESI/APCI calcd for C_{32}H_{56}N_{12}O_{11}Na ([M + Na]^+) 807.4084; measured m/z 807.4084.

4″,6″-Di-O-hexadecyl-1,3,6′,3″-tetraazidokanamycin (K4616N3).\textsuperscript{1}H NMR (300 MHz, METHANOL-D4) δ 5.24 (d, J = 3.8 Hz, 1H), 5.18 (d, J = 3.8 Hz, 1H), 4.2 – 4.1 (m, 1H), 4.1 – 4.0 (m, 1H), 3.8 – 3.3 (m, 18H ), 3.16 (t, J = 9.6 Hz, 1H), 2.4 – 2.3 (m, 1H), 1.6 – 1.5 (m, 5H), 1.4 – 1.2 (m, 52H), 0.88 (t, J = 6.9 Hz, 6H); \textsuperscript{13}C NMR (100 MHz, METHANOL-D4) δ 101.1, 98.3, 83.8, 80.2, 76.7, 74.3, 73.6, 72.6(2C), 72.2, 71.4, 71.1, 70.9, 70.5, 68.9, 66.9, 60.9, 59.6, 51.5, 32.3, 31.9(2C), 30.2, 29.7(15), 29.5(2C), 29.48(2C), 29.4(2C), 26.3, 26.1, 22.6(2C), 13.4(2C); ESI/APCI calcd for C_{50}H_{92}N_{12}O_{11}Na ([M + Na]^+) 1059.6901; measured m/z 1059.6921.

4″-O-Hexadecyl-1,3,6′,3″-tetraazidokanamycin (K416N3).\textsuperscript{1}H NMR (300 MHz, METHANOL-D4) δ 5.24 (d, J = 3.8 Hz, 1H), 5.18 (d, J = 3.8 Hz, 1H), 4.1 – 4.0 (m, 2H), 3.8 – 3.3 (m, 18H ), 3.16 (t, J = 9.6 Hz, 1H), 2.4 – 2.3 (m, 1H), 1.6 – 1.5 (m, 3H), 1.4 – 1.2 (m, 26H), 0.88 (t, J = 6.9 Hz, 3H); \textsuperscript{13}C NMR (100 MHz, METHANOL-D4) δ 102.5, 99.9, 85.1, 81.7, 77.7, 75.8, 74.9, 74.1, 74.0, 73.9, 73.6, 72.8, 72.5, 72.2, 68.3, 62.4, 61.6, 61.0, 52.9, 33.6, 33.2, 31.5, 30.9(7C), 30.7, 30.6, 27.4, 23.9, 14.6; ESI/APCI calcd for C_{34}H_{60}N_{12}O_{11}Na ([M + Na]^+) 835.4397; measured m/z 835.4416.

4″,6″-Di-O-butyl kanamycin(K4604).\textsuperscript{1}H NMR (300 MHz, D\textsubscript{2}O) δ 5.47 (d, J = 3.78 Hz, 1H), 4.96 (d, J = 3.78 Hz, 1H), 3.3 – 4.1 (m, 19H), 3.0 - 3.1 (m, 1H), 2.3 - 2.4 (m, 1H), 1.7 - 1.8 (m, 1H), 1.4 - 1.5 (m, 5H), 1.1 - 1.3 (m, 4H), 0.7 - 0.8 (m,
6H). $^{13}$C NMR (100 MHz, D$_2$O) $\delta$ 101.64, 96.87, 84.46, 79.36, 74.01, 73.51, 73.44, 73.19, 72.31, 71.07(2C), 71.00, 68.82, 68.47, 68.17, 54.51, 50.26, 48.25, 40.57, 31.51, 30.91, 28.84, 18.97, 18.79, 13.42(2C). ESI/APCI calcd for C$_{26}$H$_{53}$N$_4$O$_{11}$ ([M + H]$^+$) 597.3705; measured m/z 597.3701.

**4″-O-Buty kanamycin (K404).** $^1$H NMR (300 MHz, D$_2$O) $\delta$ 5.44 (d, $J = 3.78$ Hz, 1H), 5.00 (d, $J = 3.78$ Hz, 1H), 3.2 - 4.0 (m, 17H), 3.0 - 3.1 (m, 1H), 2.4 - 2.5 (m, 1H), 1.7 - 1.8 (m, 1H), 1.4 - 1.5 (m, 3H), 1.1 - 1.3 (m, 2H), 0.7 - 0.8 (t, $J = 7.56$ Hz, 3H); $^{13}$C NMR (100 MHz, D$_2$O) $\delta$ 101.60, 96.09, 84.12, 78.59, 73.59, 73.44, 72.51, 72.47, 72.35, 72.22, 71.90(2C), 68.88, 68.31, 59.87, 54.40, 49.97, 40.46, 31.50, 27.79, 18.68, 13.33; ESI/APCI calcd for C$_{22}$H$_{45}$N$_4$O$_{11}$ ([M + H]$^+$) 541.3079; measured m/z 541.3082.

**4″,6″-Di-O-hexyl kanamycin (K4606).** $^1$H NMR (300 MHz, D$_2$O) $\delta$ 5.52 (d, $J = 4.11$ Hz, 1H), 5.16 (d, $J = 3.45$ Hz, 1H), 3.3 - 4.1 (m, 19H), 3.0 - 3.1 (m, 1H), 2.4 - 2.5 (m, 1H), 1.8 - 1.9 (m, 1H), 1.4 - 1.5 (m, 5H), 1.1 - 1.3 (m, 12H), 0.7 - 0.8 (m, 6H); $^{13}$C NMR (100 MHz, D$_2$O) $\delta$ 100.81, 97.09, 83.89, 78.27, 73.76, 73.67, 73.28, 72.12, 71.68, 71.11, 71.03, 71.00, 68.88, 68.31, 68.26, 54.48, 50.06, 48.24, 40.56, 31.25(2C), 29.31, 28.82, 27.86, 25.51, 25.16, 22.35(2C), 13.71(2C). ESI/APCI calcd for C$_{30}$H$_{61}$N$_4$O$_{11}$ ([M + H]$^+$) 653.4331; measured m/z 653.4324.

**4″-O-Hexyl kanamycin (K406).** $^1$H NMR (300 MHz, D$_2$O) $\delta$ 5.44 (d, $J = 3.78$ Hz, 1H), 5.00 (d, $J = 3.78$ Hz, 1H), 3.2 - 4.0 (m, 17H), 3.0 - 3.1 (m, 1H), 2.4 - 2.5 (m, 1H), 1.7 - 1.8 (m, 1H), 1.4 - 1.5 (m, 3H), 1.1 - 1.3 (m, 2H), 0.7 - 0.8
(t, J = 7.56 Hz, 3H); $^{13}$C NMR (100 MHz, D$_2$O) δ 101.60, 96.09, 84.12, 78.59, 73.59, 73.44, 72.51, 72.47, 72.35, 72.22, 71.90(2C), 68.88, 68.31, 59.87, 54.40, 49.97, 40.46, 31.50, 27.79, 18.68, 13.33; ESI/APCI calcd for C$_{24}$H$_{49}$N$_4$O$_{11}$ ([M + H]$^+$) 569.3392; measured m/z 569.3391.

4″,6″-Di-O-heptyl kanamycin (K4607).$^1$H NMR (300 MHz, D$_2$O) δ 5.51 (d, J = 3.4 Hz, 1H), 4.98 (d, J = 3.4 Hz, 1H), 4.1 – 3.3 (m, 20H), 3.1 – 3.0 (m, 1H), 2.5 – 2.4 (m, 1H), 1.9 – 1.8 (m, 1H), 1.6 – 1.5 (m, 4H), 1.3 – 1.1 (m, 16H), 0.75 (t, J = 6.5 Hz, 6H); $^{13}$C NMR (100 MHz, D$_2$O) δ 100.8, 96.7, 83.9, 78.4, 73.8, 73.0, 72.3, 71.8, 71.2, 71.0, 70.9, 68.9, 68.4, 68.3, 68.2, 54.5, 50.1, 48.1, 40.5, 31.4, 31.35, 29.3, 28.8, 28.6(2C), 27.9, 25.7, 25.3, 22.2(2C), 13.7(2C). ESI/APCI calcd for C$_{32}$H$_{65}$N$_4$O$_{11}$ ([M + H]$^+$) 681.4644; measured m/z 681.4643.

4″-O-Heptyl kanamycin (K407).$^1$H NMR (300 MHz, D$_2$O) δ 5.52 (d, J = 4.1 Hz, 1H), 5.00 (d, J = 3.8 Hz, 1H), 4.0 – 3.2 (m, 18H), 3.1 – 3.0 (m, 1H), 2.6 – 2.5 (m, 1H), 1.9 – 1.8 (m, 1H), 1.5 – 1.4 (m, 2H), 1.2 – 1.1 (m, 8H), 0.75(t, J = 6.9 Hz, 3H); $^{13}$C NMR (100 MHz, D$_2$O) δ 100.6, 96.1, 84.1, 78.5, 73.9, 73.7, 72.5, 72.3, 72.25, 70.9(2C), 68.9, 68.3, 59.9, 54.4, 49.9, 47.8, 40.5, 31.2, 29.4, 28.5, 27.8, 25.2, 22.1, 13.6; ESI/APC calcd for C$_{25}$H$_{51}$N$_4$O$_{11}$ ([M + H]$^+$) 583.3549; measured m/z 583.3570.

4″,6″-Di-O-octyl kanamycin (K4608).$^1$H NMR (300 MHz, METHANOL-D4) δ 5.59 (d, J = 3.8 Hz, 1H), 5.10 (d, J = 3.8 Hz, 1H), 4.1 – 3.4 (m, 20H), 3.0 – 2.9 (m, 1H), 2.6 – 2.5 (m, 1H), 2.1 – 2.0 (m, 1H), 1.6 – 1.5 (m, 4H), 1.4 – 1.2 (m, 20H), 0.88 (t, J = 6.8 Hz, 6H); $^{13}$C NMR (100 MHz, D$_2$O) δ 100.9, 97.8, 83.8,
78.2, 73.8, 73.7, 72.0, 71.7(2C), 71.2, 68.9, 68.4, 68.0, 54.5, 50.1, 48.50, 48.24, 43.9, 40.7, 31.97(2C), 30.3, 29.5(3C), 29.2, 26.5, 26.2, 25.8, 25.2, 22.8(2C), 14.03(2C). ESI/APCI calcd for C$_{34}$H$_{69}$N$_{4}$O$_{11}$ ([M + H]$^+$) 709.4957; measured m/z 709.4962.

6″-O-Octyl kanamycin (K608). $^1$H NMR (300 MHz, D$_2$O) δ 5.53 (d, $J = 3.8$ Hz, 1H), 5.00 (d, $J = 3.8$ Hz, 1H), 4.0 – 3.2 (m, 18H), 3.1 – 3.0 (m, 1H), 2.5 – 2.4 (m, 1H), 1.9 – 1.8 (m, 1H), 1.5 – 1.4 (m, 2H), 1.4 – 1.2 (m, 10H), 0.74 (t, $J = 6.9$ Hz, 3H); $^{13}$C NMR (100 MHz, D$_2$O) δ 100.8, 96.4, 84.1, 77.9, 73.1, 72.3, 72.2, 72.0(2C), 71.1, 68.9, 68.6, 68.4, 65.9, 55.3, 50.1, 48.2, 40.7, 31.4, 31.3, 28.8, 28.7, 28.6, 25.4, 22.3, 13.8; ESI/APCI calcd for C$_{26}$H$_{53}$N$_{4}$O$_{11}$ ([M + H]$^+$) 597.3705; measured m/z 597.3705.

4″-O-Octyl kanamycin (K408). $^1$H NMR (300 MHz, D$_2$O) δ 5.44 (d, $J = 3.8$ Hz, 1H), 5.00 (d, $J = 3.8$ Hz, 1H), 4.0 – 3.2 (m, 18H), 3.1 – 3.0 (m, 1H), 2.5 – 2.4 (m, 1H), 1.9 – 1.8 (m, 1H), 1.5 – 1.4 (m, 2H), 1.2 – 1.1 (m, 10H), 0.74 (t, $J = 6.9$ Hz, 3H); $^{13}$C NMR (100 MHz, D$_2$O) δ 100.6, 96.1, 84.1, 78.4, 73.8, 73.7, 72.5, 72.3, 72.2, 72.0, 70.9, 68.8, 68.3, 59.9, 54.4, 49.9, 47.8, 40.5, 31.2, 29.3, 28.7, 28.5, 27.7, 25.2, 22.2, 13.6; ESI/APCI calcd for C$_{26}$H$_{53}$N$_{4}$O$_{11}$ ([M + H]$^+$) 597.3705; measured m/z 597.3697.

4″,6″-Di-O-nonyl kanamycin (K4609). $^1$H NMR (300 MHz, METHANOL-D4) δ 5.57 (d, $J = 3.8$ Hz, 1H), 5.09 (d, $J = 3.4$ Hz, 1H), 4.1 – 3.0 (m, 21H), 2.5 – 2.4 (m, 1H), 2.0 – 1.9 (m, 1H), 1.6 – 1.5 (m, 4H), 1.3 – 1.2 (m, 24H), 0.87 (t, $J = 6.9$ Hz, 6H); $^{13}$C NMR (101 MHz, METHANOL-D4) δ 100.8,
95.2, 84.3, 78.5, 74.0, 73.2, 72.9, 72.3, 71.9, 71.85, 71.8(2C), 71.5, 69.1, 68.9,
68.7, 55.0, 50.4, 41.1, 31.9(2C), 29.8, 29.6(3C), 29.5(2C), 29.3(2C), 27.7, 26.2,
25.9, 22.5(2C), 13.2(2C). ESI/APCI calcd for C_{36}H_{73}N_{4}O_{11} ([M + H]^+) 737.5270;
measured m/z 737.5293.

6″-O-Nonyl kanamycin (K609).\textsuperscript{1}H NMR (300 MHz, D_{2}O) δ 5.52 (d, J =
3.4 Hz, 1H), 4.99 (d, J = 3.8 Hz, 1H), 4.0 – 3.0 (m, 19H), 2.5 – 2.4 (m, 1H), 1.9 –
1.7 (m, 1H), 1.5 – 1.4 (m, 2H), 1.3 – 1.1 (m, 12H), 0.75 (t, J = 6.8 Hz, 3H); \textsuperscript{13}C
NMR (100 MHz, D_{2}O) δ 100.7, 96.6, 84.3, 78.5, 73.1, 72.3, 72.2, 72.9, 71.9,
71.0, 68.9, 68.5, 68.3, 65.9, 55.2, 50.1, 48.2, 40.6, 31.4, 28.9, 28.8, 28.7, 25.3,
25.4, 22.3, 13.6; ESI/APCI calcd for C_{27}H_{55}N_{4}O_{11} ([M + H]^+) 611.3862; measured
m/z 611.3862.

4″-O-Nonyl kanamycin (K409).\textsuperscript{1}H NMR (300 MHz, D_{2}O) δ 5.43 (d, J = 3.8 Hz,
1H), 4.99 (d, J = 3.4 Hz, 1H), 4.0 – 3.0 (m, 23H), 2.3 – 2.2 (m, 1H), 1.7 – 1.6 (m,
1H), 1.5 – 1.4 (m, 2H), 1.3 – 1.1 (m, 12H), 0.75 (t, J = 6.9 Hz, 3H); \textsuperscript{13}C NMR (100
MHz, D_{2}O) δ 100.5, 96.9, 84.9, 80.5, 74.3, 73.7, 73.2, 72.4, 72.1, 71.1, 71.0,
68.8, 68.7, 59.9, 54.5, 50.3, 48.2, 40.5, 31.3, 29.8, 29.4, 28.8, 28.7, 28.6, 25.3,
22.2, 13.6; ESI/APCI calcd for C_{27}H_{55}N_{4}O_{11} ([M + H]^+) 611.3862; measured m/z
611.3868.

4″,6″-Di-O-decyl kanamycin (K4610).\textsuperscript{1}H NMR (300 MHz, METHANOL-D4) δ 5.57 (d, J = 3.8 Hz, 1H), 5.09 (d, J = 3.4 Hz, 1H), 4.1 – 3.0 (m, 21H), 2.5 –
2.4 (m, 1H), 2.0 – 1.9 (m, 1H), 1.6 – 1.5 (m, 4H), 1.3 – 1.2 (m, 28H), 0.87 (t, J =
6.9 Hz, 6H); \textsuperscript{13}C NMR (101 MHz, METHANOL-D4) δ 100.8, 95.3, 84.5, 78.9,
74.1, 73.2, 72.9, 72.4, 71.9, 71.8(3C), 71.5, 69.1, 68.9, 68.7, 55.1, 50.4, 41.1, 31.9(2C), 29.9, 29.6(5C), 29.5(2C), 29.3(2C), 28.1, 26.2, 25.9, 22.5(2C), 13.2(2C). ESI/APCI calcd for C_{38}H_{77}N_{4}O_{11} ([M + H]^+) 765.5583; measured m/z 765.5569.

**6″-O-Decyl kanamycin (K610).** $^{1}$H NMR (300 MHz, D_{2}O) δ 5.52 (d, J = 3.4 Hz, 1H), 4.99 (d, J = 3.8 Hz, 1H), 4.0 – 3.0 (m, 19H), 2.5 – 2.4 (m, 1H), 1.9 – 1.7 (m, 1H), 1.5 – 1.4 (m, 2H), 1.3 – 1.1 (m, 14H), 0.74 (t, J = 6.8 Hz, 3H); $^{13}$C NMR (100 MHz, D_{2}O) δ 100.7, 96.6, 84.3, 78.5, 73.1, 72.3, 72.2, 71.9, 71.0(2C), 68.9, 68.5, 68.3, 65.9, 55.2, 50.1, 48.2, 40.6, 31.4, 28.9, 28.87(2C), 28.8, 28.7, 28.3, 25.4, 22.3, 13.7; ESI/APCI calcd for C_{28}H_{57}N_{4}O_{11} ([M + H]^+) 652.4081; measured m/z 652.4016.

**4″-O-Decyl kanamycin (K410).** $^{1}$H NMR (300 MHz, D_{2}O) δ 5.45 (d, J = 4.1 Hz, 1H), 5.00 (d, J = 3.8 Hz, 1H), 4.0 – 3.0 (m, 19H), 2.5 – 2.4 (m, 1H), 1.9 – 1.8 (m, 1H), 1.5 – 1.4 (m, 2H), 1.3 – 1.1 (m, 14H), 0.75 (t, J = 6.8 Hz, 3H); $^{13}$C NMR (100 MHz, D_{2}O) δ 100.6, 96.4, 84.3, 74.0, 73.7, 72.7, 72.4, 72.2, 71.1, 70.9, 68.9, 68.5, 68.4, 68.3, 55.2, 54.5, 50.1, 47.9, 40.5, 31.4, 29.4, 28.9, 28.7(2C), 28.3, 25.3, 22.2, 13.6; ESI/APCI calcd for C_{28}H_{57}N_{4}O_{11} ([M + H]^+) 652.4018; measured m/z 652.4036.

**4″,6″-Di-O-dodecyl kanamycin (K4612).** $^{1}$H NMR (300 MHz, METHANOL-D_{4}) δ 5.64 (d, J = 3.8 Hz, 1H), 5.05 (d, J = 3.8 Hz, 1H), 4.0 – 3.2 (m, 20H), 3.2 – 3.1 (m, 1H), 2.5 – 2.4 (m, 1H), 2.0 – 1.9 (m, 1H), 1.6 – 1.5 (m, 4H), 1.3 – 1.2 (m, 36H), 0.88 (t, J = 6.8 Hz, 6H); $^{13}$C NMR (101 MHz,
METHANOL-D4) δ 100.9, 98.0, 83.8, 77.0, 73.9, 73.2, 72.9, 72.1, 71.7(3C), 71.3, 69.1, 68.4, 63.6, 54.6, 50.1, 40.8, 32.2(2C), 30.2(8C), 29.8(2C), 29.3, 29.1, 29.0, 26.4(2C), 25.9, 25.3, 22.9(2C), 14.1(2C); ESI/APCI calcd for C_{42}H_{85}N_{4}O_{11} ([M + H]^+) 821.6209; measured m/z 821.6208.

6″-O-Dodecyl kanamycin (K612). $^1$H NMR (300 MHz, D$_2$O) δ 5.51 (d, $J = 4.1$ Hz, 1H), 4.99 (d, $J = 3.8$ Hz, 1H), 4.0 – 3.0 (m, 19H), 2.5 – 2.4 (m, 1H), 1.9 – 1.8 (m, 1H), 1.5 – 1.4 (m, 2H), 1.3 – 1.1 (m, 18H), 0.74 (t, $J = 6.8$ Hz, 3H); $^{13}$C NMR (100 MHz, D2O) δ 100.8, 94.1, 84.5, 78.5, 72.9, 72.3, 72.1, 71.9(2C), 70.9, 68.9, 68.4, 68.3, 65.7, 55.2, 49.9, 48.0, 40.6, 31.4, 28.9(6C), 28.7, 27.8, 25.3, 22.2, 13.6; ESI/APCI calcd for C$_{30}$H$_{61}$N$_{4}$O$_{11}$ ([M + H]$^+$) 653.4331; measured m/z 653.4342.

4″-O-Dodecyl kanamycin (K412). $^1$H NMR (300 MHz, METHANOL-D4) δ 5.53 (d, $J = 3.8$ Hz, 1H), 5.15 (d, $J = 3.8$ Hz, 1H), 4.0 – 3.0 (m, 19H), 2.5 – 2.4 (m, 1H), 2.0 – 1.9 (m, 1H), 1.5 – 1.4 (m, 2H), 1.3 – 1.1 (m, 18H), 0.86 (t, $J = 7.2$ Hz, 3H); $^{13}$C NMR (100 MHz, METHANOL-D4) δ 100.2, 94.1, 84.5, 78.7, 74.7, 73.4, 73.0, 71.9(2C), 71.7, 71.3, 69.1, 68.9, 60.7, 54.8, 50.1, 47.2, 40.9, 31.9, 29.9, 29.5(5C), 29.3, 25.8, 22.5, 13.2; ESI/APCI calcd for C$_{30}$H$_{61}$N$_{4}$O$_{11}$ ([M + H]$^+$) 653.4331; measured m/z 653.4337.

4″,6″-Di-O-tetradecyl kanamycin(K4614). $^1$H NMR (300 MHz, D$_2$O) δ 5.58 (d, $J = 3.78$ Hz, 1H), 5.10 (d, $J = 3.78$ Hz, 1H), 3.4-4.2 (m, 19H), 3.0-3.1 (m, 1H), 2.9 - 3.0, (m, 1H), 2.5 - 2.6 (m, 1H), 1.5 - 1.7 (m, 5H), 1.2 - 1.4 (m, 44H), 0.89 (t, $J = 6.5$ Hz, 6H); $^{13}$C NMR (100 MHz, D$_2$O) δ 100.82, 95.03, 84.26, 78.59,
4"-O-Tetradecyl kanamycin (K414). $^1$H NMR (300 MHz, D$_2$O) $\delta$ 5.55 (d, $J = 3.78$ Hz, 1H), 5.00 (d, $J = 3.42$ Hz, 1H), 3.4-4.2 (m, 17H), 3.29 (t, $J = 9.9$ Hz, 1H), 2.9 - 3.0 (m, 1H), 2.4 - 2.5 (m, 1H), 2.4 - 2.5 (m, 1H), 1.5 - 1.7 (m, 3H), 1.2 - 1.4 (m, 22H), 0.89 (t, $J = 6.5$ Hz, 3H); $^{13}$C NMR (100 MHz, D$_2$O) $\delta$ 100.77, 95.68, 84.84, 79.63, 74.14, 73.17, 72.92, 72.56, 71.91, 71.79(2C), 71.49, 69.04(2C), 68.67, 55.08, 50.47, 41.00, 31.78, 29.80, 29.83(6C), 29.48, 29.28, 26.25, 25.88, 22.53, 13.27.; ESI/APCI calcd for C$_{37}$H$_{65}$N$_4$O$_{11}$ ([M + H]$^+$) 681.4644; measured m/z 681.4665.

4",6"-Di-O-hexadecyl kanamycin (K4616). $^1$H NMR (300 MHz, METHANOL-D4) $\delta$ 5.56(d, $J = 3.8$ Hz, 1H), 5.09 (d, $J = 3.8$ Hz, 1H), 4.0 – 3.0 (m, 21H), 2.5 – 2.4 (m, 1H), 2.0 – 1.9 (m, 1H), 1.6 – 1.5 (m, 4H), 1.3 – 1.2 (m, 52H), 0.88 (t, $J = 6.8$ Hz, 6H); $^{13}$C NMR (101 MHz, METHANOL-D4) $\delta$ 100.8, 94.8, 84.3, 78.7, 73.9, 73.2, 72.9, 72.1, 71.9, 71.8(2C), 71.48, 71.44, 71.3, 69.1, 68.9, 68.7, 55.0, 50.4, 31.8(2C), 29.8, 29.6(18C), 29.5(2C), 29.3(2C), 26.3, 25.9, 22.5(2C), 13.2(2C); ESI/APCI calcd for C$_{50}$H$_{101}$N$_4$O$_{11}$ ([M + H]$^+$) 933.7461; measured m/z 933.7452.

4"-O-Hexadecyl kanamycin (K416). $^1$H NMR (300 MHz, METHANOL-D4) $\delta$ 5.50 (d, $J = 3.4$ Hz, 1H), 5.13 (d, $J = 3.4$ Hz, 1H), 4.0 – 3.0 (m, 19H), 2.5 –
2.4 (m, 1H), 2.0 – 1.9 (m, 1H), 1.5 – 1.4 (m, 2H), 1.3 – 1.1 (m, 26H), 0.86 (t, J = 7.2 Hz, 3H); \(^{13}\)C NMR (100 MHz, METHANOL-D4) \(\delta\) 100.2, 94.6, 85.2, 79.6, 74.8, 73.4, 73.0, 72.9(2C), 71.9, 71.8(2C), 69.1, 69.0, 60.5, 54.8, 50.3, 40.9, 31.8, 29.9, 29.6(10C), 29.3, 25.8, 22.5, 13.2; ESI/APCI calcd for C\(_{34}\)H\(_{69}\)N\(_4\)O\(_{11}\) ([M + H]+) 709.4957; measured \(m/z\) 709.4958.

3-6. References


APPENDICES
APPENDIX A. $^1\text{H}$ and $^{13}\text{C}$ Spectra for Synthesized New Compounds
$^1$H NMR of Methyl-6-O-(6-Methoxy-2-naphthalenylmethyl)-mannopyranoside (probe 1)
$^{13}$C NMR of Methyl-6-O-(6-Methoxy-2-naphthalenylmethyl)-mannopyronoside (probe 1)
$^{13}$C NMR of Methyl-6-(4-methyl-umbelliferyl)-O-$\alpha$-D-mannopyranoside (probe 3)
$^{13}$C NMR of 6-(4-Methyl-umbelliferyl)-O-D-xylofuranose (probe 4)
$^{13}$C NMR of 3-(6-Methoxy-2-naphthalenylmethyl)-O-D-xylofurnose (probe 5)
\(^1^H\) NMR of 2-(1-4-dimethoxyphenyl)tetrahydro-2H-naphthofuran (probe 10)
$^{13}$C NMR of 2-((1-(3,4-dimethoxyphenyl)ethoxy)methyl)-6-methoxynaphthalene (probe 10)
$^1$H NMR of 1-(3,4-dimethoxyphenyl)-2-(4-methyl-umbelliferyoxy)-ethanone (probe 11)
$^{13}$C NMR of 1-(3,4-dimethoxyphényl)-2-(4-methyl-umbelliféroxy)-ethanone (probe 11)
$^1$H NMR of (6-Methoxy-2-naphthalenylmethyl)-β-D-glucopyranoside (probe 14)
$^{13}$C NMR of (6-Methoxy-2-naphthalenylmethyl)-β-D-glucopyranoside (probe 14)
$^1$H NMR of (6-Methoxy-2-naphthalenylmethyl)-β-D-xylopyranoside (probe 16)
$^{13}$C NMR of (6-Methoxy-2-naphthalenylmethyl)-β-D-xylopyranoside (probe 16)
1H NMR of (6-Methoxy-2-naphthalenylmethyl)-β-D-cellubioside (probe 17)
13C NMR of (6-Methoxy-2-naphthalenylmethyl)-β-D-cellubioside (probe 17)
$^{13}$C NMR of (6-Methoxy-2-naphthalenylmethyl)-2,3,4,6-tetra-O-acetyl-β-D-glucopyranoside (8)
$^1$H NMR of (6-Methoxy-2-naphthalenylmethyl)-2,3,4,tri-O-acetyl-D-xylopyranoside (10)
$^{13}$C NMR of (6-Methoxy-2-naphthalenylmethyl)-2,3,4,6-tetra-O-acetyl-D-xylopyranoside (10)
\[ ^1H \text{NMR of Methyl-2,3,4-Tri-benzoyl-O-6-\(6\)-methoxy-2-naphthalenylmethyl)-\(\alpha\)-D-mannopyranoside (13)} \]
13C NMR of Methyl-2,3,4-Tri-benzoyl-O-6-(6-Methoxy-2-naphthalenylmethyl)-α-D-mannopyranoside (13)
1H NMR of 4''''-Di-O-butyl-1,3,6'',3'''-tetraazido kanamycin (K4604N3)
$^{13}$C NMR of 4′′,6′′-Di-O-butyl-1,3,6′,3′-tetraazido kanamycin (K4604N3)
$^1$H NMR of 4''-O-Butyl-1,3,6',3''-tetraazido kanamycin (K404N3)
$^{13}$C NMR of 4"-O-Butyl-1,3,6",3"-tetrazido kanamycin (K404N3)
$^{13}$C NMR of 4''',6''''-Di-O-hexyl-1,3,6',3''''-tetraazido kanamycin (K4606N3)
$^1$H NMR of 4"'-O-Hexyl-1,3,6',3"'-tetraazido kanamycin (K406N3)
$^{13}$C NMR of 4''-O-Hexyl-1,3,6',3''-tetraazido kanamycin (K406N3)
$^1$H NMR of 4,6'-Di-O-heptyl-1,3,6',3'-tetraazido kanamycin (K4607N3)
$^{13}$C NMR of 4”,6”-Di-O-heptyl-1,3,6”,3”-tetraazido kanamycin (K4807N3)
$^1$H NMR of 4"-O-Heptyl-1,3,6',3"-tetraazido kanamycin (K407N3)
$^{13}$C NMR of 4''',6''',Di-O-octyl-1,3,6',3'''-tetraazido kanamycin (K4608N3)
$^{13}$C NMR of 6''-O-Octyl-1,3,6',3''-tetraazido kanamycin (K608N3)
'H NMR of 4''-O-Octyl-1,3,6',3''-tetraazido kanamycin (K408N3)
$^{13}$C NMR of 4''-O-Octyl-1,3,6',3''-tetraazido kanamycin (K408N3)
'H NMR of 4''-Di-O-nonyl-1,3,8,3''-tetraazido kanamycin (K4609N3)
Standard Experiment

Current Data Parameters:
- Sample Name: NMR
- Data Type: Raw

Acquisition Parameters:
- Date: 50202006
- Time: 21:06
- Instrument: MRS400
- Frequency: 400 MHz
- Probe: B 5 mm Multinuclear
- Temperature: 300 K
- Silvov: 300 K
- NS: 400000
- DS: 2
- SNP: 3000000 Hz
- SFORES: 5000000 Hz
- KG: 0.55041000 sec
- DW: 405000000 sec
- DE: 27.14 us
- TE: 300.0 us
- D12: 0.0000000000 sec
- D13: 20.30 dB
- CPD: 50000 Hz
- FTAM: 100.00 Hz
- DL1: 0.45000000 sec
- DL2: 0.74 us
- DE: 27.14 us
- SD01: 100.00 Hz
- NUCLEUS: 100
- D11: 0.33333333

Processing Parameters:
- SP: 1528
- SR: 10227400 Hz
- MTW: 100.00 Hz
- SB: 0
- LB: 0.00 Hz
- GC: 0
- PE: 1.00

NMR Parameters:
- C1: 20.82 cm
- F1P: 156.451 ppm
- F2P: 40.0 Hz
- F3P: -15.417 ppm
- F2: -50.417 ppm
- PHD: 11.45640 ppm/Hz
- H2O: 40.0 Hz/Hz

13C NMR of 6"-O-Nonyl-1,3,6',3"-tetrazido kanamycin (K609N3)
$^{1}H$ NMR of 4''-O-Nonyl-1,3,6',3''-tetraazido kanamycin (K409N3)
$^{13}$C NMR of 4-"O-Nonyl-1,3,6,3"-tetraazido kanamycin (K409N3)
$^1$H NMR of 4",6"-Di-O-decyl-1,3,6",3"-tetraazido kanamycin (K4610N3)
$^{13}$C NMR of 4''\&,6''\&-Di-O-decyl-1,3,6',3''-tetraazido kanamycin (K4610N3)
$^{13}$C NMR of 6"-O-Decyl-1,3,6',3"-tetraazido kanamycin (K610N3)
$^{13}$C NMR of 4''-O-Decyl-1,3,6',3''-tetraazido kanamycin (K410N3)
'H NMR of 4”,6”-Di-O-dodecyl-1,3,6”,3”-tetraazido kanamycin (K4812N3)
$^{13}$C NMR of 4\textsuperscript{a},6\textsuperscript{a}-Di-O-dodecyl-1,3,6,3\textsuperscript{a}-tetraazido kanamycin (K4612N3)
^{1}H NMR of 6''-O-Dodecyl-1,3,6,3''-tetraazido kanamycin (K612N3)
$^{13}$C NMR of 6''-O-Dodecyl-1,3,6''-3''-tetraazido kanamycin (K612N3)
$^1$H NMR of 4"-O-Dodecyl-1,3,6',3"-tetraazido kanamycin (K412N3)
$^{13}$C NMR of 4"-O-Dodecyl-1,3,6,3"-tetraazido kanamycin (K412N3)
$^1$H NMR of 4",6"-Di-O-tetradecyl-1,3,6",3"-tetraazido kanamycin (K4614N3)

X: parts per Million : 1H
$^{13}$C NMR of 4"-6"-Di-O-tetradecyl-1,3,6',3'-tetraazido kanamycin (K4614N3)
$^{13}$C NMR of 4’-O-Tetradecyl-1,3,6’,3’-tetraazido kanamycin (K414N3)
$^1$H NMR of 4′,6′-Di-O-hexadecyl-1,3,6′,3′-tetraazido kanamycin (K4616N3)
$^{13}C$ NMR of 4',6'-Di-O-hexadecyl-1,3,6',3'-tetraazido kanamycin (K4616N3)
$^{13}$C NMR of 4"-O-Hexadecyl-1,3,6,3"-tetraazido kanamycin (K416N3)
$^1$H NMR of 2',3',4',2''-Tetra-0-acetyl-4',6''-di-O-butyl-1,3,6',3''-tetrazido kanamycin (K4604AC)
$^1$H NMR of 2',3',4',2",6"-Pent-O-acetyl-4"-O-hexyl-1,3,6',3"-tetraazido kanamycin (K406Ac)
$^1$H-$^1$H COSY NMR of 2',3',4',2''-Tetra-O-acetyl-4'',6''-di-O-heptyl-1,3,6',3''-tetraazido kanamycin (K4607Ac)
^1H NMR of 2',3',4',2",6"-Pent-O-acetyl-4"-O-heptyl-1,3,6',3"-tetraazido kanamycin (K407Ac)
"H-H COSY NMR of Z'3'4'4''-Pent-O-acetyl-4'-O-heptyl-1,3',5''-tetraazido kanamycin (K407A)"
$^1$H NMR of 2',3',4',2''-Tetra-O-acetyl-4'',6''-di-O-octyl-1,3,6',3''-tetraazido kanamycin (K4608Ac)
'H NMR of 2',3',4',2'',4''-Pent-O-acetyl-6''-O-octyl-1,3,6',3''-tetraazido kanamycin (K608Ac)
$^1$H NMR of 2',3',4',2''-Pent-O-acetyl-4''-O-octyl-1,3,6',3''-tetraazido kanamycin (K408Ac)
$^1$H NMR of 2',3',4',2''-Tetra-O-acetyl-4'',6''-di-O-nonyl-1,3,6',3''-tetraazido kanamycin (K4609Ac)
\( ^1H\cdot^1H\) COSY NMR of 2',3',4',2''-Tetra-O-acetyl-4'',6''-di-O-nonyl-1,3,6',3''-tetraazido kanamycin (K4609Ac)
$^{1}H-^{1}H$ COSY NMR of 2",3",4",2",4"-Pent-O-acetyl-6"-O-nonyl-1,3,6",3"-tetraazido kanamycin (K609Ac)
$^1$H NMR of 2',3',4',2'',6''-Pent-O-acetyl-4''-O-nonyl-1,3,8',3''-tetraazido kanamycin (K409Ac)
'H NMR of 2',3',4',2''',6'''-Pent-O-acetyl-4'''-O-decyl-1,3,8',3'''-tetraazido kanamycin (K410Ac)
$^1$H NMR of 2',3',4',2''-Tetra-O-acetyl-4''-di-O-dodecyl-1,3,6',3''-tetraazido kanamycin (K4612Ac)
$^1$H-$^1$H COSY NMR of 2',3',4',2''-Tetra-O-acetyl-4''''-di-O-dodecyl-1,3,6',3''-tetraazido kanamycin (K4612Ac)
'H-'H COSY NMR of 2',3',4',2",4"-Pent-O-acetyl-6"-O-dodecyl-1,3,6',3"-tetraazido kanamycin (K612Ac)
$^1$H-$^1$H COSY NMR of 2',3',4',2'',6''-Pent-O-acetyl-4''-O-dodecyl-1,3,6',3''-tetraazido kanamycin (K412Ac)
'H NMR of 2',3',4',2"-Tetra-O-acetyl-4",6"-di-O-tetradecyl-1,3,6',3"-tetraazido kanamycin (K4614Ac)
$^1$H NMR of 2',3',4',2''',4''''-Pent-O-acetyl-6''''-O-tetradecyl-1,3,6',3''''-tetrazido kanamycin (K614Ac)
$^{1}$H-$^{1}$H COSY NMR of 2',3',4',2'',4''-Pent-O-acetyl-6''-O-tetradecyl-1,3,6',3''-tetraazido kanamycin (K614Ac)
$^1$H-1H COSY NMR of 2',3',4',2"-Pent-O-acetyl-4"-O-tetradecyl-1,3,6',3"-tetraazido kanamycin (K414Ac)
$^1$H NMR of 2',3',4',2''-Tetra-O-acetyl-4'',6''-di-O-hexadecyl-1,3,6',3''-tetraazido kanamycin (K4616Ac)
$^1$H-$^1$H COSY NMR of 2',3',4',2"-Tetra-O-acetyl-4",6"-di-O-hexadecyl-1,3,6',3"-tetraazido kanamycin (K4616Ac)
$^1$H NMR of $2',3',4',2'',6''$-Pent-O-acetyl-$4''$-O-hexadecyl-$1,3,6',3''$-tetraazido kanamycin (K416Ac)
13C NMR of 4',6''-Di-O-butyl kanamycin (K4604)
'H NMR of 4"-O-Hexyl-1,3,6',3" kanamycin (K406)
^1^H NMR of 4''''-Di-O-heptyl-1,3,6''''-3''''-tetraazido kanamycin (K4607N3)
$^{13}$C NMR of 4'-6'-Di-O-heptyl kanamycin (K4607)
$^{13}C$ NMR of 4"-O-Heptyl kanamycin (K407)
'H NMR of 4",6"-Di-O-octyl kanamycin (K4608)
$^{13}$C NMR of 6"-O-Octyl kanamycin (K608)
$^1$H NMR of 4"-O-Octyl kanamycin (K408)
13C NMR of 4′-O-Octyl kanamycin (K408)
$^1$H NMR of 4'',6''-Di-O-nonyl kanamycin (K4609)
$^{13}$C NMR of 4''-O-nonyl kanamycin (K4609)
$^1$H NMR of 6'-O-Nonyl kanamycin (K609)
$^{13}$C NMR of 6'-O-Nonyl kanamycin (K609)
'H NMR of 4"-O-Nonyl kanamycin (K409)
$^{13}$C NMR of 4'-O-Nonyl kanamycin (K409)
$^1$H NMR of 4",6"-Di-O-decyl kanamycin (K4610)
**13C NMR of 6"-O-Decyl kanamycin (K810)**
$^1$H NMR of 4'-O-Decyl kanamycin (K410)
'1H NMR of 4", 8"-Di-O-dodecyl kanamycin (K4612)
$^1$H NMR of 6''-O-Dodecyl kanamycin (K612)

X : parts per Million : Hz

250
\textbf{$^{13}\text{C}$ NMR of 6''-O-Dodecyl kanamycin (K612)}
$^1$H NMR of 4",6"-Di-O-tetradecyl kanamycin (K4614)
$^{13}$C NMR of 4",8"-Di-O-tetradecyl kanamycin (K4614)
1H NMR of 4-hexadecyl kanamycin (K4616)
1H NMR of 4'-O-Hexadecyl kanamycin (K416)
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Using fluorogenic probes for the investigation of selective biomass degradation by fungi

DOI: 10.1039/C4GC01659A

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Structure-Activity Relationships for Antibacterial to Antifungal Conversion of Karamycin to Amphiphilic Analogues

Author: Mizia Foss, Madher N. Aishvadde, Qin Zhang, et al.
Publication: The Journal of Organic Chemistry
Publisher: American Chemical Society
Date: May 1, 2015
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Department of Chemistry and Biochemistry
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CURRICULUM VITAE

Qian Zhang

Department of Chemistry and Biochemistry
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Education:
Ph.D., Organic Chemistry, Utah State University, Logan, Utah July 2015
B.S., Pharmacy, Lanzhou University, P. R. China July 2010

Teaching Experience:
Teaching Assistant
Utah State University, Logan, Utah August 2010- December 2015
Including illustration, grading, holding office hours, and assigning final grades for
Organic Chemistry Lab 2315 and Organic Chemistry Lab 2325.

Research Experience:
Ph.D. Graduate Student, Advisor: Prof. Cheng-Wei Tom Chang
Department of Chemistry and Biochemistry, Utah State University
Logan, Utah, USA September 2010-July 2015
- Synthesizing fluorogenic probes for the investigation of selective biomass degradation by fungi
- Synthesizing new aminoglycosides analogs and explore the potential to revive traditional antibacterial kanamycin as new type of antifungal agents
- Synthesis of bioactive 1-alkyl-1H-naphtho[2,3-d][1,2,3]triazole-4,9-diones and N-aryl-2-aminomethylene1,3-indanedions using water as solvent
- Divergent and facile Lewis Acid-mediated synthesis of N-alkyl 2-aminomethylene-1,3-indanediones and 2-alkylamino-1,4-naphthoquinones

Undergraduate Researcher, Advisor: Prof. Xiaodong Hu
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Techniques and Expertise:

Software: Ms Office, Windows, Chemdraw

Languages: Mandarin and English


Publications:

1. **Stereoselective Potencies and Relative Toxicities of γ-Coniceine and N-Methylconine Enantiomers**, Stephen T. Lee, Benedict T. Green, Kevin D. Welch, Glenn T. Jordan, Qian Zhang, Kip E. Panter, David


Conference and Seminar:


3. ACS 248th National Meeting, August, 2014, San Francisco, USA;


5. Graduate Student Grant Writing Seminar: “Getting Started as a Successful Proposal Writer and Academician seminar” February 26th, 2013, Utah.


Professional Memberships

Member of the American Chemical Society (ACS), 2011-present.
Poster and Oral Presentation:


Honor and Award:

- 2015, PhD Completion Award, Utah State University.
- 2009 Chun-Tsung Scholar for outstanding undergraduate researcher, Lanzhou University.
- 2009, Third Class Scholarship in Lanzhou University.
- 2008, Second Class Scholarship in Lanzhou University.
- 2007, Second Class Scholarship in Lanzhou University.
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