Title
Synthesis and applications of side chain-functionalized polylactic acid-based polymers and studies toward a chemical method to degrade Alzheimer's disease-related beta-amyloid peptides

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Synthesis and Applications of Side Chain-Functionalized Polylactic Acid-based Polymers and Studies Toward a Chemical Method to Degrade Alzheimer’s Disease-Related beta-Amyloid Peptides.

A Dissertation submitted in partial satisfaction of the Requirements for the degree Doctor of Philosophy

in

Chemistry

by

Mark Rubinshtein

Committee in charge:

Professor Jerry Yang, Chair
Professor Edward Koo
Professor Joseph O’Connor
Professor Kimberly Prather
Professor James Whitesell

2011
The Dissertation of Mark Rubinshtein is approved, and it is acceptable in quality and form for publication on microfilm and electronically:

Chair

University of California, San Diego

2011
“Those who have an excessive faith in their theories or in their ideas are not only poorly disposed to make discoveries, but they also make very poor observations.”

_Claude Bernard_
# TABLE OF CONTENTS

Signature page ............................................................................................................. iii

Epigraph ....................................................................................................................... iv

Table of Contents ......................................................................................................... v

List of Abbreviations ................................................................................................... viii

List of Figures .............................................................................................................. x

List of Schemes ........................................................................................................... xvi

List of Tables ............................................................................................................... xvii

Acknowledgements ..................................................................................................... xviii

Vita .............................................................................................................................. xxiv

Abstract of the Dissertation ......................................................................................... xxvi

## Part I: Synthesis and Applications of Side Chain-Functionalized Polylactic Acid-based Polymers ........................................................................................................ 1

Chapter 1 Polylactic acid and its derivatives ................................................................ 2

1.1 Biodegradable polymers ....................................................................................... 2

1.2 Synthesis and properties of polylactic acid ....................................................... 4

1.3 Incorporating functionalization into PLA .......................................................... 7

1.4 Goals of the dissertation research ...................................................................... 10

Chapter 2 Development of facile methods to synthesize biodegradable materials from side chain-functionalized polylactic acid based polymers ........................................ 11

2.1 Introduction .......................................................................................................... 11

2.2 The Ugi and Passerini reactions in organic synthesis ........................................ 12

2.3 Passerini-type condensations for generating α-hydroxy-N-acylindoles precursors to functionalized lactide monomers .............................................................. 15
2.4 Incorporation of functionalized hemilactides into PLA copolymers........... 19
2.5 Attempting direct polymerization of α-hydroxy-N-Acylindoles............... 22
2.6 Preparatory scale production of an alkyne-functionalized polylactic acid copolymer........................................................................................................ 26
2.7 Preparation of polymeric nanoparticles from alkyne-functionalized PLAs.................................................................................................................. 27
2.8 Encapsulation of small molecules inside of functionalized PLA nanoparticles................................................................................................................. 29
2.9 Future directions for side-chain functionalized PLA-based polymers.............................................................................................................................. 31
2.10 Chapter summary..................................................................................... 32
2.11 Experimental methods.......................................................................... 33

Part II: Studies Toward a Chemical Method to Degrade Alzheimer’s Disease-Related beta-Amyloid Peptides................................................................. 47

Chapter 3 Alzheimer’s Disease: Causes, Mortality and Treatment Options........ 48
3.1 Alzheimer’s disease: an emerging health crisis...................................... 48
3.2 Alzheimer’s disease and the amyloid cascade hypothesis.................... 50
3.3 Current state of Alzheimer’s disease therapeutics................................. 52
3.4 Small-molecule based strategies and targets for AD therapy............... 54
3.5 Degradation of Aβ as a therapeutic strategy.......................................... 54
3.6 Goal of the dissertation research............................................................. 56

Chapter 4 An Enediyne-Based Target-Directed Chemical Method to Degrade Alzheimer’s-Related β-Amyloid................................................................. 57
4.1 Introduction............................................................................................ 57
4.2 A target-directed strategy toward selective peptide degradation............. 58
4.3 Enediynes as potent chemical warheads .......................................................... 59
4.4 Preparation of the BTA binding moiety ..................................................... 64
4.5 Synthesis and binding affinity of the BTA-enediyne conjugate .......... 66
4.6 Effect of the BTA-enediyne conjugate on aggregated Aβ...................... 67
4.7 Chapter summary ................................................................................. 72
4.8 Experimental methods ........................................................................ 73

Chapter 5 Efficient Synthesis and Applications of Oligoethylene glycol derivatives
of Benzothiazole Anilines .............................................................................. 85
5.1 Introduction ........................................................................................ 85
5.2 Microwave-assisted synthesis of BTA-EG₄ and BTA-EG₆ ...................... 86
5.3 Further derivatization of BTA-EG₆ ..................................................... 88
5.4 Blocking HIV-1 transmission with BTA-EG₆ .................................. 89
5.5 Self-assembled cation-selective ion channels formed from BTA-
EG₄ .................................................................................................................. 94
5.6 Chapter summary .............................................................................. 96
5.7 Experimental Methods ..................................................................... 97

Appendix A .................................................................................................... 106
References .................................................................................................... 114
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aβ</td>
<td>β-amyloid</td>
</tr>
<tr>
<td>AChE</td>
<td>acetylcholinesterase</td>
</tr>
<tr>
<td>AD</td>
<td>Alzheimer’s disease</td>
</tr>
<tr>
<td>APP</td>
<td>amyloid precursor protein</td>
</tr>
<tr>
<td>BTA</td>
<td>6-methylbenzothiazole aniline</td>
</tr>
<tr>
<td>CuAAC</td>
<td>Cu(I)-catalyzed azide-alkyne cycloaddition</td>
</tr>
<tr>
<td>DBU</td>
<td>1,8-diazabicyclo[5.4.0]undec-7-ene</td>
</tr>
<tr>
<td>DCM</td>
<td>dichloromethane</td>
</tr>
<tr>
<td>4-DMAP</td>
<td>4-dimethylaminopyridine</td>
</tr>
<tr>
<td>DMF</td>
<td>N,N-dimethylformamide</td>
</tr>
<tr>
<td>DMSO</td>
<td>dimethyl sulfoxide</td>
</tr>
<tr>
<td>EDC</td>
<td>1-ethyl-3-(3-dimethylaminopropyl)carbodiimide</td>
</tr>
<tr>
<td>ELISA</td>
<td>enzyme-linked immunosorbent assay</td>
</tr>
<tr>
<td>Et₂O</td>
<td>diethyl ether</td>
</tr>
<tr>
<td>HIV</td>
<td>human immunodeficiency virus</td>
</tr>
<tr>
<td>EtOAc</td>
<td>ethyl acetate</td>
</tr>
<tr>
<td>Mₐ</td>
<td>number average molecular weight</td>
</tr>
<tr>
<td>Mₘ</td>
<td>weight average molecular weight</td>
</tr>
<tr>
<td>MeOH</td>
<td>methanol</td>
</tr>
<tr>
<td>NFT</td>
<td>neurofibrillary tangle</td>
</tr>
<tr>
<td>NMDA</td>
<td>N-methyl-D-aspartase</td>
</tr>
<tr>
<td>PDI</td>
<td>polydispersity index</td>
</tr>
<tr>
<td>PGA</td>
<td>polyglycolic acid</td>
</tr>
<tr>
<td>PLA</td>
<td>polylactic acid / polylactide</td>
</tr>
<tr>
<td>pS</td>
<td>picoSiemens</td>
</tr>
<tr>
<td>PTSA</td>
<td>p-toluenesulfonic acid</td>
</tr>
<tr>
<td>ROP</td>
<td>ring-opening polymerization</td>
</tr>
<tr>
<td>SDS-PAGE</td>
<td>sodium dodecyl sulfate-polyacrylamide gel electrophoresis</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Description</td>
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<tr>
<td>--------------</td>
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<tr>
<td>SEVI</td>
<td>semen-derived Enhancer of viral infection</td>
</tr>
<tr>
<td>Sn(Oct)$_2$</td>
<td>stannous 2-ethylhexanoate or tin octoate</td>
</tr>
<tr>
<td>$T_g$</td>
<td>glass transition temperature</td>
</tr>
<tr>
<td>$T_m$</td>
<td>melting temperature</td>
</tr>
<tr>
<td>THF</td>
<td>tetrahydrofuran</td>
</tr>
</tbody>
</table>
LIST OF FIGURES

**Figure 1.1:** Example of a compostable food packaging made from biodegradable polymers. The bag of SunChips® is shown (A) prior to composting and (B) after 18 days of composting ................................................................. 2

**Figure 1.2:** Two examples of biodegradable polymers used for biomedical applications: (A) surgical bone screws manufactured by NP Pharm from the biodegradable polymer Lactel® and (B) a prototype of a fully absorbable, biodegradable stent produced by Abbott Vascular in conjunction with Biotronic and REVA Medical. Images obtained from NP Pharm and Advanced Medical Technologies ................................................................. 3

**Figure 1.3:** Current commercial preparation of polylactic acid (PLA). Direct polymerization of lactic acid (A) results in the formation of oligomeric material. Alternatively, dehydrative dimerization (B) leads to the formation of monomeric lactide that can then be polymerized via ring opening polymerization (C) ............... 5

**Figure 1.4:** Synthesis of a PLA derivative containing pendant protected carboxylic acid functional groups from a functionalized lactide precursor. The carboxylic acid is unmasked upon hydrogenation of the benzyl ester ......................................................... 9

**Figure 1.5:** Feijen’s preparation of various polyesteramides via copolymerization of DL-lactide with substituted morpholine-2,5-diones prepared from protected L-aspartic acid, L-lysine and L-cysteine .................................................................................. 9

**Figure 1.6:** Synthesis (A) and polymerization (B) of an alkyne-functionalized lactide ........................................................................................................... 10

**Figure 2.1:** The mechanism of the multicomponent Ugi Reaction between an aldehyde or ketone (black), amine (blue) isocyanide (green) and carboxylic acid (red) to generate a bis-amide .................................................................................. 13

**Figure 2.2:** Proposed concerted (A) or stepwise (B) mechanisms for the multicomponent Passerini reaction. Both proposed mechanisms proceed via an alkylimidic anhydride intermediate that rapidly rearranges to form an esteramide .................................................................................. 13

**Figure 2.3:** Formation of pyroglutamic acids using a convertible isocyanide. The bis-amide formed via the Ugi reaction is readily converted to the N-acylindole upon treatment with PTSA. The N-acylindole is subsequently hydrolyzed to give pyroglutamic acid and indole ........................................................................ 14
Figure 2.4: Proposed stepwise mechanism of the Passerini-like reaction for the generation of \(\alpha\)-hydroxy-\(N\)-acylindoles. This variant of the Passerini replaces the carboxylic acid component with water a catalytic amount of PTSA. The catalytic acid initiates the reaction and also affects the transformation of the esteramide intermediate to the \(\alpha\)-hydroxy-\(N\)-acylindole. In principle, the generation of the esteramide may also proceed by concerted mechanism similar to that described in Figure 2.2.

Figure 2.5: Proposed route toward generating PLAs. The \(N\)-acylindoles formed during the Passerini-type reaction may be further converted to sidechain functionalized hemilactides and incorporated into poly(\(\alpha\)-hydroxy acid) copolymers.

Figure 2.6: Modification of copolymers 6b-d using CuAAC click reactions. (A-D) Size exclusion chromatographic traces of crude samples containing CuSO\(_4\) and sodium ascorbate with: (A) PLA 6a and dansyl azide 7, (B) alkyne-functionalized polymer 6b and dansyl azide 7, (C) azide-functionalized polymer 6c and dansyl alkyne 8, or (D) azide-functionalized polymer 6d and dansyl alkyne 8. The chromatograms of crude polymer-containing solutions were monitored by differential refractive index (dRI, black) and UV absorbance (red). The UV was monitored at \(\lambda = 365\, \text{nm}\) to indicate species containing a dansyl group.

Figure 2.7: One possible pathway of \(\alpha\)-hydroxy-\(N\)-acylindole decomposition upon treatment with a thiol. Initial attack of the \(\alpha\)-hydroxy-\(N\)-acylindole by the highly nucleophilic thiol may lead to the formation of an \(\alpha\)-hydroxythioester, which can subsequently undergo self-condensation to form dimeric, trimeric and ultimately multimeric products.

Figure 2.8: ESI-MS analysis of attempted polymerization of \(\alpha\)-hydroxy-\(N\)-acylindole 3a using Cs\(_2\)CO\(_3\) as an initiator. Peaks at \(m/z = 644, 716, 788, 860\) and 932 have differences of 72, corresponding to the mass of a monomeric unit of PLA.

Figure 2.9: ESI-MS analysis of attempted polymerization of \(\alpha\)-hydroxy-\(N\)-acylindole 3b initiated with DBU in DCM. Peaks at \(m/z = 701, 811, 921, 1031, 1141, 1251, 1361, 1471, 1581,\) and 1691 have differences of 110, corresponding to the mass of a monomeric unit of poly(2-hydroxy-5-hexynoic acid).

Figure 2.10: Structures of the anticancer drugs camptothecin and doxorubicin and the Thioflavin T analogue BTA-EG\(_4\).
**Figure 2.11:** Alkyne-functionalized PLA provides a means for covalently linking small molecule-based drugs onto the polymer scaffold. Drugs may be attached (A) directly to the scaffold using an azide-functionalized drug, or (B) indirectly by exploiting a bifunctional acid-cleavable linker...

**Figure 3.1:** A portion of the amyloid precursor protein sequence (APP) represented by one-letter codes, with the sequence of Aβ₁-₄₂ shown in bold. α-Secretase cleaves APP between the Lys₁₆ and Leu₁₇ residues within the Aβ sequence, resulting in non-amyloidogenic peptide fragments. However, APP cleaved successively by β-secretase and γ-secretase produces Aβ peptides of varying length, depending on the specific cleavage site of γ-secretase...

**Figure 3.2:** Structure of the β-secretase inhibitor GRL-8234...

**Figure 3.3:** Examples of several γ-secretase modulators...

**Figure 4.1:** Bergman cycloaromatization of (A) a linear enediyne and (B) a cyclic enediyne in a 10-membered ring system to a reactive p-benzyne diradical intermediate...

**Figure 4.2:** Cartoon depicting the target-directed approach of Aβ degradation. A cyclic enediyne warhead bearing a suitable binding group is predicted to associate with Aβ peptides, delivering the enediyne moiety to the target peptide. Subsequent cyclization to the p-benzyne diradical may provide the reactive species necessary to degrade Aβ...

**Figure 4.3:** Possible mechanisms of peptides degradation by the reactive p-benzyne diradical. Abstraction of an α-hydrogen from the peptide backbone leads to peroxide formation and ultimately peptide scission to give an amide and α-oxo ketone (or aldehyde). Alternatively, the highly reactive diradical may abstract an α-hydrogen from the peptide backbone (or a hydrogen atom from an amino acid side chain) to give other degradation products...

**Figure 4.4:** Structures of the Aβ binding molecules Thioflavin T (ThT) and Pittsburgh compound B (PiB) containing the benzothiazole moiety...
Figure 4.5: Briefly, the design of this ELISA-based competition assay entails the evaluation of molecules that inhibit the interaction of Aβ fibrils (here, formed from Aβ1-40) with a monoclonal anti-Aβ IgG raised against residues 3-8 of AD-related Aβ peptide (clone 6E10). This assay is based on the hypothesis that molecules that can effectively and efficiently coat Aβ fibrils will be able to inhibit the binding of this anti-Aβ IgG to Aβ fibrils. The relative inhibition of IgG-Aβ fibril interactions by small molecules is quantified using a standard ELISA protocol. Inhibition (Inh.) of anti-Aβ IgG (clone 6E10)–Aβ interactions with compound: a) 21, 55% Maximal Inh., IC50 = 220 μM; b) 22, No Inhibition; and c) 20, 51% Maximal Inh., IC50 = 100 μM. Error bars represent ± SEM.

Figure 4.6: A Circular dichroism spectrum of a 100 μM aqueous solution of Aβ after incubation at 37°C for 4 days.

Figure 4.7: Chromatographic and electrophoretic analyses of the degradation of Aβ peptides using a targeted enediyne. (A) RP-HPLC analysis of Aβ1-40 peptides after incubation with 1 molar equivalent of 21 at 37°C after 0 days (top) and 7 days (middle), or 10 equivalents of 21 after 7 days (bottom). (B) SDS-PAGE analysis of Aβ1-40 peptides after incubation with 1 molar equivalent of 21 or 5 molar equivalents of 22 or 20 at 37°C for 7 days. Lane 1: Mark12TM standard. Lane 2: Aβ1-40 alone. Lane 3: Aβ1-40 + 1 molar equivalent 21 incubated for 7 days. Lane 4: Aβ1-40 + 5 molar equivalent 22 incubated for 7 days. Lane 5: Aβ1-40 + 5 molar equivalent 20 incubated for 7 days. SDS-PAGE gels were run using a 16% tricine gel and visualized via silver staining.

Figure 4.8: SDS-PAGE analysis of ubiquitin (8.6 kDa) alone and after incubation with 1 molar equivalent of 21 at 37°C for 7 days. Lane 1: Mark12TM standard. Lane 2: ubiquitin (90%, from Sigma-Aldrich, used without further purification). Lane 3: ubiquitin + 1 molar equivalent 21 incubated for 7 days. SDS-PAGE gels were performed using a 16% tricine gel and visualized via silver staining.

Figure 4.9: Relative cell viability of SH-SY5Y human neuroblastoma cells in the presence of aggregated Aβ peptides with or without incubation with molecule 21. Cells incubated with 21 alone were treated with a solution of 21 that was incubated for 7 days in sterile water prior to exposure to cells. Aβ samples were prepared by incubation in sterile water for 4 days and: 1) incubated further for 7 days prior to exposure to cells, 2) incubated further for 7 days, mixed with 21, and immediately exposed to cells (labeled as 0 days), or 3) incubated with 21 for 7 days prior to exposure to cells (labeled as 7 days). The data are expressed as mean values ± standard deviations. The viability of cells exposed to Aβ incubated with molecule 21 for 7 days was significantly different than the viability of cells exposed to Aβ alone (P < 0.001).
Figure 5.1: Structures of BTA-EG₄ (23) and BTA-EG₆ (24). BTA-EG₄ is synthesized using tetraethylene glycol as a starting material, while BTA-EG₆ is prepared from hexaethylene glycol.

Figure 5.2: Schematic representation of the putative role of aggregated PAP248-286 peptides (also called SEVI peptides) in HIV-1 infection. a) Primary sequence and helical representation of PAP248-286 peptides; these abundant peptides have been reported to spontaneously form aggregated amyloid fibrils in semen. b) Illustration of the SEVI amyloid-mediated infection of a T cell (pink/red) with HIV-1 (blue/purple). Also depicted is the proposed method to attenuate SEVI-mediated infection by forming bio-resilient coatings on aggregated amyloids derived from PAP248-286.

Figure 5.3: Binding of BTA-EG₆ to SEVI fibrils as determined by a previously reported centrifugation assay. Briefly, various concentrations of BTA-EG₆ in PBS were incubated overnight at room temperature in the presence or absence of SEVI fibrils. After equilibration, each solution was centrifuged, and the supernatants were separated from the pelleted fibrils. The fluorescence of BTA-EG₆ was determined from the resuspended pellets in PBS solution. Error bars represent S.D. of duplicate measurements. The $K_d$ was determined by fitting the data to a one-site specific binding algorithm: $Y = B_{\text{max}}[X/(K_dX)]$, where $X$ is the concentration of BTA-EG₆, $Y$ is the specific binding fluorescence intensity, and $B_{\text{max}}$ corresponds to the apparent maximal observable fluorescence upon binding of BTA-EG₆ to SEVI fibrils. RFI, relative fluorescence intensity.

Figure 5.4: (A) HIV-1 IIIB virions were preincubated with increasing concentrations of BTA-EG₆ (24) (0, 5.5, 11, and 22.5 μg/ml) and with or without SEVI (15 μg/ml) as indicated. The samples were then added to CEM-M7 cells. Cells were washed at 2 h, and infection was assayed at 48 h by measuring Tat-driven luciferase expression. Results shown are average values ± S.D. of triplicate measurements from one of four independent experiments that yielded equivalent results. * indicates p < 0.05 when compared with control cells exposed to HIV-1IIIB + SEVI alone by ANOVA with Tukey’s post test. RLU, relative luciferase units; Uninf, uninfected. (B) B, zoom in of panel A to show data for cells treated with HIV-IIIB virions with and without increasing concentrations of BTA-EG₆, in the absence of SEVI. BTA-EG₆ had no effect on the infectivity of HIV alone; concentrations of BTA-EG₆ are noted above for panel A.
**Figure 5.5:** HIV-1IIIB virions were preincubated with 50% pooled human semen, with or without increasing concentrations of BTA-EG$_6$ (5.5, 11, and 22.5 μg/ml). After 10 min, these stocks were diluted 15-fold into CEM-M7 cells. Cells were washed after 1 h, and luciferase expression was measured at 48 h to quantify the extent of infection. Results shown are average values ± S.D. of triplicate measurements from one of three independent experiments that yielded equivalent results. * indicates $p < 0.05$ when compared with control cells exposed to HIV-1IIIb + semen alone, by ANOVA with Tukey’s post test. RLU, relative luciferase units..............................................................................................................................

**Figure 5.6:** The cervical endothelial cell lines A2En (endocervical), 3EC1(ectocervical), and SiHa were treated for 12 h with BTA-EG$_6$ at concentrations up to 10 times greater than the IC$_{50}$. Control cultures were treated with nonoxynol-9 (non-9) at 0.1% final concentration as a positive control for induction of cell death (33). At 12 h, viability was measured by resazurin cytotoxicity assay (alamarBlue™ assay). Representative results from A2En cells are shown; results from 3EC1 and SiHa cells were very similar (not shown)............

**Figure 5.7:** (A) Original current versus time trace from single ion channel recording through self-assembled pores from BTA-EG$_4$ in a planar lipid bilayer composed of 1,2-diphtanylo-$sn$-glycero-3-phosphatidylcholine (DiphyPC) lipids. Histogram of current amplitudes reflects their number of occurrence in the corresponding current trace. (B) Normalized survival plot of the open channel lifetime determined from 174 single channel opening events under the same experimental conditions as in A). The average lifetime, $\tau$, for BTA-EG$_4$ pores was determined from a fit of the histogram to the equation, $N(t)/N(0) = \exp(-t/\tau)$, where $N(t)$ represents the number of channels with lifetimes longer than the time $t$, and $N(0)$ represents the total number of channels with observable single conductance. BTA-EG$_4$ was added at a final concentration of 20 μM to both bilayer compartments, the applied voltage was +50 mV, and the electrolyte contained 1.0 M CsCl with 10 mM HEPES buffer, pH 7.4..............................................................

**Figure 5.8:** (A) Inhibitory effect of BTA-EG$_4$ 23 on the growth of Bacillus subtilis bacteria 22 h after exposure to LB media containing various concentrations of BTA-EG$_4$. Growth was quantified by the optical density at a wavelength of 600 nm relative to untreated control cells. The concentration of BTA-EG$_4$ molecules that inhibited growth by 50% (IC$_{50}$ values) was 50 μM. Each point represents the mean of 2 experiments with 3 replicates in each experiment; error bars represent the standard error of the mean. (B) Cytotoxicity of BTA-EG$_4$ on human neuroblastoma (SH-SY5Y) cells 24 h after exposure. Each point represents the mean of 2 or 3 experiments with 6 replicates in each experiment. Error bars reflect the standard error of the mean. The IC$_{50}$ value was 65 μM..............................................................
LIST OF SCHEMES

**Scheme 2.1:** Scheme for the facile two-step conversion of α-hydroxy-\(N\)-acylindoles to the corresponding hemilactides

**Scheme 2.2:** Synthesis of poly(DL-lactide)-co-(3-(but-3-yn-1-yl)-6-methyl-1,4-dioxane-2,5-dione) (9)

**Scheme 4.1:** Synthesis of the substituted cyclic enediyne bearing a terminal carboxylic acid pendant functional group 14 from linear dibromide 10

**Scheme 4.2:** Synthesis of BTA-EG₅ amine 20

**Scheme 4.3:** Formation of the enediyne-BTA conjugate 21 and a related control molecule 22 containing the enediyne warhead but lacking an Aβ-binding group

**Scheme 5.1:** Synthesis of BTA-EG₄ 23 and BTA-EG₆ 24 from tetraethylene glycol 25 and hexaethylene glycol 28, respectively

**Scheme 5.2:** Tosylation of BTA-EG₆ 24 under different conditions. Treatment of BTA-EG₆ with TsCl in pyridine/DCM (A) affords the \(N,O\)-ditosylated product 31, while using triethylamine/4-DMAP in DCM gives toluenesulfonyl ester 33 as the major product. Subsequent substitution of 31 and 33 with sodium azide affords the organoazide BTA derivatives 32 and 34, respectively
LIST OF TABLES

Table 1.1: Common biodegradable polyesters and their uses................................. 4

Table 1.2: Physical properties of polylactic acids...................................................... 7

Table 2.1: Isolated yields of $N$-acylindoles from the Passerini-type condensation reaction.......................................................................................................................... 18

Table 2.2: Incorporation of functionalized hemilactides into PLA-based polymers..... 22

Table 2.3: Preparation of nanoparticles from commercial PLA and alkyne-functionalized PLA......................................................................................................................... 29

Table 2.4: Preparation of nanoparticles containing encapsulated small molecule drugs.......................................................................................................................... 31

Table 3.1: Percentage change in selected causes of death in the United States between 2000 and 2006................................................................................................. 50

Table 3.2: Current FDA-approved small molecule treatments for Alzheimer's Disease.......................................................................................................................... 54
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tirelessly to provide me with an education and a desire to constantly pursue knowledge and better myself. I will be forever in their debt. In addition I would like to thank my grandmother, Sonya, my sister and brother-in law, Irene and Eddie, my nieces and nephews, Bella, Alec, Stephanie and David, and my in-laws Frank and Marilyn Kretzer for their encouragement during my graduate studies. All of you were a big part of helping me get through the program and I do not even know how to begin to express my gratitude for all of your love and support.

Notes about the chapters

Chapter 2, in part, is based on material which appears in "Facile Procedure for Generating Side Chain Functionalized Poly (alpha-hydroxy acid) Copolymers from Aldehydes via a Versatile Passerini-Type Condensation" Rubinshtein, M.; James, C. R.; Young, J. L.; Ma, Y. J.; Kobayashi, Y.; Gianneschi, N. C.; Jerry Yang, J. *Organic Letters*, 2010, 12, 3560-3563. I am the primary author of this paper. In addition, Chapter 2 contains material currently being prepared for submission for publication: "Direct synthesis of side chain functionalized alpha-hydroxy acid oligomers from alpha-hydroxy-N-acylindole precursors." Rubinshtein, M.; James, C. R.; Young, J. L.; Gianneschi, N. C.; Yang, J. I am the primary author of this manuscript.

Chapter 4 is based on material currently being prepared for submission for publication: Mark Rubinshtein, Lila K. Habib, Mahealani R. Bautista, and Jerry Yang “Chemical Degradation of Alzheimer’s-Related beta-Amyloid Peptides Using a Targeted Enediyne.” I am the primary author of this manuscript.
Chapter 5 contains material that appears in "Amyloid binding small molecules efficiently block SEVI (semen-derived enhancer of infection) and semen mediated enhancement of HIV-1 infection." Olsen, J. S.; Brown, C.; Capule, C. C.; Rubinshtein, M.; Doran, T. M.; Srivastava, R. K.; Feng, C.; Nilsson, B. L.; Yang, J.; Dewhurst, S J. Biol. Chem. 2010, 285, 35488-35496. I am a co-author on this paper. Additionally, Chapter 5 contains material as it may appear in a paper recently submitted for publication: “Self-assembled, cation-selective ion channels from oligo(ethylene glycol) derivatives of benzothiazole aniline.” Prangkio, P.; Rao, D.; Lance, K.; Rubinshtein, M.; Yang, J.; Mayer, M. I am a co-author of this paper.
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PUBLICATIONS


**ABSTRACTS AND POSTERS**


ABSTRACT OF THE DISSERTATION

Synthesis and Applications of Side Chain-Functionalized Polylactic Acid-based Polymers and Studies Toward a Chemical Method to Degrade Alzheimer’s Disease-Related beta-Amyloid Peptides.

by

Mark Rubinshtein

Doctor of Philosophy in Chemistry

University of California, San Diego, 2011

Professor Jerry Yang, Chair

The first part of this dissertation focuses on new methodology to prepare functionalizable derivatives of polylactic acid (PLA), an important biodegradable and biocompatible polymer often used for drug-delivery applications. Chapter 1 provides a brief background covering the uses and properties of biodegradable polymers and outlines previous strategies to synthesize functionalized PLA. Chapter 2 presents a novel synthetic route to functionalized lactide monomers via a Passerini-type condensation reaction. This methodology provides access to a variety of functionalized PLA molecules, including those with derivatizable alkyne and azide pendant groups. In addition, the chapter explores the preparation of polymeric nanoparticles from these functionalized PLAs for potential use in drug delivery applications.

The second part of this dissertation focuses on studies toward the development of a small molecule capable of chemically degrading the Alzheimer’s related β-amyloid (Aβ) peptide. Chapter 3 provides a brief introduction to Alzheimer’s disease (AD) and
its potential impact on global health. The chapter discusses the amyloid cascade hypothesis (which implicates Aβ as a causative factor in developing AD) as well as the current drugs and therapeutic strategies used to stop or reverse the progression of this disease. Chapter 4 introduces target-directed degradation of Aβ with a small molecule as a potential approach for the treatment of AD. The chapter presents the design and synthesis of a small molecule comprised of a cyclic enediyne chemical “warhead” moiety and a benzothiazole aniline (BTA) Aβ binding group. This designed BTA-enediyne conjugate reduces the toxicity of Aβ, presumably by degrading the amyloid to lower molecular weight fragments that are less harmful than full-length Aβ. Chapter 5 describes the development of a methodology leading to an improved synthesis of oligoethylene glycol derivatives of BTA and the collaborations made possible because of the increased availability of these molecules. These compounds exhibit many interesting, biomedically relevant properties including the ability to inhibit SEVI and semen-mediated infectivity of HIV-1 and form ion channels in lipid bilayers.
Part I: Synthesis and Applications of Side Chain-Functionalized Polylactic Acid-based Polymers
Chapter 1

Polylactic Acid and its Derivatives

1.1 Biodegradable polymers

Biodegradable polymers have attracted significant attention for their wide range of potential applications. Scores of commercial products such as disposable plastics and compostable food packaging have been manufactured from these extraordinary macromolecules.\textsuperscript{1-3} The agricultural sector has also employed these materials for uses such as controlled release of fertilizers and pesticides.\textsuperscript{4, 5} Most recently, however, investigation of the synthesis and properties of biodegradable polymers has stemmed from the desire to exploit their highly favorable properties for biocompatible applications including surgical sutures\textsuperscript{6, 7} and materials for tissue engineering.\textsuperscript{8-11}

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{sunchips_bags}
\caption{Example of a compostable food packaging made from biodegradable polymers.\textsuperscript{12} The bag of SunChips® is shown (A) prior to composting and (B) after 18 days of composting.}
\end{figure}
Figure 1.2: Two examples of biodegradable polymers used for biomedical applications: (A) surgical bone screws manufactured by NP Pharm from the biodegradable polymer Lactel® and (B) A prototype of a fully absorbable, biodegradable stent produced by Abbott Vascular in conjunction with Biotronic and REVA Medical. Images obtained from NP Pharm\textsuperscript{12} and Advanced Medical Technologies.\textsuperscript{13}

Polyesters are among the most exhaustively studied and widely used biodegradable polymeric materials. Polycaprolactone and the poly(hydroxyalkanoate)s, which include poly-3-hydroxyvalerate (PHV) and poly-4-hydroxybutyrate (P4HB), are produced in large quantities and have been explored for their potential commercial and biomedical applications. However, it is the poly(\(\alpha\)-ester)s polyglycolic acid (PGA) and polylactic acid (PLA) that have garnered tremendous interest over the past several decades due to the low toxicity of the polymers and their biodegradation products. PGA, PLA and their copolymers have been chosen for biologically compatible applications since the 1960’s. The commercially available suture materials Vicryl\textsuperscript{®} and Dexcon\textsuperscript{®}, for example, are made from PGA and PLA/PGA copolymers.

Because of the low toxicity of PLA and its biodegradation products, PLA and PLA copolymers are used in numerous applications including biodegradable bone screws,\textsuperscript{14, 15} surgical stents\textsuperscript{16-19} and controlled-release drug delivery systems.\textsuperscript{8, 19, 20} PLA’s
status as an FDA-approved material\textsuperscript{21-23} and its predictable properties make it an ideal material to explore in order to expand its possible applications in the biomedical sciences.

Table 1.1: Common biodegradable polyesters and their uses.

<table>
<thead>
<tr>
<th>Type of polyester</th>
<th>structure</th>
<th>uses</th>
</tr>
</thead>
<tbody>
<tr>
<td>Polycaprolactone (PCL)</td>
<td><img src="structure1.png" alt="Structure" /></td>
<td>Specialty polyurethane production, drug encapsulation.</td>
</tr>
<tr>
<td>Poly-3-hydroxyvalerate (PHV)</td>
<td><img src="structure2.png" alt="Structure" /></td>
<td>Drug delivery systems (often in co-polymers with P4HB).</td>
</tr>
<tr>
<td>Poly-4-hydroxybutyrate (P4HB)</td>
<td><img src="structure3.png" alt="Structure" /></td>
<td>Heart valves, sutures and medical textile products.</td>
</tr>
<tr>
<td>Polyglycolic acid (PGA)</td>
<td><img src="structure4.png" alt="Structure" /></td>
<td>Surgical sutures, drug delivery devices, skin care products.</td>
</tr>
<tr>
<td>Polylactic acid (PLA)</td>
<td><img src="structure5.png" alt="Structure" /></td>
<td>Compostible food packaging, surgical bone screws, drug delivery systems.</td>
</tr>
</tbody>
</table>

1.2 Synthesis and properties of polylactic acid

Commercial production of polylactic acid begins with bacterial fermentation of natural sources such as cornstarch and cane sugar to produce lactic acid.\textsuperscript{24} Attempts at direct polymerization of lactic acid, however, results in the formation of oligomeric species that are not useful for commercial applications (Figure 1.3A). The water produced during direct polymerization acts to hydrolyze the growing polymer chain,
resulting in the formation of low molecular weight oligomers. In order to circumvent this problem, lactic acid is first dimerized to give lactide while the water generated is removed to drive the reaction (Figure 1.3B). The resulting lactide is then converted into commercially useful polymer via ring-opening polymerization (ROP) by heating it in the presence of a suitable catalyst (Figure 1.3C). Industrial production of PLA utilizes tin(II)bis-2-ethylhexanoate (tin octoate) as the catalyst due to its good solubility in molten lactide, high catalytic activity, and low rate of racemization of the polymer chain.\textsuperscript{25, 26} Other inorganic\textsuperscript{27} and organic\textsuperscript{28} catalysts have been explored, however, tin octoate remains the catalyst of choice for large-scale production of PLA.

**Figure 1.3:** Current commercial preparation of polylactic acid (PLA). Direct polymerization of lactic acid (A) results in the formation of oligomeric material. Alternatively dehydrative dimerization (B) leads to the formation of monomeric lactide that can then be polymerized via ring opening polymerization (C).
The chiral nature of lactic acid leads to the formation of several different PLA products. Dimerization of the naturally occurring L-lactic acid gives L-lactide and subsequent polymerization leads to the formation of poly(L-lactic acid) (PLLA). Similarly, D-lactic acid leads to poly(D-lactic acid). Both PLLA and PDLA are semicrystalline polymers with a melting point ($T_m$) of about 180°C and a glass transition temperature ($T_g$) of 60-65°C. Physical blends of PLLA and PDLA result in materials with higher crystallinity (and therefore a higher $T_m$) that are more suitable for applications requiring increased stiffness or resistance to high temperature. Polymerization of either racemic or meso lactide generally produces amorphous material that still has wide application as films, membranes and coatings; however, using stereoselective catalysts in polymerization can lead to the formation of semicrystalline polymers from either the racemic or meso lactide.

Degradation of poly($\alpha$-hydroxy acid)s such as PGA and PLA occurs primarily via an ester hydrolysis mechanism first proposed by Chu. Under physiological conditions (pH 7.4, 37°C), PLA is first hydrolyzed into smaller oligomers and ultimately broken down into its constituent lactic acid units, with degradation rates dependent on parameters such as molecular weight and degree of crystallinity of the particular polymer. In the case of PLA-based implantable devices used in humans, the lactic acid that is generated can be enzymatically oxidized to pyruvate by lactate dehydrogenase and further processed by various physiological mechanisms such as the Krebs (citric acid) cycle. Accordingly, PLA-based materials particularly attractive for use in biomedical applications as they break down to byproducts of low toxicity that are effectively processed by the human body.
Table 1.2: Physical properties of polylactic acids.

<table>
<thead>
<tr>
<th>compound</th>
<th>Crystallinity</th>
<th>$T_m$ (°C)</th>
<th>$T_g$ (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Poly(L-lactide) (PLLA)</td>
<td>semicrystalline</td>
<td>173-178</td>
<td>60-65</td>
</tr>
<tr>
<td>Poly(D-lactide) (PDLA)</td>
<td>semicrystalline</td>
<td>173-178</td>
<td>60-65</td>
</tr>
<tr>
<td>50:50 PLLA:PDLA</td>
<td>semicrystalline</td>
<td>230</td>
<td>n/a</td>
</tr>
<tr>
<td>$meso$-Poly(DL-lactide)$^a$</td>
<td>amorphous</td>
<td>130-135</td>
<td>44-48</td>
</tr>
<tr>
<td>$rac$-Poly(DL-lactide)$^a$</td>
<td>amorphous</td>
<td>n/a</td>
<td>38-42</td>
</tr>
<tr>
<td>$meso$-Poly(DL-lactide)$^b$</td>
<td>semicrystalline</td>
<td>152</td>
<td>34</td>
</tr>
<tr>
<td>$rac$-Poly(DL-lactide)$^b$</td>
<td>semicrystalline</td>
<td>191</td>
<td>55</td>
</tr>
</tbody>
</table>

$^a$ Prepared using standard polymerization techniques using a non-stereospecific catalyst. $^b$ Prepared using a stereospecific catalyst.

1.3 Incorporating functionalization into PLA

Although PLA is a commercially useful material, its utility is limited by its insolubility in aqueous media and lack of functionalizable side chains. Ouchi and Fujino have demonstrated the synthesis of a PLA derivative containing pendant benzyl ester functional groups, which may be easily converted to the free acid by hydrogenation (Figure 1.4). The catalytic effect of the acid groups, however, can lead to the accelerated hydrolysis of the PLA backbone.
Figure 1.4: Synthesis of a PLA derivative containing pendant protected carboxylic acid functional groups from a functionalized lactide precursor. The carboxylic acid is unmasked upon hydrogenation of the benzyl ester.

Other groups have tried copolymerizing lactide with monomers to introduce useful functional pendant groups. In particular, Feijen and coworkers have produced polyesteramides by copolymerizing DL-lactide with various substituted morpholine-2,5-diones (Figure 1.5). The morpholine-2,5-diones were readily synthesized from protected amino acids L-aspartic acid L-lysine and L-cysteine to ultimately provide PLA functionalized with acid, amine, and thiol groups, respectively. This methodology generates a viable synthetic route to functionalizable polymers using a variety of α-amino acids as starting materials, and was successfully employed by Langer to make the amine-substituted polyesteramide poly(lactic acid-co-lysine) that was further modified with RGD peptide and evaluated for tissue engineering applications. This approach has several limitations: morpholine-2,5-diones are less reactive than their lactide counterparts, which results in a lower percent conversion for the morpholine-2,5-dione monomer as compared to DL-lactide. Therefore, the morpholine-2,5-diones are not as easily incorporated into the growing polymer chain. Practical considerations also limit the scope of the morpholinedione methodology. Although many α-amino acids can, in principle, be used as starting materials for the morpholinedione monomer, only those
obtained at reasonable cost can be used to make this a commercially viable process. For example, incorporating the highly desirable terminal alkyne function group via the unnatural amino acid propargylglycine is prohibitively expensive for large-scale production: DL-propargylglycine costs over $130 per gram and the price of the enantiomerically pure L-propargylglycine is over $500 per gram—over 200 times the cost of L-lysine.

\[
\text{Feijen’s preparation of various polyesteramides via copolymerization of DL-lactide with substituted morpholine-2,5-diones prepared from protected L-aspartic acid, L-lysine and L-cysteine.}
\]

Baker and coworkers developed a method for the synthesis of terminal alkyne-functionalized PLA from the alkyne-bearing lactide (Figure 1.6). The substituted lactide is prepared in three steps. First propargyl bromide is treated with zinc metal to generate the corresponding organozinc reagent and reacted with ethyl glyoxylate to give the α-hydroxy acid ethyl ester, which is then readily hydrolyzed to give the free acid in good
yield. Dimerization of the acid in the presence of p-toluenesulfonic acid (PTSA) with simultaneous azeotropic removal of water gives the desired lactide, but in a modest 34% yield. Although this methodology permits synthesis of the alkyne-substituted lactide on a multi-gram scale at relatively low cost, the approach is not general enough to produce lactide products with a variety of derivatizable pendant groups.

![Synthesis and polymerization of alkyne-functionalized lactide](image)

**Figure 1.6:** Synthesis (A) and polymerization (B) of an alkyne-functionalized lactide.

1.4 Goals of the dissertation research

PLA and its derivatives have many demonstrated uses, particularly in FDA-approved biomedical application, and its biodegradability and biocompatibility make this material ideally suited to further exploration. This portion of the dissertation will focus on developing a general method for introducing a wide range of useful pendant groups to PLA and demonstrating potential applications of these polymeric materials. The successful synthesis of a variety of derivatized PLAs may provide access to PLA-based materials with interesting new functionality while retaining the physical properties that make them particularly useful for current commercial and biomedical applications.
Chapter 2

Development of a facile procedure for generating sidechain functionalized poly(α-hydroxy acid) copolymers from aldehydes via a versatile Passerini-type condensation

2.1 Introduction

Polylactic acid (PLA), one of the most well-known members of the poly(α-ester) class of polymers, has attracted significant interest for its potential uses in biodegradable and biocompatible material; however PLA has inherent limitations due to its insolubility in aqueous media and lack of functionalizable sidechains. Simple, general, and scalable methods to incorporate functionality in PLA-based polymers may enable new applications of these materials for biomedical and commercial uses. Although previous reports describe the preparation of PLA-based copolymers containing functionalized sidechains,43, 44 these methodologies are often laborious or require specialized reagents to obtain a specific, desired functional group in the polymer. The research presented in this chapter describes a method to produce high molecular weight PLAs with functionalized side chains via a multi-component, Passerini-type condensation reaction. This methodology has led to the synthesis of PLAs and PLA precursors functionalized with terminal alkyne, azide, oligoethylene glycol and phthalimide groups, which will allow further derivatization of these polymers. These functionalized PLAs provide an entry
into the synthesis of biologically compatible polymeric compounds with potentially useful properties for various biomedical applications.

2.2 The Ugi and Passerini reactions in organic synthesis

The Ugi\textsuperscript{45} and Passerini\textsuperscript{46} reactions are related multicomponent condensations that employ isocyanides to generate bis-amides and esteramides, respectively. A powerful tool for convergent synthesis, the Ugi reaction generates a complex product in high yield via the one-pot reaction of an aldehyde (or ketone), an amine, an isocyanide and a carboxylic acid (Figure 2.1).\textsuperscript{45} The reaction proceeds initially by forming an imine \textit{in situ} from the aldehyde or ketone and amine components. Protonation of the resulting imine generates the highly electrophilic iminium ion, which is readily attacked by the isocyanide to generate a nitrilium species. Subsequent reaction with the carboxylate forms an alkylimidic anhydride that is rapidly converted to the stable bis-amide product via Mumm rearrangement, \textsuperscript{47} which provides the driving force for the Ugi reaction.

The closely related Passerini reaction does not have an amine component and therefore proceeds by direct attack of the aldehyde or ketone by the isocyanide; however, other aspects of the reaction are similar to the Ugi. It has been proposed that the Passerini reaction may proceed via stepwise (ionic) or concerted mechanisms depending on the polarity of the solvent used. Both variations are illustrated in Figure 2.2 and account for the formation the esteramide product via an alkylimidic anhydride intermediate.
**Figure 2.1:** The mechanism of the multicomponent Ugi Reaction between an aldehyde or ketone (black), amine (blue) isocyanide (green) and carboxylic acid (red) to generate a bis-amide.

**Figure 2.2:** Proposed concerted (A) or stepwise (B) mechanisms for the multicomponent Passerini reaction. Both proposed mechanisms proceed via an alkylimidic anhydride intermediate that rapidly rearranges to form an esteramide.
Recently, Kobayashi and co-workers reported successful syntheses of various pyroglutamic acids via the Ugi reaction by employing a convertible isocyanide (1) (Figure 2.2). The reaction proceeds as expected to give the bis-amide product in high yield; however, the functionality introduced by the convertible isocyanide allows further reactivity that is centrally important to this synthesis. Treating the bis-amide with an acid such as p-toluenesulfonic acid (PTSA) leads to collapse of the acetal and subsequent cyclization to form the readily hydrolyzable N-acylindole product.

**Figure 2.3:** Formation of pyroglutamic acids using a convertible isocyanide. The bis-amide formed via the Ugi reaction is readily converted to the N-acylindole upon treatment with PTSA. The N-acylindole is subsequently hydrolyzed to give pyroglutamic acid and indole.
2.3 Passerini-type condensations for generating $\alpha$-hydroxy-$N$-acylindoles precursors to functionalized lactide monomers

Motivated by Kobayashi’s success in utilizing Ugi chemistry for the synthesis of pyroglutamic acid, we explored the possibility of using convertible isocyanide 1 in a Passerini-type condensation reaction to develop a general method for generating $\alpha$-hydroxy-$N$-acylindoles containing a variety of side chain functionality. We proposed a variation of the Passerini where the normally present carboxylic acid is replaced by water and a catalytic amount of a sulfonic acid such as PTSA. Catalytic PTSA serves two

![Diagram of the proposed mechanism](attachment:image.png)

**Figure 2.4:** Proposed stepwise mechanism of the Passerini-like reaction for the generation of $\alpha$-hydroxy-$N$-acylindoles. This variant of the Passerini replaces the carboxylic acid component with water a catalytic amount of PTSA. The catalytic acid initiates the reaction and also affects the transformation of the esteramide intermediate to the $\alpha$-hydroxy-$N$-acylindole. In principle, the generation of the esteramide may also proceed by concerted mechanism similar to that described in Figure 2.2.
purposes: 1) it initiates the reaction by protonating the aldehyde, and 2) it provides the necessary acid to affect the cyclization of the esteramide to the α-hydroxy N-acylindole. Other aspects of this reaction, however, presumably remain the same (Figure 2.4). Because of the dual role of the catalytic acid, we speculated that this reaction may be used to generate the α-hydroxy N-acylindole in a one-pot process. Once formed, we hypothesized that these functionalized α-hydroxy N-acylindoles could be readily hydrolyzed to the corresponding acids and further converted to hemilactides for subsequent incorporation into PLA-based copolymers (Figure 2.5). A significant advantage of this synthetic approach is its versatility, since this common strategy can potentially generate a large and diverse set of tailored polymers from simple, commercial or readily prepared aldehydes.

**Figure 2.5:** Proposed route toward generating PLAs. The N-acylindoles formed during the Passerini-type reaction may be further converted to sidechain functionalized hemilactides and incorporated into poly(α-hydroxy acid) copolymers.
We demonstrated that reaction of convertible isocyanide 1, acetaldehyde 2a and water afforded the N-acylindole of lactic acid 3a in good yield (Table 2.1). To investigate the general utility of these condensation conditions, we tested several aldehydes for the ability to generate sidechain functionalized N-acylindoles. The results indicate that we can introduce a range of chemical properties in good yield using this method—that is, functionality with azide-reactive (2b), alkyne-reactive (2c and 2d), hydrophilic (2d), or sterically bulky (2e) properties. We chose to incorporate sidechains from aldehydes 2b-2e, which are easily prepared from commercially available compounds, in the condensation reaction in order to provide chemical handles for post-polymerization modification or to introduce other potentially useful chemical or physical properties into the polymer. Functional groups provided by aldehydes 2b-d, for instance, would lead to PLA-based copolymers that could be modified by utilizing a Cu(I)-catalyzed azide-alkyne cycloaddition (CuAAC) reaction.\textsuperscript{49-51} Compound 2d, in addition to providing a handle for CuAAC, may also impart a degree of water solubility to a polymer that is otherwise insoluble in aqueous solution. Aldehyde 2e may also afford access to a PLA-based copolymer containing sidechains with amine handles upon deprotection of the phthalimide group.\textsuperscript{52, 53} To illustrate another potential advantage of this Passerini-type condensation for generating large-scale quantities of N-acylindoles, we prepared multiple grams of 3b from 4-pentynal from a single condensation reaction.
Table 2.1: Isolated yields of \(N\)-acylindoles from the Passerini-type condensation reaction.

![Chemical structure](image)

<table>
<thead>
<tr>
<th>compound</th>
<th>aldehyde</th>
<th>yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3a</td>
<td></td>
<td>74</td>
</tr>
<tr>
<td>3b</td>
<td></td>
<td>67</td>
</tr>
<tr>
<td>3c</td>
<td></td>
<td>69</td>
</tr>
<tr>
<td>3d</td>
<td></td>
<td>65</td>
</tr>
<tr>
<td>3e</td>
<td></td>
<td>69</td>
</tr>
</tbody>
</table>

In order to demonstrate the utility of \(N\)-acylindoles as functionalized polymeric precursors, we converted compounds 3b-d to the corresponding hemilactides 5b-d. The \(N\)-acylindoles were readily hydrolyzed using LiOH in THF/H\(_2\)O to give the corresponding \(\alpha\)-hydroxy acids 4b-d in 67-80% isolated yields; these \(\alpha\)-hydroxy acids required minimal purification and were isolated as pure products after solution phase extraction. Subsequent reaction of \(\alpha\)-hydroxy acids with 2-bromopropionyl chloride afforded hemilactides 5b-d in moderate yield as mixtures of stereoisomers (Scheme 2.1).
**Scheme 2.1:** Scheme for the facile two-step conversion of α-hydroxy N-acylindoles to the corresponding hemilactides.

### 2.4 Incorporation of functionalized hemilactides into PLA copolymers

To show these novel hemilactides 5b-d could be incorporated into PLA-based copolymers, we reacted 15 mol % of 5b-d with commercial D,L-lactide and catalytic amounts of stannous octoate (as a polymerization catalyst) and 4-tert-butylbenzyl alcohol (as a co-initiator) at 130 °C under an inert atmosphere for 2 h. We analyzed the resulting polymers 6b-d by NMR and size-exclusion chromatography (SEC) to estimate the percent conversion of lactide or hemilactide to polymer, the number-average molecular weight ($M_n$), and the polydispersity (PDIs) of the copolymers. As a control, we compared these parameters of copolymers 6b-d to pure PLA polymer 6a that was synthesized under the same polymerization conditions. Lactide and hemilactide were efficiently converted to copolymer under these reaction conditions (Table 2.2). SEC analysis of 6b-d indicated that these polymerization conditions resulted in polymers with a molecular weight range of 22-32 kDa and PDIs in the range of 1.1-1.2, which were similar in size and dispersity as pure PLA polymer 6a that was synthesized in the absence of a hemilactide as a dopant. The SEC traces may be found in Appendix A.

We next wanted to verify that copolymers 6b-d were amenable to post-polymerization modification, therefore, we subjected 6a-d to CuAAC conditions in the
presence of an excess of a dansyl derivative containing either an azide (7) or a terminal alkyne (8) group. The UV-active dansyl group incorporated into all three copolymers 6b-d under these reaction conditions (Figure 2.6), whereas pure PLA polymer 6a remained unmodified. These results strongly support that hemilactides 5b-d were indeed incorporated into polymers 6b-d and that the functionalized sidechains retained their specific reactive properties.

We estimated the percent conversion of the polymerization reactions of hemilactides into polymer 6b-d by analysis of crude reaction samples by $^1$H-NMR (Appendix A). Percent conversion was estimated by integrating the methine protons in the monomeric species (M) and the methine protons in the polymeric compound (P). The percent conversion was estimated to be $1 - \frac{[M]}{[M+P]}$. The data from $^1$H-NMR and $^{13}$C-NMR of crude samples of polymers 6b-d (Appendix A) also provided evidence that hemilactides 5b-d incorporated into polymers 6b-d. The degree of incorporation of hemilactide appeared to be nearly quantitative from comparison of the integration of methyl Hs and methine Hs within the polymers by $^1$H-NMR (within the inherent error limits of $^1$H-NMR). Additionally, we saw evidence that 5b-5d had been incorporated into the polymers by examining key regions in the $^{13}$C-NMR. For 6b, for instance, the $^{13}$C-NMR resonance at $\delta = 14.2$ ppm (which represents the propargylic carbon in hemilactide 5b) was absent and a new broad peak was observed at around 14.4 ppm, suggesting that the alkyne functionality is present on the polymeric species rather than the monomeric hemilactide form. For 6c-6d, a broadening of the $^{13}$C-NMR peak is observed at the resonances near $\delta = 51$ ppm (which represents the carbon atom bearing
the azide functionality), suggesting that the azide functionality is present in the polymeric species rather than in the monomeric hemilactide form.

Figure 2.6: Modification of copolymers 6b-d using CuAAC click reactions. (A-D) Size exclusion chromatographic traces of crude samples containing CuSO₄ and sodium ascorbate with: (A) PLA 6a and dansyl azide 7, (B) alkyne-functionalized polymer 6b and dansyl azide 7, (C) azide-functionalized polymer 6c and dansyl alkyne 8, or (D) azide-functionalized polymer 6d and dansyl alkyne 8. The chromatograms of crude polymer-containing solutions were monitored by differential refractive index (dRI, black) and UV absorbance (red). The UV was monitored at $\lambda = 365$ nm to indicate species containing a dansyl group.
Table 2.2: Incorporation of functionalized hemilactides into PLA-based polymers.

![Chemical structure](image)

<table>
<thead>
<tr>
<th>hemilactide</th>
<th>conversion (%)</th>
<th>$10^3\cdot M_n$ (g/mol)</th>
<th>PDI $(M_w/M_n)$</th>
</tr>
</thead>
<tbody>
<tr>
<td>none</td>
<td>91</td>
<td>27.4</td>
<td>1.19</td>
</tr>
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<td>94</td>
<td>22.6</td>
<td>1.11</td>
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<tr>
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<td>77</td>
<td>34.5</td>
<td>1.12</td>
</tr>
<tr>
<td><img src="image" alt="Chemical structure" /></td>
<td>71</td>
<td>23.1</td>
<td>1.17</td>
</tr>
</tbody>
</table>

2.5 Attempting direct polymerization of $\alpha$-hydroxy-$N$-acylindoles

The curious behavior of the $\alpha$-hydroxy-$N$-acylindoles led us to investigate the possibility of using these compounds as the monomeric starting material for direct polymerization to form PLA derivatives. $\alpha$-Hydroxy-$N$-acylindoles are easily hydrolyzed to form carboxylic acids and readily form esters and amides upon treatment with alcohols and amides, respectively. However, attempts to generate thioesters by treating $\alpha$-hydroxy-$N$-acylindoles with thiols led to decomposition products—likely oligomeric and polymeric species—that formed via the rapid intermolecular self-condensation reaction.
of the short-lived α-hydroxythioester intermediate (Figure 2.7). Accordingly, we speculated that treating α-hydroxy-N-acylindoles with a suitable base could remove the proton from the α-hydroxy group, generating enough nucleophilic alkoxide to initiate polymerization of the α-hydroxy-N-acylindole to directly produce a poly(α-hydroxy acid) and indole without the need to first prepare lactide-based monomers.

![Diagram](image-url)

**Figure 2.7:** One possible pathway of α-hydroxy-N-acylindole decomposition upon treatment with a thiol. Initial attack of the α-hydroxy-N-acylindole by the highly nucleophilic thiol may lead to the formation of an α-hydroxythioester, which can subsequently undergo self-condensation to form dimeric, trimeric and ultimately multimeric products.

We attempted to directly polymerize acylindole 3b using 1 molar equivalent of triethylamine as the base with anhydrous THF as the solvent. Only starting material was observed after a day of stirring at room temperature. Adding 0.1 molar equivalents of anhydrous Cs₂CO₃, however, resulted in complete disappearance of starting material after
several hours. Analysis of the reaction mixture by SEC showed that no high molecular weight polymer was produced. Electrospray Ionization Mass Spectrometry (ESI-MS) analysis of the reaction mixture, however, revealed a series of molecular ion peaks separated by $m/z = 72$, corresponding to the mass of a monomeric lactic acid unit (Figure 2.7). Thus, the SEC and ESI-MS evidence together suggest that only low molecular weight oligomers are formed during the reaction. Attempts to initiate polymerization of functionalized $\alpha$-hydroxy-$N$-acylindole $3b$ using $\text{Cs}_2\text{CO}_3$ in THF led to oligomer formation as well. We suspected that the low molecular weight could be attributed to residual water in THF, therefore we attempted to initiate polymerization of $3b$ in dry dichloromethane with the strong organic base 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU).

![Figure 2.8: ESI-MS analysis of attempted polymerization of $\alpha$-hydroxy-$N$-acylindole $3a$ using $\text{Cs}_2\text{CO}_3$ as an initiator. Peaks at $m/z = 644, 716, 788, 860$ and $932$ have differences of 72, corresponding to the mass of a monomeric unit of PLA.](image)
under the assumption that it would be easier to keep the reaction moisture-free; however, these conditions also generated oligomeric products and the expected pattern of molecular ion peaks (differences of $m/z = 110$) were observed (Figure 2.8).

![Figure 2.9: ESI-MS analysis of attempted polymerization of α-hydroxy-N-acylindole 3b initiated with DBU in DCM. Peaks at $m/z = 701, 811, 921, 1031, 1141, 1251, 1361, 1471, 1581,$ and 1691 have differences of 110, corresponding to the mass of a monomeric unit of poly(2-hydroxy-5-hexynoic acid).](image)

Unfortunately, attempts to vary base concentrations and solvents did not yield products of high molecular weight. The formation of the electron rich indole side product may react with electrophilic intermediates generated during polymerization and lead to side products that limit multimer size and yields. Additionally, as this process is a living polymerization, chain-transfer kinetics may limit the size of multimer formed. The thermodynamics of the reaction may be improved by using α-hydroxy-N-acylindoles
that have more electron-withdrawing groups on the indole ring to improve its ability as a leaving group. However, this avenue has yet to be explored.

2.6 Preparatory scale production of an alkyne-functionalized polylactic acid copolymer.

In order to demonstrate the utility of this methodology for practical applications, we synthesized polymer 9, a copolymer of DL-lactide and the alkyne-functionalized hemilactide, on the gram scale (Scheme 2.2). Commercially available DL-lactide doped with 20 mol% of the alkyne-substituted hemilactide were copolymerized using stannous octoate and 4-tert-butylbenzyl alcohol as co-initiators, in a manner nearly identical to the preparation of polymer 6b. The alkyne-functionalized copolymer was recovered in 63% yield after repeated precipitation (made possible by the large scale of the reaction) to remove monomer, low molecular weight polymer and the tin catalyst. Size-exclusion chromatography of the copolymer revealed a PDI of 1.26 and a $M_n$ of 46.9 kDa. In addition, analysis of the copolymer via $^1$H and $^{13}$C NMR spectroscopy displayed the peak

![Scheme 2.2: Synthesis of poly(DL-lactide)-co-(3-(but-3-yn-1-yl)-6-methyl-1,4-dioxane-2,5-dione) (9)](image)
broadening expected for polymeric species. The degree of incorporation of alkyne hemilactide 5b appeared to be nearly quantitative from comparison of the integration of methyl Hs and methine Hs within the polymers by 1H-NMR. Accordingly, this scaled-up synthesis demonstrates a viable methodology for the preparation of useful quantities of alkyne (and possibly other) functionalized PLA-based copolymers.

### 2.7 Preparation of polymeric nanoparticles from alkyne-functionalized PLAs

PLA and its copolymers have captured the interest of the biomedical community for their potential applications in drug delivery. Polymeric nanoparticles made from PLA and its derivatives have been successfully used as drug delivery systems that encapsulate and predictably release small molecule-based therapeutics.\(^{55,56}\) Proof-of-principle studies by Görner and coworkers have shown that PLA-based nanoparticles can encapsulate the hydrophobic anesthetic lidocaine and release it over a period of about 24 hours.\(^{57,58}\) Others have shown that a variety of small molecules including pentamidine,\(^{59}\) haloperidol,\(^{60,61}\) 9-nitrocamptothecin\(^{62}\) and others\(^{63}\) can be encapsulated in PLA and PLGA nanoparticle drug delivery systems. These demonstrated success have inspired investigation into the feasibility of making nanoparticles from PLA derivatives such as the alkyne-functionalized copolymer 9 previously described in this dissertation.

Many different methodologies are available for the preparation of polymeric nanoparticles.\(^{64}\) Among the most widely used techniques is the oil/water emulsion method, in which a polymeric species dissolved in a water-immiscible solvent such as DCM is emulsified in an aqueous solution containing a stabilizer (usually polyvinyl alcohol). Subsequent evaporation of the organic solvent gives the desired
nanoparticles. A somewhat more involved variation of this procedure includes the double emulsion method, in which a prepared oil/water emulsion is added to an aqueous phase containing a stabilizer, has been used by Faisant and coworkers to prepare polymeric nanoparticles loaded with the anticancer drug 5-fluorouracil. Alternatively, PLA nanoparticles may be prepared via nanoprecipitation. This particularly facile method of producing nanoparticles first developed by Fessi and coworkers involves the addition of a solution of PLA (and drug if desired) dissolved in a water-soluble organic solvent (acetone or acetonitrile) to an aqueous solution containing a stabilizer followed by evaporation of the organic solvent. The nanoprecipitation procedure is useful for encapsulating hydrophobic drug molecules while not very effective for trapping hydrophilic reagents, as they readily leak into the aqueous phase during the process. In addition nanoprecipitation tends to produce smaller particles with a narrower size distribution than those made using emulsion-based methods.

Initially, we attempted to prepare nanoparticles from commercially available PLA using the oil/water emulsion method. The PLA was predissolved in DCM and emulsified with an aqueous phase containing 0.3% (w/v) polyvinyl alcohol as a stabilizer. Analysis by dynamic light scattering gave poor quality data and revealed that the particles formed using this procedure were over 400 nm in diameter and had a large polydispersity. The oil/water emulsion method was repeated several times, but the data obtained was neither high quality nor reproducible. As a result, we shifted our efforts to preparing particles using a nanoprecipitation protocol.

Table 2.3 summarizes the different conditions we used to make PLA-based nanoparticles. Acetone and acetonitrile were chosen as the organic solvents due to their
volatility, miscibility with water, and ability to dissolve both PLA and small hydrophobic molecules. Although particle size varied slight based on the concentration of PLA and the nature of the organic solvent, nanoprecipitation of PLA produced consistently small particles with a narrow PDI. Functionalized PLA particles were produced with somewhat higher PDI, which may be the result of larger molecular weight distribution of the functionalized PLA compared to commercially available PLA. Interestingly, nanoparticles formed from the alkyne-functionalized PLA derivative were considerably smaller, possibly due to the increased hydrophobic effect from the longer alkyl chains.

Table 2.3: Preparation of nanoparticles from commercial PLA and alkyne-functionalized PLA.

<table>
<thead>
<tr>
<th>polymer</th>
<th>organic solvent</th>
<th>polymer concentration (mg/mL)</th>
<th>method</th>
<th>diameter (nm)</th>
<th>PDI</th>
</tr>
</thead>
<tbody>
<tr>
<td>PLA</td>
<td>DCM</td>
<td>20</td>
<td>o/w emulsion</td>
<td>425</td>
<td>0.779</td>
</tr>
<tr>
<td>PLA</td>
<td>acetone</td>
<td>10</td>
<td>nanoprecipitation</td>
<td>163</td>
<td>0.078</td>
</tr>
<tr>
<td>PLA</td>
<td>acetone</td>
<td>2.0</td>
<td>nanoprecipitation</td>
<td>111</td>
<td>0.034</td>
</tr>
<tr>
<td>PLA</td>
<td>acetone</td>
<td>1.0</td>
<td>nanoprecipitation</td>
<td>119</td>
<td>0.026</td>
</tr>
<tr>
<td>9</td>
<td>CH$_3$CN</td>
<td>2.0</td>
<td>nanoprecipitation</td>
<td>66</td>
<td>0.215</td>
</tr>
<tr>
<td>9</td>
<td>acetone</td>
<td>2.0</td>
<td>nanoprecipitation</td>
<td>76</td>
<td>0.242</td>
</tr>
</tbody>
</table>

Solvent for precipitation was a 0.3% w/v aqueous solution of poly(vinyl alcohol).

2.8 Encapsulation of small molecules inside of functionalized PLA nanoparticles

In order to exploit the possibility of using our polymeric nanoparticles as vehicles for controlled release of small molecules, we attempted to prepare nanoparticles
containing encapsulated small molecules via nanoprecipitation. We chose several small molecules with both known and potential therapeutic properties to encapsulate in the alkyne-functionalized nanoparticles: the FDA-approved chemotherapeutic agent doxorubicin, the topoisomerase I inhibitor camptothecin, and the Thioflavin T analogue BTA-EG₄ (Figure 2.10).

As our initial trial, we attempted to encapsulate doxorubicin (which we purchased as the HCl salt) in commercial PLA. Although nanoparticles were successfully made, the doxorubicin proved to be too water-soluble and did not appear to be encapsulated in the particles. Due to its hydrophobicity, we anticipated that camptothecin would be a more ideal choice for encapsulation. Indeed, it appeared that camptothecin was encapsulated in both commercial PLA and alkyne-functionalized PLA nanoparticles (Table 2.4).

Figure 2.10: Structures of the anticancer drugs camptothecin and doxorubicin and the Thioflavin T analogue BTA-EG₄.

BTA-EG₄ has demonstrated many interesting amyloid-binding and ion channel-forming properties (discussed in chapter 5 of this dissertation); however, its extremely poor water solubility may limit its efficacy as a drug. Accordingly, encapsulating this
molecule within a polymeric nanoparticle may improve its therapeutic properties. We attempted encapsulation of BTA-EG$_4$ in PLA-based polymeric nanoparticles derived from both commercial PLA and 9 and obtained nanoparticle suspensions that was highly fluorescent even after purification using a size-exclusion column and passage through a 0.45 μm syringe filter. On the contrary, aqueous suspensions of BTA-EG$_4$ did not exhibit any fluorescence after being similarly treated. It must be noted, however, that the fluorescence observed could also be attributed to small molecules that may be adsorbed to the surface of the nanoparticles, both in the case of BTA-EG$_4$ and camptothecin.

**Table 2.4:** Preparation of nanoparticles containing encapsulated small molecule drugs.

<table>
<thead>
<tr>
<th>polymer</th>
<th>organic solvent</th>
<th>polymer concentration (mg/mL)</th>
<th>Encapsulated drug</th>
<th>method</th>
<th>diameter (nm)</th>
<th>PDI</th>
</tr>
</thead>
<tbody>
<tr>
<td>PLA</td>
<td>acetone</td>
<td>1.0</td>
<td>doxorubicin</td>
<td>nanoprecipitation</td>
<td>167</td>
<td>0.060</td>
</tr>
<tr>
<td>PLA</td>
<td>acetone/methanol</td>
<td>1.0</td>
<td>doxorubicin</td>
<td>nanoprecipitation</td>
<td>215</td>
<td>0.176</td>
</tr>
<tr>
<td>PLA</td>
<td>CH$_3$CN</td>
<td>1.0</td>
<td>camptothecin</td>
<td>nanoprecipitation</td>
<td>122</td>
<td>0.030</td>
</tr>
<tr>
<td>9</td>
<td>CH$_3$CN</td>
<td>1.0</td>
<td>camptothecin</td>
<td>nanoprecipitation</td>
<td>62</td>
<td>0.130</td>
</tr>
<tr>
<td>PLA</td>
<td>acetone</td>
<td>2.0</td>
<td>BTA-EG$_4$</td>
<td>nanoprecipitation</td>
<td>133</td>
<td>0.048</td>
</tr>
<tr>
<td>PLA</td>
<td>CH$_3$CN</td>
<td>1.0</td>
<td>BTA-EG$_4$</td>
<td>nanoprecipitation</td>
<td>146</td>
<td>0.064</td>
</tr>
<tr>
<td>9</td>
<td>acetone</td>
<td>2.0</td>
<td>BTA-EG$_4$</td>
<td>nanoprecipitation</td>
<td>85</td>
<td>0.234</td>
</tr>
</tbody>
</table>

**2.9 Future Directions for side-chain functionalized PLA-based polymers**

The introduction of a derivatizable functional group to PLA has opened the door to many future areas of investigation. Alkyne-functionalized PLA nanoparticles can not
only encapsulate small molecule drugs, but also have their properties modified to affect drug loading and drug release. The presence of alkyne functionality on the PLA provides for a means of making nanoparticle-based drug delivery systems by covalently linking small molecules drugs or targeting groups either directly onto the polymeric scaffold or via a pH cleavable linker such as $N$-ethoxybenzimidazole (NEBI) (Figure 2.11).\textsuperscript{70, 71} We anticipate that these alkyne-functionalized polymers will increase their applicability in the area of polymeric nanoparticle-based drug delivery systems and expand their already important role in other biomedical applications.

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure2_11.png}
\caption{Alkyne-functionalized PLA provides a means for covalently linking small molecule-based drugs onto the polymer scaffold. Drugs may be attached (A) directly to the scaffold using an azide-functionalized drug, or (B) indirectly by exploiting a bifunctional acid-cleavable linker.}
\end{figure}

\subsection*{2.10 Chapter summary}

In summary, this chapter presents an efficient and versatile Passerini-type condensation reaction to generate $\alpha$-hydroxy acid derivatives that can be readily
incorporated into sidechain functionalized PLA-based copolymers. A particularly attractive feature of this method is the capability of incorporating a range of tailored sidechain functionality into the polymers from a variety of commercially available or readily prepared aldehydes. Furthermore, the procedure outlined in this work affords access to copolymers at potentially practical scales and makes it possible to incorporate functionality for further modification of the polymer. This new methodology, therefore, represents a significant step towards accessing biodegradable and biocompatible materials with improved functional and potentially tunable properties that may find utility in a variety of applications including polymeric nanoparticle drug delivery systems.

2.11 Experimental methods

All synthetic reagents were purchased from Aldrich, Fisher Scientific, Alfa Aesar, Fluka or TCI. DL-lactide was recrystallized twice from toluene before use. Poly(D,L-lactide) (specific viscosity: 0.55-0.75, $M_n = 75,000-125,000$), poly(vinyl alcohol) ($M_n = 13,000-23,000$, 87-89% hydrolyzed). Doxorubicin (purchased as the hydrochloride salt), and camptothecin were used without purification. BTA-EG$_4$ was prepared according to methods described in chapter 5 of this dissertation. All solvents used for reactions were obtained from Fisher scientific and dried on Alumina columns prior to use. Solvents used for polymer purification were ACS technical grade and used without further purification. Solvents used for nanoparticle formation were HPLC grade and used without further purification. Water (18.2 μΩ/cm) was filtered through a NANOPure Diamond™ (Barnstead) water purification system before use.

All NMR spectra of the monomeric lactides and small molecule precursors were
recorded on a Varian Mercury Plus 400 MHz NMR spectrometer in CDCl₃. ¹H and ¹³C NMR spectra of polymerized products 6b-6d were obtained on a Varian VNMRS NMR spectrometer equipped with a 500MHz XSens Cold Probe in CDCl₃. Low resolution MS analysis was performed on a ThermoFinnigan LCQdeca mass spectrometer with an atmospheric pressure electrospray ionization (APCI) source or an electrospray ionization (ESI) source. High resolution MS analysis was performed on a Thermo Scientific LTQ Orbitrap XL mass spectrometer with an electrospray ionization source.

Polymer polydispersities and molecular weights were determined by size-exclusion chromatography (Phenomenex Phenogel 5u 10, 1K-75K, 300 x 7.80 mm in series with a Phenomex Phenogel 5u 10, 10K-1000K, 300 x 7.80 mm (0.05 M LiBr in DMF, 0.75 mL/min 60°C)) using a Hitachi-Elite LaChrom L-2130 pump equipped with a UV detector (Hitachi- Elite LaChrom L-2420), a multi-angle light scattering detector (DAWN-HELIOS: Wyatt Technology) and a refractive index detector (Hitachi L-2490). Data analysis was performed using the ASTRA software package.

Particle size determination by dynamic light scattering was performed on a Malvern Zetasizer Nano 3690 instrument using Malvern microvolume disposable polystyrene cuvettes. All samples were passes through 0.45 µm syringe filters prior to taking measurements. Data analysis was performed using Nanosizer software (Southborough, MA). All data values were based on a minimum of six average runs.
Synthesis of aldehydes 2b-2e

4-pentynal (2b)

To a stirred solution of oxalyl chloride (3.40 g, 26.86 mmol, 2.54 mL) in 50 mL dichloromethane (DCM) under argon at -78°C was added dimethyl sulfoxide (4.40 g, 56.3 mmol, 4.0 mL) in 10 mL DCM dropwise over 10 minutes. The mixture was then stirred under argon at -78°C for 30 minutes, at which time a solution of 4-pentyn-1-ol (1.99 g, 23.6 mmol, 2.2 mL) in 20 mL DCM was added dropwise over 10 minutes. When addition was complete, the reaction mixture was stirred for a further 1h. Triethylamine (18.5 mL) was added and the reaction mixture was stirred for 1.5h at -78°C followed by 1h at RT. The reaction mixture was diluted with 100 mL DCM, washed with 5% HCl, (2 x 30 mL), DI H2O (1 x 30 mL), saturated NaCl (1 x 30 mL), dried over anhydrous sodium sulfate, filtered and concentrated in vacuo. The residue was purified by Kugelrohr distillation (600 mtorr, 40 °C) giving the aldehyde as a colorless oil (1.34 g, 69%). 1H-NMR (400 MHz, CDCl3); δ 9.79 (t, 1.2 Hz, 1H), 2.73-2.67 (tt, 9.6 Hz, 1.2 Hz, 2H), 2.53-2.47 (m, 2H), 1.99 (t, 4.8 Hz, 1H), 1.64 (s, 5H). 13C-NMR (100 MHz, CDCl3): δ = 200.34, 82.49, 69.49, 42.54, 11.84.

5-azidopentanal (2c)

To a 100 mL round bottom flask equipped with a stirbar, 5-bromo-1-pentanol (2.64 g, 15.8 mmol) and NaN₃ (3.08 mg, 47.4 mmol) were dissolved in 35 mL of DMF. The reaction flask was stirred at 90 °C for 12 h. The reaction flask was allowed to cool to room temperature upon which the solvent was removed in vacuo to yield a yellow
The residue was diluted to 150 mL with ethyl acetate (EtOAc), washed with DI H₂O (4 x 20 mL), saturated NaCl (2 x 20 mL), dried over anhydrous sodium sulfate, filtered and concentrated in vacuo to give 1.74 g of crude 5-azido-1-pentanol as a pale yellow oil which was used immediately without further purification.

To a stirred solution of oxalyl chloride (3.42, 26.9 mmol, 2.31 mL) in 120 mL DCM under argon at -78°C was added dimethyl sulfoxide (4.21 g, 53.8 mmol, 2.31 mL) dropwise over 15 minutes. The mixture was then stirred under argon at -78°C for 30 minutes, at which time a solution of crude 5-azido-1-ol (1.74 g, 13.5 mmol) in 30 mL DCM was added dropwise over 10 minutes. When addition was complete, the reaction mixture was stirred for a further 1h. Triethylamine (11.5 mL) was added and the reaction mixture was stirred for 1h at -78°C followed by 1h at 0°C. The reaction mixture was diluted with 50 mL DCM, washed with saturated NH₄Cl, (2 x 30 mL), DI H₂O (1 x 30 mL), saturated NaCl (1 x 30 mL), dried over anhydrous sodium sulfate, filtered and concentrated in vacuo. The residue was purified by column chromatography (90:10 hexanes:ethyl acetate) giving the aldehyde as a pale yellow oil (912 mg, 45% over two steps). ¹H-NMR (400 MHz, CDCl₃); δ 9.78 (t, 1.6 Hz, 1H), 3.31 (t, 6.8 Hz, 2H), 2.50 (dt, 6.8 Hz, 1.6 Hz, 2H), 1.76-1.59 (m, 4H). ¹³C-NMR (100 MHz, CDCl₃): δ = 201.92, 51.31, 43.44, 28.50, 19.44.

5-phthalimidopentanal (2d)

To a 50 mL round bottom flask equipped with a stirbar, 5-amino-1-pentanol (2.06 g, 20.0 mmol) and phthalic anhydride (2.96 g 20.0 mmol) were dissolved in 60 mL of toluene. The reaction flask was heated to reflux under Dean-Stark conditions for 3h. The
reaction flask was allowed to cool to room temperature upon which the solvent was removed in vacuo to yield a yellow residue. The residue was purified by column chromatography (100% CHCl₃) to give 5-phthalimido-1-pentanol as a white-yellow solid (3.34 g, 72%). ¹H-NMR (400 MHz, CDCl₃); δ = 7.83 (m, 2H), 7.71 (m, 2H), 3.70, (t, 7.2 Hz, 2H), 3.64 (t, 6.4 Hz, 1H), 1.75-1.58 (m, 4H), 1.46-1.40 (m, 2H). ¹³C-NMR (100 MHz, CDCl₃); δ = 168.72 (2C), 134.12 (2C), 132.33 (2C), 123.41 (2C), 62.90, 38.05 32.41, 28.59, 23.23. ESI-MS (m/z) calcd for C₁₃H₁₅NO₃ [M]+ 233.1052; found [M+H]⁺ 234.07, [M+NH₄]⁺ 250.90 and [M+Na]⁺ 256.05.

To a stirred solution of oxalyl chloride (1.45 g, 11.4 mmol, 1.08 mL) in 60 mL DCM under argon at -78°C was added dimethyl sulfoxide (1.78 g, 22.8 mmol, 1.62 mL) dropwise over 10 minutes. The mixture was then stirred under argon at -78°C for 30 minutes, at which time a solution of 5-phthalimido-1-pentanol (1.33 g, 5.71 mmol) in 12 mL DCM was added dropwise over 10 minutes. When addition was complete, the reaction mixture was stirred for a further 1h. Triethylamine (5 mL) was added and the reaction mixture was stirred for 1h at -78°C followed by 1h at 0°C. The reaction was quenched with 10 mL DI H₂O and diluted with 350 mL diethyl ether. The organic layer was separated, washed with DI H₂O (2 x 50 mL), and saturated NaCl (2 x 50 mL), dried over anhydrous sodium sulfate, and concentrated in vacuo. The residue was purified by column chromatography (70:30 hexanes:ethyl acetate) giving the aldehyde as a pale yellow oil (1.00 g, 76%). ¹H-NMR (400 MHz, CDCl₃); δ 9.76 (t, 1.2 Hz, 1H), 7.84 (m, 2H), 7.72 (m, 2H), 3.71 (t, 6.8 Hz, 2H), 2.51 (dt, 6.8 Hz, 1.2 Hz, 2H), 1.77-1.63 (m, 4H). ¹³C-NMR (100 MHz, CDCl₃); δ = 173.72, 83.01, 35.39 28.23, 22.48. ESI-MS (m/z)
calcd for C_{13}H_{13}NO_{3} [M]^+ 231.0895; found [M+H]^+ 232.11, [M+NH_4]^+ 248.91 and [M+Na]^+ 254.06.

2-(2-(2-(azidoethoxy)ethoxy)ethoxy)acetaldehyde (2e)

Tetraethylene glycol (10.0 g, 51.5 mmol) was dissolved in 500mL dry DCM and stirred at room temperature. To the reaction flask were successively added KI (1.71 g, 10.3 mmol), Ag_2O (17.9 g, 77.2 mmol), and tosyl chloride (10.8 g, 56.6 mmol) and then was stirred vigorously at room temperature for 2h. The reaction mixture was filtered through a glass frit to remove the solids and concentrated in vacuo. The residue was purified via silica column chromatography (using a gradient from 100% EtOAc to 98:2 EtOAc:MeOH as eluent) giving 2-(2-(2-(2-azidoethoxy)ethoxy)ethoxy)ethanol a colorless oil (13.2 g, 74%). ^1H-NMR (400 MHz, CDCl_3): δ = 7.74 (d, 8.0 Hz, 2H), 7.30 (d, 8.0 Hz, 2H), 4.11 (t, 4.8 Hz, 2H), 3.66-3.53 (m, 12H), 2.79 (s, 1H), 2.39 (s, 3H). ^13C-NMR (100 MHz, CDCl_3); δ = 145.04, 133.17, 130.10 (2C), 128.19 (2C), 70.95, 70.79, 70.70, 69.49, 68.88, 21.87. ESI-MS (m/z) calcd for C_{15}H_{24}O_7S [M]^+ 348.1243; found [M+H]^+ 348.96, [M+NH_4]^+ 365.94 and [M+Na]^+ 371.08.

To a 50 mL round bottom flask equipped with a stirbar, 2-(2-(2-(2-hydroxyethoxy)ethoxy)ethoxy)ethyl toluenesulfonate (2.0 g, 5.74 mmol) and NaN_3 (186 mg, 17.2 mmol) were dissolved in 15 mL of DMF. The reaction flask was stirred at 90 °C for 12 h. The reaction flask was allowed to cool to room temperature upon which the solvent was removed in vacuo to yield a yellow residue. The residue was diluted with 150 mL of ethyl acetate, washed with DI H_2O (4 x 20 mL), saturated NaCl (2 x 20 mL), dried over anhydrous sodium sulfate, filtered and concentrated in vacuo to give 763 mg
of crude 2-((2-(2-azidoethoxy)ethoxy)ethoxy)ethanol as a yellow oil which was used immediately without further purification.

To a stirred solution of oxalyl chloride (883 mg, 6.96 mmol, 0.597 mL) in 30 mL DCM under argon at -78°C was added dimethyl sulfoxide (1.09 g, 13.9 mmol, 0.989 mL) dropwise over 10 minutes. The mixture was then stirred under argon at -78°C for 30 minutes, at which time a solution of crude 2-((2-(2-azidoethoxy)ethoxy)ethoxy)ethanol (763 mg) in 5 mL DCM was added dropwise over 10 minutes. When addition was complete, the reaction mixture was stirred for a further 1h. Triethylamine (3 mL) was added and the reaction mixture was stirred for 1h at -78°C followed by 1h at 0°C. The reaction mixture was diluted with 100 mL DCM, washed with saturated NH₄Cl, (2 x 25 mL), DI H₂O (1 x 25 mL), saturated NaCl (1 x 25 mL), dried over anhydrous sodium sulfate, filtered and concentrated in vacuo. The residue was purified by column chromatography (100% ethyl acetate) giving the aldehyde as a pale yellow oil (334 mg, 27% over two steps). ¹H-NMR (400 MHz, CDCl₃); δ 9.73 (t, J = 0.8 Hz, 1H), 4.16 (d, 0.8 Hz, 2H), 3.75-3.65 (m, 10H), 3.39 (t, 5.2 Hz, 2H). ¹³C-NMR (100 MHz, CDCl₃): δ = 201.16, 77.06, 71.46, 71.04-70.81 (4C), 70.27, 50.88. HR-MS (m/z) calcd for C₂₂H₂₀N₂O₄Na [M + Na]⁺ 240.0955; found [M + Na]⁺ 240.0957.

2-hydroxy-1-(1H-indol-1-yl)hex-5-yn-1-one (3a)

1-(2,2-dimethoxyethyl)-2-isocyanobenzene (192 mg, 1.0 mmol), 4-pentynal (98.5 mg, 1.2 mmol) and water (36 mg, 36 μL) were dissolved in 5 mL dry DCM and allowed to stir in a scintillation vial at room temperature. After 5 minutes, DL-camphorsulfonic acid (46.5 mg, 0.2 mmol) was added to the vial. The reaction was allowed to stir at room
temperature for 4h, concentrated in vacuo and immediately purified by column chromatography (85:15 hexanes:EtOAc) giving the title N-acylindole 3a as a colorless oil (150 mg, 67%). $^1$H-NMR (400 MHz, CDCl$_3$): 8.46 (d, 8.0 Hz, 1H), 7.58 (d, 7.2 Hz, 1H), 7.47-7.30 (m, 3H) 6.72 (d, 4 Hz, 1H), 5.07 (m, 1H), 3.47 (d, 7.6 Hz, 1H), 2.65-2.57 (m, 1H), 2.49-2.41 (m, 1H), 2.15-2.07 (m, 2H), 1.89-1.80 (m, 1H). $^{13}$C-NMR (100 MHz, CDCl$_3$); δ = 173.49, 135.92, 130.47, 125.82, 124.69, 123.72, 121.31, 116.91, 111.11, 83.01, 70.11, 68.98, 35.02, 15.05. HR-MS (m/z) calcd for C$_{14}$H$_{13}$NO$_2$Na [M + Na]$^+$ 250.0838; found [M + Na]$^+$ 250.0841.

6-azido-2-hydroxy-1-(1H-indol-1-yl)hexan-1-one (3b)

1-(2,2-dimethoxyethyl)-2-isocyanobenzene (192 mg, 1.0 mmol), 5-azidopentanal (154 mg, 1.2 mmol) and water (36 mg, 36 μL) were dissolved in 5 mL dry DCM and allowed to stir in a scintillation vial at room temperature. After 5 minutes, DL-camphorsulfonic acid (46.5 mg, 0.2 mmol) was added to the vial. The reaction was allowed to stir at room temperature for 4h, concentrated in vacuo and immediately purified by column chromatography (80:20 hexanes:EtOAc) giving the title N-acylindole 3b as a colorless oil (190 mg, 69%). $^1$H-NMR (400 MHz, CDCl$_3$): 8.46 (d, 8.0 Hz, 1H), 7.58 (d, 7.2 Hz, 1H), 7.47-7.30 (m, 3H) 6.72 (d, 4 Hz, 1H), 4.85 (dt, 1H, Hz), 3.49 (d, 7.2 Hz, 1H), 3.28 (t, 6.4 Hz, 2H), 1.96-1.93 (m, 1H), 1.79-1.58 (m, 5H). $^{13}$C-NMR (100 MHz, CDCl$_3$); δ = 173.61, 135.92, 130.45, 125.92, 124.67, 123.59, 121.32, 116.86, 111.02, 70.38, 51.43, 37.59, 28.79, 22.55. HR-MS (m/z) calcd for C$_{14}$H$_{16}$N$_4$O$_2$Na [M + Na]$^+$ 295.1165; found [M + Na]$^+$ 295.1161.
2-(5-hydroxy-6-(1H-indol-1-yl)-6-oxohexyl)phthalimide (3c)

1-(2,2-dimethoxyethyl)-2-isocyanobenzene (192 mg, 1.0 mmol), 5-phthalimido-1-pentanol (278 mg, 1.2 mmol) and water (36 mg, 36 μL) were dissolved in 5 mL dry DCM and allowed to stir in a scintillation vial at room temperature. After 5 minutes, DL-camphorsulfonic acid (46.5 mg, 0.2 mmol) was added to the vial. The reaction was allowed to stir at room temperature for 4h, concentrated in vacuo and immediately purified by column chromatography (70:30 hexanes:EtOAc) giving the title acyl indole as a pale yellow oil (261 mg, 69%). 1H-NMR (400 MHz, CDCl3); 8.46 (d, 8.4 Hz, 1H), 7.82-7.80 (m, 2H), 7.71-7.68 (m, 2H), 7.57 (d, 8 Hz, 1H), 7.42-7.28 (m, 3H), 6.72 (d, 4 Hz, 1H), 4.85 (dt, 8 Hz, 3.2 Hz, 1H), 3.70 (t, 6.8 Hz, 2H), 3.44 (d, 7.6 Hz, 1H), 2.01-1.95 (m, 1H), 1.77-1.57 (m, 5H). 13C-NMR (100 MHz, CDCl3): δ = 173.72, 168.63, 135.94, 134.13, 132.29, 130.48, 125.81, 124.57, 123.43, 121.25, 116.87, 110.91, 83.01, 70.11, 70.40, 37.59, 35.39 28.23, 22.48. HR-MS (m/z) calcd for C22H20N2O4Na [M + Na]+ 399.1315; found [M + Na]+ 399.1310.

3-(2-(2-(2-azidoethoxy)ethoxy)ethoxy)-2-hydroxy-1-(1H-indol-1-yl)propan-1-one (3d)

1-(2,2-dimethoxyethyl)-2-isocyanobenzene (192 mg, 1.0 mmol), 2-(2-(2-(azidoethoxy)ethoxy)ethoxy)acetaldehyde (261 mg, 1.2 mmol) and water (36 mg, 36 μL) were dissolved in 5 mL dry DCM and allowed to stir in a scintillation vial at room temperature. After 5 minutes, DL-camphorsulfonic acid (46.5 mg, 0.2 mmol) was added to the vial. The reaction was allowed to stir at room temperature for 4h, concentrated in vacuo and immediately purified by column chromatography (50:50 hexanes:EtOAc)
giving the title acyl indole as a pale yellow oil (237 mg, 65%). $^1$H-NMR (400 MHz, CDCl$_3$): δ = 8.46 (d, 8.0 Hz, 1H), 7.58-7.28 (m, 4H), 6.72 (d, 7.6 Hz, 1H), 5.01 (d, 5.2 Hz, 1H), 3.90 (t, 4.4 Hz, 2H), 3.69-3.54 (m, 11H), 3.34 (t, 5.2 Hz, 2H). $^{13}$C-NMR (100 MHz, CDCl$_3$): δ = 171.16, 136.00, 130.53, 125.62, 124.81, 124.54, 121.14, 116.94, 110.30, 73.74, 71.58, 70.93-70.79 (4C), 70.19, 68.98, 50.84. HR-MS ($m/z$) calcd for C$_{17}$H$_{22}$N$_4$O$_5$Na [M + Na]$^+$ 385.1482; found [M + Na]$^+$ 385.1476.

**General procedure for polymerization 6b-6d**

All polymerizations were carried out with tin(II) 2-ethylhexanoate (stannous octoate, Sn(Oct)$_2$) (purchased from Alfa Aesar) and 4-tert-butylbenzyl alcohol (purchased from Acros) without further purification. The polymerization method is similar to that described by Baker and coworkers.$^{43}$ Freshly prepared 0.01 M solutions of Sn(Oct)$_2$ and 4-tert-butylbenzyl alcohol in anhydrous toluene were used for all polymerizations. Sn(Oct)$_2$ (0.01 M in anhydrous toluene, 796 μL) and 4-tert-butylbenzyl alcohol (0.01 M in anhydrous toluene, 796 μL) were added to a small glass vial and the solution was concentrated in vacuo. The sides of the vial were washed with small portions of additional anhydrous toluene and concentrated in vacuo to concentrate the initiators and the bottom of the vial. To the vial was then added D,L-lactide (144 mg, 1.0 mmol) and either more D,L-lactide or substituted hemilactide (0.15 mmol). The vial was then placed under vacuum. After 20 minutes, the vial was equipped with a small stirbar, flushed with N$_2$ gas, tightly sealed and heated at 130°C in a silicone oil bath for 2 h. After the reaction was completed the vials were cooled on an icebath, dissolved in CDCl$_3$ and the crude was analyzed by $^1$H and $^{13}$C NMR. A small aliquot of the CDCl$_3$
solution was concentrated \textit{in vacuo}, redissolved in DMF, filtered through a Whatman Anontop 10 0.2 μm filter and analyzed by Gel Permeation Chromatography.

**General procedure for CuAAC functionalization of polymers 6a-6d.**

An aliquot containing ~5 mg of polymer 5a-5d in CDCl$_3$ was withdrawn, concentrated in vacuo and redissolved in 0.2 mL DMF. To the polymer sample was added an excess of either dansyl azide 7 or dansyl alkyne 8 dissolved in DMF (0.5 mL of a 2.0 mg/mL solution), an excess of sodium ascorbate (1 mg) and finally ~0.3 mL of a 1 mg/mL solution of CuSO$_4$·5H$_2$O in DMF. The vials were allowed to stand for 2 h at room temperature with occasional shaking. The solutions were then filtered through a Whatman Anontop 10 0.2 μm filter and analyzed by size exclusion chromatography.

**Attempted polymerization of α-hydroxy-N-acylindole 3a**

In a dry 20 mL scintillation vial equipped with a small stirbar, acylindole 3a (19 mg, 0.1 mmol) was dissolved in 5 mL of dry THF and allowed to stir at room temperature. Anhydrous Cs$_2$CO$_3$ (33 mg, 0.1 mmol) was added to the reaction vial. The reaction was stirred for 24 h at room temperature, and directly analyzed by ESI-MS. In addition, a small aliquot of the reaction mixture was removed from the vial, redissolved in DMF and analyzed by size exclusion chromatography.
Attempted polymerization of 3b

In a dry 20 mL scintillation vial equipped with a small stirbar, acylindole 3b (22 mg, 0.1 mmol) was dissolved in 5 mL of dry DCM and allowed to stir at room temperature. A solution of DBU in anhydrous DCM (13.8 mg/mL, 100 μL, 0.010 mmol) was added to the reaction vial. The reaction was stirred for 24 h at room temperature, and directly analyzed by ESI-MS. In addition, a small aliquot of the reaction mixture was removed from the vial, redissolved in DMF and analyzed by size exclusion chromatography.

Synthesis of the copolymer poly(DL-lactide)-co-(3-(but-3-yn-1-yl)-6-methyl-1,4-dioxane-2,5-dione) (9)

Freshly prepared 0.01 M solutions of Sn(Oct)2 and 4-tert-butylbenzyl alcohol in anhydrous toluene were used for the copolymerization. Sn(Oct)2 (1.0 mM in anhydrous toluene, 694 μL, 69.4 μmol) and 4-tert-butylbenzyl alcohol (1.0 mM in anhydrous toluene, 694 μL, 69.4 μmol) were added to a small glass vial and the solution was concentrated in vacuo. The sides of the vial were washed with small portions of additional anhydrous toluene and concentrated in vacuo to concentrate the initiators and the bottom of the vial. To the vial was then added D,L-lactide (800 mg, 5.55 mmol) and the alkyne substituted hemilactide (253 mg, 1.39 mmol). The vial was then placed under vacuum. After 20 minutes, the vial was equipped with a small stirbar, flushed with N2 gas, tightly sealed and heated at 130°C in a silicone oil bath. After 2 h, the reaction vial was cooled on an icebath and the vial contents were dissolved in approximately 5 mL of
chloroform. The chloroform solution was slowly added to 75 mL of methanol and the resulting cloudy suspension was separated into four centrifuge tubes and spun at 10,000 rpm for 30 minutes. The supernatant was decanted from each tube, combined, concentrated in vacuo and the precipitation repeated with the supernatant discarded. The viscous material concentrated at the bottom of each tube was washed several times with methanol to remove remaining low molecular weight oligomers and tin catalyst, dissolved in a small volume of toluene and added dropwise to heptane to precipitate a white solid, which was collected by filtration. The recovered solid was redissolved in toluene and precipitated in heptane to give the alkyne-substituted polymer as a white solid (660 mg, 63 % yield, $M_n = 46.9$ kDa, PDI =1.26). $^1$H and $^{13}$C NMR were virtually identical to that of polymer 6b.

**General Procedure for nanoprecipitation:**

20 mg of polymeric material (either PLA or PLA-co-PLA-alkyne) is dissolved in 20 mL of the organic solvent acetone or acetonitrile) to make a 1.0 mg/mL solution. If encapsulation of a drug is desired, the drug of choice was added to the organic polymer solution. The solution is transferred to an addition funnel and added dropwise slowly to 40 mL of a rapidly stirring 0.3% w/v aqueous polyvinyl alcohol solution. An opalescent suspension is observed after addition of the organic phase is complete. After addition, 20 mL of DI H$_2$O was added to the opalescent suspension while stirring. To prepare nanoparticles using more concentrated solutions, a volume of polymer solution in organic volume is added to twice the volume of aqueous phase containing stabilizer. When addition of the organic phase is complete, enough DI water is added to the opalescent
suspension such that the organic solvent constitutes 25% of the total volume. After 5 minutes, the suspension is placed under reduced pressure for 30 minutes to remove the organic solvent. The aqueous nanoparticle suspension was then passed through a 0.45 μm syringe filter and further purified on a Sephadex NAP-25 size exclusion column (Amersham Biosciences) to remove traces of small molecules.

**Notes about this chapter**

Chapter 2, in part, is based on material which appears in "Facile Procedure for Generating Side Chain Functionalized Poly (alpha-hydroxy acid) Copolymers from Aldehydes via a Versatile Passerini-Type Condensation" Rubinshtein, M.; James, C. R.; Young, J. L; Ma, Y. J.; Kobayashi, Y.; Gianneschi, N. C.; Jerry Yang, J. *Organic Letters*, 2010, 12, 3560-3563. I am the primary author of this paper. In addition, Chapter 2 contains material currently being prepared for submission for publication: "Direct synthesis of side chain functionalized alpha-hydroxy acid oligomers from alpha-hydroxy-\(N\)-acylindole precursors." Rubinshtein, M.; James, C. R.; Young, J. L.; Gianneschi, N. C.; Yang, J. I am the primary author of this pending manuscript.
Part II: Studies Toward a Chemical Method to Degrade Alzheimer’s Disease-Related beta-Amyloid Peptides.
Chapter 3

Alzheimer’s Disease: Causes, Mortality and Treatment Options

3.1 Alzheimer’s disease: An emerging health crisis

Alzheimer’s disease (AD) is a progressive, neurodegenerative disorder and the most common form of dementia. AD begins with the mild decline of cognitive function and memory and progresses to rob the patient of his or her most human qualities, including the ability to recognize family members and friends and the capacity to care for oneself. Ultimately, AD results in the impairment of even the most basic bodily processes, resulting in patient death. Although significant research efforts are ongoing, there is currently no cure or disease-altering therapeutic for this debilitating condition.

The overwhelming majority of those afflicted with AD are elderly, and the biggest risk factor for developing AD is advanced age. One in eight people over 65 have AD, with this number increasing to over 40 percent for those 85 and older. With longer life expectancies and increasing healthcare standards around the world, the number of people with AD is expected to climb. Currently, AD is the sixth leading cause of death in the United States, with over 5 million Americans afflicted with the disease. This value is projected to reach 11-16 million by 2050. The incidence of AD is also increasing at an alarming rate relative to other top killers. Between 2000 and 2006, AD related-death has increased nearly 50 percent, while mortality from heart disease, stroke, breast cancer
and prostate cancer have declined (Table 3.1), further illustrating AD’s role a growing health concern. In addition to its human toll, AD also has a profound economic impact: direct and indirect costs associated with AD currently top $180 billion annually in the United States and are expected to rise to over $1 trillion (in 2011 dollars) by 2050.

**Table 3.1:** Percentage change in selected causes of death in the United States between 2000 and 2006.

<table>
<thead>
<tr>
<th>Cause of Death</th>
<th>Percentage change</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alzheimer’s Disease</td>
<td>+46.1</td>
</tr>
<tr>
<td>Stroke</td>
<td>-18.2</td>
</tr>
<tr>
<td>Heart Disease</td>
<td>-11.1</td>
</tr>
<tr>
<td>Breast Cancer</td>
<td>-2.6</td>
</tr>
<tr>
<td>Prostate Cancer</td>
<td>-8.7</td>
</tr>
<tr>
<td>HIV/AIDS</td>
<td>-16.3</td>
</tr>
</tbody>
</table>

The problem of AD is not confined to the United States; worldwide cases of AD are expected to balloon, particularly in China, India and other developing Asian and Pacific Rim nations. Projections estimate that by 2050, 115 million people worldwide will have AD and other dementias. The increasing worldwide numbers of those with AD may precipitate a global health crisis and reinforces the importance of developing new strategies for combating this disease.
3.2 Alzheimer’s disease and the amyloid cascade hypothesis

One of the major characteristics of AD is the prevalence of extracellular amyloid plaques and intracellular neurofibrillary tangles (NFT) in post-mortem analysis of brain tissue. The plaques are comprised mostly of aggregated β-amyloid peptide (Aβ), whereas the NFTs contain misfolded hyperphosphorylated tau protein. While NFTs are associated with a variety of tauopathies such as Pick’s Disease or ganglioglioma, aggregated Aβ plaques are uniquely present in the brain tissue of AD patients.

The Aβ peptide is a 39-43 amino acid peptide, with Aβ1-40 and Aβ1-42 being two most abundant isoforms. The N-terminus region of Aβ is rich in hydrophilic residues while the C-terminal region is highly hydrophobic (Figure 3.1). The Aβ peptide, particularly the Aβ1-42 isoform, is highly prone to aggregation and can assemble into dimeric and oligomeric species in solution as well as form insoluble fibrils that are deposited extracellularly as amyloid plaques.

The Aβ peptide is generated from the amyloid precursor protein (APP), an integral membrane protein found in high concentrations in the synapses of neurons. APP is normally processed by the non-amyloidogenic α-secretase pathway, resulting in APP cleavage between the Lys16 and Leu17 residues in the transmembrane domain. Alternatively, APP may be cleaved successively by β-secretase and γ-secretase to generate Aβ. Initial enzymatic hydrolysis of APP by β-secretase gives a membrane-anchored, carboxy-terminated stub (sAPPβ) that is further cleaved within the transmembrane domain by γ-secretase to give Aβ. Because the cleavage site of γ-
secretase is not precise, this cleavage mechanism results in Aβ peptides of varying length.\textsuperscript{82}

Figure 3.1: A portion of the amyloid precursor protein sequence (APP) represented by one-letter codes, with the sequence of Aβ\textsubscript{1-42} shown in bold. α-Secretase cleaves APP between the Lys16 and Leu17 residues within the Aβ sequence, resulting in non-amyloidogenic peptide fragments. However, APP cleaved successively by β-secretase and γ-secretase produces Aβ peptides of varying length, depending on the specific cleavage site of γ-secretase.

Strong evidence suggests that Aβ is an important factor in the development of AD. For instance, Aβ is toxic to neuronal cells,\textsuperscript{83} and mutations in the APP gene, located on chromosome 21, has been linked to early onset AD.\textsuperscript{84} Furthermore, those with Down Syndrome (trisomy 21) overproduce APP and Aβ, develop AD by the time they are in their forties.\textsuperscript{85} The causative role of Aβ in AD is the cornerstone of the amyloid cascade hypothesis.\textsuperscript{86-89} First proposed by Hardy and Selkoe in early 1990s and refined over the last 20 years, the amyloid cascade hypothesis proposes that AD is caused by an imbalance between Aβ production and clearance by natural cellular mechanisms.\textsuperscript{90-92} Accumulation of Aβ leads to the formation of various aggregated forms such as soluble oligomers and the insoluble fibrils that deposit extracellularly as plaques. The toxic aggregates (particularly the soluble oligomers) cause an inflammatory response that
triggers oxidative stress in neuronal cells leading to decline in neuronal function and ultimately cell death.

Although the exact mechanisms of Aβ toxicity are unknown, it has been proposed that Aβ induces oxidative stress in the AD brain. Aβ can generate reactive oxygen species in the presence of metal ions such Cu$^{2+}$ that result in cellular damage.$^{93, 94}$ Oxidative stress can also be manifested via interaction of Aβ with other cellular components. Recent work by Habib et al showed that inhibiting the Aβ-catalase interaction protects cells from the toxic effects of Aβ-induced oxidative stress, presumably by restoring catalase function to permit H$_2$O$_2$ dismutation.$^{95}$ Another potentially detrimental interaction occurs when Aβ binds to the enzyme amyloid-beta binding alcohol dehydrogenase (ABAD) and modifies its function, which promotes the production of reactive oxygen species and increased oxidative stress to cells.$^{94, 96, 97}$ It has also been suggested that another possible mechanism of Aβ toxicity involves the formation of pores in cell membranes and subsequent alteration of membrane permeability.$^{98}$

### 3.3 Current state of Alzheimer’s disease therapeutics

Several small molecule-based drugs that are currently FDA-approved for use in the treatment of AD disease are listed in Table 3.1; however, none of these compounds are disease-altering therapeutics.$^{99}$ Rather, these drugs treat the symptoms of AD and begin to lose their potency after a short time, sometimes accelerating the cognitive decline of patients after their initial period of efficacy.$^{100-102}$ The majority of available
drugs are a class of molecules known as acetylcholinesterase (AChE) inhibitors, which combat the decrease in the levels of the acetylcholine, a neurotransmitter that facilitates synapse function, observed in AD patients.\textsuperscript{103} The only non-AChE inhibitor currently approved for AD treatment is memantine, an $N$-methyl-D-aspartase (NMDA) antagonist. Memantine works by binding to NMDA receptors to restrict cellular influx of Ca\textsuperscript{2+} ions, which can overstimulate and damage neuronal cells.\textsuperscript{104-106} Ultimately, however, there are no current AD treatments that alter the causative factors of this debilitating condition.

\textbf{Table 3.2:} Current FDA-approved small molecule treatments for Alzheimer's Disease.

<table>
<thead>
<tr>
<th>Drug</th>
<th>Structure</th>
<th>Drug Type</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dopenizil</td>
<td><img src="image" alt="Dopenizil Structure" /></td>
<td>AChE Inhibitor</td>
</tr>
<tr>
<td>Risvastigmine</td>
<td><img src="image" alt="Risvastigmine Structure" /></td>
<td>AChE Inhibitor</td>
</tr>
<tr>
<td>Tacrine</td>
<td><img src="image" alt="Tacrine Structure" /></td>
<td>AChE Inhibitor</td>
</tr>
<tr>
<td>Galantamine</td>
<td><img src="image" alt="Galantamine Structure" /></td>
<td>AChE Inhibitor</td>
</tr>
<tr>
<td>Memantine</td>
<td><img src="image" alt="Memantine Structure" /></td>
<td>NMDA antagonist</td>
</tr>
</tbody>
</table>
### 3.4 Small-molecule based strategies and targets for AD therapy

Since Aβ is believed to be a causative factor in AD, one research strategy has focused on blocking the production of Aβ by inhibiting the function of β-secretase and γ-secretase. Ghosh and coworkers have successfully prepared GRL-8234 a peptidomimetic compound that shows tremendous potential as a clinically useful β-secretase inhibitor (Figure 3.2); GRL-8234 was found to be a potent β-secretase inhibitor with a $K_i = 1.8$ nM and significantly reduces the production Aβ in transgenic mice by up to 65%.

![Structure of the β-secretase inhibitor GRL-8234.](image)

Several compounds have been identified that inhibit γ-secretase in vitro; however, developing safe γ-secretase inhibitors remains an obstacle due to its additional cellular functions, particularly cleavage of the Notch signaling protein. Instead, γ-secretase modulation has been explored as an alternative strategy. γ-Secretase modulators selectively lower the production of Aβ$_{1-42}$ in favor of the shorter Aβ isoforms that are less prone to aggregation. Several non-steroidal anti-inflammatory drugs (NSAIDs) have been identified as γ-secretase modulators including ibuprofen and
One NSAID, (R)-Flurbiprofen, showed initial promise as an AD therapeutic; however it was ultimately discontinued in 2008 after poor results during Phase III clinical trials.116

Other attempts to modify the pathology of AD involve small molecules that direct target Aβ. Gestwicki illustrates a strategy that utilizes small molecules that recruit molecular chaperones in order to inhibit Aβ fibril formation and reduce Aβ toxicity.117 Cole and coworkers demonstrate the efficacy of curcumin, found in the spice turmeric, can inhibit Aβ formation as well as effect disaggregation of multimeric assemblies of the peptide.118 Additionally, Yang and coworker have designed a class of molecules that generate surface coatings on Aβ to prevent harmful amyloid interactions with cellular components.119, 120 This was recently shown to be an effective strategy in reducing Aβ toxicity by using surface coatings to inhibit the detrimental Aβ-catalase interaction.95

3.5 Degradation of Aβ as a therapeutic strategy

Since aggregated Aβ peptides are largely resistant to normal degradation mechanisms in cells,90-92, 121 the development of chemical methods to selectively cleave
Aβ peptides under physiological conditions could lead to an effective strategy to permanently eradicate these toxic biomolecules from the brain. Selective cleavage of Aβ using a Co(III)-cyclen complex tethered to an Aβ-binding was demonstrated by Suh and coworkers in 2007,\textsuperscript{122} and a very similar strategy was employed using a Cu(II)-cyclen complex as the active species by Wu et al.\textsuperscript{123} However, there are no other examples of small-molecule promoted Aβ cleavage in literature and currently there are no metal-free methods that effect Aβ degradation.

3.6 Goal of the dissertation research

The goal of this dissertation research is to develop a method for the selective degradation of Aβ using small molecules. Chapter 4 explores a strategy to degrade Aβ peptides in aqueous solutions at physiological temperatures with a metal-free small molecule system that utilizes a cyclic enediyne as the chemical warhead. A major challenge that was encountered was the tendency of Aβ to aggregate and be generally uncooperative, appropriately earning it the nickname “the peptide from hell.”\textsuperscript{124} During the course of this research, an improved synthesis of a class of Aβ-binding was developed and led to several collaborations due to the increased availability of these compounds. Chapter 5 presents the improved synthesis of these Aβ-binding molecules, as well as several investigations into the uses and properties of these molecules ranging from inhibition of HIV transmission to formation of ion-channels in lipid bilayers.
Chapter 4

Studies Toward an Enediyne-Based Target-Directed Chemical Method to Degrade Alzheimer’s-Related β-Amyloid Peptides

4.1 Introduction

This chapter describes a chemical approach to degrade Alzheimer’s-related Aβ peptides at physiological temperature in neutral aqueous solution. Since substantial evidence suggests that Aβ peptides play a pathological role in the development of AD, many recent therapeutic strategies have focused on neutralizing the toxicity of this peptide. Examples of strategies currently being explored to directly modify the pathology of AD using Aβ-targeting synthetic molecules include: 1) the binding of Aβ peptides and soluble Aβ oligomers in order to prevent or retard the growth of Aβ fibrils; 2) the binding of Aβ fibrils or protofibrils in order to effect disaggregation of the peptide; and 3) the generation of protein resistive surface coatings on aggregated Aβ peptides in order to ameliorate harmful protein-amyloid interactions. Although all of these therapeutic strategies show promise for disfavoring the formation or deactivating the function of toxic forms of Aβ, they all rely on natural, biological degradation pathways for eventual clearance of these pathogenic peptides. Since aggregated Aβ peptides are largely resistant to normal degradation mechanisms in cells, the development of chemical methods to selectively cleave Aβ peptides under
physiological conditions could lead to an effective strategy to permanently eradicate these toxic biomolecules from the brain.

4.2 A target-directed strategy toward selective peptide degradation

Proteins are most often degraded by living systems via enzymatic hydrolysis; however, researchers have long been interested using small, synthetically accessible molecules to affect peptide cleavage. One well-known chemical protease is cyanogen bromide, which demonstrates remarkable site-specific hydrolytic activity at the C-terminus side of methionine residues. Several groups have successfully designed small protein-cleaving molecules that can target specific proteins. Notably, Schultz and coworkers demonstrated selective cleavage of streptavidin using a synthetic molecule comprised of a biotin moiety covalently linked to a metal-chelating ethylenediaminetetraacetic acid (EDTA) unit. The extraordinarily high affinity of biotin for streptavidin allows for non-covalent binding of the small molecule to the peptide, while the EDTA chelated and delivered the redox-active Fe^{3+} and Cu^{2+} cations responsible for degradation to the cleavage site. Schepartz exploited a similar strategy for site-specific degradation of the calcium receptor protein calmodulin using trifluoperazine, a synthetic molecule with a high affinity for the protein, tethered to a EDTA unit chelating a redox-active Fe^{2+} cation. Inspired by this target-directed approach, we chose to design a small molecule bearing a chemical warhead covalently linked to a suitable binding group in an attempt to selectively degrade the Alzheimer’s-related Aβ peptide.
4.3 Enediynes as potent chemical warheads

The explosion of interest in enediyne-containing natural products began with the isolation and structural determination of the calicheamicin and esperamicin antibiotics. These classes of compounds, comprised of an unusual (Z)-cyclodeca-3-en-1,5-diyne (enediyne) moiety, were found to exhibit extraordinarily potent anticancer activity. The toxicity of these molecules has been widely attributed to their ability to effect cleavage of DNA at the minor groove.

Bergmann has previously shown that under appropriate conditions, enediynes undergo cycloaromatization to give a highly reactive \(p\)-benzyne diradical species that abstracts hydrogen from a suitable donor to give a benzene-containing product (Figure 4.1). The rate of cycloaromatization is highly dependent on the \(cd\) distance—the distance measured from the terminal \(sp\) carbon atoms in the enediyne moiety. Thermal cyclization of linear enediynes, which have \(cd\) distances on the order of 4 Å, requires temperatures in excess of 200°C; however, cyclic enediynes require considerably lower temperatures to

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\text{Figure 4.1. Bergman cycloaromatization of (A) a linear enediyne and (B) a cyclic enediyne in a 10-membered ring system to a reactive } p\text{-benzyne diradical intermediate.}
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effect cycloaromatization, presumably due to their increased strain energy. Cyclic enediynes in 10-membered ring systems, for example, have a cd distance of 3.25 Å and a cyclization half-life of about 18 hours at 37°C, while enediynes confined to 9-membered ring systems (cd < 3 Å), cyclize readily even at low temperatures and cannot be isolated.141

We chose to use a 10-membered cyclic enediyne ring moiety as a peptide degrading warhead for these studies because this class of enediynes has been previously reported to be capable of cleaving proteins142-144 and DNA145, 146 at physiological temperatures through reversible cyclization to a diradical species. Additionally, cyclic enediynes are synthetically accessible, do not require an external cofactor (such as a metal) for activation as a chemical cleaving agent and have been successfully implemented in an FDA-approved therapeutic agent.147 We hypothesized that a small molecule comprised of a cyclic enediyne “warhead” moiety covalently linked to an Aβ-binding group can selectively cleave Aβ. The binding group is expected to deliver the enediyne warhead to the target peptide, where cyclization to the reactive diradical species at physiological temperature will promote degradation of the peptide backbone (Figure 4.2).
Figure 4.2: Cartoon depicting the target-directed approach of Aβ degradation. A cyclic enediyne warhead bearing a suitable binding group is predicted to associate with Aβ peptides, delivering the enediyne moiety to the target peptide. Subsequent cyclization to the $\beta$-benzyne diradical may provide the reactive species necessary to degrade Aβ.

The exact mechanism of enediyne-promoted peptide cleavage is unclear. One plausible mechanism of enediyne-induced scission of proteins under aerobic conditions has recently been proposed by Jones and coworkers, wherein the diradical intermediate generated from the enediyne abstracts a hydrogen atom $\alpha$ to the carbonyl in the peptide backbone.\textsuperscript{148} The peptide radical formed from hydrogen abstraction proceeds to react with molecular oxygen to oxidatively cleave the polypeptide give a C-terminal amide and alpha-oxo ketone (or aldehyde in the case of glycine) fragments. Alternatively, degradation of the peptide could also occur through one or more radical abstractions of the hydrogen atoms from the amino acid side chains (Figure 4.3).\textsuperscript{149}
Figure 4.3: Possible mechanisms of peptides degradation by the reactive $p$-benzyne diradical. Abstraction of an $\alpha$-hydrogen from the peptide backbone leads to peroxide formation and ultimately peptide scission to give an amide and $\alpha$-oxo ketone (or aldehyde). Alternatively, the highly reactive diradical may abstract an $\alpha$-hydrogen from the peptide backbone (or a hydrogen atom from an amino acid side chain) to give other degradation products.

We synthesized a 10-membered cyclic enediyne ring using a modified procedure as described by Jones and coworkers (Scheme 4.1). Importantly, this molecule bears a pendant functional group that will ultimately permit covalent attachment of the enediyne warhead moiety onto a suitable A\(\beta\) targeting domain. The enediyne moiety was formed in one step from linear dibromide 10, presumably via a carbenoid-coupling reaction, to give the desired TBDMS-protected macrocyclic product 11. Jones and coworkers have reported yields of up to 95% for this cyclization; however, only poor yields were achieved using the published experimental protocol. Upon successful cyclization the TBDMS protecting group was removed with TBAF under standard conditions to afford enediyne alcohol 12.

In order to make the enediyne more suitable for covalent linkage with an A\(\beta\) binding group, we chose to convert the alcohol pendant functionality of 12 to a terminal
carboxylic acid. The acid functionality would enable the formation of a very stable amide linkage between the enediyne and a binding group bearing a free amine. We explored several methods to oxidize the alcohol, however, direct oxidation proved to be too harsh for the cyclic enediyne moiety. Accordingly we decided to use milder methods to introduce a terminal carboxylic acid onto the warhead molecule. Reacting the enediyne alcohol 12 with tert-butyl bromoacetate under phase transfer conditions gave terminal ester 13 in moderate yield.\textsuperscript{151, 152} We assumed that ester 13 would be readily deprotected under standard conditions; however, both acid deprotection with trifluoroacetic acid and basic deprotection with potassium tert-butoxide resulted in immediate decomposition of the molecule. Ultimately we removed the tert-butyl group using a method described by Danishefsky.\textsuperscript{153} Treating ester 13 with tert-butyl dimethysilyl triflate and 2,6-lutidine provided enediyne free acid 14 in 77\% yield, presumably by first forming the TBDMS ester which readily hydrolyzed during aqueous workup.

\textbf{Scheme 4.1:} Synthesis of the substituted cyclic enediyne bearing a terminal carboxylic acid pendant functional group 14 from linear dibromide 10.
4.4 Preparation of the BTA binding moiety

In order to achieve targeted degradation of Aβ using small molecules, the designed synthetic molecule must bear a moiety known for binding effectively to Aβ. Several molecules are known to bind to Aβ with high affinity and have been exploited to aid in the visualization of these aggregates in brain tissue. Two such molecules include thioflavin T (ThT), commonly used to visualize Aβ plaques and a related analogue, Pittsburgh Compound B (PiB), often used in positron emission tomography (PET) imaging of amyloids (Figure 4.4). These molecules both contain a benzothiazole aniline (BTA) ring system and are thought to bind to the multiple repeated binding sites found on Aβ.154

![Structures of Thioflavin T (ThT) and Pittsburgh Compound B (PiB)](image)

**Figure 4.4:** Structures of the Aβ binding molecules Thioflavin T (ThT) and Pittsburgh compound B (PiB) containing the benzothiazole moiety.

Yang and coworkers have previously shown that BTA analogues containing an oligoethylene glycol chain bind with high density to Aβ.119 Since the ethylene glycol group should also impart a degree of water solubility to the molecule, we chose to use BTA derivative prepared from pentaethylene glycol as the targeting group. The synthesis of BTA-EG₅ (21) is shown in Scheme 4.2. Di-tosylation of pentaethylene glycol (15) followed by reaction with excess sodium iodide readily afforded diiodide 16. However,
nucleophilic substitution of the BTA onto the oligoethylene glycol proved to be much less facile. The reaction of 6-methylbenzothiazole aniline (BTA) with 17 afforded BTA-EG₅-monoiodide 18 in 25% after 3 days of refluxing in THF. Only a minute amount (<1%) of dimeric BTA species was formed. The efficiency of this substitution reaction has recently been improved and is further discussed in chapter 5 of this thesis. Conversion of the BTA-EG₅-monoiodide to the free amine was accomplished by substitution with azide to provide 19, followed by Staudinger reduction to give BTA-EG₅-amine 20. It is important to note that due to the cyclic enediyne’s propensity to slowly cyclize, we took precautions to minimize deterioration: Accordingly, reaction times of enediyne-containing compounds at room temperature were minimized, workups were done as quickly as possible and cyclic enediyne intermediates were stored at -80°C.

Scheme 4.2: Synthesis of BTA-EG₅ amine 20.
4.5 Synthesis and binding affinity of the BTA-enediyne conjugate

We covalently attached the cyclic enediyne to BTA-EG₅-amine via an amide coupling reaction promoted by 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC) to afford the Aβ-targeting enediyne 21 (Scheme 4.3). In addition, we synthesized a similar control molecule 22 comprising a cyclic enediyne molecule that does not carry an Aβ-binding group. Our final control molecule was the BTA-EG₅-amine 20 as it contains an Aβ-binding group but lacks the enediyne warhead required for peptide degradation. We demonstrated, using a recently reported ELISA-based binding assay,¹³¹ that the BTA-containing molecules 20 and 21 associate with aggregated Aβ peptides, whereas molecule 22 does not appear to associate with Aβ (Figure 4.5).

![Scheme 4.3: Formation of the enediyne-BTA conjugate 21 and a related control molecule 22 containing the enediyne warhead but lacking an Aβ-binding group.](image)
Figure 4.5: Briefly, the design of this ELISA-based competition assay entails the evaluation of molecules that inhibit the interaction of Aβ fibrils (here, formed from Aβ1-40) with a monoclonal anti-Aβ IgG raised against residues 3-8 of AD-related Aβ peptide (clone 6E10). This assay is based on the hypothesis that molecules that can effectively and efficiently coat Aβ fibrils will be able to inhibit the binding of this anti-Aβ IgG to Aβ fibrils. The relative inhibition of IgG-Aβ fibril interactions by small molecules is quantified using a standard ELISA protocol. Inhibition (Inh.) of anti-Aβ IgG (clone 6E10)–Aβ interactions with compound: a) **21**, 55% Maximal Inh., IC$_{50}$ = 220 μM; b) **22**, No Inhibition; and c) **20**, 51% Maximal Inh., IC$_{50}$ = 100 μM. Error bars represent ± SEM.

4.6 Effect of the BTA-enediyne conjugate on aggregated Aβ

We prepared aggregated Aβ by incubating synthetic Aβ1-40 peptides for 4 days at 37 °C in deionized water. CD spectroscopy indicated that the solution of Aβ peptides contained significant β-sheet character (Figure 4.6), which is characteristic of aggregated Aβ peptides. Specifically, The CD spectrum shows a maximum at $\lambda = 197$ and a
minimum at $\lambda = 216$ nm, which confirmed the $\beta$-sheet character of A$\beta$ and is consistent with experiments conducted by Gursky and Aleshkov.$^{157}$ Deconvolution software was used to analyze the $\beta$-sheet content and was determined to be 35% using the K2D software (available at http://www.embl-heidelberg.de/~andrade/k2d) and 40.2% using SOMCD software (available at http://geneura.ugr.es/cgi-bin/somcd/index.cgi).

![Circular dichroism spectrum](image)

**Figure 4.6:** A Circular dichroism spectrum of a 100 $\mu$M aqueous solution of A$\beta$ after incubation at 37°C for 4 days.

We incubated A$\beta$ peptides in deionized water with the BTA-enediyne conjugate 21 (at 37 °C) and analyzed the peptide solution by RP-HPLC and SDS-PAGE after 7 days (Figure 5.9A). We observed that the RP-HPLC peak at 12.1 minutes, corresponding to the A$\beta$ peptide, decreased over the course of 7 days (with approximately 50% and 20% of the peak area of A$\beta$ peptide remaining after incubation with 1 mole and 10 mole equiv. of 21, respectively), while we also observed the appearance of several new peaks (Figure 4.7 A). The new HPLC peaks that appeared from incubating A$\beta$ with 21 presumably
correspond to some of the major fragments from cleavage of Aβ peptides. Attempts to characterize these new fragments by LC-MS and LC-MS-MS were inconclusive, most likely due to the expected complicated mixture of products from reaction of the α and side-chain hydrogen atoms of the amino acids in Aβ under these radical conditions.\textsuperscript{149}

Although repeated reactions of Aβ with 21 consistently resulted in the same RP-HPLC peaks, the quantitative conversion of Aβ to peptide fragments varied between experiments presumably due to the well-known heterogeneity of aged solutions of Aβ.\textsuperscript{124}

SDS-PAGE analysis of Aβ (~ 4 kDa) incubated with 1 mole equivalent of 21 for 7 days supports partial degradation of Aβ\textsubscript{1-40} to peptide fragments with a smaller mass than Aβ.

**Figure 4.7**: Chromatographic and electrophoretic analyses of the degradation of Aβ peptides using a targeted enediyne. (A) RP-HPLC analysis of Aβ\textsubscript{1-40} peptides after incubation with 1 molar equivalent of 21 at 37°C after 0 days (top) and 7 days (middle), or 10 equivalents of 21 after 7 days (bottom). (B) SDS-PAGE analysis of Aβ\textsubscript{1-40} peptides after incubation with 1 molar equivalent of 21 or 5 molar equivalents of 22 or 20 at 37°C for 7 days. Lane 1: Mark12TM standard. Lane 2: Aβ\textsubscript{1-40} alone. Lane 3: Aβ\textsubscript{1-40} + 1 molar equivalent 21 incubated for 7 days. Lane 4: Aβ\textsubscript{1-40} + 5 molar equivalent 22 incubated for 7 days. Lane 5: Aβ\textsubscript{1-40} + 5 molar equivalent 20 incubated for 7 days. SDS-PAGE gels were run using a 16% tricine gel and visualized via silver staining.
The new, lowest molecular weight band in lane 3 in Figure 4.7B presumably corresponds to a mixture of products from the degradation of Aβ peptides that could not be separated from each other under the SDS-PAGE conditions employed. We did not observe, however, any degradation of aggregated Aβ over 7 days when we incubated these aggregated peptides in the presence of 5 mole equivalents of enediyne 22 (i.e., lacking an Aβ-binding moiety, Figure 4.7B, lane 4), or in the presence of 5 mole equivalents of BTA derivative 20 (i.e., lacking the enediyne, Figure 4.7B, lane 5). As a further control, we incubated 21 with ubiquitin, an 8.6 kDa protein, and did not observe any degradation of this protein over the course of 7 days (Figure 4.8). These HPLC and SDS-PAGE studies collectively demonstrate that BTA-enediyne conjugate 21 may

![Figure 4.8: SDS-PAGE analysis of ubiquitin (8.6 kDa) alone and after incubation with 1 molar equivalent of 21 at 37 °C for 7 days. Lane 1: Mark12TM standard. Lane 2: ubiquitin (90%, from Sigma-Aldrich, used without further purification). Lane 3: ubiquitin + 1 molar equivalent 21 incubated for 7 days. SDS-PAGE gels were performed using a 16% tricine gel and visualized via silver staining.](image-url)
selectively degrade Aβ peptides over other potential proteins in solution, and that the cleavage of Aβ by the enediyne moiety in 21 is directed by the Aβ-binding BTA group.

To assess whether the cleavage of Aβ₁₋₄₀ by 21 had an effect on the toxicity of aggregated Aβ on cells, we exposed SH-SY5Y human neuroblastoma cells to aggregated Aβ₁₋₄₀ peptides, or to aggregated Aβ peptides that were incubated with 21 for 0 or 7 days (Figure 4.9). Cell viability was estimated using a standard MTT assay as describe by Mosmann.¹⁵⁸ Incubation of 20 mM Aβ peptides with 5 mole equivalents of 21 for 7 days resulted in a mixture of peptides and peptide fragments that was less toxic to these cells (~67 ± 1% retention of cell viability) compared to Aβ alone (~53 ± 2% retention of cell viability) or compared to when Aβ and 21 were mixed and immediately exposed to the cells (~42 ± 6% retention of cell viability). We did not perform these cytotoxicity studies using higher molar ratios of Aβ and 21 since 21 was significantly toxic to this cell line at concentrations above 100 μM and we needed to use a 20 μM solution of aggregated Aβ peptides to observe significant toxicity to these cells. These results indicate that 21 can, at least, partially protect cells from the toxicity of Aβ, presumably through its ability to degrade Aβ into less toxic species.
Figure 4.9: Relative cell viability of SH-SY5Y human neuroblastoma cells in the presence of aggregated Aβ peptides with or without incubation with molecule 21. Cells incubated with 21 alone were treated with a solution of 21 that was incubated for 7 days in sterile water prior to exposure to cells. Aβ samples were prepared by incubation in sterile water for 4 days and: 1) incubated further for 7 days prior to exposure to cells, 2) incubated further for 7 days, mixed with 21, and immediately exposed to cells (labeled as 0 days), or 3) incubated with 21 for 7 days prior to exposure to cells (labeled as 7 days). The data are expressed as mean values + standard deviations. The viability of cells exposed to Aβ incubated with molecule 21 for 7 days was significantly different than the viability of cells exposed to Aβ alone (P < 0.001).

4.7 Chapter summary

We demonstrate that a cyclic enediyne covalently attached to an Aβ-binding group can promote the degradation of Aβ peptides at physiological temperature in aqueous solution. This targeted enediyne exhibited partial cytoprotective properties of against Aβ toxicity in human neuroblastoma cells, and, hence, represents a first step towards a new strategy to combat AD through the chemical degradation of Aβ using synthetic molecules. A major challenge that remains is to develop such targeted
molecular warheads that can be administered safely in humans. One potential methods to improve the biocompatibility of warheads could be to use triggered enediynes\textsuperscript{159} (i.e., that become activated only when bound to Aβ peptides). Several groups have developed chemical cleaving agents based on metal-chelating cyclen motifs that demonstrate the capability to affect degradation of Aβ peptides under aqueous conditions.\textsuperscript{122,123} However, the method presented in this chapter uses a warhead that does not require activation by an external source such as a redox-active metal. A chemical method to degrade disease-related materials such as Aβ peptides under physiological conditions represents a promising new strategy for combating AD and other amyloid-related disorders.

### 4.8 Experimental Methods

All synthetic reagents were from Aldrich, Fisher Scientific, Alfa Aesar, Fluka, City Chemical, or Quanta Biosciences and were used without further purification. Aβ\textsubscript{1-40} peptides were from Biopeptide, Inc., and used without further purification. All solvents used for reactions were obtained from Fisher scientific and dried prior to use. Solvents used for chromatography were ACS technical grade and used without further purification. All intermediates containing cyclic enediyne moieties described in this chapter were stored at -80 °C. SH-SY5Y human neuroblastoma cells (Product No: CRL-2266) and 3-(4,5-dimethylthiazolyl-2)-2, 5-diphenyltetrazolium bromide (MTT) cell proliferation assay (Product No: 30-1010K) were purchased from American Type Culture Collection.

All NMR data was obtained on a 400 MHz Varian Mercury Spectrometer. Circular Dichroism (CD) experiments were performed on an Aviv Instruments Circular
Dichroism Spectrometer Model 215 using a quartz CD cell purchased from Hellma (0.2 mm path length). HPLC analyses were performed on a Hewlett-Packard (now Agilent) 1100 Series HPLC instrument. Water (18.2 $\mu$Ω/cm) was filtered through a NANOPure Diamond™ (Barnstead) water purification system before use.

1-(tert-butyl)-1,1-dimethylsilyl (5-cyclodecen-3,7-diynylmethyl) ether (11)

A flame-dried 250 mL 3-necked round bottom flask was charged with dibromide 10 (872 mg, 2.0 mmol), hexamethylphosphoramide (8.8 g, 100.0 mmol, 8.5 mL) and 110 mL dry tetrahydrofuran (THF) and allowed to stir under a N$_2$ atmosphere at -40 °C. After the temperature had stabilized, Lithium hexamethyldisilazide (LiHMDS, 0.25 M in THF, 10.0 mmol, 20 mL) was added slowly via mechanical syringe pump over 5 h. The dark brown reaction mixture was poured without warming onto 120 mL of an aqueous saturated solution of NH$_4$Cl (containing 40 g of ice) and the solution was extracted with diethylether (Et$_2$O) (4 x 40 mL). The combined organic phase was washed with cold HCl (10%, 2 x 40 mL), DI H$_2$O (40 mL), saturated NaHCO$_3$ (40 mL) and saturated NaCl (20 mL), dried over sodium sulfate and concentrated in vacuo. Care was taken to use a low vacuum setting on the rotary evaporator and to keep the reaction flask cool. The residue was eluted using a plug of silica (95:5 hexanes:Et$_2$O as eluent) gave 220 mg of crude product as a yellow oil. The crude material was taken on to the next step without further purification. $^1$H-NMR (400 MHz) of the crude in CDCl$_3$ showed the presence of the cyclized product 11 by the appearance of the olefinic protons of the enediyne at $\delta = 5.83$ ppm.
Cyclodeca-5-en-3,7-diylnmethanol (12).

Crude tert-butyl(cyclodeca-5-en-3,7-diylnmethoxy)dimethylsilane 11 (220 mg) was dissolved in 5 mL of dry THF and allowed to stir at 0 °C. A solution of tetrabutyl ammonium fluoride (1.0 M in THF, 1.2 mL, 1.2 mmol) was added dropwise to the reaction mixture over two minutes. The ice bath was removed and the solution was allowed to stir for a further 90 minutes while warming to room temperature. The reaction mixture was diluted with 75 mL of Et2O and washed with HCl (10%, 1 x 15 mL), saturated NaHCO3 (1 x 15 mL), DI H2O (1 x 15 mL), and saturated NaCl (1 x 15 mL). The organic layer was dried over Na2SO4 and concentrated in vacuo to give a yellow residue. Column chromatography (60:40 Et2O:hexane as eluent) gave the enediyne alcohol 12 as a yellow oil (54 mg, 17% over two steps from 10). 1H-NMR (400 MHz, CDCl3); δ = 5.82 (s, 2H), 3.54 (m, 2H), 2.50-2.63 (m, 2H), 2.06-2.35 (m, 3H), 1.78-1.90 (m, 2H), 1.67 (s, 1H). 13C-NMR (400 MHz, CDCl3): δ = 123.53, 123.48, 104.16, 102.97, 83.17, 83.08, 67.64, 44.59, 32.85, 24.68, 21.35. ESI-MS (m/z) calcd for C11H12O [M]+ 160.0888; found [M-H]- 159.22.

tert-butyl-2-(cyclodeca-5-en-3,7-diylnmethoxy)acetate (13).

Enediyne alcohol 12 (20 mg, 0.13 mmol) was dissolved in 0.25 mL benzene and allowed to stir in a 4 mL reaction vial at 10 °C. After 5 minutes, tetrabutyl ammonium hydrogen sulfate (21 mg, 0.06 mmol) and KOH (40% in H2O, 0.25 mL) were added to the reaction vial and the biphasic reaction mixutre was allowed to stir vigorously. After 5 minutes, tert-butyl bromoacetate (29 mg, 0.15 mmol, 22.2 μL) was rapidly added to the vial. The reaction was allowed to stir at 10 °C for 45 min, and then at room temperature
for an additional 60 min. The reaction was diluted to 30 mL with Et₂O, washed with DI H₂O (5 mL) and saturated NaCl (5 mL), dried over Na₂SO₄ and concentrated in vacuo to give a yellow oil. The residue was purified via flash column chromatography (using 90:10 hexanes:EtOAc (ethyl acetate) as eluent) giving a clear pale yellow oil (19 mg, 55%). ¹H-NMR (400 MHz, CDCl₃): δ = 5.81 (s, 2H), 3.95 (d, 1.2 Hz, 2H), 3.43 (m, 2H), 2.49-2.63 (m, 2H), 2.19-2.36 (m, 3H), 1.85-1.90 (m, 2H) 1.47 (s, 9H). ¹³C-NMR (400 MHz, CDCl₃); δ = 169.92, 123.52, 123.40, 104.24, 103.07, 83.10, 83.08, 81.92, 76.17, 69.02, 42.10, 33.03, 28.42 (3C), 25.08, 21.27. ESI-MS (m/z) calcd for C₁₇H₂₂O₃ [M]+ 274.1569; found [M+Na]+ 297.04.

2-(cyclodeca-5-en-3,7-diynylmethoxy)acetic acid (14).

An oven-dried 25 mL roundbottom flask was charged with tert-butyl ester 13 (39 mg, 0.14 mmol), 2,6-lutidine (304 mg, 2.84 mmol, 329 μL) and 10 mL dry dichloromethane (DCM) and allowed to stir under an N₂ atmosphere at 0 °C. After 15 minutes, tert-butyldimethylsilyl triflate (316 mg, 1.4 mmol, 257 μL) was added rapidly to the reaction flask. After 1 h, the icebath was removed and the solution was allowed to stir for a further 2 h while warming to room temperature. The reaction was diluted to 100 mL with Et₂O, washed with HCl (5%, 10 mL), and saturated NaCl (10 mL), dried over Na₂SO₄ and concentrated in vacuo to give a viscous yellow oil. The residue was purified via flash column chromatography (using 90:10 DCM:methanol as eluent) giving a yellow foam (24 mg, 77%). ¹H-NMR (400 MHz, CDCl₃): δ = 5.81 (s, 2H), 4.10 (s, 2H), 3.46 (m, 2H), 1.85-2.62 (m, 7H). ¹³C-NMR (400 MHz, CDCl₃); δ = 175.26, 123.51 (2C),
104.10, 102.76, 83.25 (2C), 76.34, 68.78, 41.96, 33.12, 25.01, 21.23. ESI-MS (m/z) calcd for C_{13}H_{14}O_{3} [M]^+ 218.0943; found [M-H]^- 217.10 and [2M-H]^- 434.95.

3,6,9,12-tetraoxatetradecane-1,14-diyl bis(toluenesulfonate) (16)

Pentaethylene glycol (15) (4.0 g, 26.0 mmol) and tosyl chloride (12.4 g, 65.0 mmol) in 130 mL dry DCM was allowed to stir until complete dissolution. Pyridine (10.7 mL, 132 mmol) was then added to the reaction flask and allowed to stir at room temperature for 12 h. The reaction mixture was slowly poured onto a mixture of concentrated HCl (15ml) and crushed ice (150 g). The mixture was left standing until the ice had melted and then organic layer was separated from the aqueous layer. The organic layer was washed with saturated NaCl (1 x 25 mL), dried over Na_2SO_4 and concentrated in vacuo. The residue was purified via silica column chromatography (using a gradient from 100% DCM to 55:45 DCM:ethyl acetate (EtOAc) as eluent) giving di-tosylate 16 as a clear pale yellow oil (9.8 g, 69%). ^1H-NMR (400 MHz, CDCl_3): δ = 7.77 (d, 8.0 Hz, 4H), 7.33 (d, 8.0 Hz, 4H), 4.18 (t, 4.8 Hz, 4H), 3.56-3.68 (m, 18H), 2.43 (s, 6H). ^13C-NMR (400 MHz, CDCl_3): δ = 145.04 (2C), 133.17 (2C), 130.10 (4C), 128.19 (4C), 70.95 (2C), 70.79 (2C), 70.70 (2C), 69.49 (2C), 68.88 (2C), 21.87 (2C). ESI-MS (m/z) calcd for C_{24}H_{34}O_{10}S_{2} [M]^+ 546.1593; found [M+NH_4]^+ 564.08 and [M+Na]^+ 569.13.

1,14-diido-3,6,9,12-tetraoxatetradecane (17)

3,6,9,12-tetraoxatetradecane-1,14-diyl bis(toluenesulfonate) (16) (3.03 g, 5.48 mmol) and dry sodium iodide (7.20 g, 48.0 mmol) were combined in an oven-dried round bottom flask. Anhydrous acetone (30 mL) was added to the flask. Upon dissolution of
the solid material, the reaction mixture was heated to reflux for 12 h. After cooling to room temperature, the solids were removed by filtration and washed with acetone until colorless. The mother liquor was concentrated \textit{in vacuo} and the residue was re-dissolved in EtOAc (75 mL). The organic layer was then washed with Na$_2$S$_2$O$_3$ (10\% in H$_2$O, 7 mL), saturated NaCl (1 x 10 mL), dried over Na$_2$SO$_4$ and concentrated \textit{in vacuo} to give a pale yellow oil (2.46 g, 98\%). \textsuperscript{1}H-NMR (400 MHz, CDCl$_3$): $\delta$ = 3.56 (t, 6.8 Hz, 4H), 3.46 (m, 11H), 3.08 (t, 6.8 Hz, 3H). ESI-MS ($m/z$) calcd for C$_{10}$H$_{20}$I$_2$O$_4$ [M]$^+$ 457.9451; found [M+H]$^+$ 458.84

\textbf{14-iodo-N-(4-(6-methylbenzothiazol-2-yl)phenyl)-3,6,9,12-tetraoxatetradecan-1-amine (18)}

An oven-dried 2 L round bottom flask charged with 1,14-diiodo-3,6,9,12-tetraoxatetradecane 17 (23.4g, 51.0 mmol), 6-methylbenzothiazole aniline (BTA) (88g, 360 mmol), potassium carbonate (115 g, 820 mmol) and dry THF (1 L) was allowed to stir for 15 minutes while being purged with N$_2$ gas. The reaction flask was equipped with a condenser (and a drying tube) and was heated to reflux while stirring for 7 days. The reaction flask was then cooled to room temperature and the solvent removed \textit{in vacuo}. The residue was dissolved in DCM (200 mL), and filtered to remove insoluble material. The organic phase was then washed with DI H$_2$O (2 x 30 mL), dried over Na$_2$SO$_4$, and concentrated \textit{in vacuo} to give a dark yellow/brown residue. The residue was purified via silica column chromatography (98:2 EtOAc:MeOH) to yield a yellow oil (7.2 g, 25\%).

Note: This compound should be used immediately after preparation as it slowly spontaneously cyclizes to the aza-crown ether. \textsuperscript{1}H-NMR (400 MHz, CDCl$_3$): $\delta$ = 7.88 (d,
8.4 Hz, 2H), 7.85 (d, 8.4 Hz, 1H), 7.62 (s, 1H), 7.23 (dd, 6.8 Hz, 1.4 Hz, 1H), 6.67 (d, 8.8 Hz, 2H), 3.65-3.75 (m, 17H), 3.37 (t, 5.2 Hz, 2H), 3.24 (t, 6.8 Hz, 2H), 2.47 (s, 3H). \(^{13}\text{C-}
\text{NMR (400 MHz, CDCl}_3\text{): }\delta = 168.01, 152.57, 150.82, 134.86, 134.60, 129.22 (2C), 127.81, 122.99, 122.08, 121.48, 112.89 (2C), 72.23, 70.96, 70.90, 70.85 (2C), 70.63, 70.48, 69.59, 43.40, 21.77, 3.19. \text{ESI-MS (m/z) calcd for C}_{24}\text{H}_{31}\text{IN}_{2}\text{O}_{4}\text{S [M]+ 570.1049; found [M+H]+ 571.04.}

\textbf{14-azido-N-(4-(6-methylbenzothiazol-2-yl)phenyl)-3,6,9,12-tetraoxatetradecan-1-amine (19)}

To a 50 mL round bottom flask equipped with a stirbar, 535 mg (938 mmol) BTA-EG\(_5\)-I \textbf{18} and NaN\(_3\) (186 mg, 2.86 mmol) were dissolved in 25 mL of DMF. The reaction flask was stirred at 90 °C for 17 h. The reaction flask was allowed to cool to room temperature upon which the solvent was removed \textit{in vacuo} to yield a yellow residue. The residue was diluted to 150 mL in EtOAc, washed with DI H\(_2\)O (4 x 15 mL), saturated NaCl (15 mL), dried over sodium sulfate and concentrated \textit{in vacuo}. Purification via silica column chromatography (100% EtOAc) gave the azide \textbf{19} as a viscous yellow oil (419 mg, 91%). \(^{1}\text{H-NMR (400 MHz, CDCl}_3\text{): }\delta = 7.88 (d, 8.8 Hz, 2H), 7.84 (d, 8.4 Hz, 1H), 7.62 (s, 1H), 7.23 (dd, 6.8 Hz, 1.2 Hz, 1H), 6.66 (d, 8.8 Hz, 2H), 3.72 (t, 5.2 Hz, 2H), 3.65-3.75 (m, 15H), 3.24 (t, 5.2 Hz, 4H), 2.46 (s, 3H). \(^{13}\text{C-}
\text{NMR (400 MHz, CDCl}_3\text{): }\delta = 167.99, 152.68, 150.81, 134.92, 134.56, 129.18 (2C), 127.78, 123.04, 122.09, 121.48, 112.89 (2C), 70.95, 70.88 (2C), 70.82, 70.61, 70.29, 69.59, 50.93, 43.37, 21.76. \text{IR (KBr pellet): IR: 2102.7, 1608.4, 1484.6, 1454.5, 1180.6,}

1120.4 cm\(^{-1}\). ESI-MS (m/z) calcd for C\(_{24}H_{31}N_{5}O_{4}S\) [M]\(^+\) 485.2097; found [M+H]\(^+\) 486.21.

\textbf{N'(4-(6-methylbenzothiazol-2-yl)phenyl)-3,6,9,12-tetraoxatetradecane-1,14-diamine (20)}

In a 100 mL round bottom flask equipped with a stirbar, compound 19 (383 mg, 789 mmol) and triphenylphosphine (621 mg, 2.37 mmol) were dissolved in 25 mL of THF. The reaction was allowed to stir at room temperature for 20 minutes, at which time 2.5 mL of DI H\(_2\)O was added. The reaction flask was equipped with a reflux condenser and allowed to stir at 60 °C. After 4 h, the reaction flask was allowed to cool to room temperature, concentrated \textit{in vacuo} and immediately subjected to silica column chromatography (90:10:1 DCM:MeOH:conc. NH\(_4\)OH(aq) as eluent). The combined fractions were dried over Na\(_2\)SO\(_4\), filtered and concentrated \textit{in vacuo} to give the amine 20 as a viscous yellow oil (351 mg, 97%). \(^1\)H-NMR (400 MHz, CDCl\(_3\)): \(\delta = 7.88\) (d, 8.8 Hz, 2H), 7.85 (d, 8.0 Hz, 1H), 7.63 (s, 1H), 7.23 (dd, 7.2 Hz, 1.2 Hz, 1H), 6.67 (d, 8.8 Hz, 2H), 3.73 (t, 5.2 Hz, 2H), 3.61-3.67 (m, 13H), 3.50 (t, 5.2 Hz, 2H), 3.37 (t, 5.2 Hz, 2H), 2.86 (s, 2H), 2.47 (s, 3H), 1.77 (s, 2H). \(^{13}\)C-NMR (400 MHz, CDCl\(_3\)): \(\delta = 168.01, 152.71, 150.86, 134.94, 134.57, 129.20\) (2C) 127.79, 123.05, 122.12, 121.49, 112.86 (2C), 73.58, 70.90 (2C), 70.86 (2C), 70.66, 70.54, 69.65, 43.42, 42.00, 21.78. HR-MS (m/z) calcd for C\(_{24}H_{33}N_{5}O_{4}S\) [M]\(^+\) 459.2186; found [M]\(^+\) 459.2188.
2-(cyclodeca-5-en-3,7-diynylmethoxy)acetic acid 14 (6.5 mg, 30 μmol) was dissolved in 1 mL dry DCM and allowed to stir in a dry scintillation vial at 0 °C. After 5 minutes, BTA-EG$_5$-NH$_2$ 3 (16.5 mg, 36 μmol) in 1 mL dry DCM was added to the solution of the enediyne-carboxylic acid 7. 4-Dimethylaminopyridine (DMAP) (5.5 mg, 45 μmol) and Ethyl (N,N-dimethylaminopropyl)carbodiimide hydrochloride (EDC•HCl) (8.6 mg, 45 μmol) were added to the vial. The reaction was allowed to stir at 0 °C for 2h, and then at room temperature for an additional 1h. The reaction mixture was immediately purified by silica column chromatography (using a gradient from 100% EtOAc to 97:3 EtOAc:MeOH as eluent) to give 21 as a yellow oil (3.9 mg, 20%). $^1$H-NMR (400 MHz, CDCl$_3$): δ = 7.88 (d, 8.8 Hz, 2H), 7.85 (d, 8.0 Hz, 1H), 7.63 (s, 1H), 7.23 (dd, 7.2 Hz, 1.2 Hz, 1H), 6.87, (s, 1H), 6.67 (d, 8.8 Hz, 2H), 5.82 (s, 2H), 3.94 (s, 2H), 3.73 (t, Hz, 4.8 Hz, 2H), 3.63-3.67 (m, 13H), 3.57 (t, Hz, 4.8 Hz, 2H), 3.50 (m, 2H), 3.37 (m, 4H), 2.47-2.60 (m, 5H), 2.17-2.34 (m, 3H), 1.77-1.91 (m, 2H). HR-MS (m/z) calcd for C$_{37}$H$_{45}$N$_3$O$_6$S [M]$^+$ 659.3024; found [M]$^+$ 659.3016.

2-(cyclodeca-5-en-3,7-diynylmethoxy)acetic acid 14 (6.5 mg, 30 μmol) was dissolved in 1 mL dry DCM and allowed to stir in a dry scintillation vial at 0 °C. After 5 minutes, 2,5,8,11-tetraoxatridecan-13-amine (9.3 mg, 45 μmol, from QuantabioDesign,
Ltd.) in 1 mL dry DCM was added to the solution of the enediyne-carboxylic acid 14. DMAP (5.5 mg, 45 μmol) and EDC•HCl (8.6 mg, 45 μmol) were added to the vial. The reaction was allowed to stir at 0 °C for 2h, and then at room temperature for an additional 1h. The reaction mixture was immediately purified by silica column chromatography (using a gradient from 100% DCM to 95:5 DCM:MeOH as eluent) giving compound 22 as a yellow oil (6.8 mg, 57%). ¹H-NMR (400 MHz, CDCl₃): 6.90 (s, 1H), δ = 5.82 (s, 2H), 3.95 (s, 2H), 3.37-3.66 (m, 21H), 2.50-2.61 (m, 2H), 2.27-2.35 (m, 1H), 2.15-2.20 (m, 2H), 1.80-1.89 (m, 2H). ¹³C-NMR (400 MHz, CDCl₃); δ = 169.67, 123.57, 123.47, 103.91, 102.43, 83.36, 83.29, 76.17, 72.21, 70.79-70.89 (5C), 70.62, 70.06, 59.32, 42.04, 38.83, 33.09, 25.01, 21.23. HR-MS (m/z) calcd for C₂₂H₃₃NO₆ [M]+ 407.2302; found [M]+ 407.2304.

**Circular dichroism analysis of aggregated Aβ₁₋₄₀ samples**

Optical rotation data was obtained at 1 nm intervals between 300-190 nm with an acquisition time of 3 s for each data point. Results of 4 runs were averaged and then corrected by subtracting out a blank sample containing only NANOPure water.

A 100 μM solution of Aβ₁₋₄₀ was prepared by dissolving 300 μg Aβ₁₋₄₀ in 693 μL of deionized nanopure water. The solution was transferred to a quartz cuvette, which was then capped and sealed with parafilm. The sample was incubated at 37°C for 4 days and then subjected to CD analysis.
MTT Cell Viability Assay

The viability of SH-SY5Y cells exposed to different treatments was measured based on the ability of metabolically active cells to reduce yellow tetrazolium MTT to purple formazan, which can be measured at $\lambda = 570$ nm. The assay was performed in a 96-well plate format, where each sample was measured at least in triplicate. Cells were plated at a density of 50,000 cells/well (in 100 $\mu$L of culture media consisting of 1:1 mixture of Eagle's Minimum Essential Medium (Product No. 30-2003 from ATCC) and Ham’s F12 supplemented with 10% Fetal Bovine Serum) and incubated overnight before treatment with 100 $\mu$L of various sample solutions. All incubation steps were performed in a humidified incubator at 37 °C and 5% carbon dioxide unless stated otherwise.

The cells were exposed to a final concentration of the following sample solutions: 1) 100 $\mu$M molecule 21 that had been incubated in sterile water for 7 days; 2) 20 $\mu$M aggregated Aβ prepared from a 100 $\mu$M stock solution of Aβ (incubated for 4 days) that was incubated further in sterile water for 7 days, 3) 20 $\mu$M aggregated Aβ prepared from a 100 $\mu$M stock solution of Aβ (incubated for 4 days) that was incubated further in sterile water for 7 days and mixed with 100 $\mu$M 21 immediately prior to exposure to cells; and 4) 20 $\mu$M aggregated Aβ prepared from a 100 $\mu$M stock solution of Aβ (incubated for 4 days) that was incubated with 100 $\mu$M 21 in sterile water for 7 days. All sample solutions were supplemented with an equivalent volume of 2X culture media immediately prior to dosing cells. The cells were incubated with the sample solutions for 24 hours and after the 24-hour incubation period, 20 $\mu$L of the MTT reagent was added and cells incubated
further for 3 hours. The cells were subsequently solubilized with 100 μL detergent reagent, incubated overnight at room temperature and cell viability determined by measuring the absorbance at 570 nm. All results were expressed as percent reduction of MTT relative to untreated controls (defined as 100% viability) and the average absorbance value for each treatment was subtracted with the absorbance reading of wells containing media, MTT reagent and detergent reagent.

**Statistical Analysis**

Independent two-tailed student’s t-tests were performed using Origin 7.0 (Microcal Software, Inc., Northhampton, MA) to evaluate the statistical significance of the difference between the control and experimental mean values. A p-value of < 0.05 was defined as statistically significant. All results were expressed as mean values ± standard deviations.

**Notes about this chapter**

Chapter 4 is based on material currently being prepared for submission for publication: Mark Rubinshtein, Lila K. Habib, Mahealani R. Bautista, and Jerry Yang “Chemical Degradation of Alzheimer’s-Related beta-Amyloid Peptides Using a Targeted Enediyne.” I was the primary investigator and author of this material.
Chapter 5

Efficient Synthesis and Applications of Oligoethylene glycol derivatives of Benzothiazole Anilines

5.1 Introduction

This chapter presents a highly improved general methodology for the synthesis of oligoethylene glycol derivatives of BTA. Previous syntheses of this class of compounds—such as those described in chapter 4 of this dissertation and by Inbar et al.\textsuperscript{119}—have been plagued by poor yields and long reaction times. Because of the interesting amyloid binding properties and potential utility of BTA-oligoethylene glycol (BTA-EG\textsubscript{x}) derivatives, and the interest of examining their biological activity \textit{in vivo}, it was imperative to develop a more efficient synthetic route to this family of compounds. The improved methodology we present for BTA-EG\textsubscript{x} synthesis has both significantly decreased reaction times and increased yields, and has afforded facile access to multi-gram quantities of these molecules. The increased availability of the BTA-EG\textsubscript{x} molecules has provided us with the opportunity to probe the possible uses of these compounds through collaboration with several other research groups. This chapter highlights several of the collaborative efforts that showcase the potential biomedical applications and fundamental studies of BTA-EG\textsubscript{x} compounds.
5.2 Microwave-assisted synthesis of BTA-EG\textsubscript{4} and BTA-EG\textsubscript{6}

The synthetic route we describe for the preparation of BTA-EG\textsubscript{x} compounds utilizes commercially available oligoethylene glycols as starting materials. We successfully applied the methodology to the synthesis of two specific members of the BTA-EG\textsubscript{x} family: BTA-EG\textsubscript{4}, which is derived from tetraethylene glycol, and BTA-EG\textsubscript{6}, which is made from hexaethylene glycol (Figure 5.1). These two molecules were chosen as targets due to their demonstrated ability to bind aggregated A\textsubscript{\beta} peptides with high density,\textsuperscript{119} however, this methodology can, in principle, be exploited to prepare any BTA-EG\textsubscript{x} molecule from the corresponding oligoethylene glycol.

![BTA-EG4 and BTA-EG6](image)

**Figure 5.1:** Structures of BTA-EG\textsubscript{4} (23) and BTA-EG\textsubscript{6} (24). BTA-EG\textsubscript{4} is synthesized using tetraethylene glycol as a starting material, while BTA-EG\textsubscript{6} is prepared from hexaethylene glycol.

The syntheses of BTA-EG\textsubscript{4} (23) and BTA-EG\textsubscript{6} (24), which are outlined in Scheme 5.1, begin with monotosylation of tetraethylene glycol 25 and hexaethylene glycol 26, respectively. Although others have successfully monotosylated symmetric diols by using a large excess of the diol, we instead chose to prepare the desired intermediate using a protocol described by Bouzide and Sauvé.\textsuperscript{160} Employing Ag\textsubscript{2}O as the base affords the monotosylate in 74% yield and the di-tosylate as a minor side product. In addition to the high yield of desired product and the ease of purification,
introducing this synthetic step eliminates the need to use a large excess of diol, making it particularly suitable for monotosylation of costlier oligoethylene glycol starting materials in BTA-EG\textsubscript{x} synthesis. The monotosylate is readily converted to the monoiodide via an \textit{S\textsubscript{N}2} reaction with sodium iodide.

![Scheme 5.1: Synthesis of BTA-EG\textsubscript{4} 23 and BTA-EG\textsubscript{6} 24 from tetraethylene glycol 25 and hexaethylene glycol 28, respectively.](image)

Substitution of BTA onto the monoiodide had been the most problematic step in the reaction sequence, and consequently required alteration to improve the overall efficiency of BTA-EG\textsubscript{x} synthesis. Our previous attempts to affect this transformation result in low yields, even with reaction times of one week under reflux conditions. Instead of using conventional heated methods to affect the substitution reaction, we explored the possibility of using microwave-assisted synthesis\textsuperscript{161} to generate BTA-EG\textsubscript{4} and BTA-EG\textsubscript{6}. Indeed, reacting BTA with the appropriate oligoethylene glycol monoiodide at 120°C in a microwave reactor resulted in a drastically increased yield of
BTA-EG₄ (from 25% to 56%) and a modest increase in yield for the BTA-EG₆ (from 25% to 34%) while lowering the reaction times from on the order of days to only two hours.

### 5.3 Further derivatization of BTA-EG₆

Upon successful preparation of the BTA-EG₅ using microwave-assisted synthesis, we chose to evaluate the ease of further modification of the hydroxy terminus—specifically to the azide functionality—in order to create a BTA derivative compatible with CuAAC. In order to accomplish this transformation, we attempted to first convert the hydroxy group to the toluenesulfonyl ester. However, treating BTA-EG₆ 24 with 1.1 equivalents of toluenesulfonyl chloride and pyridine in DCM led to a significant sulfonanilide formation at the aniline nitrogen. Indeed, adding 2.0 equivalents of toluenesulfonyl chloride to BTA-EG₆ with pyridine as the base led to complete consumption of the starting material and afforded the N,O-ditosylated product 31, and subsequent substitution with sodium azide produce the hydrolytically stable sulfonanilide 32 (Scheme 5.2A). Alternatively, treating BTA-EG₆ 24 with toluenesulfonyl chloride, triethylamine and catalytic 4-DMAP affords only toluenesulfonyl ester 33 in good yield. Azide 34 is readily prepared from toluenesulfonyl ester 33 via nucleophilic substitution (Scheme 5.2B). The azide derivative of BTA-EG₆ 34 is highly versatile, as it possesses click-compatible functionality and can covalently linked to a number of terminal alkyne containing partners. In addition, azide 34 can be easily converted to the primary amine via Staudinger reduction.¹⁵⁵,¹⁵⁶
Scheme 5.2: Tosylation of BTA-EG₆ 24 under different conditions. Treatment of BTA-EG₆ with TsCl in pyridine/DCM (A) affords the N,O-ditosylated product 31, while using triethylamine/4-DMAP in DCM gives toluenesulfonyl ester 33 as the major product. Subsequent substitution of 31 and 33 with sodium azide affords the organoazide BTA derivatives 32 and 34, respectively.

5.4 Blocking HIV-1 transmission with BTA-EG₆

Amyloid fibrils found in semen known as semen-derived enhancer of viral infection (SEVI) have been shown to enhance infectivity of HIV-1. Consequently, SEVI is a target for potential microbicidal agents that can decrease transmission of the virus. Our recent collaboration with Dewhurst and coworkers at the University of
Rochester explores the strategy of using BTA-EG₆ (24), a known amyloid-binding small molecule, to target SEVI and inhibit the infectivity of HIV (Figure 5.2).\textsuperscript{163}

![Diagram](image)

**Figure 5.2:** Schematic representation of the putative role of aggregated PAP248-286 peptides (also called SEVI peptides) in HIV-1 infection. a) Primary sequence and helical representation of PAP248-286 peptides,\textsuperscript{164} these abundant peptides have been reported to spontaneously form aggregated amyloid fibrils in semen. b) Illustration of the SEVI amyloid-mediated infection of a T cell (pink/red) with HIV-1 (blue/purple).\textsuperscript{165} Also depicted is the proposed method to attenuate SEVI-mediated infection by forming bio-resistant coatings on aggregated amyloids derived from PAP248-286.

In order to assess the viability of this approach, the binding affinity of BTA to SEVI fibrils was measured using a previously reported fluorescence-based assay (Figure 5.3).\textsuperscript{166} This study demonstrated that BTA-EG₆ binds to SEVI fibrils with high affinity ($K_d = 127 \pm 22$ nM). In comparison, BTA-EG₆ has approximately the same binding affinity to AD-related Aβ₁-₄₂ ($K_d = 111 \pm 32$ nM). Accordingly, the extent of the interaction between BTA-EG₆ and SEVI prompted further investigation into the potential
of this targeting strategy. Indeed, it was found that BTA-EG₆ inhibits SEVI-mediated enhancement of HIV-1 infection in a dose-dependent manner (Figure 5.4A) with an IC₅₀ value of 13 μM. At high BTA-EG₆ concentrations (22.5 μg/mL), the infectivity of HIV was lowered to values only slightly higher than those detected in the absence of SEVI. Dewhurst and coworkers showed that BTA-EG₆ had no impact on the infectivity of the virus alone, implying that the reduction in infectivity is due to the interaction of BTA-EG₆ with SEVI rather than a result of general virus infectivity (Figure 5.4). In addition to its demonstrated inhibitive effect on SEVI-mediated infectivity of HIV-1, BTA-EG₆ is able to reduce the infection-enhancing properties of human semen (Figure 5.5). Treating pooled human semen samples with BTA-EG₆ inhibited semen-mediated infection at concentrations similar to those active against SEVI.

**Figure 5.3**: Binding of BTA-EG₆ to SEVI fibrils as determined by a previously reported centrifugation assay. Briefly, various concentrations of BTA-EG₆ in PBS were incubated overnight at room temperature in the presence or absence of SEVI fibrils. After equilibration, each solution was centrifuged, and the supernatants were separated from the pelleted fibrils. The fluorescence of BTA-EG₆ was determined from the resuspended pellets in PBS solution. *Error bars* represent S.D. of duplicate measurements. The $K_d$ was determined by fitting the data to a one-site specific binding algorithm: $Y = B_{max}[X/(K_d\cdot X)]$, where $X$ is the concentration of BTA-EG₆, $Y$ is the specific binding fluorescence intensity, and $B_{max}$ corresponds to the apparent maximal observable fluorescence upon binding of BTA-EG₆ to SEVI fibrils. *RFI*, relative fluorescence intensity.
Figure 5.4: (A) HIV-1 IIIB virions were preincubated with increasing concentrations of BTA-EG6 (24) (0, 5.5, 11, and 22.5 μg/ml) and with or without SEVI (15 μg/ml) as indicated. The samples were then added to CEM-M7 cells. Cells were washed at 2 h, and infection was assayed at 48 h by measuring Tat-driven luciferase expression. Results shown are average values ± S.D. of triplicate measurements from one of four independent experiments that yielded equivalent results. * indicates p < 0.05 when compared with control cells exposed to HIV-1IIIB + SEVI alone by ANOVA with Tukey’s post test. RLU, relative luciferase units; Uninf, uninfected. (B) B, zoom in of panel A to show data for cells treated with HIV-IIIB virions with and without increasing concentrations of BTA-EG6, in the absence of SEVI. BTA-EG6 had no effect on the infectivity of HIV alone; concentrations of BTA-EG6 are noted above for panel A.

The inhibitive effect of BTA-EG6 against both SEVI and semen mediated infectivity of HIV-1 makes it a potential candidate for addition to microbicidal formulations that can be used to combat the spread of the virus. Furthermore, BTA-EG6 exhibits no toxicity or inflammation to cervical cells, even after 24 hours of exposure at concentrations ten times the IC50 (Figure 5.6). The non-toxic properties of BTA-EG6 reinforces its potential as an ingredient in microbicidal formulations, as it is well known that agents known to cause inflammation to cervical cells, such as the discontinued microbicide nonoxynol-9, may increase the risk of HIV transmission.167, 168 Accordingly,
Figure 5.5: HIV-1IIIB virions were preincubated with 50% pooled human semen, with or without increasing concentrations of BTA-EG₆ (5.5, 11, and 22.5 μg/ml). After 10 min, these stocks were diluted 15-fold into CEM-M7 cells. Cells were washed after 1 h, and luciferase expression was measured at 48 h to quantify the extent of infection. Results shown are average values ± S.D. of triplicate measurements from one of three independent experiments that yielded equivalent results. * indicates $p < 0.05$ when compared with control cells exposed to HIV-1IIIB + semen alone, by ANOVA with Tukey’s post test. RLU, relative luciferase units.

Figure 5.6: The cervical endothelial cell lines A2En (endocervical), 3EC1(ectocervical), and SiHa were treated for 12 h with BTA-EG₆ at concentrations up to 10 times greater than the IC₅₀. Control cultures were treated with nonoxynol-9 (non-9) at 0.1% final concentration as a positive control for induction of cell death. At 12 h, viability was measured by resazurin cytotoxicity assay (alamarBlue™ assay). Representative results from A2En cells are shown; results from 3EC1 and SiHa cells were very similar (not shown).
BTA-EG₆ is a viable candidate for use as an antimicrobial agent for combating the spread of HIV-1 through sexual contact.

### 5.5 Self-assembled cation-selective ion channels formed from BTA-EG₄.

The formation of ion channels in cell membranes is critical for many biological properties including signal transduction and regulation of membrane potentials. Accordingly synthetic molecules with ion-channel forming properties are garnering significant attention due to their potential use in biomedical applications. Because of its amphiphilic nature and demonstrated membrane permeability, we suspected that BTA-EG₄ might exhibit interesting activity in the presence of cell membranes. In collaboration with Mayer and coworkers at the University of Michigan, we explored the ion channel forming properties of BTA-EG₄.

Figure 5.7 shows that BTA-EG₄ indeed assembles into well-defined pores with quantized conductance levels at concentrations of 10 μM in planar bilipid layers. These channels were found to have an average conductance of 37.8 ± 1.3 pS with an average lifetime of approximately 2 s in 1.0 M CsCl. Multiples of the main conductance level observed are presumably due to simultaneously open channels in the bilayer. The measured conductance values are only slightly less than those measured for the well-characterized pores formed by known ion channel-forming peptide gramicidin A under the same experimental conditions. The pores formed by BTA-EG₄ are selective for monovalent cations: while single-channel conductance values were measured for several alkali metal chlorides (Cs, K, Na and Li) and HCl, no single-channel conductance was observed in an electrolyte solution containing 1.0 M CaCl₂. Further investigation
into the behavior of the pores formed from BTA-EG$_4$ revealed that approximately four monomeric BTA-EG$_4$ are required to form the observed ion channels.

Figure 5.7: (A) Original current versus time trace from single ion channel recording through self-assembled pores from BTA-EG$_4$ in a planar lipid bilayer composed of 1,2-diphytanoyl-sn-glycero-3-phosphatidylcholine (DiphyPC) lipids. Histogram of current amplitudes reflects their number of occurrence in the corresponding current trace. (B) Normalized survival plot of the open channel lifetime determined from 174 single channel opening events under the same experimental conditions as in A). The average lifetime, $\tau$, for BTA-EG$_4$ pores was determined from a fit of the histogram to the equation, $N(t)/N(0) = \exp(-t/\tau)$, where $N(t)$ represents the number of channels with lifetimes longer than the time $t$, and $N(0)$ represents the total number of channels with observable single conductance. BTA-EG$_4$ was added at a final concentration of 20 $\mu$M to both bilayer compartments, the applied voltage was +50 mV, and the electrolyte contained 1.0 M CsCl with 10 mM HEPES buffer, pH 7.4.

Because of the previously known antibiotic activity of the ion-channel forming peptide gramicidin A toward gram-positive bacteria,$^{174, 175}$ the antibacterial effect of BTA-EG$_4$ was evaluated. Indeed, BTA-EG$_4$ retarded the growth of a strain of gram-positive bacteria (*Bacillus subtilis*) with an IC$_{50}$ value of 50 $\mu$M and completely suppressed growth at concentrations of 100 $\mu$M (Figure 5.8A). In addition, BTA-EG$_4$
was found to be toxic to SH-SY5Y human neuroblastoma cells in a dose-dependent manner with an IC$_{50}$ value of 65 μM. Due to the ability of BTA-EG$_4$ to insert into cell membranes and exhibit similar toxicity as other ion channel-forming molecules suggests that the antibacterial activity and cellular toxicity of BTA-EG$_4$ may be directly related to its ability to form pores in cell membranes. Accordingly, the ion channel forming properties of BTA-EG$_4$ may shed light on both its mechanism of action and its potential utility (and drawbacks) for drug delivery and other biomedical applications.

![Graphs](image_url)

**Figure 5.8:** (A) Inhibitory effect of BTA-EG$_4$ on the growth of Bacillus subtilis bacteria 22 h after exposure to LB media containing various concentrations of BTA-EG$_4$. Growth was quantified by the optical density at a wavelength of 600 nm relative to untreated control cells. The concentration of BTA-EG$_4$ molecules that inhibited growth by 50% (IC$_{50}$ values) was 50 μM. Each point represents the mean of 2 experiments with 3 replicates in each experiment; error bars represent the standard error of the mean. (B) Cytotoxicity of BTA-EG$_4$ on human neuroblastoma (SH-SY5Y) cells 24 h after exposure. Each point represents the mean of 2 or 3 experiments with 6 replicates in each experiment. Error bars reflect the standard error of the mean. The IC$_{50}$ value was 65 μM.

### 5.6 Chapter Summary

Through the use of microwave-assisted chemistry, we have developed an improved synthesis for BTA-EG$_4$ and BTA-EG$_6$—two demonstrated amyloid-binding
molecules—and a general methodology to efficiently access the BTA-EG\textsubscript{x} family of compounds. The increased availability of BTA-EG\textsubscript{4} and BTA-EG\textsubscript{6} has led to several collaborative efforts geared toward investigating the properties and potential applications of these compounds. In addition to its previously known ability to bind Alzheimer’s-related A\textsubscript{β}, BTA-EG\textsubscript{6} was found to be a possible candidate for antimicrobial formulations used to combat the sexual transmission of HIV-1 due to its ability to inhibit SEVI and semen-mediated infectivity of the virus. BTA-EG\textsubscript{4} was found to form monovalent cation selective pores in lipid membranes—a finding which may explain the mechanism of its antibacterial activity and cellular toxicity. Other collaborations involving BTA-EG\textsubscript{x} are exploring their use as dendritic spine density promoters for the treatment of Alzheimer’s disease. The ability to produce both known and novel derivatives of BTA-EG\textsubscript{x} compounds in an easy and highly efficient manner will provide the quantities of material necessary to further explore their properties and may potentially lead to the discovery of a myriad of additional biomedical applications for these exceptional molecules.

5.7 Experimental methods

All synthetic reagents were from Aldrich, Fisher Scientific, Alfa Aesar, or Fluka, and were used without further purification. All solvents used for reactions were obtained from Fisher scientific and dried on Alumina columns prior to use. Solvents used for chromatography were ACS technical grade and used without further purification. Water (18.2 \(\mu\Omega/cm\)) was filtered through a NANOPure Diamond\textsuperscript{TM} (Barnstead) water
purification system before use. Microwave synthesis was performed with a Biotage Initiator Microwave synthesizer using high-pressure vessels.

All NMR spectra of BTA-EGₙ compounds and precursors were recorded on a Varian Mercury Plus 400 MHz NMR spectrometer in CDCl₃. Low resolution MS analysis was performed on a ThermoFinnigan LCQdeca mass spectrometer with an atmospheric pressure electrospray ionization (APCI) source or an electrospray ionization (ESI) source. High resolution MS analysis was performed on a Thermo Scientific LTQ Orbitrap XL mass spectrometer with an electrospray ionization source.

2-(2-(2-(2-hydroxyethoxy)ethoxy)ethoxy)ethyl toluenesulfonate (26)

In a clean, dry 1L roundbottom flask equipped with a stirbar, tetraethylene glycol (10.0 g, 51.5 mmol) was dissolved in 500mL dry DCM and stirred at room temperature. After 5 minutes, KI (1.71 g, 10.3 mmol), Ag₂O (17.9 g, 77.2 mmol), and p-toluenesulfonyl chloride (10.8 g, 56.6 mmol) were successively added to the reaction flask. The reaction mixture was stirred vigorously for 2 h, filtered through celite to remove the solids and concentrated in vacuo. The residue was purified via silica column chromatography (100% DCM to 95:5 DCM:CH₃OH) giving 2-(2-(2-(2-hydroxyethoxy)ethoxy)ethoxy)ethyl toluenesulfonate as a colorless oil (13.2 g, 74%).

¹H-NMR (400 MHz, CDCl₃): δ = 7.74 (d, 8.0 Hz, 2H), 7.30 (d, 8.0 Hz, 2H), 4.11 (t, 4.8 Hz, 2H), 3.66-3.53 (m, 12H), 2.79 (s, 1H), 2.39 (s, 3H). ¹³C-NMR (100 MHz, CDCl₃); δ = 145.04, 133.17, 130.10 (2C), 128.19 (2C), 70.95, 70.79, 70.70, 69.49, 68.88, 21.87. ESI-MS (m/z) calcd for C₁₅H₂₄O₇S [M]+ 348.1243; found [M+H]+ 348.96, [M+NH₄]+ 365.94 and [M+Na]+ 371.08.
2-(2-(2-(2-iodoethoxy)ethoxy)ethoxy)ethanol (27)

2-(2-(2-(2-hydroxyethoxy)ethoxy)ethoxy)ethyl toluenesulfonate (12.01 g, 234.5 mmol), sodium iodide (207 g, 137.9 mmol) and 200 mL dry acetone were combined in a clean, dry round bottom flask and heated to reflux with vigorous stirring. After 12h the reaction was cooled to room temperature and diluted with 100 mL ethyl acetate. The organic phase was washed with 10% Na$_2$S$_2$O$_3$, (2 x 10 mL), DI H$_2$O (1 x 20 mL), saturated NaCl (1 x 20 mL), dried over anhydrous Na$_2$SO$_4$, filtered, and concentrated in vacuo giving 2-(2-(2-(2-iodoethoxy)ethoxy)ethoxy)ethanol as a pale yellow oil (5.61 g, 54%). $^1$H-NMR (400 MHz, CDCl$_3$): $\delta$ = 3.73-3.58 (m, 14H), 3.24 (t, 2H), 2.59 (s, 1H). $^{13}$C-NMR (100 MHz, CDCl$_3$); $\delta$ = 72.70, 72.19, 70.90, 70.76, 70.58, 70.39, 61.94, 3.07.

BTA-EG$_4$ (23)

A microwave reaction tube was charged with 2-(2-(2-(2-iodoethoxy)ethoxy)ethoxy)ethanol (1.47 g, 4.83 mmol), benzothiazole aniline (3.49 g, 14.5 mmol), potassium carbonate (3.34 g, 24.2 mmol) and 20 mL dry THF. The tube was then equipped with a small stirbar, sealed and placed in a microwave reactor. The reaction was heated at 125 $^\circ$C for 2h. The reaction was cooled to room temperature and filtered to remove the solids. The solids washed several times with DCM until the filtrate was colorless. The combined organic layers were concentrated in vacuo and purified by column chromatography to give the desired BTA-EG$_4$ compound as a yellow solid (1.13g, 56%). $^1$H-NMR (400 MHz, CDCl$_3$): $\delta$ = 7.87 (d, 8.8 Hz, 2H), 7.83 (d, 8.4 Hz, 1H) 7.63 (s, 1H) 7.23 (d, 8.4 Hz, 1H), 6.68 (d, 8.8 Hz, 2H), 3.76-3.58 (m, 14H), 3.37 (t, 5.2 Hz, 2H), 2.47 (s, 3H). $^{13}$C-NMR (100 MHz, CDCl$_3$); $\delta$ = 168.03, 152.64, 150.92,
17-hydroxy-3,6,9,12,15-pentaoxaheptadecyl toluenesulfonate (29)

In a clean, dry 1L roundbottom flask equipped with a stirbar, hexaethylene glycol (15.0 g, 53.1 mmol) was dissolved in 500mL dry DCM and stirred at room temperature. After 5 minutes, KI (1.76 g, 10.6 mmol), Ag₂O (18.5 g, 79.7 mmol), and p-toluenesulfonyl chloride (11.1 g, 58.4 mmol) were successively added to the reaction flask. The reaction mixture was stirred vigorously for 2 h, filtered through celite to remove the solids and concentrated in vacuo. The residue was purified via silica column chromatography (100% DCM to 97:3 DCM:CH₃OH) giving 2-(2-(2-(2-hydroxyethoxy)ethoxy)ethoxy)ethyl toluenesulfonate as a colorless oil (15.2 g, 74%).

¹H-NMR (400 MHz, CDCl₃): δ = 7.79 (d, 8.4 Hz, 2H), 7.34 (d, 8.0 Hz, 2H), 4.16 (t, 4.8 Hz, 2H), 3.71-3.58 (m, 22H), 2.45 (s, 3H). ESI-MS (m/z) calcd for C₁₉H₃₂O₉S [M]⁺ 436.1767; found [M+H]+ 437.00 and [M+Na]+ 459.08.

17-iodo-3,6,9,12,15-pentaoxaheptadecan-1-ol (30)

17-hydroxy-3,6,9,12,15-pentaoxaheptadecyl toluenesulfonate 29 (15.2 g, 34.8 mmol), sodium iodide (15.6 g, 104.3 mmol) and 300 mL dry acetone were combined in a clean, dry roundbottom flask and heated to reflux with vigorous stirring. After 12 h the reaction was cooled to room temperature, filtered and the solids washed with acetone. The filtrate was concentrated in vacuo and redissolved in 300 mL ethyl acetate. The
organic phase was washed with 10% Na₂S₂O₃, (2 x 10 mL), DI H₂O (1 x 30 mL), saturated NaCl (1 x 30 mL), dried over anhydrous Na₂SO₄, filtered, and concentrated in vacuo giving 17-iodo-3,6,9,12,15-pentaoxaheptadecan-1-ol (30) as a pale yellow oil (10.6 g, 78%). ¹H-NMR (400 MHz, CDCl₃); δ = 3.77-3.60 (m, 22H), 3.25 (t, 7.2 Hz, 2H), 2.97 (s, 1H). ¹³C-NMR (100 MHz, CDCl₃); δ = 72.85, 72.18, 70.86, 70.81, 70.78, 70.76, 70.75, 70.73, 70.46, 70.37, 61.93, 3.23. ESI-MS (m/z) calcd for C₁₂H₂₅IO₆ [M]⁺ 392.0696; found [M+H]⁺ 393.10, [M+NH₄]⁺ 410.01 and [M+Na]⁺ 415.04.

BTA-EG₆ (24)

17-iodo-3,6,9,12,15-pentaoxaheptadecan-1-ol (10.62 g, 27.1 mmol), benzothiazole aniline (19.51 g, 81.2 mmol), potassium carbonate (18.71 g, 135.4 mmol) were split into two batches. Two identical microwave reaction tubes were charged with 17-iodo-3,6,9,12,15-pentaoxaheptadecan-1-ol (5.31 g, 13.55 mmol), benzothiazole aniline (9.76 g, 40.6 mmol), potassium carbonate (9.36 g, 24.2 mmol) and 9 mL dry THF. The tubes were then equipped with a small stirbar, sealed and placed in a microwave reactor. The reaction was heated at 125 °C for 2h, cooled to room temperature and filtered to remove the solids. The solids washed several times with several portions of DCM and the combined organic layers were concentrated in vacuo. Purification by column chromatography gave the desired BTA-EG₆ compound as a tacky yellow oil (4.16 g, 34%). ¹H-NMR (400 MHz, CDCl₃); δ = 7.83-7.79 (m, 3H), 7.54 (s, 1H), 7.17 (dd, 8.8 Hz, 1.0 Hz, 1H), 6.61 (d, 8.8 Hz, 2H), 3.67-3.51 (m, 24H), 3.28 (t, 5.2 Hz, 2H), 2.39 (s, 3H). ¹³C-NMR (100 MHz, CDCl₃); δ = 168.04, 151.82, 151.03, 134.50, 134.29, 129.14 (2C), 127.80, 121.93, 121.65, 121.39, 112.68 (2C), 72.73, 70.64 (6C), 70.40 (2C),
HR-ESI-MS (m/z) calcd for C_{26}H_{36}N_{2}O_{6}SNa [M+Na]^+ 527.2186; found [M+Na]^+ 527.2184.

17-(4-methyl-N-(4-(6-methylbenzothiazol-2-yl)phenyl)phenylsulfonamido)

3,6,9,12,15-pentaoxaheptadecyl toluenesulfonate (31)

In a clean, oven-dried 100 mL round bottom flask, BTA-EG\textsubscript{6} \textsuperscript{24} (1.15 g, 2.28 mmol) was dissolved in 20 mL DCM. The flask was cooled on an ice bath to 0°C and pyridine (361 mg, 367 \( \mu \)L, 4.56 mmol) was added. After 10 min, \( p \)-toluenesulfonyl chloride (652 mg, 3.52 mmol) was added to the reaction flask and the contents were allowed to warm up to room temperature while stirring. After 12 h, the contents of the reaction flask were concentrated in vacuo and immediately purified via flash chromatography (100% DCM to 95:5 DCM:methanol) to afford a pale yellow oil (902 mg, 65%). \( ^1 \)H-NMR (400 MHz, CDCl\textsubscript{3}): \( \delta = 7.99 \) (d, 8.4 Hz, 2H), 7.93 (d, 8.4 Hz, 1H), 7.78 (d, 8.4 Hz, 2H), 7.69 (s, 1H), 7.49 (d, 8.4 Hz, 2H), 7.33-7.18 (m, 7H), 4.14 (t, 3.2 Hz, 2H), 3.77 (t, 6.4 Hz, 2H), 3.67-3.53 (m, 20H), 2.50 (s, 3H), 2.42 (s, 3H), 2.41 (s, 3H).

\( ^{13} \)C-NMR (100 MHz, CDCl\textsubscript{3}): \( \delta = 165.94, 152.42, 145.01, 143.93, 142.04, 135.90, 135.53, 135.28, 133.26, 133.15, 130.04 \) (2C), 129.77 (2C), 129.40 (2C), 128.33, 128.20 (2C), 128.17 (2C), 127.89, 123.00, 121.65, 70.93, 70.78 (2C), 70.73 (2C), 70.69, 70.67, 70.60, 69.47, 69.20, 68.88, 60.64, 50.32, 21.88, 21.82, 21.81. ESI-MS (m/z) calcd for C\textsubscript{40}H\textsubscript{48}N\textsubscript{2}O\textsubscript{10}S\textsubscript{3} [M]^+ 812.2471; found [M+H]^+ 813.2 and [M+Na]^+ 835.2.
17-azido-N-(4-(6-methylbenzothiazol-2-yl)phenyl)-3,6,9,12,15-pentaoxaheptadecan-1-amine (32)

A clean, oven-dried roundbottom flask was charged with N,O-ditosylated compound 31 (884 mg, 1.34 mmol), NaN₃ (262 mg, 4.03 mmol) and 5 mL dry DMF and allowed to stir at 70°C. After 12 h., the contents of the reaction flask were diluted with 100 mL Et₂O and washed with DI H₂O (2 x 10 mL), saturated NaCl (1 x 10 mL), dried over anhydrous Na₂SO₄, filtered, and concentrated in vacuo to afford organoazide 32 as a yellow oil (688mg, 93%). ¹H-NMR (400 MHz, CDCl₃): δ = 7.99 (d, 8.4 Hz, 2H), 7.92 (d, 8.4 Hz, 1H), 7.69 (s, 1H), 7.49 (d, 8.4 Hz, 2H), 7.31-7.18 (m, 5H), 3.77 (t, 6.4 Hz, 2H), 3.73-3.53 (m, 20H), 3.36 (t, 4.8 Hz, 2H), 2.49 (s, 3H), 2.41 (s, 3H). ¹³C-NMR (100 MHz, CDCl₃); δ = 165.94, 152.44, 143.92, 142.07, 135.90, 135.54, 135.31, 133.27, 129.77 (2C), 129.40 (2C), 128.33, 128.18 (2C), 127.91 (2C), 123.02, 121.65, 70.90, 70.86, 70.83, 70.81, 70.79, 70.77, 70.69, 70.62, 70.24, 69.23, 50.89, 50.32, 21.83, 21.81. ESI-MS (m/z) calcd for C₃₃H₄₁N₅O₇S₂ [M]⁺ 683.2447; found [M+H]⁺ 684.22 and [M+Na]⁺ 706.19.

17-((4-(6-methylbenzothiazol-2-yl)phenyl)amino)-3,6,9,12,15-pentaoxaheptadecyl toluenesulfonate (33)

In a clean, oven-dried 25 mL roundbottom flask, BTA-EG₆ 24 (440 mg, 872 µmol) was dissolved in 5 mL dry DCM. The flask was cooled on an ice bath to 0°C and triethylamine (106 mg, 146 µL, 1.05 mmol) and 4-DMAP (21.3 mg, 174 µmol) were added. After 5 min, p-toluenesulfonyl chloride (199 mg, 1.05 mmol) was added to the reaction flask and the contents were allowed to stir at 0°C for an additional hour. The
reaction was then concentrated *in vacuo* and immediately purified via flash chromatography (100% DCM to 95:5 DCM:methanol) to afford 33 as a yellow oil (417 mg, 72%). $^1$H-NMR (400 MHz, CDCl$_3$): $\delta = 7.87$ (d, 8.8 Hz, 2H), 7.84 (d, 8.4 Hz, 1H), 7.78 (d, 8.4 Hz, 2H), 7.63 (m, 1H), 7.31 (d, 8.4 Hz, 2H) 7.23 (d, 8.4 Hz, 1H), 6.66 (d, 8.8 Hz, 2H), 4.14 (t, 5.2 Hz, 2H), 3.73 (t, 5.2 Hz, 2H), 3.66-3.57 (m, 19H), 3.37 (m, 2H), 2.47 (s, 3H), 2.42 (s, 3H). $^{13}$C-NMR (100 MHz, CDCl$_3$); $\delta = 167.96, 152.63, 150.79, 145.01, 134.86, 134.51, 133.18, 130.04 (2C), 129.12 (2C), 128.19 (2C), 127.73, 122.93, 122.04, 121.43, 112.79 (2C), 70.94, 70.83-70.72 (7C), 70.56, 69.53, 68.89, 43.31, 21.87, 21.71. ESI-MS ($m/z$) calcd for C$_{33}$H$_{42}$N$_2$O$_8$S$_2$ [M]$^+$ 658.2383; found [M+H]$^+$ 659.27 and [M+Na]$^+$ 681.23.

$N$-(17-azido-3,6,9,12,15-pentaoxaheptadecyl)-4-methyl-$N$-(4-(6-methylbenzothiazol-2-yl)phenyl)benzenesulfonamide (34)

A clean, oven-dried roundbottom flask was charged with $N,O$-ditosylated compound 31 (414 mg, 628 μmol), NaN$_3$ (123 mg, 1.89 mmol) and 5 mL dry DMF and allowed to stir at 70°C. After 12 h., the contents of the reaction flask were diluted with 100 mL Et$_2$O and washed with DI H$_2$O (2 x 10 mL), saturated NaCl (1 x 10 mL), dried over anhydrous Na$_2$SO$_4$, filtered, and concentrated *in vacuo* to afford organoazide 34 as a yellow oil (316 mg, 95%). $^1$H-NMR (400 MHz, CDCl$_3$): $\delta = ^1$H-NMR (400 MHz, CDCl$_3$): $\delta = 7.88$ (d, 8.4 Hz, 2H), 7.84 (d, 8.4 Hz, 1H), 7.63 (s, 1H), 7.23 (d, 8.4 Hz, 1H), 6.66 (d, 8.4 Hz, 2H), 4.74 (s, 1H) 3.77-3.63 (m, 23H), 3.34 (t, 5.2 Hz, 2H), 2.47 (s, 3H). $^{13}$C-NMR (100 MHz, CDCl$_3$); $\delta = 167.99, 152.62, 150.80, 134.85, 134.51, 129.13 (2C), 127.73, 122.91, 122.03, 121.43, 112.79 (2C), 70.89-70.78 (5C), 70.55, 70.24, 69.54,
HR-ESI-MS (m/z) calcd for C_{26}H_{36}N_{5}O_{5}S [M+H]^+ 530.2432; found [M+H]^+ 530.2433.

Notes about this chapter

This contains material that appears in "Amyloid binding small molecules efficiently block SEVI (semen-derived enhancer of infection) and semen mediated enhancement of HIV-1 infection." Olsen, J. S.; Brown, C.; Capule, C. C.; Rubinshtein, M.; Doran, T. M.; Srivastava, R. K.; Feng, C.; Nilsson, B. L.; Yang, J.; Dewhurst, S J. Biol. Chem. 2010, 285, 35488-35496. I am a co-author on this paper. Additionally, this chapter contains material as it may appear in a paper recently submitted for publication: “Self-assembled, cation-selective ion channels from oligo(ethylene glycol) derivatives of benzothiazole aniline.” Prangkio, P.; Rao, D.; Lance, K.; Rubinshtein, M.; Yang, J.; Mayer, M. I am a co-author on this paper.
Appendix A

Light scattering and differential refractive index data for 5a-5d

Figure A.1. Size exclusion chromatogram of unfunctionalized polymer 6a monitored by light scattering (gray) and differential refractive index (black) from retention times of 18-28 minutes. This data was used to calculate $M_n$ and $M_w/M_n$ (PDI) of the sample using ASTRA software, Wyatt Technologies, Santa Barbara, CA. This polymeric species had a value of $M_n = 27.4 \times 10^4$ g/mol and a PDI of 1.19.
Figure A.2. Size exclusion chromatogram of alkyne-functionalized polymer 6b monitored by light scattering (gray) and differential refractive index (black) from retention times of 20-32 minutes. This data was used to calculate the value $M_n$ and $M_w/M_n$ (PDI) of the sample using ASTRA software, Wyatt Technologies, Santa Barbara, CA. This polymeric species had a value of $M_n = 22.6 \times 10^4$ g/mol and a PDI of 1.11.
Figure A.3. Size exclusion chromatogram of azide-functionalized polymer 6c monitored by light scattering (gray) and differential refractive index (black) from retention times of 20-32 minutes. This data was used to calculate the value $M_n$ and $M_w/M_n$ (PDI) of the sample using ASTRA software, Wyatt Technologies, Santa Barbara, CA. This chromatogram shows at least two main populations of polymers; the polymer represented by the peak at ~26 minutes had a value of $M_n = 34.5 \times 10^4$ g/mol and a PDI of 1.12, while the polymeric species represented by the peak at ~29.5 minutes (plus the shoulder peak) had a value of $M_n = 38.8 \times 10^4$ g/mol and a PDI of 1.27.
Figure A.4. Size exclusion chromatogram of oligoethylene glycol/azide-functionalized polymer 6d monitored by light scattering (gray) and differential refractive index (black) from retention times of 20-32 minutes. This data was used to calculate the value $M_n$ and $M_w/M_n$ (PDI) of the sample using ASTRA software, Wyatt Technologies, Santa Barbara, CA. This chromatogram shows two populations of polymers; the polymer represented by the broad peak at ~26.5 min had a value of $M_n = 23.1 \times 10^4$ g/mol and a PDI of 1.17, while the polymer represented by the narrower peak at ~28.5 min had a value of $M_n = 7.38 \times 10^3$ g/mol and a PDI of 1.23.
Figure A.5: $^1$H and $^{13}$C NMR spectral data for crude polymer 6a.
Figure A.6: $^1$H and $^{13}$C NMR spectral data for crude polymer 6b.
Figure A.7. $^1$H and $^{13}$C NMR spectral data for crude polymer 6c.
Figure A.8. $^1$H and $^{13}$C NMR spectral data for crude polymer 6d.
REFERENCES


105. Danysz, W.; Parsons, C. G.; Stoffler, A., Memantine: a NMDA receptor antagonist that improves memory by restoration of homeostasis in the glutamatergic system - too little activation is bad, too much is even worse. *Neuropharmacology 2007*, 53 (6), 699-723.


116. Phase III clinical trials of (R)-flurbiprofen (trade name Flurizan) on nearly 1700 individuals concluded that the drug did not increase cognitive function of patients significantly more than placebo. Accordingly, the drug was discontinued from further development by Myriad Genetics in June, 2008.


165. Adapted from the website: uhavax.hartford.edu/ bugl/hiv.htm.


