SYNTHESIS AND NMR STUDIES OF A β-TURN MIMETIC MOLECULAR
TORSION BALANCE

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B.S. Chemistry, Lehigh University, 2006

Submitted to the Graduate Faculty of
the Kenneth P. Dietrich School of
Arts and Sciences in partial fulfillment
of the requirements for the degree of
Doctor of Philosophy

University of Pittsburgh

2012
UNIVERSITY OF PITTSBURGH
DIETRICH SCHOOL OF ARTS AND SCIENCES

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SYNTHESIS AND NMR STUDIES OF A β-TURN MIMETIC MOLECULAR TORSION BALANCE

Melissa Ann Liberatore, PhD
University of Pittsburgh, 2012

The attainment of precise measurements of the molecular forces that influence protein folding is important in order to further understand peptide dynamics and stability. A hybrid synthetic-natural peptide motif, combining an \( o,o,o' \)-trisubstituted biphenyl with an (\( ortho \)-tolyl)-amide, was synthesized in multiple formats and studied by NMR to probe the effects of amino acid substitutions on antiparallel beta-sheet configuration and stability. The potential of this “molecular torsion balance” as a beta-turn mimic was demonstrated by quantifying the rotational barriers about several axes. The free-energy rotational barrier of the aryl-aryl bond was found to be 35.7 kcal mol\(^{-1}\) at 418 K in hexanes. EXSY analysis was also used to measure barriers about the N-aryl (20.9 kcal mol\(^{-1}\) at 343 K in toluene-\( d_8 \)) and N-CO bonds (17.2 kcal mol\(^{-1}\) at 298 K in chloroform-\( d \)). The N-aryl barriers of a zwitterionic torsion balance containing a single alanine residue (19.7 kcal mol\(^{-1}\) in acetonitrile-\( d_3 \) at 343 K, and 22.6 kcal mol\(^{-1}\) in deuterated buffer pD 6.9 at 373 K) showed that rotation about this bond is slower in water, most likely due to the propensity of water to form hydrogen bonds with the charged moieties participating in a salt bridge. Torsion balances were used to study intramolecular hydrogen bond preference by analyzing \(^1\)H NMR peak data. An amino acid chain (a single alanine or glycine residue) was found to preferably hydrogen bond with an amide versus an ester carbonyl (1.4:1.0 at 298 K in toluene-\( d_8 \)), and with a secondary amide versus a tertiary amide carbonyl (observation of tertiary amide proton coalescence via variable temperature NMR, from 303 K to 343 K, in toluene-\( d_8 \)).
The overall findings suggest that this hybrid torsion balance is a valuable tool that can provide data on conformational dynamics and can examine hydrogen bond and salt bridge interactions of an antiparallel beta-sheet scaffold.
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<td>Ac</td>
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<tr>
<td>Ala</td>
<td>alanine</td>
</tr>
<tr>
<td>Bn</td>
<td>benzyl</td>
</tr>
<tr>
<td>Boc</td>
<td>tert-butyloxycarbonyl</td>
</tr>
<tr>
<td>BOP-Cl</td>
<td>bis(2-oxo-3-oxazolindinyl)phosphinic chloride</td>
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</tr>
<tr>
<td>Cbz</td>
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<td>DMAP</td>
<td>4-(dimethylamino)pyridine</td>
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<tr>
<td>DME</td>
<td>1,2-dimethoxyethane</td>
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<tr>
<td>DMF</td>
<td>N,N'-dimethylformamide</td>
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HOBt 1-hydroxybenzotriazole
HPLC high performance liquid chromatography
HRMS high resolution mass spectrometry
imid imidazole
IR infrared spectrometry
Leu leucine
LRMS low resolution mass spectrometry
Me methyl
MS mass spectrometry
NMR nuclear magnetic resonance
NOESY nuclear overhauser effect spectroscopy
Ph phenyl
pNB para-nitrobenzyl
iPr isopropyl
Py pyridine
PyBOP (benzotriazol-1-yl-oxy)tripyrrolidinophosphonium hexafluorophosphate
Q-TOF quadrupole time-of-flight
ROESY rotating-frame nuclear overhauser effect correlation spectroscopy
rt room temperature
SPhos 2-dicyclohexylphosphino-2’,6’-dimethoxybiphenyl
TAEA tris(2-aminoethyl)amine
TBAF tetrabutylammonium fluoride
TBS-Cl tert-butyldimethylsilyl chloride
TFA trifluoroacetic acid
THF tetrahydrofuran
TLC thin layer chromatography
TOCSY total correlation spectroscopy
PREFACE

I would like to thank my research advisor, Professor Craig Wilcox, for his guidance and wisdom over the past six years. Also, I would like to thank the past and present Wilcox group members, and additional students throughout the department, who have helped and supported me along the way. Thank you to Professors Dennis Curran, Michael Trakselis, and Judith Klein-Seetharaman for serving on my thesis committee, and to Professor Seth Horne for serving as my proposal mentor. Also, thank you to Damodaran Achary, Sage Bowser, and Bhaskar Godugu from the NMR and Mass Spectroscopy facilities for their help with setting up and running experiments. In addition, thank you to my undergraduate advisor from Lehigh University, Professor Robert Flowers, for giving me my first research experience.

This dissertation is first dedicated to my husband Jared. His love and strength have supported me throughout all aspects of life and school; without him, this work would not be possible. Secondly, this dissertation is dedicated to my late brother Matthew; he has immeasurably influenced my upbringing and my aspirations. I also would like to thank my parents for their love and guidance and for their encouragement of all my endeavors.
1.0 INTRODUCTION

1.1 INTRODUCTION TO THE TORSION BALANCE

The Wilcox group has introduced the “molecular torsion balance” as a tool to investigate
the various molecular forces (i.e. hydrogen bonding, salt bridging) that influence biological
phenomena, such as protein folding, drug-receptor binding, and enzyme catalysis.¹ The torsion
balance is a molecule designed to have two different configurations, set apart by gently restricted
rotation around a single bond, that represent the folded and unfolded states of a protein folding
model. These two conformations have well separated thermodynamic states that allow for
precise measurement of their relative energies. Any deviation from a 1:1 ratio of states discloses
intramolecular force effects that can be deduced by observations of NMR peak variations and
provide essential experimental data to enhance theoretical folding models.¹

The first torsion balance from the Wilcox group employs a Tröger’s base moiety
(dibenzodiazocine) to serve as the aromatic T-shaped backbone and examine the edge-to-face
aromatic interactions that affect molecular recognition; an example is shown in Figure 1.¹
The Tröger’s base is situated as the “bottom half” of the torsion balance, while the “top half” comprises an aromatic ring with two different ortho-substituents. Interconversion by rotation around the aryl–aryl bond provides the two conformational substrates for analysis. In the "unfolded" configuration, the aromatic ortho-substituent is further from the toluenic ring of the Troger’s base; the "folded" configuration is defined to be the form in which these two rings are closest in distance. Analysis by $^1$H NMR spectroscopy revealed how much of a preference the molecule had for the folded versus the unfolded states. This preference was further investigated by the addition of different electron–donating or electron–withdrawing groups to the aromatic ortho-substituent. Folding energies ranged from 0.2 to 0.8 kcal·mol$^{-1}$. Subsequent torsion balance models expanded upon this structural motif by incorporating, for example, amino acid side chain$^2$ mimics as the ortho-substituents for analysis or a water-solubilizing group on the axis of rotation to allow for measuring the effects of water on folding.$^{1,3}$ Efforts by others to utilize the torsion balance system include that of Diederich and coworkers,$^4$ in which they make use of our balance design to give evidence of an attractive noncovalent dipolar $C_{sp^2}$–F⋯C=O interaction between organic fluorine and amide groups.
1.2 DEVELOPMENT OF A NOVEL TORSION BALANCE RELEVANT TO PEPTIDES

Enzyme and drug–receptor binding rely upon several inter- and intramolecular interactions to increase binding activity and specificity. We desired to apply our torsion balance concept toward studying such interactions and therefore formulate a new balance that would allow us to investigate both amino acid interactions in an antiparallel β-sheet configuration and also short β-strand stability with changes in amino acids. This balance, emulating a β-turn, would be incorporated as part of a hybrid synthetic-natural peptide that would allow for direct comparison of the energies of these pair-wise amino-acid interactions. Thus far, the previous torsion balances had utilized the dibenzodiazocine scaffold in acquiring data for implementation in molecular recognition and protein folding models.\(^1\)\(^3\) However, previous computer modeling efforts led to the conclusion that this scaffold did not have the correct shape to match with the ends of an antiparallel β-sheet and enforce the configuration and distance required between hydrogen-bonded amino acids.

The β-sheet substructures of enzymes were examined for the interactions that provide conformational stability. For example, lysozyme has six surface salt bridges that contribute toward the stability of the protein.\(^5\) Mutational analysis can aid in an understanding of protein stability. A mutational analysis of lysozyme showed that the contributions were small when the salt bridges were readily accessible by solvent and larger (by 9 kJ·mol\(^{-1}\)) when they were 100% inaccessible and emulating a buried salt bridge.\(^5\) Another enzyme, chorismate lyase, has an unusual internal ligand-binding cavity behind two flaps.\(^6\) A computer model of the enzyme binding site and its inhibitor, vanillate, is depicted in Figure 2.
Either the lyase substrate or the inhibitor vanillate enter the binding site upon opening of the flaps. These flaps contain many hydrophobic side chains (i.e. Ile and Leu) on the interior and are in proximity to the central sheet of the enzyme, which is a hydrophobic and densely packed region. Probing the product release mechanism and binding activity by mutation and modeling has shown that three important hydrogen bonds hold the flaps together and must be broken to initiate product release. These enzyme studies and computer modeling of the binding sites have been important for determining a good structure for our model with which to measure inter- and intramolecular protein interactions.

A torsion balance could be used to directly imitate the primary and secondary structures of a binding site upon computer modeling analysis. For example, the chorismate lyase binding site could be mimicked by incorporating a Leu-Leu-Ile-Glu-Arg-NH$_2$ chain (amino acid nos. 80 to 76) on the top portion of the balance, while the bottom would have a Leu-Ala-Ala-Arg-CO$_2$H chain (amino acid nos. 88 to 91). The aromatic backbone of the balance would be devised to take

**Figure 2.** Computer modeling of the chorismate lyase binding site with vanillate inhibitor depicted; the inhibitor is nestled in the hydrophobic pocket (Ile-78, Leu-79, and Leu-80) but also forms a hydrogen bond with neighboring Arg-76.
the place of the amino acid sequence that comprises the β-turn (Ser-Ala-Asp-Gly-Glu-Pro-Trp, amino acid nos. 81-87); the correct structure of this backbone would be determined from the computer model. The completed balance could then be utilized to investigate conformational dynamics and hydrogen bond interactions between the "top" and "bottom" sequences.

We have devised a new torsion balance that relies on the rotation around an aryl-nitrogen bond\(^8\) to realize our goal; a general example is shown in Figure 3. There have been many synthetic efforts to structurally emulate a β-turn, but this balance is the first in our group and in the general scientific community to do so and can still be used to obtain conformational data similar to our previous generation balances.

![Figure 3](image-url)

**Figure 3. Example of two conformers of a β-turn mimetic torsion balance. The R groups are different and each represents an amino acid side chain.**

This new torsion balance combines the established biaryl structural unit with an (ortho-tolyl)amide unit\(^8\). This unit has been of interest over the past 40 years. The N-aryl rotation is restricted; Mislow found a barrier of \(\Delta G^\ddagger = 20.0 \text{ kcal} \cdot \text{mol}^{-1}\) at 408 K for one acyclic amide example shown in Figure 4.\(^8\) The rate constant for this rotation is \(k = 95 \text{ s}^{-1}\). For a related (ortho-tolyl)amide in which the N-CO bond was also restricted in the form of a cyclic amide, the barrier was lowered to \(\Delta G^\ddagger = 17.3 \text{ kcal} \cdot \text{mol}^{-1}\) at 346 K, with a rate constant of \(k = 51 \text{ s}^{-1}\).
Figure 4. Examples of a Mislow (a) acyclic and (b) cyclic (ortho-tolyl)amide, with rotation around the N-aryl bond depicted.

Curran and coworkers employed the use of o-haloanilide atropisomers in asymmetric radical cyclizations;\textsuperscript{9} the atropisomers utilized had an additional o-methyl substituent present in which the barriers to rotation around the N-aryl bond were 28-31 kcal\cdot mol\textsuperscript{-1} (without the additional o-methyl substituent, the barriers were less than 20 kcal\cdot mol\textsuperscript{-1}). The additional ortho-group increased the stability of the atropisomers\textsuperscript{10} at room temperature and allowed for easier separation.

This is the first time our group has incorporated a o,o,o\textsuperscript{-}tri-substituted biphenyl in a torsion balance, which is meant to structurally emulate a β-turn. The biphenyl unit contributes an additional site for restricted rotation.\textsuperscript{11} The barrier to rotation around an aryl–aryl bond is mostly dependent upon the ortho-substituents; however, a stabilizing buttressing effect can also be observed (by an increase in the rotational barrier) when meta-substituents also are present.\textsuperscript{11a, b} Based on work by Kawano,\textsuperscript{11c} we used computer simulations to estimate the barrier for rotation around the aryl-aryl bond to be $\Delta G^\ddagger = 27$ kcal\cdot mol\textsuperscript{-1} at 343 K. One biphenyl example of Kawano is shown in Figure 5,\textsuperscript{11c} with a barrier of 28.2 kcal\cdot mol\textsuperscript{-1}; this barrier was from the combination of optical rotation measurements and half-life times for racemization at various temperatures.
Figure 5. Kawano biphenyl compound with three ortho-substituents.

The combination of the \( o,o,o' \)-trisubstituted biphenyl with the N-aryl atropisomer gives a molecule that would serve as a \( \beta \)-turn mimic, by imitating the familiar bonds and dihedral angles necessary to connect and bring two antiparallel \( \beta \)-strands close enough together (to form hydrogen bonds), and also exhibit two conformational states.

There have been many efforts in designing and analyzing an appropriate antiparallel \( \beta \)-strand mimic\(^{12} \) for protein folding studies. Gellman\(^{13} \) and coworkers have reported the use of a \( \beta \)-hairpin motif containing a hydrophobic cluster of naturally occurring amino acids to probe the folding and stability of antiparallel \( \beta \)-sheets in water.\(^{13a} \) NOE and variable temperature \(^1\)H NMR data provided insight into the thermodynamic profiles of their folded and unfolded two-state system and showed that a well-defined \( \beta \)-hairpin conformation was generated by the nonpolar side chain cluster. Nowick\(^{14} \) and coworkers describe several \( \beta \)-sheet mimics that utilize specific combinations of natural and unnatural amino acids to induce folding and interaction in peptides.\(^{14a} \) In one example, shown in Figure 6, an artificial amino acid “Orn(\( i \)-PrCO-Hao)” (natural amino acid ornithine in addition to unnatural amino acid “Hao”: hydrazine, 5-amino-2-methoxybenzoic acid, and oxalic acid) was incorporated into a peptide to act as an artificial \( \beta \)-turn in a \( \beta \)-strand.
It was shown that “Orn(i-PrCO-Hao)” was able to induce the structure and interactions similar to those seen in a natural β-sheet, with minimal perturbation of the peptide sequence. The results of these studies have been useful in the design of our new peptidic mimic by demonstrating the molecular spacing needed to maintain the hydrogen bonding found in the natural peptide structure; our torsion balance would act as the artificial β-turn in the short hybrid synthetic-natural β-strand. Furthermore, longer amino acid chains can be appended to the torsion balance to more fully emulate β-strand and peptidic dynamics, by providing more hydrogen bonds for stabilization.

1.3 ASSIGNMENT OF TORSION BALANCE CONFORMERS BY TWO-DIMENSIONAL NMR ANALYSES

Structural assignment of the torsion balance targets is required to accurately determine the effects of amino acid changes on β-strand configuration and stability. We desired to use the scaffold of our novel torsion balance to create select protein folding models, which would then be studied using two-dimensional NMR spectra analyses. Relevant models will focus on small...
changes to the two ortho-sites of the top aromatic ring of the biaryl structure; one model type would incorporate different amino acid side chains on each ortho-site, while another type would have a different number of amino acids on each ortho-site (therefore differing in the number of available hydrogen bonding sites). A related model type, while also featuring different amino acids, will concentrate on observing salt-bridge effects on configuration and stability. Two-dimensional (2D) NMR spectra analyses and assignments of these models will help determine which torsion balance conformers are more stable.

Williamson\textsuperscript{15} and coworkers utilize several methods to determine the structures of specific RNA targets and their variants; these methods include both 2D and one-dimensional (1D) NMR. In one example,\textsuperscript{15a} shown in Figure 7, two oligonucleotides that were varied at only one position were studied by NMR techniques to determine and compare the secondary structures. It had been observed that the function of the corresponding tRNA was distinctly affected by the single change in discriminator base; the change involved substituting uracil for adenine at position 19. The 1D NMR spectra (not shown) depicted a shift downfield of the imino proton of the uracil variant compared to the adenine variant; the 2D NMR spectra (i.e. NOESYs), show a 'fold-back' conformer structure for only the uracil variant that is also detected at different temperatures.
Figure 7. Secondary structure of Williamson's tRNA variants and the corresponding NOESY spectra (reprinted from the literature).\textsuperscript{15a} NOESY spectra (A) and (B) show the NOEs of interest for variants A19 and U19, respectively. (Copyright (1994) National Academy of Sciences, U.S.A. Printed with permission.)

NMR assignments of other similar compounds, such as TAR RNA that is located in all viral mRNAs,\textsuperscript{15b, c} were also performed by the Williamson group. The use of NOESY experiments, in addition to COSY, TOCSY, and various \textsuperscript{13}C-detected experiments, aided in determining the conformation of the wild-type and mutant RNAs as well as some of the exchangeable imino- and amino-protons. In addition, the 2D NMR analyses were also able to suggest partial base stacking and a minor distortion in conformation. It is clear that heavy use of the aforementioned 2D NMR techniques (and others such as ROESYs\textsuperscript{15d}) can lead to a complete or near-complete assignment of a desired compound, therefore elucidating conformational data that can reveal new information about protein stability.
2.0 SYNTHESIS AND ANALYSIS OF INITIAL TORSION BALANCES

2.1 RETROSYNTHETIC ANALYSIS

The primary targets for our β-turn mimetic torsion balance synthesis, biaryl amide analogs 1 and 2, are shown in Scheme 1. These torsion balance analogs are versatile because they are differentially protected at the positions a, b, and c to facilitate an orthogonal deprotection strategy that allows for independent amino acid couplings. In addition, the methyl ester can also be cleaved to enhance the water solubility of the torsion balance.

Scheme 1. Retrosynthetic pathway for β-turn mimetic torsion balance.

The biaryl amides 1 and 2 could be synthesized by an amide coupling of the biaryl amine 3 with the Fmoc-protected amino acid chloride derivatives of either alanine or glycine, followed
by deprotection and acylation. We envisioned that biaryl amine 3 could be prepared by a Suzuki–Miyaura cross-coupling reaction\textsuperscript{16} between asymmetric bromide 4 and boronic ester 5. The preparation of 4 could be achieved efficiently from the Fischer esterification\textsuperscript{17} of starting material 4-bromo-3,5-dihydroxybenzoic acid, followed by dual application of the Williamson aryl ether synthetic protocol.\textsuperscript{18} The retrosynthetic plan for 5, shown in Scheme 2, involves a Miyaura boration\textsuperscript{19} of bromide 6; bromide 6 could be prepared from aniline 7 by successive protection, alkylation, and deprotection reactions.

**Scheme 2. Retrosynthetic pathway for boronate 5.**

![Scheme 2](image)

2.2 SYNTHESIS OF THE TORSION BALANCE

2.2.1 Synthesis of the Bromide Coupling Fragment

The synthesis of the first coupling fragment, bromide 4, is shown in Scheme 3. Methyl benzoate 9 was synthesized in 99% yield by a Fischer esterification\textsuperscript{17} on commercially available 4-bromo-3,5-dihydroxybenzoic acid 8. The literature procedure was slightly modified by using solid sodium bicarbonate to quench the reaction before workup; recrystallization was found to be unnecessary. A similar procedure using thionyl chloride in methanol\textsuperscript{20} also gave benzoate 9, but with a slightly lower yield of 90%. Reaction of 9 with 1 equiv of commercially available tert-
butyl bromoacetate by the Williamson aryl ether protocol\(^{18}\) afforded ether 10 in a moderate 35% yield; the major product in 48% yield was the symmetrical ether 11, but this was readily separated from the desired product by flash chromatography. Synthesis of the symmetrical ether 11 was also accomplished in an excellent 89% yield as the sole product by using 2 equiv of tert-butyl bromoacetate. The same reaction conditions\(^{18}\) were applied to 10 with commercially available benzyl bromoacetate to obtain 4 in an excellent 90% yield.


2.2.2 Synthesis of the Arylboronic Ester Coupling Fragment

We next synthesized the second coupling fragment, boronic ester 5. Following upon Hudson’s report of reductive monoalkylation of aromatic amines,\(^{21}\) we initially focused on synthesizing \(N\)-ethylaniline 13, shown in Scheme 4, instead of \(N\)-methylaniline 6 because the direct monoalkylation of aniline 7 would shorten the synthetic route toward targets 1 and 2 by two steps. In a model reaction, \(o\)-toluidine 12 underwent monoalkylation with palladium and ammonium formate in acetonitrile to yield \(N\)-ethyl-\(o\)-toluidine 14 as product in quantitative yield.\(^{21}\) However, when the same monoalkylation conditions were applied to commercially
available 3-bromo-2-methylaniline 7, product 13 was not obtained. The $^1$H NMR spectrum of the byproduct obtained matched that of $N$-ethyl-ortho-toluidine, demonstrating that cleavage of the bromine from 7 had occurred. We observed the formation of a fine brown solid sideproduct, not observed in the model reaction, which readily dissolved in water.

Scheme 4. Reductive monoalkylation of anilines 7 and 12.

The modified synthetic route is based on the protection and later deprotection of the aniline, and is shown in Scheme 5. Protection of aniline 7 was effected by reaction with di-tert-butyl dicarbonate (Boc anhydride, or Boc$_2$O) to afford carbamate 15, which was in turn treated with bromoethane and sodium hydride to arrive at alkylated carbamate 16. Deprotection of the Boc group with trifluoroacetic acid (TFA) produced desired aniline 13 in quantitative yield.

Scheme 5. Successful synthesis of aniline 13.
The synthesis of the \( \text{N-methylaniline} \) 6 counterpart was carried out in similar fashion and is shown in Scheme 6. Methylation of 15 with methyl iodide and sodium hydride gave carbamate 17 in quantitative yield; deprotection with TFA afforded aniline 6, also in quantitative yield.

**Scheme 6. Synthesis of \( \text{N-methylaniline} \) 6.**

With the alkylated anilines constructed, we next endeavored to carry out an amidation reaction involving a protected amino acid. Aniline 13 was initially treated with commercially available \( \text{N-acetyl-alanine} \) 18 using various standard amino acid coupling conditions, and the results, summarized in Table 1, had poor to moderate yields. Treatment of 13 and 18 with \( \text{bis(2-oxo-3-oxazolindinyl)phosphinic chloride (BOP-Cl)}, \) triethylamine (\( \text{NEt}_3 \)), in \( \text{CH}_2\text{Cl}_2 \) gave only 14\% yield of expected amide 19 (Entry 1). Reaction of 13 and 18 with \( \text{N,N'-dicyclohexylcarbodiimide (DCC)} \) and 4-(dimethylamino)pyridine (DMAP) in \( \text{CH}_2\text{Cl}_2 \) resulted in a 61\% recovery of the starting material (SM) 13 but with no product isolated (Entry 2). Employment of reagents \( \text{N-ethyl-N’-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDCI)}, \) 1-hydroxybenzotriazole (HOBt) with 13 and 18 in \( \text{CH}_2\text{Cl}_2 \) afforded 19 in the best yield at 34\% (Entry 3). Changing the solvent to DMF caused a drop in the yield to 14\% (Entry 4); likewise, using a 1:1 mixture of \( \text{CH}_2\text{Cl}_2 \) and DMF gave a similar 15\% yield (Entry 5). Use of either of the phosphate salts \( \text{o-benzotriazole-N,N,N',N'-tetramethyl-uronium-hexafluorophosphate (HBTU)} \) or \( \text{o-(7-azabenzotriazol-1-yl)-N,N,N',N'-tetramethyl-uronium-hexafluorophosphate (HATU)} \) with the corresponding triazoles and \( \text{N,N'-diisopropylethylamine} \)
(DIEA) in CH$_3$CN$^{25}$ only resulted in recovered aniline starting material (Entries 6 and 7, respectively). We propose that steric hindrance and reduced nucleophilic activity of the aniline (as a secondary alkyl (aryl) amine) contributed to the failure of these reactions.

Table 1. Conditions and results for amidation reactions between aniline 13 and N-acetyl-alanine 18.

<table>
<thead>
<tr>
<th>Entry</th>
<th>Conditions*</th>
<th>Yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>BOP-Cl, NEt$_3$, CH$_2$Cl$_2$, 2 d</td>
<td>14%$^b$</td>
</tr>
<tr>
<td>2</td>
<td>DCC, DMAP, CH$_2$Cl$_2$, 12 h</td>
<td>SM</td>
</tr>
<tr>
<td>3</td>
<td>EDCI, HOBr, CH$_2$Cl$_2$, 3 d</td>
<td>34%$^b$</td>
</tr>
<tr>
<td>4</td>
<td>EDCI, HOBr, DMF, 2 d</td>
<td>14%$^b$</td>
</tr>
<tr>
<td>5</td>
<td>EDCI, HOBr, CH$_2$Cl$_2$/DMF (1:1), 3 d</td>
<td>15%$^b$</td>
</tr>
<tr>
<td>6</td>
<td>HBTU, HOBr, DIEA, CH$_3$CN, 24 h</td>
<td>SM</td>
</tr>
<tr>
<td>7</td>
<td>HATU, HOAt, DIEA, CH$_3$CN, 24 h</td>
<td>SM</td>
</tr>
</tbody>
</table>

*The reaction temperature of Entry 1 began at 18 °C and was increased to room temperature; the temperatures of all other entries began at 0 °C and were increased to room temperature.

$^b$ Isolated yield of product; aniline starting material (SM) was also recovered.

We next turned to an approach, based on Carpino’s conditions$^{26}$, involving initial conversion of the protected amino acid 18 to the protected amino acid chloride intermediate 18-int before reaction with the amine. This reaction, shown in Scheme 7, was unsuccessful, as the
amino acid decomposed in the first step with thionyl chloride when performed at either room 
temperature or at reflux, and neither starting material nor product was observed in the $^1$H NMR 
spectrum.

**Scheme 7. Attempted synthesis of amide 19 by using Carpino’s conditions.**

According to Montalbetti, formation of amino acid halides can be difficult due to 
possible cyclization to a five-membered $N$-carboxyanhydride ring; however, this can be 
suppressed with other protecting groups, such as the 9-fluorenlymethyloxycarbonyl (Fmoc) 
groups. Therefore, $N$-acetyl-alanine was replaced by commercially available $N$-Fmoc-alanine as 
the amino acid substrate.

Treatment of $N$-Fmoc-alanine 20 and aryl amine 13 with the same conditions from Table 
1, Entry 3 (EDCI and HOBt in CH$_2$Cl$_2$) gave amide 22 in a 24% yield. In another attempt, we 
utilized a Carpino procedure involving initial conversion of the amino acid to its corresponding 
fluoride by treatment with cyanuric fluoride and DIEA in CH$_2$Cl$_2$ before reaction with the amine; 
the reaction generated 22 in a moderate yield of 22%, most likely due to facile hydrolysis of the 
amino acid fluoride.

The most successful amide coupling reactions involve amino acid chlorides from our 
previous implementation of Carpino’s procedure, and are summarized in Table 2. We 
converted $N$-Fmoc-alanine 20 to its corresponding acid chloride intermediate 20-int, with use of
thionyl chloride in CH$_2$Cl$_2$, before reaction with 13 and achieved our first good yield of 68% (Entry 1). We then expanded our scope to also include aniline 6 and N-Fmoc-glycine 21 as reagents to arrive at amides 23 (Entry 2, 96%), 24 (Entry 3, 85%), and 25 (Entry 4, 94%). Using an amino acid chloride as the substrate had the advantage of being slightly less susceptible to hydrolysis in comparison to the amino acid fluoride. Although more stable than acyl fluorides, the amino acid chlorides were used immediately upon their syntheses because hydrolysis could be seen (via $^1$H NMR) after one day. Another advantage of the acyl chloride was to increase the electrophilicity of the amino acid toward the less nucleophilic secondary aniline. It is also important to note the role of steric effects in formation of the amides 22-25; the yields of N-methyl-anilides 23 and 25 were higher in comparison to their N-ethyl- counterparts 22 and 24. In a model reaction completed with commercially available N-ethylaniline and N-Fmoc-alanine (with conversion to the acid chloride) as the substrates, the corresponding amide product was formed in 81% yield (compared to 68% for 22); this suggests that not only did the alkyl group on the nitrogen provide steric hindrance for attack, but that the aryl methyl group did as well.
Table 2. Results for amidation reactions between anilines 13 and 6 and \(N\)-Fmoc amino acids 20 and 21.

<table>
<thead>
<tr>
<th>Entry</th>
<th>Amine</th>
<th>Amino Acid</th>
<th>Product</th>
<th>R</th>
<th>R'</th>
<th>% Yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>13</td>
<td>20</td>
<td>22</td>
<td>CH(_2)CH(_3)</td>
<td>CH(_3)</td>
<td>68</td>
</tr>
<tr>
<td>2</td>
<td>6</td>
<td>20</td>
<td>23</td>
<td>CH(_3)</td>
<td>CH(_3)</td>
<td>96</td>
</tr>
<tr>
<td>3</td>
<td>13</td>
<td>21</td>
<td>24</td>
<td>CH(_2)CH(_3)</td>
<td>H</td>
<td>85</td>
</tr>
<tr>
<td>4</td>
<td>6</td>
<td>21</td>
<td>25</td>
<td>CH(_3)</td>
<td>H</td>
<td>94</td>
</tr>
</tbody>
</table>

The next step enroute to the formation of the torsion balance was a Miyaura boration,\(^{19}\) with bis(pinacolato)diboron used as the boronate reactant. The \(N\)-methylanilide 23 was first employed due to its simplicity in structure and \(^1\)H NMR interpretation in comparison with its \(N\)-ethyl counterpart 22. However, as seen in Scheme 8, no product was isolated. The crude \(^1\)H NMR spectrum revealed that the Fmoc group was cleaved during the reaction; a singlet at \(\delta = 6.08\) ppm corresponded to the cleavage sideproduct dibenzofulvene (DBF). The Fmoc group was possibly cleaved due to the high reaction temperature (100 °C) and the base (KOAc) used.
We then endeavored to synthesize 26 first by boration of aniline 6, followed by amidation; this is shown in Scheme 9. Use of the same Miyaura conditions\textsuperscript{19} afforded 5 in 64% yield; the presence of the aryl methyl group may have provided steric hindrance and influenced the yield. Boronic ester 5 was then coupled with $N$-Fmoc-alanine acid chloride, generated by the method of Carpino,\textsuperscript{26} to produce amide 26 in 72% yield.
2.2.3 Final Steps to Synthesize the Torsion Balance

At this point we envisioned that a Suzuki–Miyaura cross-coupling reaction between bromide 4 and amide 26 would produce biaryl amide 27, which could be Fmoc-deprotected and acylated to generate torsion balance 1. However, there are few examples in the literature that show cross-coupling of two substrates with a total of three ortho-substituents with and without an additional bulky meta-substituent. The attempts to synthesize 27 are summarized in Scheme 10. Small amounts of both starting materials 4 and 26 were recovered, along with boronic ester homocoupling sideproducts, but no product was isolated. An observation was the cleavage of the Fmoc group as evidenced by the formation and relatively large quantity of DBF recovered. In addition, the tert-butyl ester may have also been cleaved due to its sensitivity to high temperatures, and the reaction temperature of 130 °C (used in all entries) may have been too high.30 For subsequent Suzuki–Miyaura cross-coupling reactions, the reaction temperature used was 90 °C.

Based upon these results, the synthetic route was modified by replacing amide 26 with amine 5 for the cross-coupling; the various conditions and results are summarized in Table 3. Previously used reaction conditions of tetrakis(triphenylphosphine)Pd(0) [Pd(PPh$_3$)$_4$] in 1,2-dimethoxyethane (DME) and H$_2$O gave 3 in 28% (Entry 1). Substituting DMF as the co-solvent led to no recovered product (Entry 2). Employing different palladium catalysts, palladium (II) acetate [Pd(OAc)$_2$] and tris[dibenzyldieneacetone]dipalladium(0) [Pd$_2$(dba)$_3$], in DME, accompanied by the Buchwald ligand JohnPHOS®$^{16}$ also did not result in any product (Entries 3 and 4). However, using Pd$_2$(dba)$_3$ and JohnPHOS® in DMF afforded 3 in 10% yield (Entry 5). Switching the ligand to Buchwald’s 2-dicyclohexylphosphino-2’,6’-dimethoxybiphenyl (SPHOS®)$^{16}$ and the base from KF to K$_3$PO$_4$·H$_2$O in DME gave 3 in 31% (Entry 6). Addition of water as a co-solvent to the conditions in Entry 6 lowered the product yield to 9% (Entry 7). Substitution of isopropanol as the solvent, to suppress formation of the boronic ester homocoupling sideproduct,$^{31}$ did not afford product (Entry 8). However, use of toluene$^{16}$ as the solvent gave the best yield of 47% (Entry 9); the yield dropped to 37% upon reaction scale-up from 0.1 mmol to 2.5 mmol (based upon bromide 4) (Entry 10), most likely due to the higher probability of oxygen being present in the reaction vessel, even after degassing the system.
Table 3. Conditions and yields for Suzuki-Miyaura cross-coupling between fragments 4 and 5.

![Chemical Structures](image)

<table>
<thead>
<tr>
<th>Entry</th>
<th>Conditions (^a)</th>
<th>Yield (^b)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Pd(PPh(_3))(_4), NaHCO(_3), H(_2)O, DME</td>
<td>28%</td>
</tr>
<tr>
<td>2</td>
<td>Pd(PPh(_3))(_4), NaHCO(_3), H(_2)O, DMF</td>
<td>0%</td>
</tr>
<tr>
<td>3</td>
<td>Pd(OAc)(_2), JohnPHOS(^\circledR), K(_F), DME</td>
<td>0%</td>
</tr>
<tr>
<td>4</td>
<td>Pd(_2)(dba)(_3), JohnPHOS(^\circledR), K(_F), DME</td>
<td>0%</td>
</tr>
<tr>
<td>5</td>
<td>Pd(_2)(dba)(_3), JohnPHOS(^\circledR), K(_F), DMF</td>
<td>10%</td>
</tr>
<tr>
<td>6</td>
<td>Pd(_2)(dba)(_3), SPHOS(^\circledR), K(_3)PO(_4)-H(_2)O, DME</td>
<td>31%</td>
</tr>
<tr>
<td>7</td>
<td>Pd(_2)(dba)(_3), SPHOS(^\circledR), K(_3)PO(_4)-H(_2)O, H(_2)O, DME</td>
<td>9%</td>
</tr>
<tr>
<td>8</td>
<td>Pd(_2)(dba)(_3), SPHOS(^\circledR), K(_3)PO(_4)-H(_2)O, isopropanol</td>
<td>0%</td>
</tr>
<tr>
<td>9</td>
<td>Pd(_2)(dba)(_3), SPHOS(^\circledR), K(_3)PO(_4)-H(_2)O, toluene</td>
<td>47%</td>
</tr>
<tr>
<td>10</td>
<td>Pd(_2)(dba)(_3), SPHOS(^\circledR), K(_3)PO(_4)-H(_2)O, toluene</td>
<td>37%</td>
</tr>
</tbody>
</table>

\(^a\) Reaction scale based on 0.1 mmol of 4, except Entry 10 where the scale was 2.5 mmol; all reactions were run for 14 h at 90 °C

\(^b\) Isolated yield of product; recovered material also included both starting materials 4 and 5, the homocoupling sideproduct of 5, and the bromine-proton exchange side product of 4
With biaryl amine 3 constructed, the last steps to form the torsion balance were implemented, and are shown in Scheme 11. Again using Carpino’s amino acid chloride procedure,26 N-Fmoc-alanine acid chloride 20-int and N-Fmoc-glycine acid chloride 21-int were generated and added to 3 to produce 27 and 28 in good yields of 82% and 81%, respectively. These amides were then each treated with piperidine in DMF to cleave the Fmoc group, followed by acylation using acetic anhydride and pyridine in CH$_2$Cl$_2$ to give torsion balances 1 and 2 in yields of 99% and 97%, respectively.

Scheme 11. Final steps to synthesize torsion balance.
3.0 NMR STUDIES OF INITIAL TORSION BALANCES

3.1 EVALUATION OF TORSION BALANCE BOND ROTATIONS

3.1.1 Analysis of Carbamates and the N-CO Bond Rotation

We observed interesting conformational dynamics of carbamates 16 and 17 via $^1$H NMR analysis. At room temperature, the spectra for both carbamates showed two conformations. Both carbamates, for example, exhibit two signals in approximately a 2:1 ratio that represent the nine tert-butyl protons. This suggested that there was a higher than expected barrier to rotation around the N-CO bond. According to Smith and coworkers, $^{33}$ hindered rotation around the C-N single bond of N-phenylcarbamates gave rise to a low rotational barrier equal to 12.5 kcal·mol$^{-1}$. However, Smith’s carbamates, unlike ours, did not possess ortho-substituents. Therefore, variable temperature $^1$H NMR studies, ranging from 278 K to 323 K (5 to 50 °C), were performed to analyze the rotational barriers of our carbamates 16 and 17. The $^1$H variable temperature spectra for carbamate 17 are shown in Figure 8; the spectra for carbamate 16 were similar. Carbamate (E)-17 is thought to be the more favored rotamer because the (Z) rotamer is more prone to electron-electron repulsion.$^{33-34}$

In comparing the $^1$H variable temperature spectra of 16 and 17, we could see signal coalescence for the nine tert-butyl protons beginning at approximately 35 °C (308 K) for both
carbamates. The signals corresponding to the ethyl group of carbamate 16 were already exhibiting line broadening at room temperature (298 K). We eventually utilized only methyl carbamate 17 toward generating our torsion balances because the $^1$H NMR of the methyl carbamate displayed fewer signals; this would allow for a less complicated assignment of our larger molecules once completed. Therefore, we concentrated our determination of the N-CO rotational barrier to carbamate 17. By using both dynamic line shape analysis$^{35}$ and an EXSY experiment,$^{36}$ we found the barrier to be $\Delta G^\ddagger = 17.2$ kcal·mol$^{-1}$ at 298 K in CDCl$_3$. This barrier to isomerization is higher than for Smith’s carbamates. The phenyl group of our carbamate is rotated out of resonance with the nitrogen and this may allow the nitrogen to become more electron-donating toward the carbonyl. By comparing the variable temperature $^1$H NMR spectra for 16 and 17, we saw that coalescence begins to occur at approximately the same temperature, and so we can deduce that the barrier of carbamate 16 is approximately 17 kcal·mol$^{-1}$ as well, and that the difference between the methyl and ethyl groups did not exert a significant change in the dynamics.
Figure 8. Carbamates (Z)-17 and (E)-17 and a selected area of the variable temperature $^1$H NMR spectra from 278 K to 323 K (5 to 50 °C, chloroform-$d$, 500 MHz). Protons of interest are denoted.
We performed a related model study to investigate if the $^1$H NMR spectrum of an aryl carbamate without an ortho- substituent would also reveal two signals for the nine tert-butyl protons (at 298 K). Our model carbamate was tert-butyl 3-bromophenyl(methyl)carbamate — an analog of 17 with a proton instead of a methyl group in the ortho- position — which was synthesized by treatment of commercially available 3-bromoaniline with previously utilized Boc protection and methylation conditions. However, we observed only one sharp peak for the tert-butyl group at $\delta = 1.46$ ppm. In comparison to Smith’s carbamates, the presence of the aryl o-methyl group of our carbamates 16 and 17 accounts for the increase in rotational barrier of almost 5 kcal·mol$^{-1}$, and allows for the observation of two conformer peaks for each carbamate at room temperature.

3.1.2 Analysis of Torsion Balance Amides and the N-Aryl Bond Rotation

Although amides 23 and 25 could not be applied toward the synthesis of torsion balances 1 and 2, they could potentially supply information about the rotational barrier of the N-aryl bond; rotation around this bond will provide the conformational switch necessary for our studies. Four conformers of 1 and two conformers of 2 were observed (by $^1$H NMR at room temperature), and so it was speculated that rotation around either the N-aryl or the N-CO bond contributed toward the two additional conformers; rotation around the aryl-aryl bond was estimated to have a higher barrier and therefore not contribute much or at all toward the conformational switch.

In a study performed to further examine the rotational barriers present in the torsion balance targets, the $^1$H NMR spectra of 23 and 25 was first obtained at room temperature to compare to those of 1 and 2, respectively. To complement the $^1$H NMR findings of 1, the $^1$H NMR spectrum of 23 at room temperature displayed evidence of four conformers. The spectra of
analogs 2 and 25 had similar results. Therefore, we subjected both 23 and 25 to variable temperature $^1$H NMR experiments,\textsuperscript{35b} ranging from 5 to 50 °C (278 to 323 K). For both molecules, we were able to observe broadening of all peaks starting at 35 °C (308 K). To provide a more accurate account of the torsion balance bottom half, however, we needed to replace the bulky Fmoc protecting group with the acyl group. This exchange would also simplify the $^1$H NMR analysis by eliminating the three non-aromatic Fmoc protons from the δ = 4.0–4.5 ppm range.

The synthesis of acyl amides 29 and 30 is shown in Scheme 12, and is similar in fashion to the final steps toward formation of 1 and 2. Amides 23 and 25 were each treated with piperidine in DMF to cleave the Fmoc group, followed by acylation using acetic anhydride and pyridine in CH$_2$Cl$_2$\textsuperscript{32} to give 29 and 30 in yields of 89% and 82%, respectively.

\textbf{Scheme 12. Synthesis of torsion balance precursor amides 29 and 30.}

\begin{center}
\begin{tikzpicture}
\node (a) at (0,0) {\includegraphics[width=0.4\textwidth]{scheme12.png}};
\node[below=0.5cm of a] {1. Piperidine, DMF};
\node[below=0.75cm of a] {2. Ac$_2$O, Pyridine, CH$_2$Cl$_2$};
\node at (2.25,0) {\includegraphics[width=0.4\textwidth]{scheme12.png}};
\node at (0.25,-2.5) {23 $R$ = CH$_3$, $R'$ = CH$_3$};
\node at (0.25,-3) {25 $R$ = CH$_3$, $R'$ = H};
\node at (2.75,-2.5) {29 $R$ = CH$_3$, $R'$ = CH$_3$, 89%};
\node at (2.75,-3) {30 $R$ = CH$_3$, $R'$ = H, 82%};
\end{tikzpicture}
\end{center}

The $^1$H NMR spectra of these amides were less complicated because of the absence of the Fmoc group, and so we subjected both amides 29 and 30 to variable temperature $^1$H NMR studies,\textsuperscript{35b} ranging from 25 to 80 °C (298 to 353 K); the results of the studies for both 29 and 30 were similar, and so only those for 29 are shown (Figure 9). We observed broadening of the set of three quintets at δ = 4.40–5.10 ppm (representing the alanine α-methine proton) and the set of
four doublets at $\delta = 0.90$–1.30 ppm (representing the three $\alpha$-methyl protons of alanine) as the temperature approached 70 °C. These results showed that the interconversion between the conformers was fast; however it was possible that rotation around both the N-aryl and the N-CO bonds contributed to the conformational changes detected for this amide.

We subjected 29 to dynamic line shape$^{35}$ and EXSY$^{36}$ analyses in order to determine the barrier to rotation around the N-aryl bond, because it is rotation around the N-aryl bond that should give rise to the desired conformational states of our torsion balances 1 and 2 (as proposed in Figure 3). The line shape simulation verified that the peak broadening was due to both N-aryl and N-CO bond rotations; we observed interference of the minor conformer peaks (most notably at $\delta = 5.10$ and 1.30 ppm, representing the alanine $\alpha$-methyl and methine groups) with the major peaks. Therefore, it was determined that the EXSY analysis would be the most reliable method because it focused more on cross-peak integration of the target conformer peaks. The cross-peaks of the two largest alanine $\alpha$-methyl isomer peaks (at $\delta = 1.10$ and 0.90 ppm) were used to calculate the barrier to rotation around the N-aryl bond to be $\Delta G^\ddagger = 20.9$ kcal·mol$^{-1}$ at 343 K in toluene-$d_8$.

The small peaks at, for example, $\delta = 1.30$ ppm (two doublets) and 5.10 ppm (one quintet), observed in the $^1$H NMR spectra of 29 suggested that a small amount of a “minor anti amide” was present due to rotation around the N-CO amide bond. These rotamers and their suggested pathways to formation are shown in Scheme 13 (glycine analog 30 showed similar results and is also depicted). The two major conformers 29a and 29b, present together in 93% by $^1$H NMR, arise from the higher barrier to rotation around the N-aryl bond of $\Delta G^\ddagger = 21$ kcal·mol$^{-1}$ and possess a racemization half life of 2.2 minutes at 298 K. The two minor conformers arising from
the N-CO bond rotation, 29c and 29d, are present together in 7\% by $^1$H NMR and have a lower barrier of $\Delta G^\ddagger = 17$ kcal·mol$^{-1}$.

**Scheme 13. Possible rotations around N-aryl and N-CO bonds of amides 29 and 30.**
Figure 9. Variable temperature $^1$H NMR spectra of amide 29 from 298 K to 353 K (25 to 80 °C, toluene-$d_8$, 500 MHz). Protons of interest are denoted as follows and coincide with Scheme 13: a and a’ correspond to the two major conformers that arise from N-aryl bond rotation; a’’ and a’’’ correspond to the two minor conformers that arise from N-CO bond rotation.
3.1.3 Analysis of Biaryl Amine and the Torsion Balance Aryl-Aryl Bond Rotation

An essential rotational barrier to analyze was that of the aryl-aryl bond. The barriers in earlier torsion balances were usually measured by NMR spectroscopy. In our case, we initially subjected biaryl amine 3 to variable temperature $^1$H NMR experiments, in similar fashion to those used for amides 29 and 30. Amine 3 was dissolved in toluene-$d_8$ and subjected to a temperature range of -20 to 50 °C. The $^1$H NMR spectra are shown in Figure 10; the diastereotopic protons of the two enantiomeric forms of 3 are shown also in Scheme 14. From the analysis of the $^1$H NMR spectrum (300 MHz) of 3 in chloroform-$d$ and comparison to the analogous spectrum (700 MHz) of the core target 59 in acetonitrile-$d_3$, the diastereotopic protons of 3 were determined to be present at 4.48 ppm (a and a', one singlet) and 4.63 ppm (b and b', one singlet). Diastereotopic protons b and b' are more downfield most likely because the nearby benzyl group is an electron withdrawing group, which would lead to deshielding of the neighboring nuclei and cause a downfield shift in the $^1$H spectrum.

Scheme 14. Enantiomers of biaryl amine 3 and rotation around the aryl-aryl bond.
Figure 10. Variable temperature $^1$H NMR spectra of amine 3 from 253 K to 323 K (-20 to 50 °C, toluene-$d_8$, 500 MHz). Dichloromethane (DCM) impurity is denoted. The diastereotopic protons of interest, coinciding with Scheme 14, are designated as a, a’, b and b’.

Only peak shifting was observed for the denoted diastereotopic protons and no peak broadening was detected. Kinetic equilibrium experiments were attempted via HPLC. Resolution of 3 would allow the rotation rate to be measured directly through observing
racemization via $^1$H NMR. Resolution on both a Chiralcel and Whelk analytical HPLC column was unsuccessful.

Therefore, amine 31, a derivative of 3 with a phenolic group, was synthesized$^{16}$ to obtain better chiral recognition by the HPLC analytical column. The synthesis is shown in Scheme 15; the reaction conditions are identical to those used in the formation of 3 with the exception of the bromide substrate 10. The yield for 31 was low at 9%; the low yield is most likely due to the presence of not only an amine, but also a phenol that is acidic enough to experience deprotonation during the course of the reaction and interfere with the C–C bond coupling.


To increase the yield of the Suzuki–Miyaura coupled product, bromide 10 was first protected with tert-butyl dimethylsilyl chloride (TBS-Cl) and imidazole$^{38}$; bromide 32 was furnished in a good 68% yield. Bromide 32 was combined with boronic ester 5 in a Suzuki–Miyaura cross coupling reaction$^{16}$, shown in Scheme 16, and then the crude was subjected to TBS deprotection with TBAF$^{38b}$ to give amine 31 in an improved 31% yield. The orthomethylene protons of 31 were present at 4.49 ppm (one singlet, 300 MHz, chloroform-d), which
is a similar chemical shift value in comparison to that of the analogous protons of 3 (at 4.48 ppm, near the tert-butyl ester group).

**Scheme 16. Syntheses of TBS protected benzoate 32 and use toward biaryl amine 31.**

![Scheme 16](image)

The enantiomers of derivative 31 were separated by chiral preparatory HPLC. From a racemic mixture of 18 mg, the first eluting enantiomer (31-FEE) was obtained in 5.4 mg (93.6% ee) with a retention time (RT) of 14.7 min, and the second eluting enantiomer (31-SEE) was obtained in 7.1 mg (90.4% ee) with a retention time of 18.4 min. Therefore, derivative 31 was utilized in the kinetic experiment to measure the rotational barrier around the aryl–aryl bond. The two enantiomers of 31 are shown in Scheme 17, and the HPLC separation is depicted in Figure 11, depicting an expected 1:1 ratio of the enantiomers.

The kinetic experiment involved using a solution of 1.0 mg of an enantioenriched sample of 31-SEE in 1.0 mL hexanes that was placed in a sealable thick–walled tube and heated at 145 °C (418 K). Aliquots of 10 μL were removed at 1 h or 2 h increments, with the exception of an overnight run, and submitted to chiral HPLC analysis.
Scheme 17. Enantiomers of biaryl amine 31.

Figure 11. Analytical HPLC analysis of amine 31. The two peaks (enantiomers) were separated successfully by chiral preparatory HPLC, and enantiomer 31-SEE (18.4 min retention time) was used in the kinetic experiment.

A plot of the logarithmic decrease in enantiomeric excess (ee) versus time is shown in Figure 12, and showed a first–order rate for racemization.\textsuperscript{37} Using the Arrhenius kinetics, the rate of racemization was $k_{\text{rac}} = 0.363 \text{ ms}^{-1}$ at 418 K, which translates to a free energy rotational barrier of the aryl-aryl bond for 31 of 35.7 kcal mol$^{-1}$ (149.5 kJ mol$^{-1}$) at 418 K in hexanes, with a racemization half life of 2.2 days at 418 K.
Figure 12. Equilibrium plot for amine 31 based upon the logarithmic decrease in ee from 31-SEE (denoted as S in the y-axis label) to 31-FEE (denoted as F in the y-axis label) over time.

3.2 EVALUATION OF HYDROGEN BOND SELECTIVITY OF INITIAL TORSION BALANCES

3.2.1 Hydrogen Bond Selectivity and Folding Preferences of Initial Torsion Balances

It was necessary to test our torsion balance scaffold for the proper antiparallel β-sheet configuration and hydrogen bonding distance before further appending amino acids to construct the β-turn mimic. Therefore, both torsion balances 1 and 2 were first examined for any selectivity in hydrogen bond formation between the amino acid proton and either the benzyl ester or tert-butyl ester carbonyl. Because the rotational barrier of the aryl-aryl bond was large with a half life of 2.2 days at 418 K, it is only rotation around the N-aryl bond that would provide the conformational switch, as illustrated in Figure 2. Although our β-turn mimic will not contain the aforementioned ester carbonyls once more amino acids are added to the balance, the analysis
with the ester carbonyls should offer insight into the selectivity of the amino acid for one side
over another when considering the constraints of the biphenyl core.

Torsion balance 2 was examined first based upon its simplicity in having only two
distinct conformers arising from rotation around the N-aryl bond, compared to balance 1 which
has four. The two conformers are shown in Scheme 18.

Scheme 18. Two conformers of glycine torsion balance 2 by rotation around the N-aryl
bond, with diastereotopic glycine protons H_a and H_b denoted.

Before our examination into the folding preferences of 2, we carried out a variable
temperature $^1$H NMR study, in similar fashion to 29, to check for an observable sign of N-aryl
bond rotation. We noted the beginnings of peak broadening of the six diastereotopic protons at
70 °C (343 K), which coincided with our analysis of 29 and showed that rotation around the N-
aryl bond dominated.

For the selectivity studies, we compared $^1$H NMR spectra of 2 at 298 K when dissolved
in either chloroform-$d$ or toluene-$d_8$; this comparison is shown in Figure 13. The two N-methyl
group singlets are ultimately the best indicator of the two conformers present; the chemical shifts
for these signals are more deshielded in chloroform-$d$ ($\delta = 3.29$ ppm) than in toluene-$d_8$ ($\delta = 3.06$
ppm). In both solvents, the two singlets are seen in almost a 1:1 conformer ratio: in chloroform-$d_4$ the ratio is 1.04:1.00, while in toluene-$d_8$ the ratio is 1.00:1.23 with a slight change toward the more upfield peak. However, the essentially 1:1 ratio observed from both spectra shows that there is no significant selectivity of either ester in forming a hydrogen bond with the amino acid proton.

![NMR spectra](image)

**Figure 13.** Ester hydrogen bonding selectivity of the glycine torsion balance 2 can be studied from the ratios of the denoted $N$-methyl protons in chloroform-$d_4$ and toluene-$d_8$ (500 MHz). Both ratios were essentially 1:1, meaning that there was little selectivity.

An assignment of the diastereotopic ortho-methylene protons of 2 was performed. From the analysis of the $^1$H and COSY NMR spectra (500 MHz) of 2 in chloroform-$d_4$, the two ortho-methylene protons nearest to the benzyl ester group were determined to be present at 4.70-4.81
ppm (one AB quartet and one singlet), and the two nearest to the tert-butyl ester group were present at 4.52-4.66 ppm (one AB quartet and one multiplet). These data are on par with the assignments from biaryl 3. The $^1$H NMR spectrum (500 MHz) of 2 in toluene-$d_8$, however, showed two singlets (4.15 and 4.16 ppm) and both AB quartets overlapping (4.45-4.81 ppm).

With the hydrogen-bonding selectivity measured, our next goal was to determine if the amino acid itself had a preference for the left or right side of the biphenyl scaffold by rotation around the N-aryl bond. We utilized balance 1 because the L-alanine chain provided an additional site of chirality and therefore four conformers; these are shown in Scheme 19.

**Scheme 19. Four conformers of alanine torsion balance 1.**
Because rotation around the aryl-aryl bond is slow, the four conformers are not at equilibrium at 298 K. The conformers $1-R_aR_aS$ and $1-R_aS_aS$ are in equilibrium with each other because N-aryl bond rotation is fast; likewise, $1-S_aR_aS$ and $1-S_aS_aS$ are in equilibrium with each other. However, because the biphenyl core was a racemate before amide coupling and cannot rotate, the combined amount of $1-R_aR_aS$ and $1-R_aS_aS$ must equal the amount of $1-S_aR_aS$ and $1-S_aS_aS$. In similar fashion to balance 2, balance 1 was dissolved in either chloroform-$d$ or toluene-$d_8$ at 298 K; this comparison is shown in Figure 14.

![Figure 14](image)

Figure 14. Differences in folding preference exhibited by alanine torsion balance 1 can be seen from the ratios of the N-methyl protons (denoted) and others in chloroform-$d$ and toluene-$d_8$ (500 MHz).
From the $^1$H NMR analysis, we observed a conformer ratio of 1.4:1.6:1.0:1.1 in chloroform-$d$ and a ratio of 1.0:1.0:4.5:4.5 in toluene-$d_8$. Toluene-$d_8$ promotes a higher degree of hydrogen bonding compared to chloroform-$d$. Deuterated chloroform is known to compete for hydrogen bonding sites because it is acidic in comparison to its relatively low polarity.\(^{39}\) Given our analysis of balance 2, the conformers $1-R_aR_aS$ and $1-S_aR_aS$ are present in an equal amount (either the signal ratios 1.0:1.0 or 4.5:4.5), as are the conformers $1-R_aS_aS$ and $1-S_aS_aS$.

An assignment of the diastereotopic ortho-methylene protons of 1 was attempted; however, excessive overlap of the AB quartets and multiplets was observed in the $^1$H NMR spectra (4.00-5.00 ppm in chloroform-$d$ and toluene-$d_8$, 500 MHz). In addition, the multiplets of the alanine $\alpha$-proton were also present in this region. In the future, a set of experiments that includes HMQC and HMBC NMR spectra would facilitate a more definitive assignment.

We then performed another $^1$H NMR study with 1 to better understand the behavior of the peaks (the ratios and chemical shifts of a particular moiety, for example the N-methyl group depicted in Figure 14) when subjected to a second solvent. Balance 1 was dissolved in chloroform-$d$ at room temperature, and then 0.09 mL aliquots of toluene-$d_8$ were added until the solution was comprised of 50% toluene-$d_8$. We initially hypothesized that with the introduction of toluene-$d_8$, the set of four singlets would shift upfield without mixing or crossing over of peaks, while the peak integrations increased or decreased accordingly. However, upon addition of the first aliquot, we observed the peaks beginning to converge. With additional aliquots, the peaks at first shifted upfield and continued to converge, followed by the emergence of two small singlets and a much larger upfield singlet. Therefore, this mixing of the singlets revealed how different solvents can have a profound effect on not only the chemical shifts, but also the populations of hydrogen-bonded conformers.
The selectivity observed in toluene-$d_8$ cannot be based on the preference of the amino acid proton to hydrogen bond to one ester over another, as established by the analysis of torsion balance 2. There must be a preference of the amino acid to be on the left or right side (perhaps influenced by proximity of the alanine $\alpha$-methyl to the aryl group of the biphenyl core). An EXSY experiment was performed at 343 K. We did not observe ester selectivity from the EXSY analysis; therefore, it is likely that the selectivity for the amino acid to be on either side was more influenced by potential negative interactions between the aforementioned $\alpha$-methyl and aryl groups, which might have been exacerbated by the hydrogen bond. Molecular modeling predicted that without the hydrogen bond present, there should be less selection upon N-aryl rotation and the ratios would be closer to 1:1:1:1, and so this remains to be tested.

3.2.2 Hydrogen Bond Selectivity Analysis with Torsion Balance Derivatives

In our NMR analyses of the torsion balances 1 and 2, we were not able to detect a higher selectivity of the amino acid proton towards the carbonyl oxygen of either the tert-butyl or the benzyl esters. Therefore, we next changed one ester carbonyl to an amide in order to test whether we could observe the expected hydrogen bond selectivity toward the amide carbonyl versus the ester carbonyl.

3.2.2.1 Synthesis of the Dimethylamide Torsion Balance Derivatives and NMR Analysis

The synthesis of the torsion balance derivatives 35 and 36 is shown in Scheme 20. Biaryl amides 27 and 28 were both subjected to hydrogenolysis conditions using 10% palladium on carbon and a balloon filled with hydrogen gas and at atmospheric pressure to afford carboxylic acids 33 and 34 in essentially quantitative yields. These acids were then treated with
dimethylamine hydrochloride and standard coupling reagents EDCI, HOBr, and triethylamine (NEt₃) in acetonitrile for 80 h at room temperature; this resulted in both the amide coupling and also the cleavage of the Fmoc protecting group in preparation for acylation. Although this shortened the synthetic route by one step, it resulted in lower yields because the dramatic increase in polarity of these molecules had made purification by flash chromatography more difficult. The intermediates were recovered, still containing some impurities, and treated with acetic anhydride and pyridine to yield biaryl amide derivatives 35 and 36 in two-step yields of 52% and 48%, respectively. A tertiary amide in the form of dimethylamide was chosen over a secondary amide (i.e. methylamide) because there would be no ambiguity over whether the amide functionality was participating as a hydrogen bond donor as well as a hydrogen bond acceptor.

The torsion balance derivatives $35$ and $36$ were examined by NMR studies for any preference in carbonyl selectivity. If a carbonyl selectivity was in effect, we predicted that the equally sized peaks (ratios 4.5:4.5 or 1.0:1.0 from torsion balance 1) would become unequal. The selectivity was first analyzed with $35$; the four possible conformations are shown in Scheme 21. Similar to alanine balance 1, all four conformers of $35$ are not in equilibrium with each other. Based upon the analysis of the four N-methyl signals (the N-methyl adjacent to the amino acid), we observed the four isomers of $35$ to be present in a ratio of approximately 1.0:3.0:6.0:7.0 in toluene-$d_8$ (1.7:1.4:1.3:1.0 in chloroform-$d$), in comparison to the 1.0:1.0:4.5:4.5 selectivity observed for balance 1.

Scheme 21. Four conformers of alanine torsion balance derivative $35$. 

![Scheme 21. Four conformers of alanine torsion balance derivative $35$.](image)
The $^1$H NMR spectra of 35 with either chloroform-$d$ or toluene-$d_8$ are shown in Figure 15. There is a stronger preference for an amide proton to hydrogen bond with the dimethylamide carbonyl than with the tert-butyl ester carbonyl. We therefore propose that the conformer present in the highest population (the largest signal in toluene-$d_8$) has both the hydrogen bond between the amino proton and the amide carbonyl, as well as little interaction between the alanine $\alpha$-methyl and the aryl group.

Figure 15. Differences in folding preference exhibited by alanine torsion balance derivative 35 can be seen from the ratios of the denoted $N$-methyl protons in chloroform-$d$ and toluene-$d_8$ (500 MHz).
An assignment of the diastereotopic ortho-methylene protons of 35 was also attempted; however, similar to alanine balance 1, excessive overlap of the AB quartets and multiplets was observed in the $^1$H NMR spectra (4.10-5.10 ppm in chloroform-$d$ and toluene-$d_8$, 500 MHz). In addition, the multiplets of the alanine $\alpha$-proton were also present in this region. Therefore, a future set of experiments that includes HMQC and HMBC NMR spectra would facilitate a more definitive assignment.

3.2.2.2 Introduction of a Beta-Alanine Torsion Balance Analog and its Dimethylamide Derivative

We also subjected derivative 36 to the same studies as 35, and we noted a carbonyl preference of 1.4:1.0 in toluene-$d_8$ in comparison to the ratio of 1.0:1.2 for balance 2. Further analyses involving 36 and other balances were done in comparison with $\beta$-alanine derivatives. It was important to discern if the amino acid proton on the (o-tolyl)amide portion of the balance was spaced appropriately in relation to the ester (or amide) carbonyls on the benzoate portion to ensure hydrogen bond formation. The choice of $\beta$-alanine was made because it provided an additional methylene spacer to separate the nitrogen proton from the $\beta$-turn core. The syntheses of the $\beta$-alanine amides 38, 39, and dimethylamide 41 were performed in similar fashion to their alanine and glycine analogs, and are shown in Schemes 22 and 23. Using Carpino’s amino acid chloride procedure, $^26$ N-Fmoc-$\beta$-alanine acid chloride was generated from N-Fmoc-$\beta$-alanine 37 and added to 3 to produce biaryl amide 38 in an 82% yield.

Amide 38 was then treated with DBU in CH₂Cl₂ to cleave the Fmoc group, and the product was acylated using acetic anhydride and pyridine in CH₂Cl₂ to give torsion balance analog 39 in a moderate 50% yield. DBU was substituted for piperidine to compare its potency to cleave the Fmoc group, and it was found to be equally effective. Amide 39 was then subjected to hydrogenolysis conditions to afford carboxylic acid 40 in quantitative yield, after which 40 was treated with dimethylamine hydrochloride and standard coupling reagents EDCI, HOBt, and triethylamine (NEt₃) in acetonitrile for 80 h at room temperature to give dimethylamide 41 in 74% yield. The route used to synthesize 41, although containing an extra step in comparison with 35 and 36, gave less trouble in terms of purification via flash chromatography.

We compared the derivatives 36 and 41 in both chloroform-$d$ and toluene-$d_8$ to test for any carbonyl selectivity. This comparison was appropriate because both derivatives existed as two conformations (based upon rotation around the N-aryl bond) and they only differed by a methylene spacer. The conformers are shown in Scheme 24, and the $^1$H NMR comparison at 298 K in toluene-$d_8$ is shown in Figure 16. We observed that both dimethylamides 36 and 41 exhibited the same selectivity ratio of 1.0:1.9 in favor of one of the carbonyls. The similarity in ratios is most likely due to the lack of an amino acid side chain that could interact with the aryl group of the biaryl core.
Scheme 24. Two conformers each of glycine 36 and β-alanine 41 torsion balance derivatives.

Figure 16. Folding preference exhibited by glycine derivative 36 and β-alanine derivative 41 in toluene-$d_8$ at 298 K (500 MHz). The $N$-methyl protons are denoted; both derivatives have conformer ratios of 1.0:1.9.
Assignments of the diastereotopic *ortho*-methylene protons of 36 and 41 were performed. From the analysis of the $^1$H and NOESY NMR spectra (500 MHz) of 36 in toluene-$d_8$, the two *ortho*-methylene protons nearest to the dimethylamide group were determined to be present at 4.46-4.81 ppm (one AB quartet at 4.46-4.81 ppm and one singlet at 4.64 ppm) and the two nearest to the *tert*-butyl ester group were present at 4.15-4.19 ppm (one singlet at 4.15 ppm and one multiplet at 4.19-4.19 ppm). For the analysis of 41 (500 MHz, toluene-$d_8$), the two *ortho*-methylene protons nearest to the dimethylamide group were determined to be present at 4.32-4.38 ppm (one multiplet) and the two nearest to the *tert*-butyl ester group were present at 4.12-4.17 ppm (one multiplet). The diastereotopic protons nearest to the dimethylamide group are more downfield most likely due to the increased basicity and enhanced resonance of the amide functionality.

In a final study, we compared target balance 2 to its *β*-alanine derivative 39 to again test for any hydrogen bond selectivity. The conformers are shown in Scheme 25, and the $^1$H NMR comparison at 298 K in toluene-$d_8$ is shown in Figure 17.

**Scheme 25. Two conformers each of glycine balance 2 and its *β*-alanine 39 derivative.**
Figure 17. Folding preference exhibited by glycine balance 2 and β-alanine derivative 39 in toluene-$d_8$ at 298 K (500 MHz). The N-methyl protons are denoted.

We observed that derivative 39 showed a slightly higher selectivity of 1.0:1.7 between the ester carbonyls in contrast with 2, which showed a much lower selectivity of 1.0:1.2. It is possible that the extra methylene spacer in 39 is placing the acyl group in close proximity to the tert-butyl group (depicted by $39-R_a,S_a$) and creating a negative steric effect that would promote selectivity of the amino acid for the benzyl ester carbonyl.

An assignment of the diastereotopic ortho-methylene protons of 39 was performed. From the analysis of the $^1$H NMR spectrum (300 MHz) of 39 in chloroform-$d$, the two ortho-methylene protons nearest to the benzyl ester group were determined to be present at 4.67 ppm (one broad singlet), and the two nearest to the tert-butyl ester group were present at 4.50 ppm.
(one broad singlet). These data are on par with the assignments from glycine balance 2.
Interestingly, the $^1$H NMR spectrum (500 MHz) of 39 in toluene-$d_8$ showed two overlapping singlets and also a large multiplet between 4.00 and 4.50 ppm.

### 3.3 CONCLUSION, AND PROGRESSION TOWARD THE ADVANCED BETA-STRAND TORSION BALANCE

We have described the attainment of an initial set of new molecular torsion balance targets that can be applied toward the study of dynamics in the antiparallel $\beta$-sheet motif. This balance utilizes the restricted rotation around an aryl–aryl bond and an N-aryl bond to impose a two–state nature of folding. Our $^1$H and EXSY NMR analyses of the torsion balance have revealed interesting effects of solvent on hydrogen-bonding conformers and have allowed for suitable calculation of rotational barriers. Overall, our scaffold has the correct shape to simulate intramolecular interactions between the ends of an antiparallel $\beta$-sheet.

Our goal for the hybrid synthetic-natural peptidic torsion balance is to append additional amino acids to the biaryl core 27 and 28 by selective deprotection and amide couplings at the position of choice. A small peptide generally contains six amino acids or less, and so having a chain of two or three amino acids long on the (o-tolyl)amide portion of the torsion balance, in addition to having chains of one or two amino acids on either side of the benzoate portion of the torsion balance would be a good initial target to mimic peptidic dynamics.

We introduced a second amino acid in the form of glycine benzyl ester to each torsion balance; the synthesis is shown in Scheme 26. Carboxylic acids 33 and 34 were each treated with
glycine benzyl ester hydrochloride, in addition to solid sodium bicarbonate, EDCI, and HOBr to yield amides 42 and 43 in 99% and 93%, respectively.

**Scheme 26. Synthesis of biaryl amides 42 and 43 via amide coupling.**

![Scheme 26](attachment:scheme_26.png)

The $^1$H NMR spectra of these bis(amine acid) torsion balances were examined to determine the amount of conformers present; similar to biaryl amides 27 and 28, we observed four configurations for alanine-glycine amide 42 and two for glycine-glycine amide 43. We hope to overcome the issue of having more than two conformers present when performing studies to monitor the changes of amino acids on peptide folding and stability. With further amino acid couplings, the hydrogen bonding between the strands will hopefully restrict rotation around the N-aryl bond or the N-CO bond. The hydrogen bonding from these additional amino acids would allow for comparisons in β-sheet stability (with the smaller balances) and lock in the torsion balance conformations; this would provide new experimental data relevant to the dynamics of the β-turn of a small peptide.
4.0 DESIGN AND SYNTHESIS OF ADVANCED TORSION BALANCE TARGETS.

4.1 RETROSYNTHETIC ANALYSIS

We next planned to expand upon the torsion balance and create target molecules to test how specific changes of amino acids or functional groups would affect peptide folding in antiparallel β-sheets. The first torsion balance design, amide 44, would test the preference of an amino acid chain to form a hydrogen bond with either a secondary amide or a tertiary amide functional group. Amide 44, shown in Scheme 27, could be synthesized by an amide coupling\(^{26}\) of biaryl amine 45 with the Fmoc-protected glycine acid chloride derivative, followed by deprotection and acylation.\(^{32}\) Biaryl amine 45 could be prepared by a Suzuki–Miyaura cross-coupling reaction\(^{16}\) between asymmetric bromide 46 and boronic ester 5. The generation of 46 could be realized from a Williamson ether synthesis\(^{18}\) of a dimethyl bromoacetamide with asymmetric ether 10.
Scheme 27. Retrosynthetic pathway for priority target amide 44.

The second set of torsion balances, amides 47 and 48, are shown in Scheme 28 and would share the same asymmetrical precursor compounds. Amide 47 would test for the preference of an amino acid chain to hydrogen bond more strongly when there are one or two sites for hydrogen bonding in a chain; amide 48 would test the potential of an amino acid chain to form hydrogen bonds more strongly with an alanine or leucine side chain. Amides 47 and 48 would be synthesized by an amidation reaction of acid precursor 49 with either methylamine hydrochloride\textsuperscript{41} or methylated leucine\textsuperscript{44}, respectively. Acid 49 would be prepared by performing several successive deprotections and amidation reactions with biaryl amine 3. The amidation reagents would include a methylated alanine\textsuperscript{44}, N-acetyl-alanine\textsuperscript{45}, and Fmoc-protected glycine acid chloride derivative\textsuperscript{26}. 

The third set of torsion balances, amides 50 and 51, zwitterions 52 and 53, and controls 54 and 55, are shown in Scheme 29 and would share some of the same symmetrical precursor compounds. Amide 50 would test the preference of an amino acid chain to hydrogen bond when a restrictive steric effect is involved (a potentially negative interaction between the methyl of an alanine and a neighboring aryl methyl group). Amide 50 would be prepared by performing several successive deprotections and amidation reactions with symmetrical alanine torsion balance 56. The amidation reagents would include a methylated alanine\(^4^4\) and \(N\)-acetyl-alanine\(^4^5\). Amide 56 would be synthesized by an amide coupling\(^2^6\) of symmetrical biaryl amine 58 and Fmoc-protected alanine acid chloride derivative. Amine 58 would be generated by a Suzuki–Miyaura cross-coupling reaction\(^1^6\) between symmetrical bromide 11 and boronic ester 5.

Amide 51 would test the effect of salt-bridging between ammonium and carboxylate moieties of amino acid chains on the rate of rotation around the N-aryl bond. Amide 51 would be
prepared from deprotections and amidation reactions with symmetrical glycine torsion balance 57. The amidation reagents would include a benzyl protected alanine45 and N-Cbz-alanine45. Amide 57 would be synthesized by an amide coupling of symmetrical biaryl amine 58 and Fmoc-protected glycine acid chloride derivative.26

Zwitterions 52 and 53 would test the effect of salt-bridging between an amino acid ammonium and a simple carboxylate moiety on the rate of rotation around the N-aryl bond. These zwitterions would be produced by performing both Fmoc- and tert-butyl ester deprotections45,46 on symmetrical alanine 56 and glycine 57 torsion balances, respectively.

Control amides 54 and 55 would aid in the test for the effect of salt-bridging between an amino acid ammonium and a simple carboxylate moiety on the rate of rotation around the N-aryl bond. These controls would be produced by performing Fmoc-deprotection and acylation,32 followed by tert-butyl ester deprotection46 on symmetrical alanine 56 and glycine 57 torsion balances, respectively.
Scheme 29. Retrosynthetic pathway for torsion balance targets amide 50, amide 51, zwitterions 52 and 53, and controls 54 and 55.

52 R = H
54 R = (C=O)CH₃
53 R = H
55 R = (C=O)CH₃
The fourth torsion balance type, biaryl amine 59, is shown in Scheme 30 and would be used as a core tool for either solution- or solid-phase peptide synthesis with the introduction of a tert-butyl aryl ester moiety. Amine 59 would be prepared by a Suzuki–Miyaura cross-coupling reaction\textsuperscript{16} between asymmetric bromide 60 and boronic ester 5. The generation of 60 would be realized from two Williamson aryl ether syntheses\textsuperscript{18} with asymmetric phenol 61, using para-nitrobenzyl bromoacetate and benzyl bromoacetate with asymmetric ether 10, upon selective removal of an allyl group. Phenol 61 would be furnished from a Fisher esterification\textsuperscript{17} of commercially available acid 8, then protection of the phenolic groups, with allyl bromide,\textsuperscript{47} followed by conversion of the methyl ester to a tert-butyl ester\textsuperscript{48}.

Scheme 30. Retrosynthetic pathway for core target 59.
4.2 SYNTHESIS OF ADVANCED TORSION BALANCE TARGETS

4.2.1 Synthesis of the Priority Target 44

The synthesis of the priority target 44 is shown in Scheme 31. Reaction of ether 10 with dimethylacetamide 62 by a Williamson aryl ether protocol\(^\text{18}\) afforded bromide 46 in a good 79% yield. A Suzuki–Miyaura cross-coupling reaction\(^\text{16}\) between bromide 46 and boronic ester 5 produced biaryl amine 45 in 24% yield. Carpinolo's amino acid chloride procedure\(^\text{26}\) was then used to generate N-Fmoc-glycine acid chloride 21-int, which was combined with 45 to give amide 63 in 83% yield. Amide 63 was then subjected to a one-pot Fmoc deprotection and acylation, using DBU\(^\text{43}\) in DMF followed by acetic anhydride and pyridine,\(^\text{32}\) to supply amide 64 in an excellent 88% yield. Cleavage of the tert-butyl ester with TFA\(^\text{46}\) and amidation\(^\text{49}\) with methylamine hydrochloride, EDCI, HOBT, and DIEA gave the priority target 44 in 17% yield.
Scheme 31. Synthesis of priority target 44.

Dimethyl bromoacetamide 62 was generated by reacting bromoacetyl chloride 65 with dimethylamine hydrochloride and triethylamine. The procedure used was found in a lone patent, and the moderate 39% yield of 62 is on par with that literature; the synthesis of 62 is shown in Scheme 32.
4.2.2 Synthesis of the Asymmetrical Targets 47 and 48

The syntheses of target 47 and target 48 are shown in Scheme 33; the overall synthesis is efficient because these two target torsion balance compounds differ only by one amidation step at the end of the route. Amide 28 was first subjected to Fmoc deprotection using DBU in DMF; the DBU was removed by flash chromatography, and the crude was then reacted with N-acetyl-alanine 18, (benzotriazol-1-yl)tripyrrlidinophosphonium hexafluorophosphate (PyBOP) and DIEA to produce amide 66 in a moderate two step yield of 58%. In a previous one-pot deprotection-amidation trial with DBU, no amidation product was isolated and only a biaryl side product was found by analysis of the 1H NMR spectrum. In another trial with tris (2-aminoethyl)amine (TAEA) as the amine base used instead, the benzyl ester was also cleaved. Therefore, it was determined that DBU should be used in the deprotection, but should be removed from the crude product before amidation. The tert-butyl ester of 66 was cleaved with TFA to yield the free acid, and then an amidation reaction with methylated alanine 67, EDCI, HOBt and sodium bicarbonate was performed to give amide 68 in a one-pot yield of 62%. Amide 68 was then treated with standard hydrogenation conditions to provide acid 49 in an excellent 92% yield. Acid 49 was subjected to amidation reactions to generate both final targets 47 and 48: treatment with methylamine hydrochloride, EDCI, HOBt and DIEA produced target
47 in 6% yield, and treatment with methylated leucine 69, EDCI, HOBT and sodium bicarbonate supplied target 48 in 45% yield.

Scheme 33. Synthesis of target 47 and target 48.
The methylated alanine 67 and leucine 69 were synthesized from N-Cbz-alanine 70 and N-Cbz-leucine 71, respectively. These protected amino acids were subjected to amidation conditions with methylamine hydrochloride, EDCI, HOBT and DIEA to provide N-methyl-N-Cbz-alanine 72 and N-methyl-N-Cbz-leucine 73 both in quantitative yields. Subsequently, these modified amino acids were submitted to hydrogenation conditions to furnish 67 and 69 in 85% and 65% yields, respectively; this synthesis is shown in Scheme 34. The yields of 72 and 73 were noticeably much higher than that of 44 and 47; it is hypothesized that steric hindrance via the torsion balance scaffold could be a contributing factor.

Scheme 34. Synthesis of N-methylated Cbz-protected amino acids 67 and 69.

The targets 47 and 48 were examined by HPLC in an effort to separate the diastereomers present. We aimed to separate the conformers and then perform 2D NMR analyses to determine their absolute configuration. Both targets were run through Chiralcel OD and Whelk chiral columns, using various mixtures of hexanes, isopropanol and methanol, with no successful separation. RegisPack and RegisCell columns were also tried, with methanol and carbon dioxide as eluents, and were unsuccessful. Use of a reverse-phase Phenomenex Luna C-18 column with water and acetonitrile produced slightly promising analytical results (the best was on a 20-35% acetonitrile gradient), as depicted in Figure 18; however, the separation was too poor for the full-preparative scale, and so targets 47 and 48 were recovered as their respective isomeric mixtures.
Figure 18. Best analytical HPLC trace of target 48 conformer peaks; target 47 was similar in appearance.

We then examined the suitability of precursor carboxylic acid 49 for HPLC separation. By using the Phenomenex reverse-phase column with a 20-32% acetonitrile gradient, we were able to obtain an analytical result, illustrated in Figure 19, that showed enough baseline separation between the conformers (at 13 and 15 min). Full-preparative HPLC runs yielded approximately 2 mg of each constituent, which was enough for 2D NMR analysis.

Figure 19. Analytical HPLC chromatogram of the precursor carboxylic acid 49; the two conformer peaks are at 13 and 15 minutes and eluted out with a 20-32% acetonitrile gradient.
4.2.3 Synthesis of the Symmetrical Targets 50-53 and Controls 54-55

The symmetrical targets were our next aim, and were generated from a symmetrical biaryl amine. Biaryl amine 58, shown in Scheme 35, was synthesized from symmetrical bromide 11 and boronic ester 5 in a modest 26% yield. The Suzuki–Miyaura cross-coupling conditions\textsuperscript{16} used were identical to those used to furnish previously synthesized asymmetrical biaryl amine 3.

Scheme 35. Synthesis of symmetrical Suzuki-coupled starting material 58.

The synthesis of target 50 is shown in Scheme 36, and is similar to that of 47 and 48. Carpino's amino acid chloride procedure\textsuperscript{26} was used to generate N-Fmoc-alanine acid chloride 20-int, which was combined with biaryl amine 58 to give amide 56 in a good 84% yield. Amide 56 was then treated with TAEA\textsuperscript{45} to remove the Fmoc group, and then coupled with N-acetyl-alanine 18 using PyBOP\textsuperscript{45} and DIEA to give amide 74 in 83% yield. Cleavage of the tert-butyl ester with TFA,\textsuperscript{46} followed by an amide coupling\textsuperscript{44} with methylated alanine 67, EDCI, HOBt, and sodium bicarbonate produced target 50 in a good one-pot yield of 56%.
Scheme 36. Synthesis of target 50.

The synthesis of target 51, which showcases a final global deprotection, is depicted in Scheme 37. Carpino's amino acid chloride procedure\textsuperscript{26} was utilized to generate N-Fmoc-glycine acid chloride 21-int, which was then combined with biaryl amine 58 to give amide 57 in an excellent 92% yield. Amide 57 was then treated with TAEA\textsuperscript{45} to remove the Fmoc group, and then coupled with N-Cbz-alanine 70 using PyBOP\textsuperscript{45} and DIEA to give amide 75 in 77% yield. Cleavage of the tert-butyl ester with TFA\textsuperscript{46} and amidation\textsuperscript{44} with commercially available L-alanine benzyl ester hydrochloride, EDCI, HOBr, and sodium bicarbonate produced amide 76 in a good one-pot yield of 78%. Global deprotection of 76, using standard hydrogenation conditions,\textsuperscript{40} gave target 51 in 85% yield.
The synthesis of alanine and glycine zwitterion targets 52 and 53, respectively, is depicted in Scheme 38. Symmetrical amides 56 and 57 were subjected to Fmoc deprotection using TAEA\textsuperscript{45} to reveal the free amine; the crude was then treated with TFA\textsuperscript{46} to cleave the tert-butyl groups and yield the alanine 52 and glycine 53 zwitterion targets in 87% and 89%, respectively. The order of deprotections was reversed and found to have no significant effect on the yield; DBU in DMF\textsuperscript{43} was substituted for TAEA in CH\textsubscript{2}Cl\textsubscript{2}, also with minimal effect.
Scheme 38. Synthesis of zwitterion targets 52 and 53.

Scheme 39. Synthesis of control targets 54 and 55.

The synthesis of the alanine and glycine control targets 54 and 55, respectively, is shown in Scheme 39. Amides 56 and 57 were subjected to a one-pot Fmoc deprotection and acylation, using DBU in DMF followed by acetic anhydride and pyridine, to supply amides 77 and 78 in excellent 90% and 89% yields, respectively. Cleavage of the tert-butyl ester with TFA resulted with the control targets 54 and 55 in 100% and 99% yield, respectively. There was a small amount (<5% by $^1$H NMR) of an acetic anhydride derivative impurity present with the alanine target 54 that remained even after extraction and column chromatography were performed; this impurity did not interfere with the subsequent 2D NMR studies.
4.2.4 Synthesis of the Core Target 59

The core target 59 was devised with solid-phase peptide synthesis in mind — the tert-butyl aryl ester of a torsion balance could be concurrently deprotected with the resin (i.e. FMP resin\(^5\)) to which it was attached. This synthetic plan would be useful in the event a target torsion balance became less organic-soluble (with the addition of more amino acid moieties) and therefore more difficult to synthesize via solution phase, and also would allow for enhanced water solubility for NMR studies.

Our plan was to synthesize 59 by initially generating the tert-butyl ester analog 79 of methyl ester 9, and subsequently generate an asymmetrical bromide similar to 4 via Williamson ether protocols. Several reaction conditions from the literature were tried, as depicted in Scheme 40 — di-tert-butyl dicarbonate and magnesium perchlorate (Mg(ClO\(_4\))\(_2\)),\(^5\)\(^2\) tert-butanol and DMAP,\(^4\)\(^6\) tert-butanol, EDCI and DMAP,\(^5\)\(^3\) tert-butanol, sulfuric acid and magnesium sulfate (MgSO\(_4\)),\(^5\)\(^4\) tert-butanol, 1,1'-carbodiimidazole (CDI) and DBU,\(^5\)\(^5\) and N,N-dimethylformamide di-tert-butyl acetal\(^5\)\(^6\) — all reactions resulted in no isolation of product. We propose that these reactions were unsuccessful because either the two phenol moieties were deprotonated first before esterification, or because the position of the bromine on the aromatic ring did not allow for easy esterification. It is reported that 2,6-dibromo-3,5-dihydroxybenzoic acid was converted to its tert-butyl ester in 50% yield using the Lewis acid Mg(ClO\(_4\))\(_2\);\(^5\)\(^2\)\(^a\) our benzoic acid substrate 8 was different only with the bromine at the para-position (to the carboxylic acid moiety) instead of at the ortho-position.
Scheme 40. Attempts to synthesize tert-butyl 4-bromo dihydroxybenzoate 79.

Our next effort involved protecting the phenolic groups before esterification, shown in Scheme 41. A global protection with benzyl bromide, followed by selective ester cleavage with sodium hydroxide, afforded 80 in a two-step 99% yield.\textsuperscript{57} Acid 80 was then subjected to tert-butyl esterification with tert-butanol, CDI and DBU to furnish ester 81 in quantitative yield.\textsuperscript{57} Deprotection of the benzyl ethers with standard hydrogenation conditions\textsuperscript{40} led to an orange oil, and \textsuperscript{1}H NMR revealed that benzoate 79 was present in 60% yield, with a side product that had an additional aromatic proton in the ortho-position (between both phenolic groups). This side product is hypothesized to result from cleavage of the bromine due to an aryl halide hydrogenolysis side reaction that could occur in the presence of a palladium catalyst.\textsuperscript{52b} A TBS-protected analog of 80 was alternatively generated in 96% yield from a one-pot reaction of 8 with TBS-Cl and imidazole in DMF\textsuperscript{38} followed by acetic acid in a THF/water mixture;\textsuperscript{58} however, we reasoned that this compound would be immediately deprotected by DBU in the next esterification step. Esterification with di-tert-butyl dicarbonate and Mg(ClO\textsubscript{4})\textsubscript{2}\textsuperscript{52} was unsuccessful.
Scheme 41. Second attempted synthesis of benzoate 79.

The purified benzoate 79 was then utilized in an attempt to perform a Williamson ether synthesis\(^\text{18}\) on one of the two phenolic groups, similar to synthesis of 10, and using 1 equiv of benzyl bromoacetate; the result was a complex mixture of product and symmetrical side product that was very difficult to separate by standard flash chromatography. We revised the synthetic plan to use a different protecting group strategy and tert-butyl esterification method. Allyl groups were chosen as hydroxy-protecting groups because single allyl ether cleavage was possible with a decent yield and would not result in bromine cleavage. Methyl groups had been considered as well; however, in a model reaction, selective methyl ether cleavage using BCl\(_3\)\(^\text{59}\) was unsuccessful.

A successful synthesis leading to the ester 61 is depicted in Scheme 42. Commercially available 4-bromo 3,5-dihydroxybenzoic acid was subjected to a Fisher esterification\(^\text{17}\) to form the methyl aryl ester moiety, and then alkylation was performed with allyl bromide to protect the phenolic groups;\(^\text{47}\) the resulting methyl ester 82 was obtained in a quantitative two-step yield. Conversion of the methyl ester to the tert-butyl ester 83 was performed in an excellent 96\% yield with 10 equiv of both tert-butyl acetate (tBuOAc) and potassium tert-butoxide (tBuOK) in THF in under 1 h.\(^\text{48}\) The tert-butyl ester 83 was then treated with 1 equiv of sodium borohydride (NaBH\(_4\)) and tetrakis(triphenylphosphate)Pd(0) [Pd(PPh\(_3\))\(_4\)] to cleave only one allyl ether group\(^\text{60}\) and give asymmetrical bromide 61 in 48\% yield, in addition to unreacted starting
material 83 (30%). In other trials of this same reaction, the yields of 61 were noticeably lower when the Pd(PPh$_3$)$_4$ catalyst was not fresh and was deep orange-brown in color instead of yellow. In another test, 2 equiv of sodium borohydride were used to obtain 79 in 82% yield.

Scheme 42. Revised synthesis toward tert-butyl esterification.

The remaining synthetic steps culminating with the core target 59 are shown in Scheme 43. Bromide 61 was subjected to Williamson ether conditions with benzyl bromoacetate to produce bromide 84 in 96% yield. The remaining allyl group of bromide 84 was removed with NaBH$_4$ and Pd(PPh$_3$)$_4$ to afford bromide 85 in 84% yield. Bromide 85 was treated with pNB bromoacetate 86 in a Williamson ether synthesis to generate asymmetrical bromide 60 in 96% yield. A Suzuki–Miyaura cross-coupling reaction between bromide 60 and boronic ester 5 produced biaryl amine 87 in 31% yield. Amine 87 was then treated with 3 equiv of TBAF — 1 equiv was added every 15 min until a deep burgundy-purple color was observed and the starting material had disappeared via TLC monitoring — to selectively cleave the pNB ester and furnish the final core target amine 59 in a modest 29% yield. When this reaction was repeated with more than three equivalents of TBAF, complete cleavage of the benzyl ester moiety was also observed by analysis of the crude $^1$H NMR spectrum.
Scheme 43. Remaining synthesis toward core target 59.

\[ \text{p-Nitrobenzyl (pNB) bromoacetate 86 was prepared by an esterification reaction}^{62} \text{ with bromoacetic acid 88 and } para\text{-nitrobenzyl alcohol 89, using DCC and DMAP as coupling reagents. The } p\text{NB bromoacetate 86, depicted in Scheme 44, was furnished in 62\% yield. Commercially available methyl bromoacetate and allyl chloroacetate had been previously used to generate the methyl ester and allyl ester analogs, respectively, of 60; however, attempts to cleave the methyl ester with NaOH}^{63} \text{ after Suzuki–Miyaura coupling to give 59 resulted in additional cleavage of the benzyl ester, and Suzuki–Miyaura cross coupling}^{16} \text{ of the allyl analog with boronic ester 5 resulted in no isolation of product by analysis of the } ^1\text{H NMR spectrum. Therefore, the } p\text{NB ester was the best choice for our orthogonal deprotection strategy toward 59.} \]
We attempted to resolve the enantiomers of core target 59 by both crystallization with chiral amines and also chiral HPLC to aid in our absolute configuration assignments. Two chiral amines, (+)-quinidine and (S)-(−)-α-methylbenzylamine, were each combined with the racemic mixture of 59 in unsuccessful crystallization efforts. The crude oily mixture of the (+)-quinidine salt crystallization attempt was submitted for HPLC analysis on the Chiralcel OD chiral column and the Phenomenex Luna C-18 reverse-phase column. The Chiralcel run was ineffective, and the Phenomenex run, shown in Figure 20, was the best found. Several full-preparative runs yielded less than 1 mg of each diastereomeric salt (both around 13 min), which was determined to be not enough for the subsequent amine salt cleavage step. Most of the diastereomeric salt had appeared to dissociate upon contact with the column to the original racemic mixture (at 19 min).
Figure 20. Analytical HPLC trace of the core target 59; the diastereomeric salt conformer peaks are grouped tightly together at 13 minutes, using a gradient of 40-45% acetonitrile.
5.0 NMR STUDIES OF ADVANCED TORSION BALANCE TARGETS

All of the advanced torsion balance targets, with the exception of the small zwitterion targets 52-55, were characterized and assigned by $^1$H and $^{13}$C NMR spectroscopy. As previously mentioned, individual assignments of each diastereomer of 47 and 48 were not possible because the separation methods employed were unsuccessful; therefore the assignments were performed by allotting one set of proton and carbon peaks to each moiety of each balance.

5.1 ANALYSIS OF PRIORITY TARGET

Our first advanced torsion balance, amide 44, was designed to test the preference of an amino acid chain to form a hydrogen bond with either a secondary amide or a tertiary amide functional group in a peptide. Balance 44 has three possible conformers and was not a candidate for HPLC or crystallization separation techniques; these conformers are shown in Scheme 45. Conformers 44a and 44b differ by rotation around the N-aryl bond; 44c arises from the rotation around the N-CO bonds to present a different pattern of hydrogen bonding. We hypothesize that either hydrogen bonding motif depicted in 44b or 44c (with the secondary amide functional group) would be preferred over 44a.
Scheme 45. Three possible conformers of amide target 44.

![Scheme 45](image)

The assignment of the $^1$H and $^{13}$C signals of amide 44 were assisted by 2D NMR techniques, including COSY, TOCSY, HMQC, and HMBC experiments. The COSY and TOCSY experiments were especially useful in determining the amide proton assignments. The allocation of signals to atom location is depicted in Figure 21 and Table 4. If an assignment could not be determined ("nd"), it is noted in the table. There was typical overlap observed in the aromatic region, and there was also overlap of the aryl methyl (location no. 19) and acetyl methyl (location no. 25) protons; some but not all of these peaks were resolved with the aid of HMQC and HMBC spectra.

![Figure 21](image)

**Figure 21.** Representation of balance 44 with numbering of atom location (hydrogen, carbon, or nitrogen) for structure assignment.
An assignment of the diastereotopic ortho-methylene protons (location nos. 9 and 26) of 44 was performed, and can be seen in Table 4. The assignments in chloroform-d were facilitated by the corresponding HMQC and HMBC spectra. The protons nearest to the dimethylamide group were more downfield (location no. 26, 4.87-4.71 ppm in chloroform-d) in comparison to the protons closer to the methylamide group (location no. 9, 4.55-4.47 ppm in chloroform-d), as

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<th>$^1$H, ppm, Toluene-d$_8$</th>
<th>$^{13}$C, ppm</th>
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<td>2.55, 2.44, 2.32, 1.95 (four s, 6H)</td>
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</table>
expected. The exact assignments in toluene-$d_8$ were less clear due to overlap of the multiplets; an HMBC spectrum in toluene-$d_8$ would be useful for the future.

An expansion of the TOCSY 2D NMR spectrum is displayed in Figure 22. The two amide protons, seen along the x-axis, have two large broad singlets each, corresponding to the two major conformers 44a and 44b. The small broad singlet corresponds to minor conformer 44c, which arises from an additional rotation around the N-CO bond.

![TOCSY NMR spectrum](image)

**Figure 22.** Expansion of TOCSY NMR spectrum in chloroform-$d$ at room temperature (700 MHz). The expansion shows the correlation of the two amide protons with their respective neighbors, which was necessary for assignment.

Comparison $^1$H NMR studies were performed in chloroform-$d$ and toluene-$d_8$ as the solvents of choice. In similar fashion to the investigations of initial torsion balances 1 and 2, target 44 displayed interesting upfield chemical shifts and a unique spreading-out of various N-methyl peaks. The N-dimethyl peaks (location no. 28) for example, depicted by four singlets,
were spread between the range of 2.55 to 1.95 ppm in toluene-$d_8$, compared with the smaller range in chloroform-$d$ of 2.95-2.92 ppm. The comparison of these $^1$H NMR spectra is shown in Figure 23. These data reveal that hydrogen bonding with either the secondary or tertiary amide functional group can be heavily influenced by solvation, and that both groups have the ability to form intramolecular hydrogen bonds with the glycine moiety.

Figure 23. Expansion of $^1$H NMR spectra comparison of target 44 in chloroform-$d$ (top) and toluene-$d_8$ (bottom) (700 MHz). The N-methyl peaks display the largest changes in chemical shift over a range of 2 ppm.
High temperature $^1$H NMR studies were performed in toluene-$d_8$, ranging from room temperature (303 K) to 343 K; these studies are shown in Figure 24.

![Figure 24](image)

Figure 24. High temperature $^1$H NMR studies performed in toluene-$d_8$ (700 MHz). The increase in temperature to 343 K shows a complete coalescence of the four N-dimethyl peaks, while the other N-methyl peaks showed less broadening and coalescence, if any.

There was a distinct coalescence of the four N-dimethyl peaks, while the other N-methyl peaks showed much less broadening and coalescence, if any. This coalescence demonstrates that there is fast rotation occurring around the neighboring amide bond of the N-dimethyl moiety, which is expected because we previously observed barriers of such N-CO bonds approximately to be 17
kcal mol\(^{-1}\). However, the N-methyl (location no. 12) peaks do not show very much coalescence, most likely due to the N-methyl moiety forming intramolecular hydrogen bonds with the protected glycine chain, and therefore raising the barrier. An HMQC spectrum (not shown) was also obtained at 343 K, which depicted the disappearance of only the four N-dimethyl peaks.

The identification of exchangeable protons was also of interest in the advanced torsion balance targets. A small sample of amide 44 was dissolved in chloroform-\(d\) with a single drop of neutral pH deuterated water (D\(_2\)O). The sample was sonicated for five minutes at room temperature; the \(^1\)H NMR spectrum acquired revealed no change. Then sonication was performed for 1 h at room temperature, after which a \(^1\)H NMR spectrum was obtained (shown in Figure 25); it was observed that both amide protons (with two broad singlets each) were readily exchanged. Therefore, these results revealed that both protons of interest were somewhat accessible to solvent, mimicking protons that are slightly buried in a protein instead of residing completely on the surface. Both protons most likely can participate in intramolecular hydrogen bonding that would be observed in a \(\beta\)-sheet motif secondary structure.
Figure 25. Expansion of $^1$H NMR spectra (400 MHz) depicting "before and after" exchange with deuterated water at 298 K. After sonication of the NMR sample for 1 h with D$_2$O, all of the amide protons (between 6.6 and 5.9 ppm) had disappeared almost completely.

Low temperature $^1$H NMR studies were also performed to determine if any expected intramolecular hydrogen bonding was occurring. According to the procedure of Gellman and coworkers,$^{65}$ a negative-slope trend ranging from 213 K to 273 K illustrates the presence of hydrogen bonding. Chemical shifts for the amide proton peaks in CD$_2$Cl$_2$ were graphed to show small or negligible intramolecular hydrogen bonding; the two amide protons (location nos. 11 and 23) have two peaks each, corresponding to the two major conformers 44a and 44b. These trends can be seen in Figure 26. In accordance with the deuterium exchange study, the data
indicate that there is some hydrogen bonding occurring between the glycine moiety and one of the amide carbonyls, but that the interaction may be weaker than expected. The secondmost upfield amide proton peak exhibits different behavior which could suggest that this amide proton is in a particular conformation that acts most like a proton on the surface of a protein.

Figure 26. Low temperature $^1$H NMR studies (methylene chloride-$d_2$, 400 MHz) were performed on target 44 to probe for intramolecular hydrogen bonding. The two amide protons were studied (location nos. 11 and 23; each amide proton has two $^1$H NMR peaks, corresponding to the two major conformers 44a and 44b). A small trend in negative slope was observed that represents the occurrence of intramolecular hydrogen bonding; however, the secondmost upfield amide peak shows negligible hydrogen bonding.

5.2 ASSIGNMENTS OF TARGETS 47, 48, AND PRECURSOR 49

Amide 47 was designed to test the possibility that an amino acid chain may in our model hydrogen bond more strongly when there are one or two sites for hydrogen bonding in a chain; amide 48 would test the partiality of an amino acid chain to form hydrogen bonds more strongly with an alanine or leucine side chain. Each of these two targets were asymmetrical and therefore had four diastereomers; as discussed previously, attempts to separate each diastereomer were
unsuccessful. However, assignments of the $^1$H and $^{13}$C NMR spectra peaks to each moiety in each balance was accomplished. The four diastereomers of targets 47 and 48 are illustrated in Schemes 46 and 47, respectively.

**Scheme 46. Four diastereomers of target 47.**
Scheme 47. Four diastereomers of target 48.

In addition, Figure 27 shows the allocation of signals to atom location, and Table 5 (5A and 5B for balance 47 and 48, respectively) displays the assignments of both targets in order to showcase their similarities.

Figure 27. Representation of balances 47 (left) and 48 (right) with numbering of atom location (hydrogen or carbon) for structure assignment.
Table 5. Target 47 (5A, left) and 48 (5B, right) $^1$H and $^{13}$C NMR assignments (700 MHz) in MeOD- $d_4$.

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<th>$^{13}$C, ppm</th>
<th>No.</th>
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<th>$^{13}$C, ppm</th>
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<td>2.74-2.69 (singlets, 3H)</td>
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</table>
The aromatic proton peaks of 47, 48, and also 49 (shown later), were difficult to assign because of the overlap observed in their $^1$H, COSY, ROESY, HMQC, and HMBC NMR spectra; therefore their $^1$H assignments are listed as part of an aromatic multiplet or could not be assigned (not determined, "nd"). The multiplets of the diastereotopic ortho-methylene protons of 47 did not overlap (location nos. 9 and 30, at 4.66-4.58 ppm and 4.57-4.50 ppm, respectively in methanol-$d_4$) and were therefore easier to assign (in comparison to those of 48 and 49). The ortho-methylene protons nearest to the alanine group (location no. 9) are more downfield most likely because there are two amide functionalities nearby, compared to location no. 30 which only has one adjacent amide. Due to each compound having four conformers (or two for the HPLC-separated conformer pairs of 49), and therefore many $^{13}$C peaks corresponding to each carbon atom, the $^{13}$C values are given as a single average value based upon the combined HMQC, HMBC and $^{13}$C NMR analyses; it has not been determined which individual $^{13}$C peak belongs to a specific diastereomer. In addition, only $^1$H and ROESY NMR spectral data were obtained for the conformer pairs of acid 49, in the interest of viewing any difference in splitting patterns or chemical shift of the diastereotopic protons. A ROESY NMR spectrum was obtained of 48 in CD$_3$CN but did not provide any new information about any intramolecular hydrogen bonding or any new NOEs; however, the leucine methine (location no. 34) was more obvious as a multiplet at 1.30 ppm.

A low temperature $^1$H NMR experiment was performed on 48 in similar fashion to balance 44, in order to determine if any hydrogen bonding could be observed. This experiment, also obtained in CD$_2$Cl$_2$, was not as successful; the presence of six amide protons in the $^1$H NMR spectrum provided much overlap which did not allow for many distinct chemical shifts. A J-resolved 2D NMR experiment was next performed on balance 48 in CD$_2$Cl$_2$, shown in Figure 28,
which gave a projection of the $^1$H NMR spectrum and allowed for identification of any $J$-coupled protons. The resulting spectrum showed the collapse of all six amide proton peaks, in addition to the glycine methylene and all alanine methine peaks. Although all of the amide proton peaks were decoupled, there was less overlap of the remaining peaks for identification.

Figure 28. A $J$-resolution 2D NMR experiment (methylene chloride-$d_2$, 700 MHz) was performed on target 48; the projected 1D NMR can be seen at the top, illustrating the collapse (disappearance) of all amide protons, glycine methylene protons, and alanine methine peaks.

The conformers of precursor acid 49, shown in Scheme 49, could be assigned to their $^1$H and $^{13}$C NMR peaks; however, no absolute assignment for each individual diastereomer could be made. The separate data of these conformer pairs is shown in Table 6 and is accompanied by the allocation of signals to atom location, shown in Figure 29.
Scheme 48. Four diastereomers of precursor acid 49.

Figure 29. Representation of balance 49 with numbering of atom location (hydrogen or carbon) for structure assignment.
Table 6. Precursor acid $^{13}$H NMR assignments (400 MHz) in MeOD-\textit{d}_4 of HPLC-separated conformer pairs.

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A ROESY NMR spectrum was obtained for precursor $^{49}$ in CD$_3$CN at 343 K; however, no EXSY crosspeaks were observed (two mixing times, 0.2 s and 0.1 s, were used to confirm this observation). ROESYs at room temperature in CD$_3$CN for both sets of diastereomers revealed
better crosspeaks, in the same fashion as for 47 and 48 (ROESY NMR spectra are available in the Appendix), and were used in the assignments.

5.3 ASSIGNMENT OF AMIDE TARGET 50

Amide 50, shown in Scheme 50, was designed to test the preference of an amino acid chain to hydrogen bond when a restrictive steric effect is involved (a potentially negative interaction between the methyl of an alanine and a neighboring aryl methyl group). Due to the presence of a symmetrical element in this torsion balance, assignments of the $^1$H and $^{13}$C NMR data were easier. The atom locations and assignments are given in both Figure 30 and Table 7. The ROESY NMR spectrum obtained showed various crosspeaks; however, it was difficult to pinpoint a particular NOE that would strongly suggest one (preferred) set of intramolecular hydrogen bonds over its symmetrical counterpart.

Scheme 49. Conformers of target 50.
Figure 30. Representation of balance 50 with numbering of atom location (hydrogen or carbon) for structure assignment.

Table 7. Target 50 $^1$H and $^{13}$C NMR assignments (700 MHz) in MeOD-$d_4$.

<table>
<thead>
<tr>
<th>No.</th>
<th>$^1$H, ppm, MeOD</th>
<th>$^{13}$C, ppm</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>--</td>
<td>166.0</td>
</tr>
<tr>
<td>2</td>
<td>3.97, 3.96 (singlets, 3H)</td>
<td>52.0</td>
</tr>
<tr>
<td>3</td>
<td>--</td>
<td>134.0</td>
</tr>
<tr>
<td>4</td>
<td>7.44-7.43 (singlets, 2H)</td>
<td>107.0</td>
</tr>
<tr>
<td>5</td>
<td>--</td>
<td>124.0</td>
</tr>
<tr>
<td>6</td>
<td>--</td>
<td>156.0</td>
</tr>
<tr>
<td>7</td>
<td>4.63-4.48 (m, 4H)</td>
<td>68.0</td>
</tr>
<tr>
<td>8</td>
<td>--</td>
<td>169.0</td>
</tr>
<tr>
<td>9</td>
<td>4.35-4.30 (m, 2H)</td>
<td>48.0</td>
</tr>
<tr>
<td>10</td>
<td>1.38-1.11 (doublets, m, 6H)</td>
<td>18.0</td>
</tr>
<tr>
<td>11</td>
<td>--</td>
<td>173.0</td>
</tr>
<tr>
<td>12</td>
<td>2.77-2.70 (singlets, 6H)</td>
<td>26.0</td>
</tr>
<tr>
<td>13</td>
<td>--</td>
<td>128.0</td>
</tr>
<tr>
<td>14</td>
<td>7.44-7.35 (m, 1H)</td>
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</tr>
<tr>
<td>15</td>
<td>--</td>
<td>136.0</td>
</tr>
<tr>
<td>16</td>
<td>7.44-7.35 (m, 1H)</td>
<td>130.0</td>
</tr>
<tr>
<td>17</td>
<td>--</td>
<td>142.0</td>
</tr>
<tr>
<td>18</td>
<td>7.44-7.35 (m, 1H)</td>
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</tr>
<tr>
<td>19</td>
<td>2.07-2.04 (singlets, 3H)</td>
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</tr>
<tr>
<td>20</td>
<td>3.50-3.23 (singlets, 3H)</td>
<td>38.0</td>
</tr>
<tr>
<td>21</td>
<td>--</td>
<td>174.0</td>
</tr>
<tr>
<td>22</td>
<td>4.35-4.30 (m, 1H)</td>
<td>50.0</td>
</tr>
<tr>
<td>23</td>
<td>1.38-1.11 (doublets, m, 3H)</td>
<td>18.0</td>
</tr>
<tr>
<td>24</td>
<td>--</td>
<td>168.0</td>
</tr>
<tr>
<td>25</td>
<td>4.35-4.30 (m, 1H)</td>
<td>50.0</td>
</tr>
<tr>
<td>26</td>
<td>1.38-1.11 (doublets, m, 3H)</td>
<td>18.0</td>
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<tr>
<td>27</td>
<td>--</td>
<td>172.0</td>
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<tr>
<td>28</td>
<td>2.00-1.96 (singlets, 3H)</td>
<td>21.0</td>
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5.4 ASSIGNMENT OF AMIDE ZWITTERION TARGET 51

Amide zwitterion 51 was designed to test the effect of salt-bridging between ammonium and carboxylate moieties of amino acid chains on the rate of rotation around the N-aryl bond. This torsion balance, shown in Scheme 51, is also symmetrical but has the additional ability to form salt-bridges. The atom locations and assignments of the $^1$H and $^{13}$C NMR data are given in both Figure 31 and Table 8. The ROESY obtained for 51 was similar in appearance and complexity to that of amide 50.

Scheme 50. Conformers of target 51.

Figure 31. Representation of balance 51 with numbering of atom location (hydrogen or carbon) for structure assignment.
Table 8. Target 51 $^1$H and $^{13}$C NMR assignments (700 MHz) in MeOD-$d_4$.

<table>
<thead>
<tr>
<th>No.</th>
<th>$^1$H, ppm, MeOD</th>
<th>$^{13}$C, ppm</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>--</td>
<td>167.0</td>
</tr>
<tr>
<td>2</td>
<td>3.98-3.97 (two s, 3H)</td>
<td>51.0</td>
</tr>
<tr>
<td>3</td>
<td>--</td>
<td>133.0</td>
</tr>
<tr>
<td>4</td>
<td>7.47-7.46 (two s, 2H)</td>
<td>107.0</td>
</tr>
<tr>
<td>5</td>
<td>--</td>
<td>123.0</td>
</tr>
<tr>
<td>6</td>
<td>--</td>
<td>156.0</td>
</tr>
<tr>
<td>7</td>
<td>4.64-4.51 (m, 4H)</td>
<td>67.0</td>
</tr>
<tr>
<td>8</td>
<td>--</td>
<td>167.0</td>
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<td></td>
<td></td>
<td>47.0</td>
</tr>
<tr>
<td>9</td>
<td>4.39, 4.27 (two quintets, 2H)</td>
<td>48.0</td>
</tr>
<tr>
<td>10</td>
<td>1.33-1.28 (m, 6H)</td>
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</tr>
<tr>
<td>11</td>
<td>--</td>
<td>175.0</td>
</tr>
<tr>
<td>12</td>
<td>--</td>
<td>127.0</td>
</tr>
<tr>
<td>13</td>
<td>7.46-7.44 (m, 1H)</td>
<td>129.0</td>
</tr>
<tr>
<td>14</td>
<td>--</td>
<td>135.0</td>
</tr>
<tr>
<td>15</td>
<td>7.39-7.35 (m, 1H)</td>
<td>129.0</td>
</tr>
<tr>
<td>16</td>
<td>--</td>
<td>142.0</td>
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<tr>
<td>17</td>
<td>7.39-7.35 (m, 1H)</td>
<td>132.0</td>
</tr>
<tr>
<td>18</td>
<td>2.07, 2.06 (two s, 3H)</td>
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<td>3.29, 3.27 (two s, 3H)</td>
<td>36.0</td>
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<td>20</td>
<td>--</td>
<td>168.0</td>
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<tr>
<td>21</td>
<td>3.73-3.66 (m, 2H)</td>
<td>42.0</td>
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<tr>
<td>22</td>
<td>--</td>
<td>169.0</td>
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<tr>
<td>23</td>
<td>3.98-3.97 (m, 1H)</td>
<td>52.0</td>
</tr>
<tr>
<td>24</td>
<td>1.53-1.52 (dd, 3H)</td>
<td>27.0</td>
</tr>
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</table>

5.5 ANALYSIS OF ZWITTERION TARGETS 52 AND 53

Zwitterion targets 52 and 53 would test the effect of salt-bridging between an amino acid ammonium and a simple carboxylate moiety on the rate of rotation around the N-aryl bond. These targets were designed as simple tools to investigate phenomena observed, for example, in
thermophilic proteins,\textsuperscript{66} where the presence of salt-bridges contribute to their stability and to a particular organism's overall ability to survive abnormal temperature conditions. These aforementioned targets and their corresponding control compounds are shown in Scheme 52 and Scheme 53. Each target has either one alanine or one glycine, and the controls are capped with an acyl group to subdue any potential hydrogen bonding with the ammonium moiety.

Scheme 51. Alanine zwitterion 52 and corresponding control 54.

\[
\begin{align*}
52-R_x,S_x, R &= H \\
54-R_x,S_x, R &= (C=O)CH_3
\end{align*}
\]

Scheme 52. Glycine zwitterion 53 and corresponding control 55.

\[
\begin{align*}
53-R_x, R &= H \\
55-R_x, R &= (C=O)CH_3
\end{align*}
\]
5.5.1 Evaluation of Zwitterion Dynamics and Rates

We observed interesting conformational dynamics of zwitterions 52 and 53 via $^1$H NMR analysis, more so for alanine zwitterion 52. At room temperature, the spectra for 52 showed four conformations, while 53 had two (as expected). Target 52 exhibited clear signals (in deuterated methanol) for the alanine methyl group, with two large doublets corresponding to the two major conformers, and two small downfield doublets for the minor rotamers. Therefore, variable temperature $^1$H NMR studies, ranging from room temperature to 373 K (25 to 100 °C), were performed on 52 and its control 54 in different pD buffers (pD's 2.8, 6.9 and 9.4) to investigate any effects that pH may have on dynamics. The charged structures of alanine zwitterion 52 in different buffers are depicted in Figure 32; control 54 was only subjected to pD 6.9 buffer due to the presence of the added acyl group. The $^1$H NMR spectra results are shown in Figure 33, with corresponding integration values listed in Table 9.

In comparing the $^1$H variable temperature spectra of 52 and 54, we could see signal broadening for all alanine methyl peaks at 373 K in comparison to those at room temperature; in addition, all high temperature spectra showed chemical shifts that were more than 0.5 ppm
downfield from the room temperature data. There was no coalescence of the major conformer peaks, which is compatible with our expectation of a higher barrier for the N-aryl rotation; coalescence of the minor conformer peaks was more evident and is also on par with our expectation that rotation around the N-CO bond has a lower rotational barrier (estimated to be 17 kcal·mol$^{-1}$ at 298 K, from our previous work).

![Figure 33](image.png)

**Figure 33.** Variable temperature and buffer $^1$H NMR spectra (600 MHz) of alanine $\alpha$-methyl protons of zwitterion 52 in three pD buffers 2.8, 6.9, and 9.4 (left), and control 54 only in pD buffer 6.9 (right). Both compounds were subjected to either room temperature or 373 K and all showed chemical shift changes and peak broadening.
The integrations of all downfield major conformer peaks are all larger and similar in value in comparison with their upfield counterparts (Table 9), whether at 373 K or room temperature, which shows the prevalence of salt bridging throughout the experiment. However, the noticeable downfield chemical shifts around 2.0 ppm at 373 K and larger integration values of the more downfield peaks (i.e. 0.661 vs. 0.588 for pD 9.4) could suggest that at higher temperatures, the zwitterion molecule has an even higher tendency to form salt bridges and is therefore more thermodynamically stable. Even the control compound 54 displayed similar salt bridge trends with the presence of the acyl group. The data for pD 2.8 at 373 K appears contradictory because while the chemical shifts are more downfield than the others, the integration of the downfield peak (1.00) is slightly less than the upfield peak (1.04). Also, additional methyl doublets were observed in all of these $^1$H spectra in D$_2$O buffer (however, not in MeOD-$d_4$, not shown) at room temperature; these peaks were investigated and will be discussed later.

Table 9. Integration ratios of alanine $\alpha$-methyl major conformer peaks for zwitterion 52 and control 54, corresponding to Figure 32 (listed in "upfield/downfield" format).

<table>
<thead>
<tr>
<th></th>
<th>Zwitterion 52</th>
<th>Control 54</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zwitterion 52</td>
<td></td>
<td></td>
</tr>
<tr>
<td>pD</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.8</td>
<td>1.04/1.0</td>
<td>0.773/1.0</td>
</tr>
<tr>
<td>6.9</td>
<td>0.862/1.0</td>
<td>0.785/1.0</td>
</tr>
<tr>
<td>9.4</td>
<td>0.661/1.0</td>
<td>0.588/1.0</td>
</tr>
</tbody>
</table>

Zwitterion 52 was also compared to amide zwitterion 51 in order to ascertain any similarities in dynamics. High temperature NOESYs were obtained for 52 in either buffer pD 6.9 or CD$_3$CN, and high temperature ROESYs were obtained for 51 in either buffer pD 6.9 or CD$_3$CN; these data, focusing on crosspeaks of interest, are displayed in Figure 34.
Figure 34. EXSY analyses were performed on alanine zwitterion 52 and amide zwitterion 51 to obtain rate calculations. The alanine α-methyl protons of 52 were examined using NOESYs (600 MHz, 1.4 s mixing time) in (A) D₂O buffer 6.9 at 373 K and (B) CD₃CN at 343 K. The glycine protons of 51 (highlighted in boxes) were examined using ROESYs (600 MHz, 0.2 s mixing time) in (C) D₂O buffer 6.9 at 343 K and (D) CD₃CN at 343 K.

Using EXSY analysis, the rates for 52 were calculated to be 22.6 kcal·mol⁻¹ at 373 K in D₂O buffer pD 6.9 and 19.7 kcal·mol⁻¹ at 343 K in CD₃CN, and the rate for 51 was calculated to
be 18.6 kcal mol\(^{-1}\) at 343 K in CD\(_3\)CN. The corresponding rate in the buffer solution could not be determined because there was interference from regular NOEs present with the exchange crosspeaks; in the future, a method to saturate the NMR sample with oxygen gas may aid in removing all unwanted NOE peaks from the experiment. It is interesting to note that the rate in water is much higher, which may be due to solvation effects where the water molecules readily interact with the charged moieties of the zwitterion compound.

### 5.5.2 Observation of an Alanine Zwitterion Dimer

As previously mentioned, there were unexpected doublets present with the alanine \(\alpha\)-methyl peaks in our buffer experiments with zwitterion 52. Examination of this compound via low resolution mass spectrometry (LRMS) showed a surprising mass at twice the value of the regular molecular weight. Although these peaks were not visible in methanol, LRMS data (depicted in Figures 35 and 36) showed this doubled-mass value when the compound was dissolved in either neutral water, buffer pH 4.0, or methanol. Therefore, we surmised that there must be a dimer present (along with the monomer) for zwitterion 52. Similar data (not shown) for glycine zwitterion 53 was obtained and also displayed the doubled value in molecular weight. Zwitterion 52 was also dissolved in deuterated DMSO — DMSO is known to disrupt intramolecular hydrogen bonding — however, there was little difference observed with the extra doublets via \(^1\)H NMR examination.
Figure 35. ESI negative ion mode LRMS of zwitterion 52 in methanol, directly injected into the source of the mass spectrometry instrument. The peaks of interest showing the monomer and dimer are highlighted at masses of 473.0581 and 947.2698, respectively.
Figure 36. ESI negative ion mode LRMS of 52 in water. The peaks of interest showing the monomer and dimer are highlighted at masses of 473 and 948, respectively. The ESI positive ion mode LRMS, not shown, also displayed monomer and dimer peak masses.

The formation of the dimer is not completely understood; however, some of the data suggest that strong hydrophobic interactions may be a cause. The zwitterion is small and compact enough where the charged moieties strongly attract another zwitterion and prohibit any water molecules from interfering; also, the torsion balance's aryl backbone may be a factor. A possible depiction of the alanine zwitterion 52 dimer, with electrostatic interactions highlighted, is shown in Figure 37. The zwitterion compounds were either gels or foams, and so x-ray crystallography could not be used to confirm or refute our hypothesis.
Figure 37. MOE illustration of the electrostatic map of alanine zwitterion 52, depicting a possible dimer based upon hydrophobic interactions.

Another control was synthesized with no unprotected amino acid coupled to the \( o \)-tolyl amine moiety; this synthesis occurred by deprotection of biaryl amine 58 with TFA\(^{46} \) to give acid 90 in quantitative yield; this acid is depicted in Scheme 53. This acid 90 was designed to observe if dimer would be present if carboxylates alone were present in the balance scaffold; it was determined that no dimer had formed because \(^1\)H and NOESY NMR analyses did not show any additional proton peaks or any NOEs.

Scheme 53. Synthesis of control acid 90.
The fourth torsion balance type, biaryl amine 59, would be used as a core tool for either solution- or solid-phase peptide synthesis with the presence of a tert-butyl aryl ester moiety. Ideally, the enantiomers of this balance 59 would have been separated by traditional methods (HPLC, salting-out by crystal formation) in order to lend additional information to the absolute configuration assignments of the other targets; either the x-ray crystallography or vibrational circular dichroism (VCD) techniques could have been employed on a pure enantiomer to determine whether it had an R or S stereochemistry. However, our resolution attempts were unsuccessful, and we were not able to obtain pure enantiomers of 59.

The balance 59 in its racemic form was analyzed by 2D NMR techniques to determine as much about the structure as possible. The HMBC and HMQC were the most helpful, because we were able to utilize these spectra to assign the diastereotopic o-methylene groups. These groups are shown in Scheme 54 and are denoted as a, a', b and b'.

**Scheme 54. Diastereotopic o-methylene groups of interest in target 59.**

![Scheme 54](image)

The 2D crosspeaks of the o-methylenes are highlighted in Figure 38. The HMQC depicted all three methylenes of 59 in close proximity to each other (within 1 ppm). The HMBC gave the
final assignment of the peak representing the o-methylene protons b and b'. These protons were in the same plane as the benzyl methylene and were slightly more upfield in the $^{13}$C axis in comparison to the other peak representing methylene protons a and a'. Therefore, the peak at 4.68 ppm represents the protons b and b', and the peak at 4.60 ppm represents the protons a and a'.

**Figure 38.** HMQC (left) and HMBC expansion (right) of target 59 (acetonitrile-$d_3$, 700 MHz), with focus on the o-methylenes and also the adjacent benzyl methylene. The HMBC shows the o-methylene protons b and b' at 4.68 ppm in alignment with the benzyl methylene protons at 5.16 ppm; both are highlighted by boxes.
5.7 CONCLUSION, AND FUTURE WORK

It is widely known that the most stabilizing interactions in protein folding are hydrogen bonds and salt bridges. Out of all of the secondary structures, the β-sheet is the least understood, especially pertaining to their stabilizing interactions and their folding preferences (parallel versus antiparallel). Therefore, a hybrid synthetic-natural peptide motif, combining an o,o,o’-trisubstituted biphenyl with an (ortho-tolyl)-amide, was synthesized in multiple formats and studied by NMR to probe the effects of amino acid substitutions on antiparallel beta-sheet configuration and stability.

The potential of this “molecular torsion balance” as a beta-turn mimic was demonstrated by quantifying the rotational barriers about several axes. The free-energy rotational barrier of the aryl-aryl bond was found to be 35.7 kcal mol$^{-1}$ at 418 K in hexanes. EXSY analysis was also used to measure barriers about the N-aryl (20.9 kcal mol$^{-1}$ at 343 K in toluene-$d_8$) and N-CO bonds (17.2 kcal mol$^{-1}$ at 298 K in chloroform-$d$). The N-aryl barriers of a zwitterionic torsion balance containing a single alanine residue (19.7 kcal mol$^{-1}$ in acetonitrile-$d_3$ at 343 K, and 22.6 kcal mol$^{-1}$ in deuterated buffer pD 6.9 at 373 K) showed that rotation about this bond is slower in water, most likely due to the propensity of water to form hydrogen bonds with the charged moieties participating in a salt bridge.

Torsion balances were used to study intramolecular hydrogen bond preference by analyzing $^1$H NMR peak data. An amino acid chain (a single alanine or glycine residue) was found to preferably hydrogen bond with an amide versus an ester carbonyl (1.4:1.0 at 298 K in toluene-$d_8$), and with a secondary amide versus a tertiary amide carbonyl (observation of tertiary amide proton coalescence via variable temperature NMR, from 303 K to 343 K, in toluene-$d_8$).
The overall findings suggest that this hybrid torsion balance is a valuable tool that can provide data on conformational dynamics and can examine hydrogen bond and salt bridge interactions of an antiparallel β-sheet scaffold.

In the future, it is of great interest to investigate and obtain as many absolute structural assignments of the advanced torsion balance targets as possible. Separation of the enantiomers 59-S and 59-R would allow for specific stereochemistry and therefore more enhanced proton assignment; also, generating a larger quantity of the separated conformers of precursor acid 49 would allow for the synthesis of conformationally pure advanced targets 47 and 48, which could also be used to obtain folding ratios and preferences.

It would be wise to further analyze the torsion balance scaffold first by using CD spectroscopy. The balance would need at least two intramolecular hydrogen bonds (i.e. target 48) for analysis with the CD spectrometer. This experiment would tell us if the scaffold was actually in the β-sheet conformation, compared with α-helix or random-coil conformations. Another experiment would involve selective bromination of the “top” aromatic ring, shown in Scheme 55. It is of interest to know if the o-methylene ether side chains are aligned properly to promote hydrogen bonding (at 90°, relative to the plane of the aromatic ring). Modeling suggests that 90° is the best angle to provide alignment. We propose incorporation of bromine atoms ortho- to the aryl methyl ether and o-methylenes to see if additional intramolecular hydrogen bonding is occurring between the amino acids of the torsion balance (observed by NOESY or ROESY NMR analyses).
Adding another three amino acids to the balance scaffold would allow for three hydrogen bonds to be made, which would allow for a solidification of the β-sheet alignment and provide additional stability. In addition, charged amino acid side chains (i.e. Arg, Glu) should be considered for torsion balance studies in order to answer more questions regarding salt bridges: what is the likelihood of the charged side chains forming a salt bridge, and would the β-sheet conformation be conserved? If the termini of the amino acid chains are already charged (i.e. amide zwitterion 51), would one or more salt bridges arise? The pH studies should also be expanded upon; one experiment of interest would be to generate a pD buffer of even greater acidity than was previously used in this work (used here was pD 2.8, therefore a value of at least 2.0 would be desired), in order to assure that the zwitterion targets truly had two carboxylic acid moieties instead of carboxylates.

A new related target could be created that would measure the preference of a β-strand to form a hydrogen bond or a salt-bridge; an example of a small balance is shown in Scheme 56. This balance and the others previously mentioned would enhance and expand upon the database available about β-sheet conformational dynamics and preferences for hydrogen bond and salt bridge formations.
Scheme 56. Torsion balance to measure preference of hydrogen bond over salt-bridge.
Supporting Information: All $^1$H and $^{13}$C NMR spectra were recorded on a 300, 400, 500, 600, or 700 MHz spectrometer (Bruker Avance). The chemical shifts are given in parts per million (ppm) on the delta scale ($\delta$), and the coupling constant values ($J$) are in hertz. Chemical shifts were referenced to TMS ($^1$H) or solvent ($^{13}$C). Abbreviations for NMR data assignments\textsuperscript{67} are as follows: (s) singlet, (d) doublet, (t) triplet, (q) quartet, (dd) doublet of doublets, (dt) doublet of triplets, (dq) doublet of quartets, (tt) triplet of triplets, (m) multiplet, (br) broad, (app) apparent, and (ABq) AB quartet. All NMR spectra were obtained at room temperature (20–27 °C) unless otherwise noted.

High and low resolution mass spectra were recorded on a VG 7070 spectrometer. IR spectra were collected on an Avatar 380 Nicolet FT-IR spectrometer. Samples for IR were prepared as a thin film on a NaCl plate by dissolving the sample in CH$_2$Cl$_2$ and evaporating the CH$_2$Cl$_2$. Melting points were determined using a Mel-Temp apparatus and are uncorrected. Optical rotations were measured on a Perkin-Elmer 241 polarimeter at the Na D-line ($\lambda = 589$ nm) using a 1-dm cell at 25 °C and CHCl$_3$ or MeOH as the solvent unless noted otherwise.

Analytical chiral normal phase HPLC analysis was conducted using either an (S,S)-Whelk-O 1 column (Pirkle, 250 mm x 4.6 mm ID) or a Chiralcel OD column (Daicel, 250 mm x 4.6 mm ID) typically eluting with hexanes:i-PrOH at 1.0 mL/min, 10 μg per injection. Preparatory chiral normal phase HPLC resolutions were performed on either an an (S,S)-Whelk-
O 1 column (Pirkle, 25 mm x 21.1 mm ID) or a Chiralcel OD column (Daicel, 250 mm x 20.0 mm ID) typically eluting with hexanes:i-PrOH at 10.0 mL/min, 20 μg per injection. All HPLC injections were monitored with a Waters model 440 UV detector at wavelength 254 nm, when the solvent system was hexanes:i-PrOH.

Analytical reverse phase HPLC analysis was conducted using a Luna 5u C18(2) 100 Å column (Phenomenex, 250 mm x 4.6 mm ID) typically eluting with H₂O (0.1% TFA):CH₃CN (0.1% TFA) at 1.0 mL/min, 1-2 μg per injection. Preparatory reverse phase HPLC resolutions were performed on a Luna 10u C18(2) 100 Å column (Phenomenex, 250 mm x 21.2 mm ID) typically eluting with H₂O (0.1% TFA):CH₃CN (0.1% TFA) at 10.0 mL/min, 10 μg per injection. All HPLC injections were monitored with either a Hitachi Elite LaChrom UV detector model L-2400 or diode array detector model L-2455 at wavelengths 226 nm and 254 nm, when the solvent system was H₂O (0.1% TFA):CH₃CN (0.1% TFA).

Line-shape and EXSY analyses were performed using the iNMR lineshape analysis software.³⁵, ³⁶, ⁶⁹ NMR simulations were conducted on the tert-butyl protons of carbamates 13 and 17, the diastereotopic glycine methylene and alanine α-methyl protons of amides 29-30, and the diastereotopic o-methylene protons of biaryl amine 3. The simulated spectra were overlaid with the experimental spectra and were used to determine conformer ratios and rotational barriers. EXSY analyses were also performed on zwitterions 51-53 and control 54. The cross peaks and diagonal peaks of either the diastereotopic glycine methylene protons or the alanine α-methyl protons were integrated and used to calculate rotational barriers.

Thin-layer chromatography (TLC) was performed using silica gel analytical glass plates (Merck, 60F-254, 0.25 mm). Light absorption by compounds was observed using ultraviolet light (254 nm). Columns used for flash chromatography are prepared with silica gel 60 (Merck,
When a solvent elution gradient method was employed for flash chromatography, the gradient was changed upon TLC confirmation that no compound was eluting out.

Dry solvents were distilled shortly before use from an appropriate drying agent under a nitrogen atmosphere. Tetrahydrofuran (THF) and diethyl ether (Et₂O) were distilled from sodium and benzophenone. CH₂Cl₂ was distilled from CaH₂. Methanol (anhydrous) was used as obtained. N,N-dimethylformamide (DMF) and dimethylsulfoxide (DMSO) were purchased in sure-seal bottles (Acros) and used without purification. Other commercially available reagents and solvents were reagent grade and used without further purification.

An oil bubbler was attached to reaction vessels in which the reactions required either the maintenance of a nitrogen atmosphere or the evacuation and addition of nitrogen. Ambient temperature and pressure for “room temperature” reactions varied between 20–27 °C and 720–770 mmHg, respectively.

All percent yields reported are for compounds that have a purity of approximately 95% or better as determined by NMR analysis, and the compound was used for subsequent reactions without further purification unless otherwise noted.

### 6.1 HPLC KINETIC EQUILIBRIUM EXPERIMENTAL PROCEDURE

The enantiomers of derivative 31 were separated by chiral preparatory HPLC (using the Chiralcel OD column) after the analytical HPLC run (shown in Figure 9). From a racemic mixture of 18 mg, the first eluting enantiomer (31-FEE) was obtained in 5.4 mg (93.6% ee) with
a retention time of 14.7 min, and the second eluting enantiomer (31-SEE) was obtained in 7.1 mg (90.4% ee) with a retention time of 18.4 min.

A solution of 1.0 mg of an enantioenriched sample of 31-SEE in 1.0 mL hexanes was placed in a scalable thick-walled tube and heated at 145 °C (418 K) by using a Thermo-Watch 115V and an oil bath setup. Aliquots of 10 μL were removed at 1 h or 2 h increments, with the exception of an overnight run, and measured using chiral analytical HPLC analysis (using the Chiralcel OD column and a solvent system of 95:5 hexanes:isopropanol). The % ee (shown below in Table 4, from 31-SEE to 31-FEE) was measured from each aliquot and plotted against time (10^6 s) in order to calculate the rotational barrier, according to Lapierre^37a and Eliel^37b.

An initial trial that used a solvent system of 9:1 hexanes/isopropanol proved unsuccessful because additional peaks appeared in the HPLC chromatogram after a few hours had progressed in the experiment.

<table>
<thead>
<tr>
<th>Time (s)</th>
<th>% ee</th>
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Table 10. Kinetic equilibrium data for 31-SEE.
6.2 PREPARATION OF BUFFER SOLUTIONS USED AS NMR SOLVENTS FOR TORSION BALANCE ANALYSES

Preparation of Stock Solutions

Deuterium chloride (35% wt in D₂O) was purchased from Aldrich. To a 5 mL volumetric flask was added 0.043 mL of the DCI solution, which was diluted to 5 mL with D₂O. This stock solution was 0.10 M in DCI and was used to adjust the pD of the buffer solutions.

Sodium deuteroxide (40% wt in D₂O) was purchased from Cambridge Isotopes. To a 5 mL volumetric flask was added 0.035 mL of the concentrated NaOD solution, which was diluted to 5 mL with D₂O. This stock solution was 0.10 M in NaOD and used to adjust the pD of the buffer solutions.

Preparation of the 0.050 M Potassium Deuterium Phthalate Buffer

A solution of 2.0 g of potassium hydrogen phthalate (KHP) in 20 mL of D₂O was dried by lyophilization. The solid was redissolved in another 20 mL portion of D₂O, and the solvent was again removed by lyophilization. A solution of 0.1026 g of the deuterated potassium salt and 2.2 mL of the 0.10 M DCI stock solution was diluted to 10 mL with D₂O in a 10 mL volumetric flask. The solution was 0.050 M in phthalate and was used as an NMR solvent for the torsion balances. The pD of the solution was calculated as 2.8, which was determined from a reading taken from a glass electrode pH meter.

Preparation of the 0.050 M Deuterated Phosphate Buffer

A solution of 1.4196 g (0.0100 mol) of Na₂HPO₄ and 1.3609 g (0.0100 mol) of KH₂PO₄ in 10 mL of D₂O was dried by lyophilization. The solid was redissolved in another portion of D₂O, and the solvent was again removed by lyophilization. The deuterated solid was transferred to a 100 mL volumetric flask and diluted to 100 mL with D₂O. This stock solution was 0.200 M.
in phosphate and diluted to 0.050 M by standard methods. The 0.050 M solution was used as an NMR solvent for the torsion balances. The pD of the solution was calculated as 6.9.\(^\text{69}\)

**Preparation of the 0.050 M Deuterated Borate Buffer**

A solution of 0.0478 g of sodium tetraborate and 0.4 mL of the 0.10 M DCl stock solution was diluted to 10 mL with D\(_2\)O in a 10 mL volumetric flask. The solution was 0.050 M in borate and was used as an NMR solvent for the torsion balances. The pD of the solution was calculated as 9.4.\(^\text{69}\)

### 6.3 EXPERIMENTAL PROCEDURES

![Chemical Structure]

**General procedure A for generating Fmoc-protected amino acid chlorides:** To a solution of \(N\)-(9-fluorenylmethoxycarbonyl)-X\(_\text{aa}\) (1.0 equiv.) in CH\(_2\)Cl\(_2\) (0.176 M) was added thionyl chloride (10 equiv.), and the reaction mixture was refluxed for 30–45 min. The solution was cooled to room temperature, and the volatile components were removed under reduced pressure. The residue was dissolved in minimal CH\(_2\)Cl\(_2\) followed by hexanes to produce an off-white precipitate. The solid was filtered and dried in vacuo to afford the amino acid chloride product (83–90%) as an off-white solid. Amino acids (X\(_\text{aa}\)) used include L-alanine, glycine, and \(\beta\)-alanine. Only IR spectra were obtained to confirm these highly reactive intermediates: Fmoc-Ala-Cl **20-int** (thin film, cm\(^{-1}\)) 3328, 3044, 2947, 2888, 2786, 1948, 1911, 1780, 1693, 1534, 1478, 1450, 1382, 1340, 1282, 1261, 1125, 1103, 1085, 1040, 963, 939, 903, 875, 780, 756, 738; Fmoc-Gly-Cl **21-int** (thin film, cm\(^{-1}\)) 3315, 3066, 2967, 2947, 1811, 1702, 1540, 1477, 1448, 1395, 1349, 1271, 1182, 1104, 1087, 1051, 991, 955, 919, 780, 758, 742;
Fmoc-β-Ala-Cl 37-int (thin film, cm⁻¹) 3349, 3066, 2956, 2885, 1912, 1790, 1695, 1537, 1476, 1448, 1407, 1257, 1159, 1102, 1084, 1049, 1010, 960, 776, 758, 740.

Methyl 4-bromo-3,5-dihydroxybenzoate (9) To 5.0 g (21.5 mmol) of carboxylic acid 8 in 33.1 mL of methanol was added 1.98 mL (37.1 mmol) of H₂SO₄ dropwise, and the reaction mixture was refluxed for 16 h. The reaction was quenched by addition of NaHCO₃ (6.24 g, 74.3 mmol), and the volatile components were removed under reduced pressure. The residue was diluted with ethyl acetate (120 mL) and water (120 mL); the organic layer was extracted, and the aqueous layer was washed with additional ethyl acetate (3 × 120 mL). The organic layers were dried over MgSO₄ and filtered, and the volatile components were removed under reduced pressure to give 5.27 g (99.4%) of 9 as a white solid: Rₐ 0.34 (hexanes/ethyl acetate, 3:2); mp 225–227 °C; IR (thin film, cm⁻¹) 3416, 3329, 1701, 1594, 1421, 1353, 1270, 1233, 1118, 1033, 990, 907, 857, 760, 705; ¹H NMR (300 MHz, DMSO) δ 10.52 (s, 2H), 7.00 (s, 2H), 3.79 (s, 3H); ¹³C NMR (300 MHz, DMSO) δ 166.3, 156.0, 129.7, 107.4, 103.9, 52.7; HRMS (EI) m/z calcd for C₈H₇O₄Br 245.9528, found 245.9519.

Methyl 4-bromo-3-(2-tert-butoxy-2-oxoethoxy)-5-hydroxybenzoate (10). In a round-bottom flask, 5.2 g (21.1 mmol) of benzoate 9, 3.11 mL (21.1 mmol) of tert-butyl bromoacetate, 6.1 g (44.2 mmol) of K₂CO₃, and 0.27 g (1.1 mmol) of 18-crown-6 were dissolved in acetone (105.2 mL), and the reaction mixture was refluxed for 24 h. The volatile components were removed under reduced pressure, and the residue was diluted with ethyl acetate (250 mL) and water (250
mL); the organic layer was extracted, and the aqueous layer was washed with additional ethyl acetate (3 × 250 mL). The organic layers were dried over MgSO₄ and filtered, and the volatile components were removed under reduced pressure to give a crude yellow oil. The oil was purified by flash chromatography (SiO₂, hexanes/ethyl acetate, elution gradient 5:1/3:2/0:1) to give 2.63 g (34.6%) of 10 as a white solid: R₉ 0.44 (hexanes/ethyl acetate, 3:2); mp 117–118 °C; IR (thin film, cm⁻¹) 3393, 2923, 2852, 1724, 1590, 1497, 1438, 1354, 1247, 1158, 1117, 1011, 904, 870, 843, 767; ¹H NMR (300 MHz, CDCl₃) δ 7.38 (d, J = 2 Hz, 1H), 7.03 (d, J = 2 Hz, 1H), 5.80 (s, 1H), 4.65 (s, 2H), 3.91 (s, 3H), 1.50 (s, 9H); ¹³C NMR (500 MHz, CDCl₃) δ 166.9, 166.0, 154.9, 153.7, 130.6, 110.4, 105.7, 105.2, 82.9, 66.4, 52.5, 28.0; HRMS (EI) m/z calcd for C₁₄H₁₇O₈Br 360.0209, found 360.0205.

Methyl 4-bromo-3,5-bis(2-tert-butoxy-2-oxoethoxy)benzoate (11). In a round-bottom flask, 0.25 g (1.0 mmol) of benzoate 9, 0.45 mL (3.0 mmol) of tert-butyl bromoacetate, 0.41 g (3.0 mmol) of K₂CO₃, and 0.26 g (1.0 mmol) of 18-crown-6 were dissolved in acetone (25.0 mL), and the reaction mixture was refluxed for 16 h. The volatile components were removed under reduced pressure, and the residue was diluted with CH₂Cl₂ (50 mL) and water (50 mL); the organic layer was extracted, and the aqueous layer was washed with additional CH₂Cl₂ (3 × 50 mL). The organic layers were dried over MgSO₄ and filtered, and the volatile components were removed under reduced pressure to give a crude yellow oil. The oil was purified by flash chromatography (SiO₂, hexanes/ethyl acetate elution gradient 3:1) to give 0.39 g (89%) of 11 as a white solid: R₉ 0.47 (hexanes/ethyl acetate, 7:3); mp 103-105 °C; IR (thin film, cm⁻¹) 3090, 2980, 1753, 1725, 1588, 1423, 1394, 1369, 1339, 1304, 1239, 1161, 1132, 1030, 1000, 951, 900,
844, 763; $^1$H NMR (400 MHz, CDCl$_3$) δ 7.16 (s, 2H), 4.67 (s, 4H), 3.92 (s, 3H), 1.51 (s, 18H);
$^{13}$C NMR (400 MHz, CDCl$_3$) δ 167.0, 166.0, 155.8, 129.9, 108.0, 107.1, 82.8, 66.6, 52.5, 28.0;
HRMS (Q-TOF) $m/z$ calcd for [M+Na]$^+$ C$_{20}$H$_{27}$O$_8$BrNa 497.0787, found 497.0819. Benzoate 11 was also generated as the major product (48%) in the reaction used to furnish benzoate 10.

**Methyl 3-(2-(benzyloxy)-2-oxoethoxy)-4-bromo-5-(2-tert-butoxy-2-oxoethoxy) benzoate (4).**

In a round-bottom flask, 2.56 g (7.09 mmol) of benzoate 10, 1.35 mL (8.51 mmol) of benzyl bromoacetate, 1.17 g (8.51 mmol) of K$_2$CO$_3$, and 0.46 g (1.77 mmol) of 18-crown-6 were dissolved in acetone (35.4 mL), and the reaction mixture was refluxed for 24 h. The volatile components were removed under reduced pressure, and the residue was diluted with CH$_2$Cl$_2$ (120 mL) and water (120 mL); the organic layer was extracted, and the aqueous layer was washed with additional CH$_2$Cl$_2$ (3 × 120 mL). The organic layers were dried over MgSO$_4$ and filtered, and the volatile components were removed under reduced pressure to give a crude yellow oil. The oil was purified by flash chromatography (SiO$_2$, hexanes/ethyl acetate, elution gradient 7:1/6:1/3:1) to give 3.37 g of impure product, which was recrystallized twice with hexanes to yield 3.26 g (90.2%) of 4 as a white solid: R$_f$ 0.48 (hexanes/ethyl acetate, 3:2); mp 68–70 °C; IR (thin film, cm$^{-1}$) 2978, 1753, 1724, 1587, 1423, 1369, 1337, 1241, 1194, 1133, 1029, 1000, 844, 763; $^1$H NMR (300 MHz, CDCl$_3$) δ 7.35 (s, 5H), 7.15 (d, $J = 2$ Hz, 1H), 7.14 (d, $J = 2$ Hz, 1H), 5.25 (s, 2H), 4.81 (s, 2H), 4.66 (s, 2H), 3.88 (s, 3H), 1.50 (s, 9H); $^{13}$C NMR (500 MHz, CDCl$_3$) δ 167.8, 166.9, 165.9, 155.8, 155.7, 149.9, 149.1, 143.4, 141.7, 140.0, 135.0, 130.5, 130.0, 128.7, 128.6, 128.6, 128.5, 128.5, 108.2, 107.4, 107.2, 82.9, 67.3, 67.2, 66.7, 66.3,
60.7, 52.5, 28.0; HRMS (Q-TOF) m/z calcd for [M+Na]+ C_{23}H_{25}O_8NaBr 531.0630, found 531.0663.

 tert-Butyl 3-bromo-2-methylphenylcarbamate (15). In a round-bottom flask, 4.0 mL (32.5 mmol) of amine 7 and 14.2 g (64.9 mmol) of Boc anhydride were dissolved in THF (48.4 mL), and the reaction mixture was refluxed for 4 h. The volatile components were removed under reduced pressure; the residue was diluted with ethyl acetate (100 mL), washed with 1.0 M HCl (2 × 100 mL) and water (100 mL), dried over MgSO₄ and filtered, and the volatile components were removed under reduced pressure to give a crude light pink solid. The solid was purified by recrystallization (ethanol/water) to yield 5.87 g (95.6%) of 15 as light pink crystals: R⁰ 0.69 (hexanes/ethyl acetate, 3:2); mp 98–99 °C; IR (thin film, cm⁻¹) 3247, 2977, 1680, 1573, 1523, 1450, 1434, 1388, 1364, 1287, 1247, 1160, 1080, 1055, 1000, 845, 775, 753; ¹H NMR (300 MHz, CDCl₃) δ 7.72 (d, J = 8 Hz, 1H), 7.32 (d, J = 8 Hz, 1H), 7.05 (t, J = 8 Hz, 1H), 6.32 (broad s, 1H), 2.36 (s, 3H), 1.52 (s, 9H); ¹³C NMR (300 MHz, CDCl₃) δ 153.0, 137.4, 128.3, 127.4, 125.3, 121.0, 80.8, 28.3, 17.6; HRMS (EI) m/z calcd for C_{12}H_{16}NO_{2}Br 285.0364, found 285.0368.

 tert-Butyl 3-bromo-2-methylphenyl(ethyl)carbamate (16). To a stirred solution of 0.80 g (2.8 mmol) of carbamate 15 in DMF (6.0 mL) at 0 °C was added 0.34 g (8.4 mmol, 60% in mineral oil) of NaH, previously washed with hexanes (3 × 10 mL) to remove the mineral oil, over a 15 min period. A solution of 0.63 mL (8.4 mmol) of ethyl bromide in DMF (2.0 mL) was then added dropwise at 0 °C over a 15 min period, and the reaction mixture was stirred at room
temperature for 3.5 h. The mixture was diluted with ethyl acetate (30 mL) and water (30 mL); the aqueous layer was extracted, and the organic layer was washed with additional water (40 mL). The aqueous layers were washed with ethyl acetate (3 × 40 mL); all organic layers were dried over MgSO₄ and filtered, and the volatile components were removed under reduced pressure to give a crude yellow oil. The oil was purified by flash chromatography (SiO₂, hexanes/ethyl acetate, 20:1) to give 0.85 g (96.6%) of 16 as a colorless oil: R⁰ 0.51 (hexanes/ethyl acetate, 4:1); IR (thin film, cm⁻¹) 2975, 2932, 1702, 1591, 1563, 1462, 1391, 1307, 1255, 1179, 1155, 1114, 1080, 1011, 992, 948, 858, 781, 715; ¹H NMR (300 MHz, CDCl₃) δ 7.46-7.42 (m, 1H), 7.14-6.93 (m, 2H), two protons of two conformers [3.75-3.60 (doublet of quartets, J = 7, 7 Hz), 3.55-3.29 (doublet of quartets, J = 7, 7 Hz)], 2.26 (s, 3H), nine protons of two conformers in 1:2 ratio [1.50 (s), 1.31 (s)]; 1.08 (broad t, J = 7 Hz, 3H); ¹³C NMR (300 MHz, CDCl₃) δ 154.8, 142.9, 137.2, 131.8, 128.2, 127.6, 126.2, 80.4, 45.1, 28.9, 18.8, 14.0; HRMS (EI) m/z calcd for C₁₄H₂₀NO₂Br 313.0677, found 313.0669.

**tert-Butyl 3-bromo-2-methylphenyl(methyl)carbamate (17).** To a stirred solution of 5.0 g (17.5 mmol) of carbamate 15 in DMF (45.0 mL) at 0 °C was added 2.1 g (52.5 mmol, 60% in mineral oil) of NaH, previously washed with hexanes (3 × 20 mL) to remove the mineral oil, over a 15 min period. A solution of 3.27 mL (52.5 mmol) of methyl iodide in DMF (5.0 mL) was then added dropwise at 0 °C over a 15 min period, and the reaction mixture was stirred at room temperature for 3.5 h. The mixture was diluted with ethyl acetate (100 mL) and water (100 mL); the aqueous layer was extracted, and the organic layer was washed with additional water (2 × 50 mL). The aqueous layers were washed with ethyl acetate (3 × 100 mL); all organic layers were
dried over MgSO₄ and filtered, and the volatile components were removed under reduced pressure to give a crude red oil. The oil was purified by flash chromatography (SiO₂, hexanes/ethyl acetate, 3:2) to give 5.23 g (99.7%) of 17 as a red oil: Rf 0.65 (hexanes/ethyl acetate, 3:2); IR (thin film, cm⁻¹) 3521, 3390, 3068, 2976, 2930, 2720, 1927, 1693, 1592, 1563, 1464, 1358, 1305, 1255, 1164, 1102, 1074, 1036, 1002, 976, 901, 866, 825, 789, 767, 718; ¹H NMR (300 MHz, CDCl₃) δ 7.40 (app d, J = 8 Hz, 1H), 7.14-6.93 (m, 2H), 3.09 (s, 3H), 2.24 (s, 3H), nine protons of two conformers in 1:2 ratio [1.48 (s), 1.30 (s)]; ¹³C NMR (300 MHz, 323 K, CDCl₃) δ 155.1, 144.4, 136.6, 131.9, 127.9, 127.2, 126.1, 80.5, 37.5, 28.8, 18.5; HRMS (EI) m/z calcd for C₁₃H₁₈NO₂Br 299.0521, found 299.0513.

3-Bromo-N₂₂-dimethylbenzenamine (6). To a stirred solution of 5.23 g (17.4 mmol) of carbamate 17 in CH₂Cl₂ (26.4 mL) at room temperature was added 26.4 mL of trifluoroacetic acid, and the reaction mixture was stirred for 2 h. The mixture was dissolved in chilled saturated NaHCO₃ solution (100 mL) and CH₂Cl₂ (100 mL); the organic layer was extracted and washed with additional chilled saturated NaHCO₃ solution (100 mL). The aqueous layers were washed with CH₂Cl₂ (3 × 100 mL); all organic layers were dried over MgSO₄ and filtered, and the volatile components were removed under reduced pressure to give 3.48 g (99.8%) of 6 as a yellow oil: Rf 0.46 (hexanes/ethyl acetate, 4:1); IR (thin film, cm⁻¹) 3446, 3077, 2985, 2907, 2815, 2733, 2592, 1900, 1805, 1673, 1595, 1503, 1464, 1424, 1378, 1310, 1283, 1205, 1169, 1128, 1063, 996, 829, 762, 704; ¹H NMR (300 MHz, CDCl₃) δ 7.07-6.97 (m, 2H), 6.58 (dd, J = 2, 2 Hz, 1H), 3.70 (broad s, 1H), 2.91 (s, 3H), 2.29 (s, 3H); ¹³C NMR (300 MHz, CDCl₃) δ 148.4, 127.8, 125.5, 121.2, 121.0, 108.2, 31.0, 16.7; HRMS (EI) m/z calcd for C₈H₁₀NBr 198.9997, found 198.9999.
3-Bromo-N-ethyl-2-methylbenzenamine (13). To a stirred solution of 0.85 g (2.7 mmol) of carbamate 16 in CH$_2$Cl$_2$ (4.1 mL) at room temperature was added 4.1 mL of trifluoroacetic acid, and the reaction mixture was stirred for 1 h. The mixture was dissolved in chilled saturated NaHCO$_3$ solution (30 mL) and CH$_2$Cl$_2$ (30 mL); the organic layer was extracted and washed with additional chilled saturated NaHCO$_3$ solution (30 mL). The aqueous layers were washed with CH$_2$Cl$_2$ (3 ¥ 30 mL); all organic layers were dried over MgSO$_4$ and filtered, and the volatile components were removed under reduced pressure to give 0.58 g (99.9%) of 13 as a light brown oil: R$_f$ 0.56 (hexanes/ethyl acetate, 4:1); IR (thin film, cm$^{-1}$) 3433, 3074, 2969, 2927, 2871, 2591, 2434, 1898, 1805, 1712, 1573, 1458, 1380, 1313, 1279, 1204, 1163, 1119, 1093, 1062, 998, 937, 910, 841, 796, 761, 702; $^1$H NMR (300 MHz, CDCl$_3$) $\delta$ 7.01-6.94 (m, 2H), 6.56 (dd, $J$ = 3, 3 Hz, 1H), 3.48 (broad s, 1H), 3.19 (q, $J$ = 7 Hz, 2H), 2.27 (s, 3H), 1.33 (t, $J$ = 7 Hz, 3H); $^{13}$C NMR (300 MHz, CDCl$_3$) $\delta$ 147.5, 127.8, 125.6, 121.0, 120.9, 108.7, 38.7, 16.8, 14.9; HRMS (EI) m/z calcd for C$_9$H$_{12}$NBr 213.0153, found 213.0150.

N,2-Dimethyl-3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl) benzenamine (5). To a flame-dried round-bottom flask were added 3.48 g (17.4 mmol) of amine 6, 4.86 g (19.1 mmol) of bis(pinacolato)diboron, 0.38 g (0.52 mmol) of [1,1’-bis(diphenylphosphino)ferrocene] dichloropalladium(II), and 5.12 g (52.2 mmol) of potassium acetate. DMSO (84.3 mL), and the solution was degassed using the freeze-pump-thaw method three times under a nitrogen atmosphere. The flask was sealed; the reaction mixture was stirred at 100 °C for 14 h, and cooled
to room temperature. The mixture was diluted with CH$_2$Cl$_2$ (150 mL) and water (150 mL); the organic layer was extracted, and the aqueous layer was washed with additional CH$_2$Cl$_2$ (3 × 150 mL). The organic layers were dried over MgSO$_4$ and filtered, and the volatile components were removed under reduced pressure to give a brown crude oil. The oil was purified twice by flash chromatography (SiO$_2$, hexanes/ethyl acetate, elution gradient (two columns) 20:1/5:1, then 6:1/4:1) to give 2.76 g (64.2%) of 5 as a green solid: R$_f$ 0.41 (hexanes/ethyl acetate, 4:1); mp 87–90 °C; IR (thin film, cm$^{-1}$) 3441, 3065, 2978, 2928, 2816, 1724, 1654, 1582, 1507, 1469, 1449, 1379, 1351, 1310, 1286, 1206, 1146, 1129, 1083, 1019, 964, 872, 852, 816, 787, 736; $^1$H NMR (300 MHz, CDCl$_3$) δ 7.24–7.13 (m, 2H), 6.75 (dd, J = 7, 2, Hz, 1H), 3.64 (broad s, 1H), 2.90 (s, 3H), 2.37 (s, 3H), 1.35 (s, 12H); $^{13}$C NMR (300 MHz, CDCl$_3$) δ 147.2, 129.0, 128.8, 128.4, 128.3, 126.1, 124.3, 111.7, 83.4, 31.0, 24.9, 15.6; HRMS (Q-TOF) m/z calcd for [M+H]$^+$ C$_{14}$H$_{23}$NO$_2$B 248.1822, found 248.1805.

(S)-(9H-Fluoren-9-yl)methyl 1-((3-bromo-2-methylphenyl) (ethyl)amino)-1-oxopropan-2-ylcarbamate (22). To 0.09 g (0.41 mmol) of amine 13 in 4.0 mL of CHCl$_3$ was added 0.15 g (0.44 mmol) of Fmoc-alanine acid chloride 20-int (generated according to General Procedure A and used immediately thereafter) in 2.5 mL of CHCl$_3$ followed by 4.0 mL of saturated NaHCO$_3$ solution, and the reaction mixture was stirred at room temperature for 20 min. The solution was diluted with CH$_2$Cl$_2$ (30 mL) and saturated NaHCO$_3$ solution (30 mL), the organic layer was extracted, and the aqueous layer was washed with CH$_2$Cl$_2$ (3 × 30 mL). The organics were dried over MgSO$_4$ and filtered, and the volatile components were removed under reduced pressure to give a light brown crude oil. The oil was purified by flash chromatography (SiO$_2$, hexanes/ethyl
acetate, elution gradient 40:1/20:1/10:1/5:1/0:1) to give 0.14 g (67.8%) of 22 as a white foam: R_f 0.40 (hexanes/ethyl acetate, 3:2); IR (thin film, cm⁻¹) 3305, 3065, 2979, 2935, 1718, 1653, 1589, 1561, 1529, 1451, 1411, 1377, 1334, 1248, 1112, 1072, 1035, 999, 783, 760, 739; ¹H NMR (300 MHz, CDCl₃) δ 7.77 (d, J = 7 Hz, 2H), 7.71-7.57 (m, 3H), 7.41 (t, J = 7 Hz, 2H), 7.32 (t, J = 7 Hz, 2H), 7.24-7.07 (m, 2H), 5.63 (app d, J = 8 Hz, 1H), 4.41-4.16 (m, 3H), two protons of conformers [4.03 (doublet of quartets, J = 7, 7 Hz), 3.93 (doublet of quartets, J = 7, 7 Hz), 3.47 (doublet of quartets, J = 7, 7 Hz), 2.97 (doublet of quartets, J = 7, 7 Hz)], three protons of conformers [2.32 (s), 2.31 (s)], three protons of conformers [1.19 (d, J = 7 Hz), 1.14 (t, J = 7 Hz), 1.11 (t, J = 7 Hz)]; ¹³C NMR (300 MHz, CDCl₃) δ 172.9, 172.7, 155.6, 155.3, 144.0, 143.9, 141.3, 140.7, 140.4, 140.2, 137.2, 135.8, 133.3, 133.1, 130.1, 129.5, 128.1, 127.7, 127.1, 127.0, 126.8, 126.6, 125.2, 120.1, 120.0, 120.0, 67.0, 66.9, 47.9, 47.6, 47.2, 47.1, 44.4, 43.9, 19.4, 18.7, 18.4, 18.2, 12.7, 12.2; HRMS (Q-TOF) m/z calcd for [M+Na]^+ C₂₇H₂₇N₂O₃NaBr 529.1103, found 529.1123. [α]D²⁵ = +45.4, c = 1.00, CHCl₃.

(S)-(9H-Fluoren-9-yl)methyl 1-((3-bromo-2-methylphenyl) (methyl)amino)-1-oxopropan-2-ylcarbamate (23). To 0.08 g (0.4 mmol) of amine 6 in 4.0 mL of CHCl₃ was added 0.15 g (0.44 mmol) of Fmoc-alanine acid chloride 20-int (generated according to General Procedure A and used immediately thereafter) in 2.5 mL of CHCl₃ followed by 4.0 mL of saturated NaHCO₃ solution, and the reaction mixture was stirred at room temperature for 20 min. The solution was diluted with CH₂Cl₂ (30 mL) and saturated NaHCO₃ solution (30 mL), the organic layer was extracted, and the aqueous layer was washed with CH₂Cl₂ (3 × 30 mL). The organics were dried
over MgSO₄ and filtered, and the volatile components were removed under reduced pressure to give a light brown crude oil. The oil was purified by flash chromatography (SiO₂, hexanes/ethyl acetate, elution gradient 40:1/20:1/10:1/6:1/4:1) to give 0.19 g (96.3%) of 23 as a white foam: Rᵣ 0.34 (hexanes/ethyl acetate, 3:2); IR (thin film, cm⁻¹) 3419, 3066, 2927, 1718, 1655, 1561, 1531, 1450, 1392, 1248, 1107, 1070, 1029, 996, 789, 759, 739; ¹H NMR (300 MHz, CDCl₃) δ 7.76 (d, J = 7.2 Hz, 2H), 7.68-7.55 (m, 3H), 7.40 (t, J = 7 Hz, 2H), 7.32 (t, J = 7 Hz), 7.20-7.09 (m, J = 7 Hz, 2H), one proton of conformers [5.73 (app t, J = 7 Hz), 5.58 (s)], 4.34 (app d, J = 7 Hz, 2H), 4.28-4.15 (m, 1H), one proton of conformers [4.45-4.29 (m), 4.10 (quintet, J = 7 Hz)], three protons of conformers [3.34 (s), 3.33 (s), 3.21 (s), 3.19 (s)], three protons of conformers [2.34 (s), 2.33 (s), 2.26 (s), 2.24 (s)], three protons of conformers [1.53 (app t, J = 7 Hz), 1.22 (d, J = 7 Hz), 1.13 (d, J = 7 Hz)]; ¹³C NMR (300 MHz, CDCl₃) δ 173.1, 172.9, 155.6, 155.3, 144.0, 143.9, 142.2, 142.1, 141.3, 136.8, 135.6, 133.3, 133.1, 128.6, 128.1, 128.0, 127.7, 127.3, 127.1, 126.9, 126.7, 125.2, 120.0, 66.9, 66.8, 47.7, 47.4, 47.2, 36.8, 29.7, 19.4, 18.5, 18.3, 17.9; HRMS (EI) m/z calcd for C₂₆H₂₅N₂O₃Br 492.1049, found 492.1044. [α]²⁵_D = +46.9, c = 1.00, CHCl₃.

(9H-Fluoren-9-yl)methyl 2-((3-bromo-2-methylphenyl) (ethyl)amino)-2-oxoethylcarbamate (24). To 0.08 g (0.36 mmol) of amine 13 in 4.0 mL of CHCl₃ was added 0.14 g (0.5 mmol) of Fmoc-glycine acid chloride 21-int (generated according to General Procedure A and used immediately thereafter) in 2.5 mL of CHCl₃ followed by 4.0 mL of saturated NaHCO₃ solution, and the reaction mixture was stirred at room temperature for 20 min. The solution was diluted with CH₂Cl₂ (30 mL) and saturated NaHCO₃ solution (30 mL), the organic layer was extracted,
and the aqueous layer was washed with CH$_2$Cl$_2$ ($3 \times 30$ mL). The organics were dried over MgSO$_4$ and filtered, and the volatile components were removed under reduced pressure to give a light brown crude oil. The oil was purified by flash chromatography (SiO$_2$, hexanes/ethyl acetate, elution gradient 20:1/10:1/5:1/2:1) to give 0.15 g (85.3%) of 24 as a white foam: $R_f$ 0.27 (hexanes/ethyl acetate, 3:2); IR (thin film, cm$^{-1}$) 3320, 3065, 2974, 2935, 1723, 1667, 1588, 1561, 1512, 1450, 1415, 1379, 1341, 1246, 1167, 1125, 1062, 1038, 1000, 919, 783, 760, 739; $^1$H NMR (300 MHz, CDCl$_3$) δ 7.76 (d, $J = 7$ Hz, 2H), 7.63 (app t, $J = 8$, 7 Hz, 3H), 7.40 (t, $J = 7$ Hz, 2H), 7.31 (t, $J = 7$ Hz, 2H), 7.15 (t, $J = 8$ Hz, 1H), 7.09 (d, $J = 8$ Hz, 1H), one proton of conformers [5.87 (s), 5.75 (s), 5.49 (s)], 4.33 (d, $J = 7$ Hz, 2H), 4.21 (m, $J = 7$ Hz, 1H), 4.14 (doublet of quartets, $J = 7$, 7 Hz, 1H), 3.69 (dd, $J = 4$, 17 Hz, 1H), 3.43 (dd, $J = 4$, 17 Hz, 1H), 3.28 (doublet of quartets, $J = 7$, 7 Hz, 1H), 2.31 (s, 3H), 1.15 (t, $J = 7$ Hz, 3H); $^{13}$C NMR (500 MHz, CDCl$_3$) δ 167.8, 156.1, 143.9, 141.3, 139.6, 136.6, 133.5, 128.5, 128.2, 127.7, 127.1, 127.0, 125.2, 120.0, 67.1, 47.1, 43.8, 43.5, 18.2, 12.7; HRMS (EI) $m/z$ calcd for C$_{26}$H$_{25}$N$_2$O$_3$Br 492.1049, found 492.1044.

(9H-Fluoren-9-yl)methyl-2-((3-bromo-2-methylphenyl)(methyl)amino)-2-oxoethyl carbamate (25). To 0.08 g (0.437 mmol) of amine 6 in 4.0 mL of CHCl$_3$ was added 0.14 g (0.44 mmol) of Fmoc-glycine acid chloride 21-int (generated according to General Procedure A and used immediately thereafter) in 2.5 mL of CHCl$_3$ followed by 4.0 mL of saturated NaHCO$_3$ solution, and the reaction mixture was stirred at room temperature for 20 min. The solution was diluted with CH$_2$Cl$_2$ (30 mL) and saturated NaHCO$_3$ solution (30 mL), the organic layer was extracted, and the aqueous layer was washed with CH$_2$Cl$_2$ ($3 \times 30$ mL). The organics were dried
over MgSO\textsubscript{4} and filtered, and the volatile components were removed under reduced pressure to give a light brown crude oil. The oil was purified by flash chromatography (SiO\textsubscript{2}, hexanes/ethyl acetate, elution gradient 20:1/10:1/5:1/3:1/1:1) to give 0.18 g (93.6\%) of 25 as a white foam: R\textsubscript{f} 0.20 (hexanes/ethyl acetate, 3:2); IR (thin film, cm\textsuperscript{-1}) 3330, 3065, 2928, 1717, 1664, 1590, 1562, 1516, 1449, 1392, 1327, 1249, 1168, 1113, 1032, 998, 760, 738, 721; \textsuperscript{1}H NMR (300 MHz, CDCl\textsubscript{3}) δ 7.79 (d, J = 7 Hz, 2H), 7.62 (app d, J = 7 Hz, 3H), 7.43 (t, J = 7 Hz, 2H), 7.34 (t, J = 7 Hz, 2H), 7.23-7.08 (m, 2H), one proton of conformers [5.74 (s), 5.46 (s)], 4.36 (d, J = 7 Hz, 2H), 4.24 (m, 1H), 3.75 (dd, J = 4, 17 Hz, 1H), 3.49 (dd, J = 4, 17 Hz, 1H), 3.26 (s, 3H), 2.34 (s, 3H); \textsuperscript{13}C NMR (300 MHz, CDCl\textsubscript{3}) δ 168.3, 156.2, 145.9, 143.9, 141.3, 139.6, 136.2, 135.6, 134.7, 133.6, 133.5, 133.2, 130.1, 129.5, 129.1, 128.6, 127.9, 127.7, 127.2, 127.1, 126.9, 126.6, 126.3, 125.7, 125.2, 124.6, 124.3, 124.0, 121.0, 120.3, 120.1, 120.0, 119.8, 119.3, 67.1, 47.1, 43.3, 36.5, 29.7, 17.8; HRMS (EI) m/z calcd for C\textsubscript{25}H\textsubscript{23}N\textsubscript{2}O\textsubscript{3}Br 478.0892, found 478.0890.

![Methyl 2-(2-(benzyloxy)-2-oxoethoxy)-6-(2-(tert-butoxy)-2-oxoethoxy)-2'-methyl-3'-(methyl amino)-[1,1'-biphenyl]-4-carboxylate](image)

Methyl 2-(2-(benzyloxy)-2-oxoethoxy)-6-(2-(tert-butoxy)-2-oxoethoxy)-2'-methyl-3'-(methyl amino)-[1,1'-biphenyl]-4-carboxylate (3). In a resealable oven-dried Schlenk flask containing a magnetic stir bar, 1.27 g (2.50 mmol) of benzoate 4, 0.93 g (3.75 mmol) of boronic ester 5, 0.092 g (0.100 mmol) of tris[dibenzylideneacetone]dipalladium(0), 0.164 g (0.400 mmol) of 2-dicyclohexylphosphino-2’,6’-dimethoxybiphenyl (SPhos ligand), and 1.73 g (7.50 mmol) of K\textsubscript{3}PO\textsubscript{4} monohydrate were added and dried under reduced pressure for 2 min. Toluene (15 mL) was added, and the solution was degassed using the freeze-pump-thaw method three times under a nitrogen atmosphere. The Schlenk flask was sealed; the reaction mixture was vigorously stirred...
at 90 °C for 15 h, and cooled to room temperature. The mixture was diluted with CH₂Cl₂ (225 mL) and water (225 mL); the organic layer was extracted, and the aqueous layer was washed with additional CH₂Cl₂ (3 × 225 mL). The organic layers were dried over MgSO₄ and filtered, and the volatile components were removed under reduced pressure to give a brown crude oil. The oil was purified by flash chromatography (SiO₂, hexanes/ethyl acetate, elution gradient 5:1/4:1/3:1) to give 0.502 g (36.5%) of 3 as a brown oil/foam: Rf 0.38 (hexanes/ethyl acetate, 3:2); IR (thin film, cm⁻¹) 3440, 2930, 2815, 1752, 1722, 1587, 1513, 1493, 1436, 1368, 1327, 1233, 1191, 1134, 1080, 1029, 996, 844, 786, 753; ¹H NMR (500 MHz, CDCl₃) δ 7.41–7.25 (m, 5H), 7.25–7.15 (m, 3H), 6.66 (d, J = 8 Hz, 1H), 6.63 (d, J = 8 Hz, 1H), 5.17 (s, 2H), 4.63 (s, 2H), 4.48 (s, 2H), 3.90 (s, 3H), 3.65 (broad s, 1H), 2.92 (s, 3H), 1.89 (s, 3H), 1.44 (s, 9H); ¹³C NMR (300 MHz, CDCl₃) δ 168.6, 167.8, 166.4, 156.3, 156.1, 147.0, 141.6, 135.2, 132.7, 130.1, 128.6, 128.4, 128.0, 125.6, 121.5, 119.3, 108.8, 106.8, 106.5, 82.3, 66.9, 66.1, 65.6, 52.3, 31.0, 28.0, 14.0; HRMS (Q-TOF) m/z calcd for [M+Na]⁺ C₃₁H₃₅NO₈Na 572.2260, found 572.2312.

Methyl 3'-(S)-2-(((9H-fluoren-9-yl)methoxy)carbonyl)amino)-N-methylpropanamido)-2-(2-benzlyoxy)-2-oxoethoxy)-6-(2-(tert-butoxy)-2-oxoethoxy)-2'-methyl-[1,1'-biphenyl]-4-carboxylate (27). To 0.24 g (0.437 mmol) of carboxylate 3 in 4.0 mL of CHCl₃ was added 0.17 g (0.6 mmol) of Fmoc-alanine acid chloride 20-int (generated according to General Procedure A and used immediately thereafter) in 2.5 mL of CHCl₃ followed by 4.0 mL of saturated NaHCO₃ solution, and the reaction mixture was stirred at room temperature for 20 min. The solution was
diluted with CH₂Cl₂ (60 mL) and saturated NaHCO₃ solution (60 mL), the organic layer was extracted, and the aqueous layer was washed with CH₂Cl₂ (3 × 60 mL). The organics were dried over MgSO₄ and filtered, and the volatile components were removed under reduced pressure to give a light brown crude oil. The oil was purified by flash chromatography (SiO₂, hexanes/ethyl acetate, elution gradient 10:1/5:1/2:1/1:1) to give 0.30 g (82%) of 27 as a white foam: Rf 0.25 (hexanes/ethyl acetate, 3:2); IR (thin film, cm⁻¹) 3303, 3064, 2927, 2855, 1752, 1722, 1653, 1581, 1501, 1450, 1393, 1368, 1329, 1236, 1193, 1135, 1080, 1030, 997, 863, 845, 806, 759, 739; ¹H NMR (500 MHz, CDCl₃) δ 7.81-7.74 (m, J = 8 Hz, 2H), 7.66-7.57 (d, J = 8 Hz; m; d, J = 8 Hz; 2H), 7.45-7.38 (m, 2H), 7.38-7.26 (m, 7H), 7.26-7.17 (m, 4H), 7.16-7.09 (m, 1H), 5.81-5.73 (m, J = 9 Hz, 1H), 5.23-5.16 (m, 2H), six protons of conformers [4.91 (d, J = 12 Hz), 4.83 (d, J = 16 Hz), 5.02 (d, J = 12 Hz), 4.75-4.63 (m), 4.55-4.46 (m)], four protons [4.40-4.30 (m), 4.30-4.15 (m)], three protons of conformers [3.93 (s), 3.91 (s), 3.90 (s)], three protons of conformers [3.32 (s), 3.30 (s), 3.28 (s), 3.25 (s)], three protons of conformers [2.05 (s), 2.04 (s), 2.01 (s), 1.99 (s)], nine protons of conformers [1.47 (s), 1.46 (s), 1.35 (s)], three protons of conformers [1.23 (t, J = 8 Hz), 1.11 (d, J = 7 Hz), 1.06 (d, J = 7 Hz)]; ¹³C NMR (500 MHz, CDCl₃) δ 173.4, 173.1, 168.7, 168.3, 168.2, 168.2, 168.0, 167.5, 167.4, 167.3, 166.2, 156.3, 156.1, 156.0, 155.7, 155.5, 155.0, 144.1, 144.1, 144.0, 144.0, 141.3, 141.0, 136.0, 135.7, 135.6, 135.2, 135.1, 135.1, 131.3, 130.9, 130.8, 128.7, 128.6, 128.4, 128.3, 128.1, 127.7, 127.1, 127.0, 126.5, 126.3, 123.6, 123.6, 123.3, 120.0, 106.3, 106.0, 82.5, 82.0, 67.2, 67.0, 66.9, 66.8, 66.5, 65.9, 65.3, 47.9, 47.9, 47.2, 36.8, 36.7, 28.1, 28.0, 28.0, 19.7, 17.9; HRMS (Q-TOF) m/z calcd for [M+Na]⁺ C₄₉H₅₀N₂O₁₁Na 865.3312, found 865.3275. [α]D²⁵°C = +40.6, c = 1.00, CHCl₃.
Methyl-3'-(2-(((9H-fluoren-9-yl)methoxy)carbonyl)amino)-N-methylacetamido)-2-(2-
(benzyloxy)-2-oxoethoxy)-6-(2-(tert-butoxy)-2-oxoethoxy)-2'-methyl-[1,1'-biphenyl]-4-
 carboxylate (28). To 0.24 g (0.44 mmol) of carboxylate 3 in 4.0 mL of CHCl₃ was added 0.18 g
(0.60 mmol) of Fmoc-glycine acid chloride 21-int (generated according to General Procedure A
and used immediately thereafter) in 2.5 mL of CHCl₃ followed by 4.0 mL of saturated NaHCO₃
solution, and the reaction mixture was stirred at room temperature for 20 min. The solution was
diluted with CH₂Cl₂ (60 mL) and saturated NaHCO₃ solution (60 mL), the organic layer was
extracted, and the aqueous layer was washed with CH₂Cl₂ (3 × 60 mL). The organics were dried
over MgSO₄ and filtered, and the volatile components were removed under reduced pressure to
give a light brown crude oil. The oil was purified by flash chromatography (SiO₂, hexanes/ethyl
acetate, elution gradient 10:1/5:1/3:1/2:1/1:1) to give 0.29 g (81%) of 28 as a white foam: R_f 0.20
(hexanes/ethyl acetate, 3:2); IR (thin film, cm⁻¹) 3392, 2926, 2855, 1751, 1722, 1660, 1581,
1421, 1369, 1329, 1299, 1234, 1193, 1134, 1028, 998, 864, 845, 806, 735; ¹H NMR (500 MHz,
CDCl₃) δ 7.77 (t, J = 8 Hz, 2H), 7.63 (d, J = 8 Hz, 1H), 7.60 (d, J = 8H, 1H), 7.45-
7.26 (m, 10H), 7.23 (app s, 2H), 7.18 (app t, J = 6 Hz, 2H), one proton of conformers [5.74 (app s), 5.56 (broad
s)], two protons of conformers [5.20 (s), 5.13 (d, J = 13 Hz), 5.07 (d, J = 13 Hz)], two protons of
conformers [4.78 (d, J = 17 Hz), 4.71 (s), 4.70 (d, J = 17 Hz)], two protons of conformers [4.60
(d, J = 17 Hz), 4.56-4.50 (m)], 4.39-4.19 (m, 3H), three protons of conformers [3.93 (s), 3.92
(s)], two protons of conformers [3.80-3.70 (td, J = 5, 14 Hz), 3.70-3.60 (td, J = 5, 14 Hz)], three
protons of conformers [3.33 (s), 3.30 (s)], three protons of conformers [2.07 (s), 2.04 (s)], nine protons of conformers [1.48 (s), 1.42 (s)]; \(^{13}\)C NMR (500 MHz, CDCl\(_3\)) \(\delta\) 168.8, 168.2, 167.5, 167.4, 166.2, 156.1, 155.8, 155.6, 144.0, 144.0, 141.3, 140.3, 135.8, 135.7, 135.1, 135.0, 131.3, 130.9, 128.7, 128.6, 128.5, 128.4, 128.3, 127.7, 127.1, 126.9, 125.3, 125.2, 123.3, 120.0, 106.5, 106.4, 106.0, 82.5, 82.3, 67.0, 66.9, 65.9, 65.7, 65.3, 65.1, 52.4, 47.2, 47.1, 43.4, 36.3, 28.1, 28.0, 14.5; HRMS (Q-TOF) \(m/z\) calcd for [M+Na]\(^{+}\) C\(_{48}\)H\(_{48}\)N\(_2\)O\(_{11}\)Na 851.3156, found 851.3071.

Methyl-3'-(S)-2-acetamido-N-methylpropanamido)-2-(2-(benzoyloxy)-2-oxoethoxy)-6-(2-(tert-butoxy)-2-oxoethoxy)-2'-methyl-[1,1'-biphenyl]-4-carboxylate (1). To 0.042 g (0.05 mmol) of carboxylate 27 in a round-bottom flask was added 0.22 mL of a 20% piperidine/DMF solution, and the reaction mixture was stirred at room temperature for 20 min. Then 0.76 mL (8.04 mmol) of acetic anhydride and 2.32 mL (28.7 mmol) of pyridine were added, and the solution was stirred at room temperature for 30 min. The volatile components were removed under reduced pressure to give a yellow crude oil. The oil was purified by flash chromatography (SiO\(_2\), hexanes/ethyl acetate/methanol elution gradient 1:1:0/0:1:0/0:9:1/0:4:1) to give 0.033 g (99%) of 1 as a slightly impure (by \(^{1}\)H NMR, <5%) white foam: \(R_f\) 0.63 (100% ethyl acetate); IR (thin film, cm\(^{-1}\)) 3329, 2932, 1751, 1723, 1645, 1580, 1423, 1392, 1369, 1329, 1234, 1192, 1134, 1028, 996, 731; \(^{1}\)H NMR (500 MHz, CDCl\(_3\)) \(\delta\) 7.40-7.27 (m, 7H), 7.26-7.12 (m, 3H), one proton of conformers [6.57 (d, \(J = 8\) Hz), 6.54 (d, \(J = 8\) Hz), 6.47 (d, \(J = 8\) Hz), 6.44 (d, \(J = 8\) Hz),], 5.22-5.14 (m, 2H), five protons of conformers [4.96 (s), 4.93 (s), 4.80 (d, \(J = 10\) Hz), 4.77 (d, \(J = 8\) Hz),]}
10 Hz), 4.73-4.63 (m), 4.59-4.48 (m), 4.39 (quintet, $J = 7$ Hz)], three protons of conformers [3.93 (s), 3.93 (s), 3.92 (s), 3.91 (s)], three protons of conformers [3.30 (s), 3.28 (s), 3.25 (s), 3.22 (s)], six protons of conformers [2.11 (s), 2.03 (s), 2.03 (s), 2.01 (s), 1.99 (s), 1.99 (s), 1.93 (s), 1.86 (s)], nine protons of conformers [1.48 (s), 1.47 (s), 1.47 (s), 1.46 (s)], three protons of conformers [1.19 (d, $J = 7$ Hz), 1.17 (d, $J = 7$ Hz), 1.07 (d, $J = 7$ Hz), 1.02 (d, $J = 7$ Hz)]; $^{13}$C NMR (500 MHz, CDCl$_3$) $\delta$ 173.5, 173.5, 173.1, 169.1, 168.9, 168.4, 168.4, 168.3, 168.2, 168.1, 167.5, 167.4, 167.3, 166.3, 166.2, 156.1, 159.5, 159.9, 155.7, 155.4, 140.9, 140.8, 140.8, 140.7, 136.0, 135.7, 135.6, 135.4, 135.2, 135.0, 135.0, 131.2, 131.2, 130.9, 130.8, 130.8, 128.3, 128.5, 128.5, 128.5, 128.4, 128.3, 127.9, 127.6, 127.0, 126.9, 126.5, 126.5, 126.5, 123.5, 123.5, 123.2, 106.5, 106.3, 106.0, 105.9, 105.8, 105.8, 82.5, 82.0, 67.0, 66.6, 65.9, 65.7, 65.4, 65.3, 65.3, 65.1, 64.9, 52.4, 52.4, 47.5, 46.5, 46.5, 46.3, 42.5, 36.9, 36.8, 36.7, 36.7, 28.0, 28.0, 26.4, 25.5, 24.5, 23.4, 23.3, 23.3, 21.5, 19.4, 19.4, 17.6, 17.5, 14.9, 14.9, 14.6, 14.6; HRMS (Q-TOF) $m/z$ calcd for [M+Na]$^+$ C$_{36}$H$_{42}$N$_2$O$_{10}$Na 685.2737, found 685.2675. $[\alpha]_D^{25\circ} = +32.4$ (c = 0.98, CHCl$_3$).

Methyl-3'-[(acetamido-N-methylacetamido)-2-(2-(benzyloxy)-2-oxoethoxy)-6-(2-(tert-butoxy)-2-oxoethoxy)-2'-methyl-[1,1'-biphenyl]-4-carboxylate (2). To 0.041 g (0.05 mmol) of carboxylate 28 in a round-bottom flask was added 0.22 mL of a 20% piperidine/DMF solution, and the reaction mixture was stirred at room temperature for 20 min. Then 0.76 mL (8.04 mmol) of acetic anhydride and 2.32 mL (28.7 mmol) of pyridine were added, and the solution was stirred at room temperature for 30 min. The volatile components were removed under reduced
pressure to give a yellow crude oil. The oil was purified by flash chromatography (SiO₂, hexanes/ethyl acetate/methanol elution gradient 1:1:0/0:1:0/0:9:1/0:4:1) to give 0.031 g (97%) of 2 as a slightly impure (by ¹H NMR, <5%) white foam: Rᵢ 0.58 (100% ethyl acetate); IR (thin film, cm⁻¹) 3338, 2932, 1751, 1723, 1655, 1580, 1423, 1393, 1369, 1329, 1235, 1193, 1135, 1029, 996, 844, 806, 733; ¹H NMR (500 MHz, CDCl₃) δ 7.38-7.33 (m, 3H), 7.33-7.26 (m, 4H), 7.22 (s), 7.17 (dd, J = 1, 3 Hz, 1H), 7.14 (d, J = 7 Hz, 1H), 6.52-6.47 (broad app t, 1H), 5.21-5.13 (m, 2H), four protons of conformers [4.82 (d, J = 17 Hz), 4.73 (d, J = 17 Hz), 4.70 (s), 4.64 (d, J = 17 Hz), 4.57-4.50 (m)], three protons of conformers [3.92 (s), 3.92 (s)], two protons of conformers [3.78-3.71 (app dd, J = 4, 19 Hz), 3.70-3.60 (td, J = 4, 19 Hz)], three protons of conformers [3.31 (s), 3.28 (s)], six protons of conformers [2.10 (s), 2.02 (s), 2.00 (s), 1.98 (s)], nine protons of conformers [1.47 (s), 1.47 (s)]; ¹³C NMR (300 MHz, CDCl₃) δ 169.7, 168.8, 168.8, 168.5, 168.2, 167.7, 167.4, 166.2, 156.0, 155.8, 155.8, 155.5, 140.1, 140.1, 135.7, 135.7, 135.2, 135.0, 131.3, 130.9, 128.6, 128.6, 128.4, 128.4, 127.0, 126.8, 123.2, 123.2, 106.4, 106.2, 105.9, 105.7, 82.5, 82.3, 67.0, 66.9, 65.9, 65.5, 65.3, 65.0, 52.4, 47.5, 42.5, 42.2, 42.2, 36.3, 28.0, 26.4, 25.5, 24.5, 23.1, 21.5, 14.5, 14.5; HRMS (EI) m/z calcd for C₃₅H₄₀N₂O₁₀ 648.2683, found 648.2672.

(S)-2-Acetamido-N-(3-bromo-2-methylphenyl)-N-methylpropanamide (29). To 0.049 g (0.10 mmol) of carbamate 23 in a round-bottom flask was added 0.44 mL (0.89 mmol) of a 20% piperidine/DMF solution, and the reaction mixture was stirred at room temperature for 20 min. Then 1.52 mL (16.1 mmol) of acetic anhydride and 4.62 mL (57.1 mmol) of pyridine were added, and the solution was stirred at room temperature for 30 min. The volatile components
were removed under reduced pressure to give a brown crude oil. The oil was purified by flash chromatography (SiO$_2$, hexanes/ethyl acetate/methanol elution gradient 1:10:0/0:1:0/0:9:1) to give 0.028 g (89.4%) of 29 as an off-white foam: R$_f$ 0.16 (100% ethyl acetate); IR (thin film, cm$^{-1}$) 3305, 3069, 2931, 1648, 1590, 1558, 1460, 1391, 1374, 1281, 1151, 1110, 1034, 997, 792, 767, 720; $^1$H NMR (300 MHz, CDCl$_3$) δ 7.60 (d, $J = 8$ Hz, 1H), 7.27-7.07 (m, 2H), one proton of conformers [6.65 (s), 6.41 (s)], one proton of conformers [5.08 (quintet, $J = 7$ Hz), 4.53 ($J = 7$ Hz), 4.29 ($J = 7$ Hz)], three protons of conformers [3.35 (s), 3.34 (s), 3.19 (s), 3.16 (s)], three protons of conformers [2.31 (s), 2.29 (s), 2.22 (s), 2.21 (s)], three protons of conformers [1.96 (s), 1.96 (s)], three protons of conformers [1.49 (app t), 1.17 (d, $J = 7$ Hz), 1.07 (d, $J = 7$ Hz)]; $^{13}$C NMR (500 MHz, CDCl$_3$) δ 173.2, 173.1, 169.3, 169.0, 142.1, 141.9, 136.8, 135.5, 133.3, 133.1, 132.3, 128.6, 128.0, 128.0, 127.2, 126.9, 126.6, 126.2, 46.1, 45.9, 36.8, 36.8, 23.3, 23.2, 19.2, 18.5, 18.1, 17.8; HRMS (EI) m/z calcd for C$_{13}$H$_{17}$N$_2$O$_2$Br 312.0473, found 312.0470. 

$^{[\alpha]}_D^{25^\circ} = +27.1$ (c = 1.00, CHCl$_3$).

2-Acetamido-N-(3-bromo-2-methylphenyl)-N-methylacetamide (30). To 0.048 g (0.10 mmol) of carbamate 25 in a round-bottom flask was added 0.44 mL (0.89 mmol) of a 20% piperidine/DMF solution, and the reaction mixture was stirred at room temperature for 20 min. Then 1.52 mL (16.1 mmol) of acetic anhydride and 4.62 mL (57.1 mmol) of pyridine were added, and the solution was stirred at room temperature for 30 min. The volatile components were removed under reduced pressure to give a brown crude oil. The oil was purified by flash chromatography (SiO$_2$, hexanes/ethyl acetate/methanol elution gradient 1:10:0/0:1:0/0:9:1) to
give 0.025 g (82.2%) of 30 as an off-white foam: Rf 0.14 (100% ethyl acetate); IR (thin film, cm⁻¹) 3318, 3074, 2927, 1655, 1560, 1462, 1440, 1392, 1328, 1285, 1111, 1041, 998, 792, 721; ¹H NMR (300 MHz, CDCl₃) δ 7.59 (dd, J = 2, 8 Hz, 1H), 7.17-7.08 (m, 2H), 6.50 (broad s, 1H), two protons of conformers [4.26 (d, J = 4 Hz), 3.73 (dd, J = 4, 18 Hz), 3.45 (dd, J = 4, 18 Hz)], three protons of conformers [3.26 (s), 3.20 (s)], three protons of conformers [2.28 (s), 2.22 (s)], three protons of conformers [2.06 (s), 2.00 (s)]; ¹³C NMR (500 MHz, CDCl₃) δ 170.0, 168.3, 141.1, 139.3, 136.2, 133.6, 132.5, 128.7, 128.0, 127.2, 126.9, 126.3, 42.1, 36.5, 23.0, 17.8; HRMS (EI) m/z calcd for C₁₂H₁₅NO₂Br 298.0317, found 298.0321.

Methyl-2-(2-(tert-butoxy)-2-oxoothoxy)-6-hydroxy-2′-methyl-3′-(methylamino)-[1,1′-biphenyl]-4-carboxylate (31). In a resealable oven-dried Schlenk flask containing a magnetic stir bar, 0.25 g (0.68 mmol) of benzoate 10, 0.25 g (1.03 mmol) of boronic ester 5, 0.025 g (0.03 mmol) of tris[dibenzyldieneacetone]dipalladium(0), 0.045 g (0.11 mmol) of 2-dicyclohexylphosphino-2′,6′-dimethoxybiphenyl (SPhos ligand), and 0.472 g (2.05 mmol) of K₃PO₄ monohydrate were added and dried under reduced pressure for 2 min. Toluene (4.1 mL) was added, and the solution was degassed using the freeze-pump-thaw method three times under a nitrogen atmosphere. The Schlenk flask was sealed; the reaction mixture was vigorously stirred at 90 °C for 15 h, and cooled to room temperature. The mixture was diluted with CH₂Cl₂ (60 mL) and water (60 mL); the organic layer was extracted, and the aqueous layer was washed with additional CH₂Cl₂ (3 × 60 mL). The organic layers were dried over MgSO₄ and filtered, and the
volatile components were removed under reduced pressure to give a brown crude oil. The oil was purified by flash chromatography (SiO$_2$, hexanes/ethyl acetate, elution gradient 10:1/6:1) to give 0.018 g (6.6%) of 31 as a yellow oil: $R_f$ 0.39 (hexanes/ethyl acetate, 3:2); IR (thin film, cm$^{-1}$) 3440, 2928, 1750, 1586, 1497, 1436, 1368, 1340, 1235, 1158, 1119, 1009, 843, 786, 770, 752, 724; $^1$H NMR (300 MHz, CDCl$_3$) $\delta$ 7.37 (d, $J = 1$ Hz, 1H), 7.28 (t, $J = 8$ Hz, 1H), 7.05 (d, $J = 1$ Hz, 1H), 6.72 (d, $J = 8$ Hz, 1H), 6.63 (d, $J = 8$ Hz, 1H), 5.07 (s, 1H), 4.49 (s, 2H), 3.92 (s, 3H), 3.78 (broad s, 1H), 2.95 (s, 3H), 1.91 (s, 3H), 1.45 (s, 9H); $^{13}$C NMR (500 MHz, CDCl$_3$) $\delta$ 168.7, 166.7, 155.6, 154.0, 148.0, 130.7, 130.5, 127.7, 122.0, 118.4, 110.2, 109.8, 104.4, 82.3, 65.9, 52.3, 30.9, 28.0, 13.7; HRMS (El) $m/z$ calcd for C$_{22}$H$_{27}$NO$_6$ 401.1838, found 401.1837.

Methyl 4-bromo-3-(2-(tert-butoxy)-2-oxoethoxy)-5-(((tert-butyldimethylsilyl)oxy) benzoate (32). To a solution of 0.18 g (0.5 mmol) of benzoate 10 in DMF (2.0 mL) was added 0.11 g (0.68 mmol) of tert-butyldimethylsilyl chloride and 0.09 g (1.25 mmol) of imidazole. The yellow solution was stirred for 24 h at room temperature. The residue was diluted with CH$_2$Cl$_2$ (15 mL) and water (15 mL); the organic layer was extracted, and the aqueous layer was washed with additional CH$_2$Cl$_2$ (3 $\times$ 15 mL). The organics were dried over MgSO$_4$ and filtered, and the volatile components were removed under reduced pressure to give a brown crude oil. The oil was purified by flash chromatography (SiO$_2$, hexanes/ethyl acetate elution gradient 9:1/6:1) to give 0.16 g (67.9%) of 32 as a white solid: $R_f$ 0.62 (hexanes/ethyl acetate, 7:3); mp 58-60 $^\circ$C; IR (thin film, cm$^{-1}$) 2954, 2932, 2859, 1756, 1727, 1581, 1423, 1393, 1368, 1347, 1297, 1243, 1220, 1161, 1125, 1029, 836, 783, 767; $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 7.17 (s, 1H), 7.03 (s, 1H), 4.60 (s, 2H), 3.84 (s, 3H), 1.44 (s, 9H), 1.01 (s, 9H), 0.24 (s, 6H); $^{13}$C NMR (400 MHz, CDCl$_3$) $\delta$
167.0, 166.0, 155.8, 154.0, 129.6, 114.1, 111.3, 106.3, 82.5, 66.5, 52.3, 27.9, 25.7, 18.3; HRMS (Q-TOF) m/z calcd for [M+Na]+ C_{20}H_{31}O_{6}NaSiBr 497.0971, found 497.0975.

2-((3’-((S)-2-(((9H-Fluoren-9-yl)methoxy)carbonyl)amino)-N-methylpropanamido)-6-(2-(tert-butoxy)-2-oxoethoxy)-4-(methoxycarbonyl)-2'-methyl-[1,1'-biphenyl]-2-yl)oxy)acetic acid (33). To 0.118 g (0.14 mmol) of carboxylate 27 and 0.012 g of 10% Pd/C in a round-bottom flask was added 1.7 mL of a 9:1 MeOH/H_2O solution. The flask was sealed with a septum; a balloon attached to a syringe needle and filled with hydrogen gas was inserted. The reaction mixture was stirred at room temperature for 4 h, after which the solution was filtered through a pad of Celite and further eluted with ethyl acetate (200 mL). The volatile components were removed under reduced pressure to give 0.105 g (100%) of 33 as an off-white foam: R_f 0.20 (100% ethyl acetate); IR (thin film, cm^-1) 3328, 2929, 1722, 1651, 1580, 1504, 1449, 1396, 1369, 1327, 1236, 1135, 1029, 997, 759, 740; 1H NMR (500 MHz, CDCl_3) δ 7.78 (t, J = 8 Hz, 2H), 7.63-7.57 (m, 2H), 7.45-7.28 (m, 7H), two protons [7.25 (app d, J = 8 Hz), 7.21 (dd, J = 1, 8 Hz), 7.20-7.15 (m)], one proton of conformers [6.22 (d, J = 8 Hz), 5.94-5.74 (m)], 4.70-4.48 (m, 4H), four protons of conformers [4.41-4.34 (m), 4.34-4.26 (m), 4.19 (quintet, J = 8 Hz)], three protons of conformers [3.96 (s), 3.95 (s), 3.94 (s), 3.93 (s)], three protons of conformers [3.31 (s), 3.30 (s), 3.29 (s), 3.26 (s)], three protons of conformers [2.07 (s), 2.05 (s), 2.03 (s), 1.99 (s)], nine protons of conformers [1.48 (s), 1.47 (s), 1.46 (s), 1.35 (s)], three protons of conformers [1.28 (app d, J = 7 Hz), 1.24 (d, J = 7 Hz), 1.16 (d, J = 7 Hz)]; 13C NMR (300 MHz, CDCl_3) δ
167.9, 167.4, 167.3, 166.3, 166.2, 156.1, 155.9, 155.8, 155.6, 155.1, 144.0, 143.9, 143.7, 141.2, 140.7, 140.6, 136.0, 135.8, 135.6, 135.0, 131.5, 131.1, 131.0, 130.8, 127.7, 127.5, 127.3, 127.1, 126.9, 126.6, 125.2, 124.0, 123.4, 119.9, 107.7, 106.8, 106.3, 82.6, 82.5, 82.1, 67.4, 67.1, 66.8, 65.9, 65.8, 52.4, 48.0, 47.6, 47.1, 47.0, 37.1, 36.8, 36.8, 29.7, 28.0, 27.9, 19.6, 19.3, 14.9, 14.7, 14.5; HRMS (EI) m/z calcd for C_{42}H_{44}N_{2}O_{11} 752.2945, found 752.2913. [\alpha]_{D}^{25^\circ} = +34.6, c = 1.00, CHCl₃.

\[ \text{2-((3'-((9H-Fluoren-9-yl)methoxy)carbonyl)amino)-N-methylacetamido)-6-(2-(tert-butoxy)-2-oxoethoxy)-4-(methoxycarbonyl)-2'-methyl-[1,1'-biphenyl]-2-yl)oxy)acetic acid (34).} \]

To 0.125 g (0.15 mmol) of carboxylate 28 and 0.013 g of 10% Pd/C in a round-bottom flask was added 1.8 mL of a 9:1 MeOH/H₂O solution. The flask was sealed with a septum; a balloon attached to a syringe needle and filled with hydrogen gas was inserted. The reaction mixture was stirred at room temperature for 4 h, after which the solution was filtered through a pad of Celite and further eluted with ethyl acetate (200 mL). The volatile components were removed under reduced pressure to give 0.11 g (98.5%) of 34 as an off-white foam: R_f 0.17 (100% ethyl acetate); IR (thin film, cm⁻¹) 3405, 2931, 1723, 1663, 1580, 1517, 1422, 1369, 1329, 1239, 1136, 1057, 1029, 998, 844, 806, 760, 738; ¹H NMR (500 MHz, CDCl₃) δ 7.78 (d, J = 8 Hz, 2H), 7.61 (app d, J = 5 Hz, 2H), 7.42 (t, J = 8 Hz, 2H), 7.38-7.25 (m, 6H), 7.16 (app d, J = 7 Hz, 1H), one proton of conformers [6.17 (app t, J = 4 Hz), 5.77 (broad s)], 4.60-4.48 (m, 4H), 4.46-4.38 (m, 2H), 4.22 (t, J = 7 Hz, 1H), three protons of conformers [3.96 (s), 3.92 (s)], 3.76-3.65 (m, 2H), three protons of conformers [3.32 (s), 3.29 (s)], three protons of conformers [2.05
(s), 2.02 (s)], nine protons of conformers [1.48 (s), 1.42 (s)]; $^{13}$C NMR (300 MHz, CDCl$_3$) δ 169.7, 168.4, 167.4, 166.2, 157.1, 155.9, 155.8, 143.7, 143.7, 141.2, 139.9, 136.0, 135.7, 135.4, 131.6, 131.1, 127.7, 127.1, 127.0, 125.2, 125.2, 124.0, 123.5, 120.0, 119.8, 106.7, 82.6, 82.3, 67.7, 66.3, 65.8, 52.5, 47.1, 46.9, 43.1, 36.6, 36.3, 28.0, 28.0, 14.6, 14.4; HRMS (Q-TOF) m/z calcd for [M+Na]$^+$ C$_{41}$H$_{42}$N$_2$O$_{11}$Na 761.2686, found 761.2667.

Methyl 3'-(S)-2-acetamido-N-methylpropanamido)-2-(2-(tert-butoxy)-2-oxoethoxy)-6-(2-(dimethylamino)-2-oxoethoxy)-2'-methyl-[1,1'-biphenyl]-4-carboxylate (35). To a round-bottom flask equipped with a condenser and nitrogen gas inlet were added 0.044 g (0.06 mmol) of acid 33, 0.005 g (0.06 mmol) of dimethylamine hydrochloride, 0.011 g (0.06 mmol) of N-ethyl-N′-(3-dimethylaminopropyl)carbodiimide hydrochloride, 0.008 g (0.06 mmol) of N-hydroxybenzotriazole, 0.03 mL (0.19 mmol) of triethylamine, and acetonitrile (1.02 mL). The reaction mixture was stirred at room temperature for 80 h, and the volatile components were removed under reduced pressure to give a crude brown solid. The solid was purified by flash chromatography (SiO$_2$, ethyl acetate/methanol, elution gradient 1:0/9:1/4:1/1:1) to give 0.044 g (0.079 mmol) of impure oil. To a solution of the oil dissolved in CH$_2$Cl$_2$ (0.61 mL) at room temperature was added 0.02 mL (0.16 mmol) of acetic anhydride, and the reaction mixture was stirred for 30 min. The solution was basified dropwise with saturated K$_2$CO$_3$ solution (pH 9) and extracted with CH$_2$Cl$_2$ (3 × 5 mL). The organic layers were washed with water (2 × 4 mL), dried over MgSO$_4$ and filtered, and the volatile components were removed under reduced pressure to
give a light brown crude oil. The oil was purified by flash chromatography (SiO\textsubscript{2}, hexanes/ethyl acetate/methanol, elution gradient 1:1:0/1:0/0:9:1) to give 0.018 g (51.6\%) of \textbf{35} as a yellow oil: \textit{R}\textsubscript{f} 0.10 (100\% ethyl acetate); IR (thin film, cm\textsuperscript{-1}) 3323, 2931, 1722, 1650, 1580, 1502, 1422, 1393, 1369, 1324, 1236, 1127, 1024, 996, 845, 807, 732; \textsuperscript{1}H NMR (500 MHz, CDCl\textsubscript{3}) \textdelta 7.36-7.23 (m, 3H), 7.23-7.12 (m, 2H), one proton of conformers [6.59-6.51 (m, \textit{J} = 8 Hz), 6.46 (d, \textit{J} = 8 Hz)], five protons of conformers [4.96 (d, \textit{J} = 15 Hz), 4.84 (d, \textit{J} = 15 Hz), 4.78 (d, \textit{J} = 17 Hz), 4.75 (d, \textit{J} = 15 Hz), 4.71-4.61 (m), 4.59-4.47 (m), 4.39 (quintet, \textit{J} = 7 Hz), 4.33 (quintet, \textit{J} = 7 Hz)], three protons of conformers [3.95 (s), 3.95 (s), 3.93 (s)], three protons of conformers [3.30 (s), 3.29 (s), 3.26 (s), 3.24 (s)], six protons of conformers [2.94 (s), 2.93 (s), 2.92 (s), 2.91 (s), 2.90 (s), 2.87 (s), 2.84 (s)], six protons of conformers [2.06 (s), 2.01 (s), 2.01 (s), 2.00 (s), 1.93 (s), 1.92 (s)], nine protons of conformers [1.47 (s), 1.47 (s), 1.46 (s)], three protons of conformers [1.19 (t, \textit{J} = 6 Hz), 1.08 (d, \textit{J} = 6 Hz), 1.05 (d, \textit{J} = 6 Hz)], \textsuperscript{13}C NMR (500 MHz, CDCl\textsubscript{3}) \textdelta 173.5, 173.2, 173.1, 173.0, 169.1, 169.0, 168.5, 168.4, 168.1, 167.8, 167.5, 167.5, 167.3, 167.1, 167.0, 166.9, 166.5, 166.4, 166.3, 156.4, 156.1, 155.9, 155.8, 155.8, 155.7, 155.5, 140.9, 140.8, 136.0, 136.0, 135.9, 135.8, 135.2, 131.4, 131.2, 131.1, 131.0, 130.9, 130.9, 130.6, 130.4, 127.7, 127.5, 127.0, 126.9, 126.9, 126.8, 126.4, 126.4, 123.4, 123.3, 123.0, 106.7, 106.6, 106.5, 106.4, 106.3, 106.1, 106.1, 106.0, 82.5, 82.5, 81.9, 67.6, 67.3, 66.9, 65.9, 65.8, 65.7, 65.4, 52.4, 52.4, 46.6, 46.5, 46.3, 46.2, 36.9, 36.8, 36.7, 36.4, 36.3, 36.1, 35.7, 35.6, 35.5, 29.7, 28.0, 28.0, 23.4, 23.3, 19.4, 19.2, 17.8, 17.5, 15.0, 15.0, 14.7; HRMS (EI) \textit{m/z} calcd for C\textsubscript{31}H\textsubscript{41}N\textsubscript{3}O\textsubscript{9} 599.2843, found 599.2837. \textit{[\alpha]}\textsubscript{D}\textdegree\textsuperscript{25} = +40.3, c = 1.00, CHCl\textsubscript{3}. 

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Methyl 3’-(2-acetamido-N-methylacetamido)-2-(2-(tert-butoxy)-2-oxoethoxy)-6-(2-(dimethyl amino)-2-oxoethoxy)-2'-methyl-[1,1'-biphenyl]-4-carboxylate (36). To a round-bottom flask equipped with a condenser and nitrogen gas inlet were added 0.049 g (0.07 mmol) of acid 34, 0.005 g (0.07 mmol) of dimethyamine hydrochloride, 0.013 g (0.07 mmol) of N-ethyl-N’-(3-dimethylaminopropyl)carbodiimide hydrochloride, 0.009 g (0.07 mmol) of N-hydroxybenzotriazole, 0.031 mL (0.22 mmoL) of triethylamine, and acetonitrile (1.15 mL). The reaction mixture was stirred at room temperature for 80 h, and the volatile components were removed under reduced pressure to give a crude brown solid. The solid was purified by flash chromatography (SiO₂, ethyl acetate/methanol, elution gradient 9:1/4:1/1:1) to give 0.048 g (0.09 mmol) of impure oil. To a solution of the oil dissolved in CH₂Cl₂ (0.68 mL) at room temperature was added 0.02 mL (0.18 mmol) of acetic anhydride, and the reaction mixture was stirred for 30 min. The solution was basified dropwise with saturated K₂CO₃ solution (pH 9) and extracted with CH₂Cl₂ (3 × 5 mL). The organic layers were washed with water (2 × 4 mL), dried over MgSO₄ and filtered, and the volatile components were removed under reduced pressure to give a light brown crude oil. The oil was purified by flash chromatography (SiO₂, hexanes/ethyl acetate/methanol, elution gradient 1:1:0/0:1:0/0:9:1) to give 0.019 g (47.8%) of 36 as a yellow oil: Rf 0.09 (100% ethyl acetate); IR (thin film, cm⁻¹) 3338, 2931, 1749, 1722, 1657, 1580, 1503, 1422, 1369, 1325, 1237, 1128, 1025, 996, 844, 807, 733; ¹H NMR (500 MHz, CDCl₃) δ 7.34-7.27 (m, 3H), 7.20 (app s, 1H), 7.17-7.12 (m, 1H), one proton of conformers [6.52 (app t), 6.49
(app t), 4.79 (s, 1H), three protons of conformers [4.73 (d, J = 14 Hz), 4.69 (d, J = 14 Hz), 4.63 (d, J = 17 Hz), 4.56-4.48 (m)], 3.93 (s, 3H), two protons of conformers [3.78-3.60 (m, J = 4, 18 Hz), 3.54 (dd, J = 4, 18 Hz)], three protons of conformers [3.31 (s), 3.29 (s)], six protons of conformers [2.93 (s), 2.91 (s), 2.89 (s), 2.89 (s)], six protons of conformers [2.05 (s), 2.04 (s), 2.00 (s)], nine protons of conformers [1.47 (s), 1.47 (s)]; $^{13}$C NMR (500 MHz, CDCl$_3$) δ 169.7, 169.6, 168.9, 168.6, 167.7, 167.4, 167.3, 167.0, 166.4, 166.4, 156.1, 155.9, 155.8, 155.6, 155.3, 140.1, 140.1, 136.0, 135.8, 135.7, 131.4, 131.3, 131.0, 126.9, 126.9, 126.8, 126.8, 123.1, 123.0, 106.6, 106.4, 106.2, 106.0, 82.5, 82.3, 67.2, 67.0, 65.9, 65.5, 53.5, 52.4, 42.2, 42.1, 36.4, 36.3, 35.7, 35.6, 29.7, 28.0, 23.1, 14.6; HRMS (EI) m/z calcd for C$_{30}$H$_{39}$N$_3$O$_9$ 585.2686, found 585.2686.

Methyl 3’-(3-((((9H-Fluoren-9-yl)methoxy)carbonyl)amino)-N-methylpropanamido)-2-(benzyloxy)-2-oxoethoxy)-6-(2-(tert-butoxy)-2-oxoethoxy)-2’-methyl-[1,1’-biphenyl]-4-carboxylate (38). To 0.20 g (0.37 mmol) of carboxylate 3 in 4.0 mL of CHCl$_3$ was added 0.15 g (0.55 mmol) of Fmoc-β-alanine acid chloride 37-int (generated according to General Procedure A and used immediately thereafter) in 2.5 mL of CHCl$_3$ followed by 4.0 mL of saturated NaHCO$_3$ solution, and the reaction mixture was stirred at room temperature for 20 min. The solution was diluted with CH$_2$Cl$_2$ (60 mL) and saturated NaHCO$_3$ solution (60 mL), the organic layer was extracted, and the aqueous layer was washed with CH$_2$Cl$_2$ (3 × 60 mL). The organics were dried over MgSO$_4$ and filtered, and the volatile components were removed under reduced
pressure to give a light brown crude oil. The oil was purified by flash chromatography (SiO₂, hexanes/ethyl acetate, elution gradient 4:1/2:1/1:1) to give 0.25 g (81.9%) of 38 as a white foam:

Rᵥ 0.14 (hexanes/ethyl acetate 3:2); IR (thin film, cm⁻¹) 3411, 2950, 1752, 1721, 1648, 1581, 1504, 1421, 1391, 1369, 1329, 1238, 1193, 1135, 1078, 999, 759, 739; ¹H NMR (500 MHz, CDCl₃) δ 7.77 (t, J = 7 Hz, 2H), 7.62 (d, J = 7 Hz, 1H), 7.59 (d, J = 7 Hz, 1H), 7.44-7.38 (m, 2H), 7.38-7.28 (m, 7H), 7.28-7.23 (m, 3H), 7.21 (d, J = 7 Hz, 1H), 7.16-7.11 (m, 1H), 5.80-5.72 (m, 1H), 5.22-5.09 (m, 2H), 4.71-4.60 (m, 2H), 4.57-4.44 (m, 2H), 4.34 (d, J = 8 Hz, 1H), 4.32 (d, J = 8 Hz, 1H), 4.21 (quartet, J = 8 Hz, 1H), three protons of conformers [3.94 (s), 3.94 (s)], 3.52-3.40 (m, 2H), three protons of conformers [3.29 (s), 3.26 (s)], 2.39-2.19 (m, 2H), three protons of conformers [1.98 (s), 1.97 (s)], nine protons of conformers [1.47 (s), 1.45 (s)]; ¹³C NMR (300 MHz, CDCl₃) δ 172.2, 168.1, 167.9, 167.4, 167.2, 166.1, 156.3, 155.9, 155.7, 144.1, 142.0, 141.3, 135.4, 135.1, 130.9, 130.8, 128.6, 126.6, 128.5, 128.4, 127.6, 127.0, 126.7, 125.2, 123.6, 120.0, 106.7, 106.5, 106.2, 106.1, 82.5, 82.4, 77.7, 77.5, 77.3, 76.9, 67.0, 66.6, 65.9, 65.8, 65.3, 52.4, 47.2, 37.1, 35.9, 34.2, 28.0, 14.5; HRMS (Q-TOF) m/z calcd for [M+Na]⁺ C₄₉H₆₀N₂O₁₁Na 865.3312, found 865.3262.

Methyl 3′-(3-acetamido-N-methylpropanamido)-2-(2-(benzyloxy)-2-oxoethoxy)-6-(2-(tert-butoxy)-2-oxoethoxy)-2′-methyl-[1,1′-biphenyl]-4-carboxylate (39). To 0.231 g (0.27 mmol) of carboxylate 38 dissolved in CH₂Cl₂ (0.83 mL) was added 0.37 mL (2.47 mmol) of 1,8-diazabicyclo[5.4.0]undec-7-ene, and the reaction mixture was stirred at room temperature for 20
min. The volatile components were removed under reduced pressure, and the residue was dissolved in CH₂Cl₂ (2.11 mL). Then 0.05 mL (0.55 mmol) of acetic anhydride and 0.04 mL (0.55 mmol) of pyridine were added, and the solution was stirred at room temperature for 30 min. The volatile components were removed under reduced pressure; methanol (5.0 mL) and water (5.0 mL) were added to the residue, upon which a white solid formed and dissolved back into solution. The aqueous solution was extracted with ethyl acetate (3 × 15 mL); the organics were dried over MgSO₄ and filtered, and the volatile components were removed under reduced pressure to give a yellow crude oil. The oil was purified by flash chromatography (SiO₂, hexanes/ethyl acetate/methanol elution gradient 1:1:0/0:1:0/9:1/0:4:1) to give 0.091 g (50%) of 39 as a white foam: Rᶠ 0.18 (100% ethyl acetate); IR (thin film, cm⁻¹) 3404, 2951, 1750, 1723, 1652, 1581, 1531, 1422, 1392, 1369, 1329, 1235, 1192, 1135, 1029, 997, 845, 807, 770, 733, 699, 452; ¹H NMR (500 MHz, CDCl₃) δ 7.46-7.19 (m, 9H), 7.11 (app ddd, J = 1, 4, 8 Hz, 1H), one proton of conformers [6.54 (broad s), 6.01 (broad s)], 5.24-5.16 (m, 2H), 4.69 (app s, 2H), 4.54-4.49 (m, 2H), three protons of conformers [3.97 (s), 3.93 (s), 3.93 (s)], 3.54-3.38 (m, 2H), three protons of conformers [3.30 (s), 3.27 (s), 3.24 (s)], 2.31-2.14 (m, 2H), six protons of conformers [1.98 (s), 1.98 (s), 1.96 (s), 1.95 (s), 1.94 (s)], nine protons of conformers [1.50 (s), 1.48 (s), 1.47 (s)]; ¹³C NMR (300 MHz, CDCl₃) δ 172.6, 169.8, 168.1, 168.0, 167.3, 167.2, 166.1, 155.8, 155.7, 155.6, 141.8, 135.3, 135.1, 135.0, 130.9, 128.6, 128.5, 128.4, 128.3, 127.0, 126.7, 123.5, 106.6, 106.4, 106.1, 106.0, 82.5, 82.4, 67.0, 65.9, 65.8, 65.3, 65.2, 52.4, 36.3, 35.9, 35.2, 33.8, 28.0, 28.0, 23.3, 15.45, 14.4; HRMS (Q-TOF) m/z calcd for [M+Na]⁺ C₃₆H₄₂N₂O₁₀Na 685.2737, found 685.2714.
2-((3’-(3-Acetamido-N-methylpropanamido)-6-(2-(tert-butoxy)-2-oxoethoxy)-4-(methoxy carbonyl)-2’-methyl-[1,1’-biphenyl]-2-yl)oxy)acetic acid (40). To 0.07 g (0.11 mmol) of carboxylate 39 and 0.008 g of 10% Pd/C in a round-bottom flask was added 1.26 mL of a 9:1 MeOH/H₂O solution. The flask was sealed with a septum; a balloon attached to a syringe needle and filled with hydrogen gas was inserted. The reaction mixture was stirred at room temperature for 4 h, after which the solution was filtered through a pad of Celite and further eluted with ethyl acetate (150 mL). The volatile components were removed under reduced pressure to give 0.060 g (99.7%) of 40 as an off-white foam: R<sub>f</sub> 0.09 (100% ethyl acetate); IR (thin film, cm⁻¹) 3385, 2932, 2361, 1750, 1723, 1652, 1581, 1436, 1421, 1393, 1369, 1329, 1236, 1135, 1028, 997; <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>) δ 7.37-7.20 (m, 4H), 7.12 (d, <i>J</i> = 7 Hz, 1H), one proton of conformers [6.89 (broad s), 6.60 (broad s), 6.02 (broad s)], 4.67-4.45 (m, 4H), three protons of conformers [3.97 (s), 3.96 (s)], 3.68-3.56 (m, 1H), 3.34-3.22 (m, 4H), two protons of conformers [2.58-2.49 (m), 2.40-2.31 (m), 2.21-2.14 (m)], six protons of conformers [1.99 (s), 1.98 (s), 1.96 (s), 1.95 (s)], nine protons of conformers [1.49 (s), 1.47 (s)]; <sup>13</sup>C NMR (300 MHz, CDCl<sub>3</sub>) δ 172.7, 172.5, 172.0, 169.7, 167.4, 167.3, 166.2, 155.9, 155.8, 155.6, 141.7, 135.5, 135.3, 131.7, 131.6, 131.1, 131.0, 128.2, 127.7, 126.9, 126.7, 123.7, 123.4, 108.6, 107.7, 106.9, 106.7, 106.3, 82.8, 82.5, 82.4, 68.9, 66.2, 65.8, 53.4, 52.5, 52.4, 37.4, 36.3, 35.9, 35.6, 35.2, 33.7, 32.9, 28.0, 23.2, 23.0, 15.4, 14.5; HRMS (Q-TOF) <i>m/z</i> calcd for [M+Na]<sup>+</sup> C<sub>29</sub>H<sub>36</sub>N<sub>2</sub>O<sub>10</sub>Na 595.2268, found 595.2229.
Methyl-3'-(3-acetamido-N-methylpropanamido)-2-(2-(tert-butoxy)-2-oxoethoxy)-6-(2-(dimethylamino)-2-oxoethoxy)-2'-methyl-[1,1'-biphenyl]-4-carboxylate (41). To a round-bottom flask equipped with a condenser and nitrogen gas inlet were added 0.040 g (0.07 mmol) of acid 40, 0.008 g (0.07 mmol) of dimethylamine hydrochloride, 0.013 g (0.07 mmol) of N-ethyl-N'(3-dimethylaminopropyl)carbodiimide hydrochloride, 0.009 g (0.07 mmol) of N-hydroxybenzotriazole, 0.03 mL (0.23 mmol) of triethylamine, and acetonitrile (1.73 mL). The reaction mixture was stirred at room temperature for 80 h, and the volatile components were removed under reduced pressure to give a crude brown solid. The solid was purified by flash chromatography (SiO$_2$, hexanes/ethyl acetate/methanol, 1:1:0/0:1:0/0:9:1) to give 0.031 g (73.6%) of 41 as a colorless oil: R$_f$ 0.11 (100% ethyl acetate); IR (thin film, cm$^{-1}$) 3324, 2931, 1749, 1722, 1654, 1580, 1421, 1391, 1369, 1325, 1237, 1128, 996, 844, 808, 733; $^1$H NMR (500 MHz, CDCl$_3$) $\delta$ 7.32-7.24 (m, 3H), 7.20 (app dd, $J = 1$, 6 Hz, 1H), 7.11 (app dd, $J = 1$, 6 Hz, 1H), one proton of conformers [6.75 (app t, $J = 6$ Hz), 6.65 (app t, $J = 6$ Hz)], 4.74-4.66 (m, 2H), 4.56-4.47 (m, 2H), 3.94 (s, 3H), 3.54-3.37 (m, 2H), three protons of conformers [3.25 (s), 3.24 (s)], six protons of conformers [2.92 (s), 2.92 (s), 2.87 (s)], 2.31-2.10 (m, 2H), six protons of conformers [1.99 (s), 1.95 (s), 1.94 (s)], nine protons of conformers [1.46 (s), 1.46 (s)]; $^{13}$C NMR (500 MHz, CDCl$_3$) $\delta$ 172.7, 172.3, 170.4, 170.2, 167.4, 167.3, 167.1, 167.0, 166.4, 166.3, 156.0, 155.9, 155.7, 141.7, 135.6, 135.5, 135.3, 135.3, 131.0, 131.0, 130.9, 126.9, 126.9, 126.7, 126.5, 125.2, 123.3, 123.3, 118.2, 110.6, 106.7, 106.5, 106.4, 106.3, 82.5, 82.5, 67.2, 66.8, 65.9, 65.8,
52.4, 36.3, 36.2, 36.0, 35.9, 35.7, 35.3, 35.3, 33.7, 29.7, 28.0, 28.0, 23.3, 23.3, 14.5; HRMS (El) m/z calcd for C_{31}H_{41}N_{3}O_{9} 599.2843, found 599.2847.

Methyl 3’-((S)-2-(((9H-Fluoren-9-yl)methoxy)carbonyl)amino)-N-methylpropanamido)-2-(2-((benzyloxy)-2-oxoethyl)amino)-2-oxoethoxy)-6-(2-(tert-butoxy)-2-oxoethoxy)-2’-methyl-[1,1’-biphenyl]-4-carboxylate (42). A solution of 0.040 g (0.053 mmol) of acid 33 and 0.011 g (0.053 mmol) of glycine benzyl ester hydrochloride in CH₂Cl₂/DMF (3:1, 0.28 mL) was treated sequentially at 0 °C with 0.005 g (0.053 mmol) of NaHCO₃, 0.008 g (0.058 mmol) of N-hydroxybenzotriazole, and 0.011 g (0.058 mmol) of N-ethyl-N’-(3-dimethylaminopropyl) carbodiimide hydrochloride. The reaction mixture was stirred at 0 °C for 2 h and at room temperature for 16 h, and then quenched with H₂O (1.0 mL). The aqueous layer was extracted with ethyl acetate (3 × 5 mL), and the combined organic layers were washed with H₂O (7 mL) and brine (7 mL), dried over MgSO₄ and filtered, and the volatile components were removed under reduced pressure to give a yellow crude oil. The oil was purified by flash chromatography (SiO₂, hexanes/ethyl acetate, 1:1/0:1) to give 0.047 g (99%) of 42 as a yellow oil/foam: Rf 0.74 (100% ethyl acetate); IR (thin film, cm⁻¹) 3408, 2929, 1749, 1721, 1657, 1580, 1531, 1450, 1419, 1392, 1369, 1238, 1193, 1130, 1029, 998, 738; ¹H NMR (300 MHz, CDCl₃) δ 7.86-7.70 (m, 3H), 7.70-7.50 (m, 2H), 7.48-7.13 (m, 13H), one proton of conformers [6.92 (app t), 6.50 (app t), 6.39 (app t, J = 5 Hz)], one proton of conformers [5.79 (d, J = 8 Hz), 5.73 (d, J = 8 Hz), 5.64 (d, J = 8 Hz), 5.56 (d, J = 9 Hz)], 5.16-5.00 (m, 2H), 4.72-4.02 (m, 10H), 4.01-3.90 (m,
3H), three protons of conformers [3.43 (s), 3.29 (s), 3.28 (s), 3.27 (s), 3.23 (s)], three protons of conformers [2.07 (s), 2.04 (s), 2.03 (s), 2.01 (s), 1.93 (s), 1.90 (s), 1.89 (s)], nine protons of conformers [1.46 (s), 1.45 (s), 1.33 (s)], three protons of conformers [1.21 (d, J = 7 Hz), 1.15 (d, J = 7 Hz), 1.11 (d, J = 7 Hz), 1.05 (d, J = 7 Hz)]; 13C NMR (500 MHz, CDCl₃) δ 173.5, 173.2, 169.1, 169.0, 168.8, 168.6, 167.9, 167.8, 167.2, 167.2, 166.1, 156.2, 155.8, 155.7, 155.6, 155.2, 155.0, 144.0, 143.9, 143.8, 143.8, 143.7, 141.4, 141.3, 141.2, 140.7, 136.0, 135.8, 135.5, 135.4, 135.3, 135.1, 135.0, 131.5, 131.3, 130.7, 130.5, 130.1, 128.7, 128.7, 128.6, 128.5, 128.5, 128.4, 128.3, 128.2, 128.1, 127.7, 127.3, 127.1, 126.9, 126.8, 126.6, 126.5, 126.5, 126.5, 126.3, 126.2, 124.0, 123.1, 120.1, 120.0, 117.0, 111.3, 107.0, 106.7, 82.7, 82.6, 82.2, 68.7, 67.6, 67.6, 67.3, 67.2, 67.0, 66.9, 66.9, 66.8, 65.9, 65.4, 52.6, 52.5, 47.9, 47.4, 47.3, 47.3, 47.1, 47.1, 47.0, 40.9, 40.7, 40.7, 36.8, 36.6, 36.5, 29.7, 28.0, 27.9, 19.7, 19.5, 18.0, 14.9, 14.8, 14.5; \([\alpha]^{25}_D = +22.4, c = 1.00, \text{CHCl}_3\).

Methyl 3'-(2-(((9H-Fluoren-9-yl)methoxy)carbonyl)amino)-N-methylacetamido)-2-(2-((benzyloxy)-2-oxoethyl)amino)-2-oxoethoxy)-6-(2-((tert-butoxy)-2-oxoethoxy)-2'-methyl-[1,1'-biphenyl]-4-carboxylate (43). A solution of 0.042 g (0.057 mmol) of acid 34 and 0.012 g (0.057 mmol) of glycine benzyl ester hydrochloride in CH₂Cl₂/DMF (3:1, 0.30 mL) was treated sequentially at 0 °C with 0.005 g (0.057 mmol) of NaHCO₃, 0.009 g (0.063 mmol) of N-hydroxybenzotriazole, and 0.012 g (0.063 mmol) of N-ethyl-N'-((3-dimethylaminopropyl)-carbodiimide hydrochloride. The reaction mixture was stirred at 0 °C for 2 h and at room temperature for 16 h, and then quenched with H₂O (1.0 mL). The aqueous layer was extracted
with ethyl acetate (3 x 5 mL), and the combined organic layers were washed with H₂O (7 mL) and brine (7 mL), dried over MgSO₄ and filtered, and the volatile components were removed under reduced pressure to give a yellow crude oil. The oil was purified by flash chromatography (SiO₂, hexanes/ethyl acetate, 1:1/0:1) to give 0.047 g (93.2%) of 43 as a yellow oil/foam: Rf 0.60 (100% ethyl acetate); IR (thin film, cm⁻¹) 3408, 3065, 2930, 1748, 1721, 1667, 1581, 1529, 1420, 1393, 1369, 1328, 1239, 1193, 1132, 1059, 999, 917, 844, 806, 738; ¹H NMR (300 MHz, CDCl₃) δ 7.85-7.71 (m, 3H), 7.63-7.51 (m, 3H), 7.46-7.10 (m, 13H), one proton of conformers [6.51 (t, J = 5 Hz), 6.45 (t, J = 5 Hz)], one proton of conformers [5.74 (t, J = 5 Hz), 5.63 (t, J = 5 Hz)], 5.15 (s, 1H), one proton of conformers [5.07 (d, J = 12 Hz), 5.01 (d, J = 12 Hz)], 4.64-4.42 (m, 4H), 4.40-3.91 (m, 9H), two protons of conformers [3.69 (quartet of doublets, J = 5, 18 Hz), 3.45 (dd, J = 5, 18 Hz)], three protons of conformers [3.33 (s), 3.31 (s), 3.27 (s)], three protons of conformers [2.03 (s), 2.00 (s), 1.90 (s)], nine protons of conformers [1.47 (s), 1.46 (s), 1.40 (s)]; ¹³C NMR (500 MHz, CDCl₃) δ 169.2, 169.0, 168.8, 167.8, 167.4, 167.2, 166.0, 157.8, 156.2, 156.1, 155.9, 155.8, 155.1, 154.9, 143.8, 141.5, 141.2, 140.7, 140.7, 140.6, 135.7, 135.5, 135.4, 135.3, 135.2, 135.1, 134.8, 131.4, 131.0, 130.9, 130.9, 130.1, 129.4, 129.1, 128.7, 128.6, 128.6, 128.4, 128.3, 127.8, 127.6, 127.3, 127.3, 127.4, 127.1, 126.7, 126.6, 125.2, 125.2, 124.4, 123.2, 120.4, 120.1, 120.0, 116.8, 111.5, 107.0, 106.9, 90.6, 82.7, 82.6, 82.5, 67.7, 67.6, 67.3, 67.2, 67.1, 65.9, 65.7, 65.6, 64.7, 52.6, 47.1, 47.0, 43.4, 43.1, 40.9, 40.7, 36.4, 36.3, 29.7, 28.0, 28.0, 14.6, 14.5, 14.5.

2-Bromo-N,N-dimethylacetamide (62). To 1.05 mL (12.7 mmol) of bromoacetyl chloride 65 and 1.25 g (15.2 mmol) of dimethylamine hydrochloride in a round-bottom flask equipped with a condenser and nitrogen gas inlet were added CH₂Cl₂ (72.2 mL) and 4.4 mL (33.3 mmol) of
triethylamine, and the mixture was stirred for 2 h at room temperature. The mixture was washed with 2.0 M HCl (40 mL), 2.0 M NaOH (40 mL) and brine (40 mL); the organic layer was dried over MgSO$_4$ and filtered, and the volatile components were removed under reduced pressure to give 0.82 g (38.6%) of 62 as a yellow oil: R$_f$ 0.52 (hexanes/ethyl acetate, 4:1); IR (thin film, cm$^{-1}$) 3419, 2945, 1736, 1649, 1503, 1467, 1405, 1259, 1201, 1148, 1081, 1024, 980, 931, 794, 750; $^1$H NMR (300 MHz, CDCl$_3$) δ 4.08 (s, 2H), 3.10 (s, 3H), 2.99 (s, 3H); $^{13}$C NMR (300 MHz, CDCl$_3$) δ 166.4, 41.2, 37.6, 35.9; HRMS (Q-TOF) m/z calcd for [M+Na]$^+$ C$_{13}$H$_8$NOBrNa 187.9687, found 187.9660.

Methyl-4-bromo-3-(2-(tert-butoxy)-2-oxoethoxy)-5-(2-(dimethylamino)-2-oxoethoxy)benzoate (46). In a round-bottom flask, 1.08 g (3.0 mmol) of benzoate 10, 0.55 g (3.3 mmol) of dimethylacetamide 62, and 0.5 g (3.6 mmol) of K$_2$CO$_3$ were dissolved in acetone (15.0 mL), and the reaction mixture was refluxed for 18 h. The volatile components were removed under reduced pressure, and the residue was diluted with ethyl acetate (30 mL) and water (30 mL); the organic layer was extracted, and the aqueous layer was washed with additional ethyl acetate (3 × 30 mL). The organic layers were dried over MgSO$_4$ and filtered, and the volatile components were removed under reduced pressure to give a crude yellow oil. The oil was purified by flash chromatography (SiO$_2$, hexanes/ethyl acetate elution gradient 7:1/4:1/2:1/0:1) to give 1.54 g (78.8%) of 46 as a white solid: R$_f$ 0.13 (hexanes/ethyl acetate, 1:1); mp 110-112 °C; IR (thin film, cm$^{-1}$) 3480, 2980, 2951, 1750, 1723, 1667, 1586, 1501, 1423, 1368, 1332, 1242, 1158, 1127, 1064, 1026, 845, 808, 761; $^1$H NMR (400 MHz, CDCl$_3$) δ 7.15 (s, 1H), 7.03 (s, 1H), 4.74 (s, 2H), 4.57 (s, 2H), 3.80 (s, 3H), 3.02 (s, 3H), 2.88 (s, 3H), 1.40 (s, 9H); $^{13}$C NMR (400 MHz,
CDCl$_3$ δ 166.9, 166.6, 165.8, 155.7, 155.5, 130.0, 107.6, 107.1, 106.9, 82.7, 68.0, 66.4, 52.4, 36.6, 35.6, 27.9; HRMS (Q-TOF) m/z calcd for [M+Na]$^+$ C$_{18}$H$_{24}$NO$_7$BrNa 468.0634, found 468.0623.

Methyl-2-(2-(tert-butoxy)-2-oxoethoxy)-6-(2-(dimethylamino)-2-oxoethoxy)-2'-methyl-3'-(methylamino)-[1,1'-biphenyl]-4-carboxylate (45). In a resealable oven-dried Schlenk flask containing a magnetic stir bar, 1.45 g (3.25 mmol) of benzoate 46, 1.21 g (4.88 mmol) of boronic ester 5, 0.12 g (0.13 mmol) of tris(dibenzylideneacetone)dipalladium(0), 0.21 g (0.52 mmol) of 2-dicyclohexylphosphino-2′,6′-dimethoxybiphenyl (SPhos ligand), and 2.25 g (9.76 mmol) of K$_3$PO$_4$ monohydrate were added and dried under reduced pressure for 2 min. Toluene (6.5 mL) was added, and the solution was degassed using the freeze-pump-thaw method three times under a nitrogen atmosphere. The Schlenk flask was sealed; the reaction mixture was vigorously stirred at 90 °C for 16 h, and cooled to room temperature. The mixture was diluted with CH$_2$Cl$_2$ (20 mL) and water (20 mL); the organic layer was extracted, and the aqueous layer was washed with additional CH$_2$Cl$_2$ (3 × 20 mL). The organic layers were dried over MgSO$_4$ and filtered, and the volatile components were removed under reduced pressure to give a brown crude oil. The oil was purified by flash chromatography (SiO$_2$, hexanes/ethyl acetate elution gradient 12:1/10:1/5:1) to give 0.38 g (24.3%) of 45 as an orange oil: R$_f$ 0.33 (100% ethyl acetate); IR (thin film, cm$^{-1}$) 3583, 3431, 2979, 2931, 2815, 1750, 1721, 1665, 1587, 1493, 1421, 1368, 1350, 1324, 1235, 1160, 1127, 1077, 1019, 996, 754, 723; $^1$H NMR (400 MHz, CDCl$_3$) δ 7.36 (d, $J = 1$ Hz, 1H),
7.20 (s, 1H), 7.18 (d, J = 8 Hz, 1H), 6.64 (t, J = 8 Hz, 2H), 4.64 (s, 2H), 4.49 (s, 2H), 3.93 (s, 3H), 3.68 (broad s, 1H), 2.94 (s, 3H), 2.89 (s, 3H), 2.78 (s, 3H), 1.91 (s, 3H), 1.46 (s, 9H); $^{13}$C NMR (400 MHz, CDCl$_3$) δ 167.8, 167.4, 166.5, 156.3, 156.1, 147.0, 133.0, 130.3, 125.9, 125.6, 121.4, 119.3, 108.6, 107.4, 106.7, 82.3, 68.4, 66.0, 52.3, 36.4, 35.7, 31.0, 28.0, 14.0; HRMS (Q-TOF) m/z calcd for [M+H]$^+$ C$_{26}$H$_{35}$N$_2$O$_7$ 487.2444, found 487.2446.

![Chemical structure of the compound](image)

Methyl 3'-(2-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)-N-methylacetamido)-2-(2-(tert-butoxy)-2-oxoethoxy)-6-(2-(dimethylamino)-2-oxoethoxy)-2'-methyl-[1,1'-biphenyl]-4-carboxylate (63). To 0.38 g (0.79 mmol) of carboxylate 45 in 5.0 mL of CHCl$_3$ was added 0.27 g (0.87 mmol) of Fmoc-glycine acid chloride 21-int (generated according to General Procedure A and used immediately thereafter) in 3.0 mL of CHCl$_3$ followed by 3.0 mL of saturated NaHCO$_3$ solution, and the reaction mixture was stirred at room temperature for 20 min. The solution was diluted with CH$_2$Cl$_2$ (20 mL) and saturated NaHCO$_3$ solution (20 mL), the organic layer was extracted, and the aqueous layer was washed with CH$_2$Cl$_2$ (3 × 20 mL). The organics were dried over MgSO$_4$ and filtered, and the volatile components were removed under reduced pressure to give a light brown crude oil. The oil was purified by flash chromatography (SiO$_2$, hexanes/ethyl acetate elution gradient 2:3/1:10) to give 0.28 g (82.8%) of 63 as a white gel/foam: $R_f$ 0.38 (100% ethyl acetate); IR (thin film, cm$^{-1}$) 3410, 2947, 1721, 1663, 1580, 1502, 1421, 1368, 1325, 1238, 1128, 1056, 997, 756; $^1$H NMR (500 MHz, CDCl$_3$) δ 7.72 (d, J = 7 Hz, 2H), 7.58 (broad s, 2H), 7.37-7.13 (m, 9H), one proton of conformers [5.86 (broad s), 5.79 (broad s)],
4.72-4.46 (m, 4H), 4.32-4.25 (m, 2H), 4.20-4.18 (m, 1H), 3.89 (s, 3H), 3.70-3.54 (m, 2H), three protons of conformers [3.29 (s), 3.27 (s)], six protons of conformers [2.87 (s), 2.82 (s), 2.81 (s), 2.78 (s)], three protons of conformers [2.06 (s), 2.05 (s)], nine protons of conformers [1.45 (s), 1.39 (s)]; $^{13}$C NMR (500 MHz, CDCl$_3$) δ 168.6, 167.3, 167.1, 166.9, 166.3, 166.2, 156.2, 156.1, 156.0, 155.8, 144.0, 143.9, 141.2, 140.4, 136.0, 135.8, 135.8, 131.3, 131.1, 127.6, 127.6, 127.0, 127.0, 126.8, 126.8, 125.2, 125.1, 125.1, 123.4, 119.9, 119.9, 106.9, 106.5, 106.4, 82.3, 82.2, 67.4, 67.2, 67.0, 66.1, 65.8, 52.2, 47.2, 43.3, 36.2, 36.2, 35.5, 28.0, 28.0, 14.5; HRMS (Q-TOF) m/z calcd for [M+H]$^+$ C$_{43}$H$_{48}$N$_3$O$_{10}$ 766.3340, found 766.3338.

Methyl-3’-(2-acetamido-N-methylacetamido)-2-(2-(tert-butoxy)-2-oxoethoxy)-6-(2-(dimethylamino)-2-oxoethoxy)-2’-methyl-[1,1’-biphenyl]-4-carboxylate (64). To 0.27 g (0.36 mmol) of carboxylate 63 in a round-bottom flask were added 0.48 mL (3.22 mmol) of 1,8-diazabicyclo[5.4.0]undec-7-ene and DMF (1.08 mL), and the reaction mixture was stirred at room temperature for 30 min. Then 0.07 mL (0.72 mmol) of acetic anhydride and 0.06 mL (0.72 mmol) of pyridine were added, and the solution was stirred at room temperature for 30 min. The volatile components were removed under reduced pressure to give a brown crude oil. The oil was purified by flash chromatography (SiO$_2$, hexanes/ethyl acetate/methanol elution gradient 5:1:0/1:1:0/1:10:0/0:1:0/9:1) to give 0.18 g (87.5%) of 64 as a yellow gel/foam: R$_f$ 0.22 (100% ethyl acetate); IR (thin film, cm$^{-1}$) 3408, 2934, 1749, 1721, 1655, 1580, 1422, 1369, 1325, 1237, 1128, 1026, 996, 751; $^1$H NMR (400 MHz, CDCl$_3$) δ 7.29-7.25 (m, 3H), 7.17-7.11 (m, 2H), one
proton of conformers [6.62 (t, \( J = 4 \) Hz), 6.57 (t, \( J = 4 \) Hz)], four protons of conformers [4.75 (s); 4.71,4.63 (ABq, \( J_{AB} = 14 \) Hz); 4.60,4.49 (ABq, \( J_{AB} = 16 \) Hz); 4.52,4.47 (ABq, \( J_{AB} = 16 \) Hz)], 3.90 (s, 3H), 3.74-3.49 (m, \( J = 18 \) Hz, 4 Hz, 2H), three protons of conformers [3.27 (s), 3.25 (s)], six protons of conformers [2.89 (s), 2.87 (s), 2.86 (s)], three protons of conformers [2.02 (s), 2.00 (s), 1.96 (s)], 1.96 (s, 3H), nine protons of conformers [1.43 (s), 1.43 (s)]; \(^{13}\)C NMR (400 MHz, CDCl\(_3\)) \( \delta \) 169.8, 169.7, 168.9, 168.6, 167.4, 167.7, 167.4, 167.0, 166.4, 166.3, 156.1, 155.8, 155.8, 155.6, 140.1, 135.9, 135.9, 135.7, 135.7, 131.3, 131.3, 131.0, 130.9, 126.9, 126.9, 126.8, 126.7, 123.1, 123.0, 106.6, 106.4, 106.1, 106.0, 82.5, 82.2, 67.2, 67.0, 65.8, 65.5, 52.4, 42.2, 42.1, 36.5, 36.3, 36.3, 35.6, 35.6, 28.0, 23.0, 14.5; HRMS (Q-TOF) \( m/z \) calcd for [M+H]\(^+\) C\(_{30}\)H\(_{40}\)N\(_3\)O\(_9\) 586.2765, found 586.2770.

Methyl-3'-(2-acetamido-N-methylacetamido)-2-(2-(dimethylamino)-2-oxoethoxy)-2'-methyl-6-(2-(methylamino)-2-oxoethoxy)-[1,1'-biphenyl]-4-carboxylate (44). To a solution of 0.15 g (0.25 mmol) of carboxylate 64 in CH\(_2\)Cl\(_2\) (0.38 mL) at room temperature was added 0.38 mL of trifluoroacetic acid, and the reaction mixture was stirred for 2 h. The volatile components were removed under reduced pressure to give a brown crude oil. To this crude oil, in a round-bottom flask equipped with a condenser and nitrogen gas inlet, were added 0.018 g (0.27 mmol) of methylamine hydrochloride, 0.052 g (0.27 mmol) of \( N \)-ethyl-\( N' \)-(3-dimethylaminopropyl)-carbodiimide hydrochloride, 0.037 g (0.27 mmol) of \( N \)-hydroxybenzotriazole, 0.07 mL (0.37 mmol) of \( N \),\( N \)-diisopropylethylamine, and CH\(_2\)Cl\(_2\) (1.24 mL). The reaction mixture was stirred
at room temperature for 16 h, and then the reaction mixture was diluted with ethyl acetate (10 mL) and washed with 1.0 M HCl (3 mL) and saturated NaHCO₃ solution (3 mL). The organic layers were combined, dried over MgSO₄ and filtered, and the volatile components were removed under reduced pressure to give a reddish brown crude oil. The oil was purified by flash chromatography (SiO₂, hexanes/ethyl acetate/methanol elution gradient 1:9:0/0:1:0:9:1/0:4:1/0:0:1) to give 0.023 g (17.2%) of 44 as an off-white foam/gel: Rf 0.12 (ethyl acetate/methanol, 3:1); IR (thin film, cm⁻¹) 3359, 2923, 2853, 1723, 1658, 1462, 1377, 1260, 1120; ¹H NMR (400 MHz, CDCl₃) δ 7.40-7.23 (m, 4H), 7.22 (d, J = 8 Hz, 1H), one proton of conformers [6.52 (broad s), 6.47 (broad s)], one proton of conformers [6.21 (broad s), 6.08 (broad s), 5.89 (broad d, J = 4 Hz), four protons of conformers [4.83 (s), 4.77-4.73 (m), 4.56-4.44 (m)], 3.95 (s, 3H), two protons of conformers [3.78,3.55 (ABq of doublets, J = 18, 4 Hz), 3.70,3.57 (ABq of doublets, J = 18, 4 Hz)], three protons of conformers [3.37 (s), 3.30 (s), 3.28 (s)], six protons of conformers [2.94 (s), 2.92 (s), 2.91 (s)], three protons of conformers [2.73 (d, J = 5 Hz), 2.70 (d, J = 5 Hz)], six protons of conformers [2.08 (s), 2.04 (s), 2.01 (s), 2.00 (s), 1.91 (s)]; ¹³C NMR (400 MHz, CDCl₃) δ 169.9, 169.7, 168.6, 168.5, 168.1, 168.1, 167.0, 166.6, 166.2, 166.1, 156.2, 155.9, 155.4, 154.9, 140.7, 140.7, 136.1, 136.0, 135.9, 135.7, 131.7, 131.7, 131.0, 127.4, 127.2, 127.1, 127.0, 123.3, 123.1, 107.6, 107.4, 107.2, 107.1, 68.5, 68.1, 66.6, 66.4, 60.4, 53.5, 52.6, 42.1, 41.6, 36.4, 36.3, 36.1, 35.6, 35.6, 25.7, 25.7, 23.1, 23.0, 21.1, 14.6, 14.5, 14.2; HRMS (Q-TOF) m/z calcd for [M+H]⁺ C₂₇H₃₅N₄O₈ 543.2455, found 543.2450.
Methyl-3’-(2-((S)-2-acetamidopropanamido)-N-methylacetamido)-2-(2-(benzyl oxy)-2-oxo ethoxy)-6-(2-(tert-butoxy)-2-oxoethoxy)-2’-methyl-[1,1’-biphenyl]-4-carboxylate (66). To 1.33 g (1.61 mmol) of carboxylate 28 in a round-bottom flask were added 2.17 mL (14.5 mmol) of 1,8-diazabicyclo[5.4.0]undec-7-ene and DMF (4.83 mL), and the reaction mixture was stirred at room temperature for 30 min. The reaction mixture was purified by flash chromatography (SiO₂, ethyl acetate/methanol elution gradient 9:1/4:1/1:1) to give a crude oil of the free amine that was confirmed by ¹H NMR (300 MHz, CDCl₃) δ 7.33-7.09 (m, 10H), 5.16 (s, 2H), 4.65 (d, J = 10 Hz, 2H), 4.48 (d, J = 9 Hz, 2H), three protons of conformers [3.91 (s), 3.90 (s)], three protons of conformers [3.26 (s), 3.22 (s)], 3.14-3.06 (m, 2H), 2.39 (broad s, 2H), three protons of conformers [2.03 (s), 2.02 (s), 1.99 (s), 1.96 (s)], 1.44 (s, 9H). The crude product and 0.21 g (1.61 mmol) of N-acetyl alanine 18 were dissolved in CH₂Cl₂ (6.43 mL), and then 0.34 mL (1.93 mmol) of N,N-diisopropylethylamine was added via syringe. To avoid epimerization, 0.84 g (1.61 mmol) of (benzotriazol-1-yloxy)tripyrrolidinophosphonium hexafluorophosphate was then immediately added to the reaction mixture, and the solution was stirred at room temperature for 1 h. The volatile components were removed under reduced pressure to give a brown crude oil. The oil was purified by flash chromatography (SiO₂, hexanes/ethyl acetate/methanol elution gradient 1:9:0/0:1:0/0:9:1/0:4:1) to furnish a crude oil, which was dissolved in ethyl acetate (15 mL) and washed with saturated NH₄Cl solution (4 × 10 mL) to remove excess N,N-diisopropylethylamine and give 0.45 g (58.3%) of 66 as an off-white gel/foam: Rf 0.49 (ethyl acetate/methanol, 9:1); IR
(thin film, cm⁻¹) 3316, 2980, 2933, 1751, 1656, 1580, 1525, 1423, 1395, 1370, 1329, 1299, 1236, 1135, 1029, 997, 845, 805, 753; ¹H NMR (400 MHz, CDCl₃) δ 7.32-6.92 (m, 10H), 5.10-5.07 (m, 2H), 4.70-4.37 (m, 4H), three protons of conformers [3.85 (s), 3.83 (s)], 3.76-3.54 (m, 2H), 3.31-3.14 (m, 3H), 2.02-1.87 (m, 6H), 1.39 (s, 9H), 1.29-1.26 (m, 3H); ¹³C NMR (400 MHz, CDCl₃) δ 172.6, 170.8, 168.7, 168.4, 168.1, 167.5, 167.3, 167.2, 166.1, 155.9, 155.8, 155.5, 155.1, 140.5, 140.2, 135.6, 135.3, 135.1, 135.0, 131.3, 131.2, 130.9, 128.5, 128.4, 128.3, 127.3, 126.9, 126.5, 126.3, 123.4, 123.2, 106.8, 106.4, 106.0, 105.9, 82.5, 67.7, 66.9, 65.8, 65.7, 65.2, 55.1, 52.4, 52.1, 49.1, 41.9, 41.3, 36.6, 36.3, 36.1, 27.9, 22.9, 18.4, 16.9, 14.6, 14.4, 14.2; HRMS (Q-TOF) m/z calcd for [M+H]+ C₃₈H₄₆N₅O₁₁ 720.3132, found 720.3186. [α]D²⁵ = -10.9, c = 1.00, CHCl₃.

Methyl-3'-((S)-2-acetamidopropanamido)-N-methylacetamido-2-(2-(benzyloxy)-2-oxoethoxy)-2'-methyl-6-((S)-1-(methylamino)-1-oxopropan-2-yl)amino)-2-oxoethoxy)-[1,1'-biphenyl]-4-carboxylate (68). To a solution of 0.44 g (0.61 mmol) of carboxylate 66 in CH₂Cl₂ (0.93 mL) at room temperature was added 0.93 mL of trifluoroacetic acid, and the reaction mixture was stirred for 2 h. The volatile components were removed under reduced pressure to give a crude oil of the free amine that was confirmed by ¹H NMR (400 MHz, CDCl₃) δ 12.45 (broad s, 1H), 7.76 (broad s, 1H), 7.63 (broad s, 1H), 7.33-7.14 (m, 10H), 5.26-5.13 (m, 2H), 4.73-4.62 (m, 5H), 3.92 (s, 3H), 3.75-3.69 (m, 2H), 3.39-3.27 (m, 3H), 2.01 (broad s, 6H), 1.44-1.30 (m, 3H). A solution of this crude oil and 0.075 g (0.73 mmol) of N-methylated alanine 67 in CH₂Cl₂/DMF (1:1, 3.22 mL) was treated sequentially at 0 °C with 0.062 g (0.73 mmol) of
NaHCO₃, 0.099 g (0.73 mmol) of N-hydroxybenzotriazole, and 0.14 g (0.73 mmol) of N-ethyl-N’-(3-dimethylaminopropyl)carbodiimide hydrochloride. The reaction mixture was stirred at 0 °C for 2 h and at room temperature for 16 h, and then quenched with H₂O (2.0 mL). The aqueous layer was extracted with ethyl acetate (3 × 10 mL), and the combined organic layers were washed with H₂O (10 mL) and brine (10 mL), dried over MgSO₄ and filtered, and the volatile components were removed under reduced pressure to give a brown crude oil. The oil was purified by flash chromatography (SiO₂, hexanes/ethyl acetate/methanol elution gradient 1:1:0/1:9:0/0:1:0/9:1/0:8:1) to give 0.29 g (62%) of 68 as an off-white gel/foam: Rf 0.52 (ethyl acetate/methanol, 9:1); IR (thin film, cm⁻¹) 3303, 3073, 2940, 1657, 1535, 1420, 1329, 1243, 1203, 1135, 999, 803, 753; ¹H NMR (400 MHz, MeOD) δ 7.50-7.27 (m, 10H), 5.15 (d, J = 7 Hz, 2H), 4.76-4.72 (m, 2H), two protons of conformers [4.58 (s), 4.65-4.49 (m, J = 14 Hz)], 4.36-4.35 (m, 2H), three protons of conformers [3.92 (s), 3.90 (s)], 3.72-3.55 (m, 2H), three protons of conformers [3.33 (s), 3.27 (s), 3.24 (s), 3.18 (s)], three protons of conformers [2.74 (s), 2.73 (s), 2.69 (s)], six protons of conformers [2.05 (s), 2.01 (broad s)], six protons of conformers [1.35 (app t, J = 6 Hz), 1.28 (d, J = 7 Hz), 1.22 (app t, J = 6 Hz)]; ¹³C NMR (400 MHz, MeOD) δ 174.2, 174.1, 174.0, 173.8, 173.4, 173.3, 173.2, 172.5, 171.8, 169.2, 169.1, 168.6, 168.4, 168.4, 166.3, 166.2, 161.9, 161.5, 155.8, 155.8, 155.7, 155.5, 140.9, 140.9, 135.6, 135.6, 135.6, 135.5, 135.4, 135.4, 131.2, 131.2, 131.1, 131.0, 130.9, 128.2, 128.1, 128.0, 128.0, 127.1, 127.0, 126.5, 125.8, 123.5, 123.5, 123.4, 118.3, 117.1, 115.4, 110.4, 107.1, 107.0, 106.9, 106.4, 106.3, 72.3, 67.4, 67.3, 67.2, 66.5, 65.0, 64.8, 62.9, 53.6, 51.8, 51.4, 49.8, 49.3, 41.4, 35.5, 35.4, 35.4, 25.2, 25.1, 25.0, 21.3, 21.1, 17.4, 16.6, 16.6, 16.6, 16.4, 16.1, 13.6; HRMS (Q-TOF) m/z calcd for [M+Na]⁺ C₃⁸H₄₅N₅O₁₁Na 770.3013, found 770.3008. [α]D²⁵ = -9.97, c = 1.00, MeOH.
2-((3′-(2-((S)-2-Azetidopropanamido)-N-methylacetamido)-4-(methoxycarbonyl)-2′-methyl-6-((S)-1-(methylamino)-1-oxopropan-2-yl)amino)-2-oxoethoxy)-[1,1′-biphenyl]-2-yl)oxy)acetic acid (49). To 0.28 g (0.37 mmol) of carboxylate 68 and 0.028 g of 10% Pd/C in a round-bottom flask was added 4.4 mL of MeOH. The flask was sealed with a septum; a balloon attached to a syringe needle and filled with hydrogen gas was inserted. The reaction mixture was stirred at room temperature for 4 h, after which the solution was filtered through a pad of silica gel (SiO\textsubscript{2}) and further eluted with ethyl acetate (150 mL). The volatile components were removed under reduced pressure to give 0.23 g (91.8%) of 49 as a light brown gel/foam: R\textsubscript{f} 0.01 (ethyl acetate/methanol, 3:1); IR (thin film, cm\textsuperscript{-1}) 3314, 3090, 2938, 1656, 1544, 1419, 1329, 1248, 1205, 1134, 1000, 840, 802, 753, 722; \textsuperscript{1}H NMR (400 MHz, MeOD) δ 7.51-7.31 (m, 5H), 4.73-4.49 (m, 4H), 4.35-4.34 (m, 2H), 3.92 (s, 3H), 3.73-3.52 (m, 2H), three protons of conformers [3.27 (s), 3.25 (s), 3.24 (s)], three protons of conformers [2.73 (s), 2.69 (s)], 2.06 (s, 3H), 2.00 (s, 3H), 1.36-1.21 (m, 6H); \textsuperscript{13}C NMR (400 MHz, MeOD) δ 174.2, 173.4, 173.2, 172.0, 171.5, 169.3, 169.2, 168.5, 166.4, 161.6, 156.3, 155.9, 155.7, 155.6, 155.4, 140.9, 135.8, 135.7, 135.7, 135.5, 135.4, 131.1, 131.1, 128.2, 127.0, 126.5, 125.8, 123.5, 123.5, 123.4, 118.3, 117.1, 115.5, 110.5, 106.7, 106.4, 72.3, 67.4, 67.3, 67.2, 65.6, 64.9, 64.9, 62.9, 53.6, 51.8, 51.4, 49.8, 49.7, 49.3, 49.2, 41.4, 35.5, 35.5, 35.4, 25.3, 25.1, 25.1, 21.3, 21.1, 20.1, 18.4, 17.5, 17.4, 16.8, 16.7, 16.5, 16.4, 16.1, 13.6; HRMS (Q-TOF) m/z calcd for [M+H]\textsuperscript{+} C\textsubscript{31}H\textsubscript{40}N\textsubscript{5}O\textsubscript{11} 658.2724, found 658.2747. [α]\textsubscript{D}\textsuperscript{25}= -11.0, c = 1.00, MeOH.
Methyl-3'-(2-((S)-2-acetamidopropanamido)-N-methylacetamido)-2'-methyl-2-(2-((S)-1-(methylamino)-1-oxopropan-2-yl)amino)-2-oxoethoxy)-6-(2-(methylamino)-2-oxoethoxy)-[1,1'-biphenyl]-4-carboxylate (47). To a round-bottom flask equipped with a condenser and nitrogen gas inlet were added 0.12 g (0.18 mmol) of acid 49, 0.019 g (0.27 mmol) of methylamine hydrochloride, 0.053 g (0.27 mmol) of N-ethyl-N''-(3-dimethylaminopropyl)-carbodiimide hydrochloride, 0.037 g (0.27 mmol) of N-hydroxybenzotriazole, 0.06 mL (0.33 mmoL) of N,N-diisopropylethylamine, and CH$_2$Cl$_2$ (0.91 mL). The reaction mixture was stirred at room temperature for 16 h, and then the reaction mixture was diluted with ethyl acetate (10 mL) and washed with 1.0 M HCl (3 mL) and saturated NaHCO$_3$ solution (3 mL). The organic layers were combined, dried over MgSO$_4$ and filtered, and the volatile components were removed under reduced pressure to give a brown crude oil. The oil was purified by flash chromatography (SiO$_2$, two columns, hexanes/ethyl acetate elution gradient both 1:0/19:1/9:1) to give 0.007 g (5.7%) of 47 as a light brown gel: $R_f$ 0.39 (ethyl acetate/methanol, 3:1); IR (thin film, cm$^{-1}$) 3583, 3317, 2924, 2854, 1723, 1659, 1548, 1417, 1328, 1259, 1121, 1044, 800, 753; $^1$H NMR (400 MHz, MeOD) $\delta$ 7.45-7.35 (m, 5H), 4.63-4.55 (m, 4H), 4.41-4.30 (m, 2H), 3.98 (s, 3H), 3.69-3.55 (m, 2H), three protons of conformers [3.31 (s), 3.29 (s), 3.28 (s)], six protons of conformers [2.75 (s), 2.75 (s), 2.74 (s), 2.72 (s), 2.71 (s), 2.69 (s), 2.68 (s)], six protons of conformers [2.08 (s), 2.07 (s), 2.02 (s), 2.01 (s), 2.01 (s)], 1.39 (d, $J = 7$ Hz, 3H), 1.25 (d, $J = 6$ Hz, 3H); $^{13}$C NMR (700 MHz, MeOD) $\delta$ 173.8, 173.5, 173.2, 173.1, 171.7, 169.5, 169.4, 169.2,
Methyl-3'-((2-((S)-2-acetamidopropanamido)-N-methylacetamido)-2'-methyl-2-(2-((S)-4-methyl-1-(methylamino)-1-oxopentan-2-yl)amino)-2-oxoethoxy)-6-(2-((S)-1-(methyl amino)-1-oxopropan-2-yl)amino)-2-oxoethoxy)-[1,1'-biphenyl]-4-carboxylate (48). A solution of 0.10 g (0.15 mmol) of acid 49 and 0.026 g (0.18 mmol) of N-methylated leucine 69 in CH$_2$Cl$_2$/DMF (1:1, 0.80 mL) was treated sequentially at 0 °C with 0.015 g (0.18 mmol) of NaHCO$_3$, 0.025 g (0.18 mmol) of N-hydroxybenzotriazole, and 0.035 g (0.18 mmol) of N-ethyl-N'-(3-dimethylaminopropyl)carbodiimide hydrochloride. The reaction mixture was stirred at 0 °C for 2 h and at room temperature for 16 h, and then quenched with H$_2$O (2.0 mL). The aqueous layer was extracted with ethyl acetate (3 × 10 mL), and the combined organic layers were washed with H$_2$O (10 mL) and brine (10 mL), dried over MgSO$_4$ and filtered, and the volatile components were removed under reduced pressure to give a brown crude oil. The oil was purified by flash chromatography (SiO$_2$, two columns, ethyl acetate/methanol elution gradient both 1:0/9:1/8:1/7:1) to give 0.054 g (45.4%) of 48 as an off-white gel/foam: R$_f$ 0.32 (ethyl acetate/methanol, 3:1); IR (thin film, cm$^{-1}$) 3301, 3079, 2955, 1722, 1654, 1538, 1417, 1328,
1242, 1131, 805, 754; $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 7.48-6.89 (m, 9H), 6.60-6.46 (m, 1H), one proton of conformers [6.43 (d, $J = 8$ Hz), 6.26 (d, $J = 8$ Hz)], 4.57-4.36 (m, 7H), three protons of conformers [3.96 (s), 3.94 (s)], 3.69-3.62 (m, 2H), three protons of conformers [3.34 (s), 3.28 (s)], six protons of conformers [2.75 (s), 2.70 (s)], six protons of conformers [2.01 (s), 1.99 (s), 1.97 (s)], 1.47-1.42 (m, 2H), 1.35 (d, $J = 7$ Hz, 3H), 1.30-1.20 (m, 3H), 1.14 (t, $J = 7$ Hz, 1H), 0.87-0.79 (m, 6H); $^{13}$C NMR (400 MHz, CDCl$_3$) $\delta$ 172.9, 172.8, 178.8, 178.7, 172.7, 172.6, 172.4, 172.3, 172.1, 171.8, 171.7, 171.6, 171.2, 170.5, 170.4, 170.3, 168.7, 168.6, 168.5, 168.2, 167.7, 167.6, 167.4, 167.3, 167.2, 165.9, 156.1, 155.9, 155.9, 155.8, 154.8, 154.7, 154.7, 141.4, 141.3, 141.3, 135.6, 135.5, 135.5, 135.4, 135.3, 135.2, 135.2, 131.9, 131.8, 130.7, 130.5, 127.9, 127.8, 127.7, 127.6, 127.5, 126.9, 123.6, 123.6, 123.5, 123.4, 123.3, 108.5, 108.3, 108.2, 108.0, 107.4, 107.4, 107.3, 107.3, 72.3, 68.9, 68.4, 68.3, 67.5, 67.4, 64.1, 60.4, 53.5, 52.6, 51.4, 50.9, 50.8, 49.0, 49.0, 48.9, 48.8, 48.7, 48.5, 48.0, 44.1, 41.8, 41.8, 41.7, 41.6, 41.2, 41.0, 40.8, 37.0, 36.8, 36.3, 36.2, 36.1, 29.7, 29.3, 26.4, 26.2, 26.2, 26.1, 25.8, 24.9, 24.8, 24.7, 24.6, 24.5, 24.5, 24.0, 23.4, 23.4, 23.2, 23.1, 23.0, 22.9, 22.9, 22.8, 22.8, 22.7, 22.0, 22.0, 21.9, 21.4, 21.3, 21.2, 21.1, 19.1, 18.8, 18.6, 18.4, 18.4, 18.3, 18.1, 15.5, 15.4, 15.1, 14.7, 14.7, 14.2; HRMS (Q-TOF) m/z calcd for [M+H]$^+$ C$_{38}$H$_{54}$N$_7$O$_{11}$ 784.3881, found 784.3872. $[^{25}C]D$ $\alpha$ = +3.53, c = 1.00, MeOH.

(S)-Benzyl (1-(methylamino)-1-oxopropan-2-yl)carbamate (72). To a round-bottom flask equipped with a condenser and nitrogen gas inlet were added 3.0 g (13.4 mmol) of acid 70, 1.0 g (14.8 mmol) of methylamine hydrochloride, 2.8 g (14.8 mmol) of N-ethyl-N’-(3-dimethylaminopropyl)carbodiimide hydrochloride, 2.0 g (14.8 mmol) of N-hydroxybenzotriazole, 3.5 mL (20.2 mmol) of N,N-diisopropylethylamine, and CH$_2$Cl$_2$ (67.2
mL). The reaction mixture was stirred at room temperature for 16 h, and then the reaction mixture was diluted with ethyl acetate (20 mL) and washed with 1.0 M HCl (20 mL), saturated NaHCO₃ solution (20 mL) and brine (20 mL). The organic layers were combined, dried over MgSO₄ and filtered, and the volatile components were removed under reduced pressure to give 3.16 g (99.5%) of 72 as a white solid: Rf 0.04 (hexanes/ethyl acetate, 7:3); mp 127-129 °C; IR (thin film, cm⁻¹) 3299, 3092, 3035, 2980, 2940, 2805, 1654, 1539, 1453, 1411, 1375, 1327, 1247, 1163, 1121, 1067, 1029, 737; ¹H NMR (400 MHz, CDCl₃) δ 7.29 (broad s, 1H), 7.22 (s, 5H), 6.43 (d, J = 7 Hz, 1H), 5.02,4.95 (ABq, JAB = 12 Hz, 2H), 4.23 (broad t, J = 7 Hz, 1H), 2.64 (d, J = 5 Hz, 3H), 1.27 (d, J = 7 Hz, 3H); ¹³C NMR (400 MHz, CDCl₃) δ 173.1, 156.1, 136.2, 128.5, 128.2, 128.0, 67.0, 50.6, 26.2, 18.7; HRMS (Q-TOF) m/z calcd for [M+H]⁺ C₁₂H₁₇N₂O₃ 237.1239, found 237.1224. [α]D²⁵°C = -19.7, c = 1.00, CHCl₃.

(S)-Benzyl (4-methyl-1-(methylamino)-1-oxopentan-2-yl)carbamate (73). To a round-bottom flask equipped with a condenser and nitrogen gas inlet were added 1.57 g (5.90 mmol) of acid 71, 0.44 g (6.22 mmol) of methylamine hydrochloride, 1.24 g (6.49 mmol) of N-ethyl-N′-(3-dimethylaminopropyl)carbodiimide hydrochloride, 0.88 g (6.49 mmol) of N-hydroxybenzotriazole, 1.54 mL (8.85 mmol) of N,N-diisopropylethylamine, and CH₂Cl₂ (29.5 mL). The reaction mixture was stirred at room temperature for 16 h, and then the reaction mixture was diluted with ethyl acetate (20 mL) and washed with 1.0 M HCl (20 mL), saturated NaHCO₃ solution (20 mL) and brine (20 mL). The organic layers were combined, dried over MgSO₄ and filtered, and the volatile components were removed under reduced pressure to give 1.58 g (100%) of 73 as a white solid: Rf 0.09 (hexanes/ethyl acetate, 7:3); mp 124-126 °C; IR
(S)-2-Amino-N-methylpropanamide (67). To 3.1 g (13.1 mmol) of carboxylate 72 and 0.40 g of 10% Pd/C in a round-bottom flask was added 110.0 mL of MeOH. The flask was sealed with a septum; a balloon attached to a syringe needle and filled with hydrogen gas was inserted. The reaction mixture was stirred at room temperature for 24 h (the reaction was monitored by TLC for completion and the balloon was refilled with hydrogen gas after 12 h), after which the solution was filtered through a pad of silica gel (SiO$_2$) and further eluted with ethyl acetate (300 mL). The volatile components were removed under reduced pressure to give a crude oil. The oil was purified by flash chromatography (SiO$_2$, ethyl acetate/methanol elution gradient 1:0/9:1/1:1:0:1) to give 1.14 g (85.3%) of 67 as a yellow oil: R$_f$ 0.06 (ethyl acetate/methanol, 9:1); IR (thin film, cm$^{-1}$) 3350, 2979, 2160, 1655, 1562, 1453, 1413, 1374, 1318, 1270, 1161, 1041, 962, 849; $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 7.68 (broad s, 1H), 3.47 (q, $J = 7$ Hz, 1H), 2.73 (d, $J = 5$ Hz, 3H), 2.28 (broad s, 2H), 1.27 (d, $J = 7$ Hz, 3H); $^{13}$C NMR (400 MHz, CDCl$_3$) $\delta$ 176.3, 128.3, 50.4, 25.6, 21.3; HRMS (EI) m/z calcd for [M]$^+$ C$_{15}$H$_{23}$N$_2$O$_3$ 279.1709, found 279.1704. $[\alpha]_D^{25}$ = -25.2, c = 1.00, MeOH.
(S)-2-Amino-N,4-dimethylpentanamide (69). To 1.5 g (5.4 mmol) of carboxylate 73 and 0.15 g of 10% Pd/C in a round-bottom flask was added 64.2 mL of MeOH. The flask was sealed with a septum; a balloon attached to a syringe needle and filled with hydrogen gas was inserted. The reaction mixture was stirred at room temperature for 24 h (the reaction was monitored by TLC for completion and the balloon was refilled with hydrogen gas after 12 h), after which the solution was filtered through a pad of silica gel (SiO$_2$) and further eluted with ethyl acetate (300 mL). The volatile components were removed under reduced pressure to give a crude oil. The oil was purified by flash chromatography (SiO$_2$, hexanes/ethyl acetate/methanol elution gradient 1:1:0/0:1:0:9:1:1/0:1:0:1) to give 0.53 g (67.7%) of 69 as a yellow oil: R$_f$ 0.11 (ethyl acetate/methanol, 9:1); IR (thin film, cm$^{-1}$) 3295, 3088, 2956, 1655, 1468, 1411, 1368, 1267, 1161, 898, 845; $^1$H NMR (400 MHz, CDCl$_3$) δ 7.45 (broad s, 1H), 3.38 (d, $J = 9$ Hz, 1H), 2.75 (d, $J = 5$ Hz, 3H), 2.12 (broad s, 2H), 1.66 (m, 2H), 1.31 (t, $J = 9$ Hz, 1H), 0.91 (d, $J = 6$ Hz, 3H), 0.88 (d, $J = 6$ Hz, 3H); $^{13}$C NMR (400 MHz, CDCl$_3$) δ 175.9, 53.4, 43.9, 25.8, 24.8, 23.3, 21.4; HRMS (Q-TOF) $m/z$ calcd for [M+H]$^+$ C$_7$H$_{17}$N$_2$O 145.1341, found 145.1299. [α]$_D^{25}$ = +9.59, c = 1.00, MeOH.

Di-tert-butyl-2,2'-(4-(methoxycarbonyl)-2'-methyl-3'-(methylamino)-[1,1'-biphenyl]-2,6-diyl)bis(oxy))diacetate (58). In a resealable oven-dried Schlenk flask containing a magnetic stir bar, 5.11 g (10.7 mmol) of benzoate 11, 4.0 g (16.1 mmol) of boronic ester 5, 0.35 g (0.38 mmol)
of tris[dibenzylideneacetone]dipalladium(0), 0.62 g (1.50 mmol) of 2-dicyclohexylphosphino-2',6'-dimethoxybiphenyl (SPhos ligand), and 6.50 g (28.2 mmol) of K₃PO₄ monohydrate were added and dried under reduced pressure for 2 min. Toluene (18.8 mL) was added, and the solution was degassed using the freeze-pump-thaw method three times under a nitrogen atmosphere. The Schlenk flask was sealed; the reaction mixture was vigorously stirred at 90 °C for 16 h, and cooled to room temperature. The mixture was diluted with CH₂Cl₂ (40 mL) and water (40 mL); the organic layer was extracted, and the aqueous layer was washed with additional CH₂Cl₂ (3 × 40 mL). The organic layers were dried over MgSO₄ and filtered, and the volatile components were removed under reduced pressure to give a brown crude oil. The oil was purified by flash chromatography (SiO₂, hexanes/ethyl acetate elution gradient 9:1/6:1/5:1) to give 1.25 g (25.8%) of 58 as a brown oil: Rf 0.34 (hexanes/ethyl acetate, 7:3); IR (thin film, cm⁻¹) 3440, 2979, 2933, 2815, 1751, 1724, 1588, 1513, 1492, 1437, 1393, 1369, 1329, 1233, 1161, 1133, 1029, 997, 942, 844, 754, 723; ¹H NMR (400 MHz, CDCl₃) δ 7.24-7.21 (m, 3H), 6.69 (t, J = 8 Hz, 2H), 4.50 (s, 4H), 3.94 (s, 3H), 3.73 (broad s, 1H), 2.93 (s, 3H), 1.96 (s, 3H), 1.49 (s, 18H); ¹³C NMR (400 MHz, CDCl₃) δ 167.8, 166.5, 156.3, 147.1, 132.9, 129.9, 126.0, 125.4, 121.5, 119.3, 108.7, 106.6, 82.2, 66.1, 52.2, 30.9, 28.0, 14.1; HRMS (Q-TOF) m/z calcd for [M+H]⁺ C₂₈H₃₈NO₈ 516.2597, found 516.2597.
Di-tert-butyl-2,2'-(3'-(S)-2-(((9H-fluoren-9-yl)methoxy)carbonylamino)-N-methyl propanamido)-4-(methoxycarbonyl)-2'-methyl-[1,1'-biphenyl]-2,6-diyl)bis(oxy))diacetate (56). To 0.50 g (0.97 mmol) of carboxylate 58 in 11.0 mL of CHCl₃ was added 0.35 g (1.07 mmol) of Fmoc-alanine acid chloride 20-int (generated according to General Procedure A and used immediately thereafter) in 7.0 mL of CHCl₃ followed by 11.0 mL of saturated NaHCO₃ solution, and the reaction mixture was stirred at room temperature for 20 min. The solution was diluted with CH₂Cl₂ (50 mL) and saturated NaHCO₃ solution (50 mL), the organic layer was extracted, and the aqueous layer was washed with CH₂Cl₂ (3 × 50 mL). The organics were dried over MgSO₄ and filtered, and the volatile components were removed under reduced pressure to give a light brown crude oil. The oil was purified by flash chromatography (SiO₂, hexanes/ethyl acetate elution gradient 5:1/3:1) to give 0.66 g (84.1%) of 56 as a brown gel/foam: Rf 0.13 (hexanes/ethyl acetate, 7:3); IR (thin film, cm⁻¹) 3306, 2980, 1750, 1722, 1651, 1581, 1504, 1450, 1423, 1394, 1369, 1331, 1235, 1134, 1079, 1031, 998, 941, 844, 805, 757; ¹H NMR (400 MHz, CDCl₃) δ 7.71 (d, J = 7 Hz, 2H), 7.61 (broad s, 2H), 7.34-7.16 (m, 9H), one proton of conformers [6.23 (d, J = 8 Hz), 6.09 (d, J = 8 Hz), 5.90 [(broad s)], 4.70-4.50 (m, 4H), 4.36-4.32 (m, 2H), 4.21-4.16 (m, 2H), three protons of conformers [3.89 (s), 3.88 (s)], 3.29 (s, 3H), three protons of conformers [2.12 (s), 2.09 (s)], 18 protons of conformers [1.45 (s), 1.33 (s)], three protons of conformers [1.25 (d, J = 6 Hz), 1.17 (d, J = 6 Hz)]; ¹³C NMR (400 MHz, CDCl₃) δ 175.3, 173.6, 173.4, 168.0, 167.5, 167.4, 167.4, 166.2, 166.2, 156.2, 155.9, 155.8, 155.7, 155.1,
144.1, 144.0, 144.0, 143.9, 143.8, 141.2, 140.9, 136.1, 135.8, 135.7, 135.3, 131.4, 131.0, 130.8,
130.6, 127.7, 127.1, 126.9, 126.5, 125.3, 123.4, 123.1, 120.0, 106.2, 106.1, 82.4, 82.3, 81.9, 66.9,
66.8, 65.8, 65.5, 53.6, 52.3, 47.9, 47.7, 47.1, 36.8, 36.6, 28.0, 27.9, 21.0, 19.6, 18.7, 17.6,
15.0, 14.8, 14.2; HRMS (Q-TOF) \( m/z \) calcd for \([M+H]^+\) \( C_{46}H_{53}N_2O_{11} \) 809.3649, found
809.3668. \( \alpha \)\( ^{25}_D \) = +35.5, c = 1.00, CHCl₃.

![Chemical structure](image)

**Di-tert-butyl-2,2'-(3'-(S)-2-(S)-2-acetamidopropanamido)-N-methylpropanamido)-4-
(methoxycarbonyl)-2'-methyl-[1,1'-biphenyl]-2,6-diyl)bis(oxy))diacetate (74).** To 0.67 g (0.83 mmol) of carboxylate 56 in a round-bottom flask were added 5.0 mL (33.1 mmol) of tris(2-aminoethyl)amine and CH₂Cl₂ (5.0 mL); the reaction mixture was stirred at room temperature for 30 min and was monitored by TLC for verification of deprotection. Then ethyl acetate (15 mL) was added, and the organic layer was extracted with brine (10 mL). The organic layer was further extracted with phosphate buffer (pH = 5.5, 50 mM, 3 × 15 mL), then dried over MgSO₄ and filtered. The volatile components were removed under reduced pressure to give a crude oil of the free amine that was confirmed by \(^1\)H NMR (300 MHz, CDCl₃) δ 7.32-7-13 (m, 7H), 4.50-4.43 (m, 4H), 3.91 (s, 3H), one proton of conformers [3.49 (m), 3.32 (m)], three protons of conformers [3.14 (s), 3.12 (s)], 2.02 (s, 3H), 18 protons of conformers [1.44 (s), 1.43 (s)], three protons of conformers [1.12 (d, \( J = 7 \) Hz), 1.06 (d, \( J = 7 \) Hz)]. Then 0.44 g (0.74 mmol) of the crude free amine and 0.15 g (1.12 mmol) of N-acetyl alanine 18 were dissolved in CH₂Cl₂ (4.5 mL), and 0.24 mL (1.34 mmol) of N,N-diisopropylethylamine was added via syringe. To avoid
epimerization, 0.58 g (1.12 mmol) of (benzotriazol-1-yloxy)trippyrrolidinophosphonium hexafluorophosphate was then immediately added to the reaction mixture, and the solution was stirred at room temperature for 1 h. The volatile components were removed under reduced pressure to give a brown crude oil. The oil was purified by flash chromatography (SiO$_2$, hexanes/ethyl acetate/methanol elution gradient 1:9:0/1:0:9:1) to furnish a crude oil, which was dissolved in ethyl acetate (30 mL) and washed with saturated NH$_4$Cl solution ($4 \times 10$ mL) to remove excess $N,N$-diisopropylethylamine and give 0.33 g (82.5%) of 74 as an off-white gel/foam: $R_f$ 0.33 (ethyl acetate/methanol, 9:1); IR (thin film, cm$^{-1}$) 3303, 2980, 2935, 1751, 1725, 1647, 1580, 1538, 1424, 1394, 1370, 1330, 1234, 1133, 1029, 997, 845, 806, 754; $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ one proton of conformers [7.41 (d, $J = 6$ Hz), 7.34 (d, $J = 7$ Hz)], 7.29-7.09 (m, 5H), one proton of conformers [6.96 (broad s), 6.76 (d, $J = 6$ Hz)], 4.67-4.31 (m, 6H), 3.84 (s, 3H), three protons of conformers [3.23 (s), 3.19 (s)], three protons of conformers [2.00 (s), 1.95 (s)], 1.89 (s, 3H), 1.38 (s, 18H), three protons of conformers [1.31 (d, $J = 7$ Hz), 1.24 (d, $J = 7$ Hz)], three protons of conformers [1.12 (d, $J = 6$ Hz), 1.03 (d, $J = 6$ Hz)]; $^{13}$C NMR (400 MHz, CDCl$_3$) $\delta$ 173.0, 172.7, 171.9, 171.3, 170.0, 169.6, 168.1, 167.4, 167.4, 167.3, 166.2, 166.1, 156.0, 155.8, 155.7, 155.6, 140.8, 135.9, 135.7, 135.5, 135.1, 131.4, 130.8, 130.7, 130.5, 127.5, 126.9, 126.8, 126.4, 123.2, 123.0, 106.3, 106.2, 106.0, 106.0, 82.4, 82.3, 82.1, 65.9, 65.8, 65.8, 65.4, 60.3, 53.5, 52.3, 52.2, 48.8, 48.7, 46.4, 46.3, 36.8, 36.6, 28.0, 27.9, 27.9, 23.0, 22.9, 20.9, 19.1, 18.8, 17.0, 14.9, 14.6, 14.1; HRMS (Q-TOF) $m/z$ calcd for [M+H]$^+$ C$_{36}$H$_{50}$N$_3$O$_{11}$ 700.3445, found 700.3447. $[\alpha]_D^{25^\circ C} = +29.4$, c = 1.00, CHCl$_3$. 

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Methyl-3'-(((S)-2-((S)-2-acetamidopropanamido)-N-methylpropanamido)-2'-methyl-2,6-
bis(2-((S)-1-(methylamino)-1-oxopropan-2-yl)amino)-2-oxoethoxy)-[1,1'-biphenyl]-4-
carboxylate (50). To a solution of 0.29 g (0.41 mmol) of carboxylate 74 in CH₂Cl₂ (0.63 mL) at
room temperature was added 0.63 mL of trifluoroacetic acid, and the reaction mixture was stirred
for 2 h. The volatile components were removed under reduced pressure to give a crude oil of the
free acid that was confirmed by ¹H NMR (300 MHz, CDCl₃) δ 12.37 (broad s, 2H), one proton
of conformers [7.77 (s), 7.72 (d, J = 7 Hz)], one proton of conformers [7.53 (broad s), 7.43
(broad s), 7.38-7.18 (m, 5H), 4.71-4.58 (m, 5H), 4.44 (quintet, J = 8 Hz, 1H), 3.94 (s, 3H), three
protons of conformers [3.29 (s), 3.26 (s)], six protons of conformers [2.06 (s), 2.01 (s), 1.96 (s)],
six protons of conformers [1.38 (d, J = 7 Hz), 1.28 (m), 1.22 (d, J = 8 Hz)]. A solution of this
crude oil and 0.10 g (0.99 mmol) of N-methylated alanine 67 in CH₂Cl₂/DMF (1:1, 2.18 mL)
was treated sequentially at 0 °C with 0.084 g (0.99 mmoL) of NaHCO₃, 0.14 g (0.99 mmol) of
N-hydroxybenzotriazole, and 0.19 g (0.99 mmol) of N-ethyl-N'-((3-dimethylaminopropyl)
carbodiimide hydrochloride. The reaction mixture was stirred at 0 °C for 2 h and at room
temperature for 16 h, and then quenched with H₂O (4.0 mL). The aqueous layer was extracted
with ethyl acetate (3 × 20 mL), and the combined organic layers were washed with H₂O (10 mL)
and brine (10 mL), dried over MgSO₄ and filtered, and the volatile components were removed
under reduced pressure to give a brown crude oil. The oil was purified by flash chromatography
(SiO₂, two columns, ethyl acetate/methanol elution gradient, 9:1/4:1 then 8:1/7:1/6:1) to give
0.17 g (55.6%) of 50 as an off-white gel/foam: \( R_f \) 0.76 (ethyl acetate/methanol, 9:1); IR (thin film, cm\(^{-1}\)) 3304, 3075, 2986, 2936, 1721, 1654, 1535, 1417, 1374, 1327, 1244, 1131, 865, 753; \( ^1\)H NMR (300 MHz, MeOD) \( \delta \) 7.52-7.28 (m, 5H), 4.64-4.61 (m, 4H), 4.38-4.32 (m, 4H), 3.95 (s, 3H), three protons of conformers [3.29 (s), 3.23 (s)], six protons of conformers [2.73 (s), 2.70 (s)], six protons of conformers [2.06 (s), 2.03 (s), 2.00 (s), 1.97 (s)], 12 protons of conformers [1.50 (d, \( J = 7 \) Hz), 1.38 (d, \( J = 7 \) Hz), 1.30 (d, \( J = 7 \) Hz), 1.24 (d, \( J = 7 \) Hz), 1.12 (d, \( J = 7 \) Hz)]; \( ^{13}\)C NMR (400 MHz, MeOD) \( \delta \) 175.4, 173.6, 173.6, 173.4, 173.3, 173.2, 172.6, 171.7, 171.6, 170.3, 170.2, 169.2, 168.4, 168.3, 166.2, 156.0, 155.8, 155.6, 155.5, 141.8, 141.6, 141.6, 135.8, 135.5, 135.5, 135.5, 134.9, 131.3, 130.9, 130.6, 128.0, 127.4, 127.0, 126.9, 126.0, 125.3, 123.7, 117.3, 110.5, 107.5, 107.2, 67.8, 67.3, 63.1, 60.2, 51.9, 49.8, 49.1, 46.5, 46.0, 37.7, 36.2, 35.9, 25.4, 25.3, 25.2, 21.4, 21.4, 19.8, 18.2, 17.8, 17.7, 17.6, 17.5, 17.3, 16.9, 16.5, 15.7, 14.3, 14.0, 13.3; HRMS (Q-TOF) \( m/z \) calcd for [M+H]\(^+\) \( C_{36}H_{50}N_7O_{11} \) 756.3568, found 756.3617. \( [\alpha]_{D}^{25} = -2.48, c = 1.00, \text{MeOH} \).

**Di-tert-butyl-2,2'-((3'-((9H-fluoren-9-yl)methoxy)carbonyl)amino)-N-methyl acetamido)-4-(methoxycarbonyl)-2'-methyl-[1,1'-biphenyl]-2,6-diylbis(oxy))diacetate** (57).

To 0.50 g (0.97 mmol) of carboxylate 58 in 11.0 mL of CHCl\(_3\) was added 0.34 g (1.07 mmol) of Fmoc-glycine acid chloride 21-int (generated according to General Procedure A and used immediately thereafter) in 7.0 mL of CHCl\(_3\) followed by 11.0 mL of saturated NaHCO\(_3\) solution, and the reaction mixture was stirred at room temperature for 20 min. The solution was diluted
with CH$_2$Cl$_2$ (50 mL) and saturated NaHCO$_3$ solution (50 mL), the organic layer was extracted, and the aqueous layer was washed with CH$_2$Cl$_2$ (3 × 50 mL). The organics were dried over MgSO$_4$ and filtered, and the volatile components were removed under reduced pressure to give a light brown crude oil. The oil was purified by flash chromatography (SiO$_2$, hexanes/ethyl acetate elution gradient 3:1/1:1) to give 0.71 g (92%) of 57 as a light yellow gel/foam: $R_f$ 0.39 (hexanes/ethyl acetate 1:1); IR (thin film, cm$^{-1}$) 3413, 2978, 1750, 1724, 1664, 1581, 1512, 1423, 1394, 1369, 1330, 1234, 1133, 1056, 1030, 998, 845, 758; $^1$H NMR (300 MHz, CDCl$_3$) $\delta$ 7.73 (d, $J = 7$ Hz, 2H), 7.62 (d, $J = 7$ Hz, 2H), 7.39-7.12 (m, 9H), one proton of conformers [5.89 (broad s), 5.72 (broad s)], 4.64-4.49 (m, 4H), 4.39-4.18 (m, 3H), 3.91 (s, 3H), 3.66 (broad s, 2H), 3.31 (s, 3H), 2.10 (s, 3H), 18 protons of conformers [1.46 (s), 1.41 (s)]; $^{13}$C NMR (400 MHz, CDCl$_3$) $\delta$ 168.9, 167.5, 167.4, 166.2, 156.1, 156.0, 155.7, 144.0, 144.0, 141.2, 140.3, 135.9, 135.8, 131.3, 130.8, 127.7, 127.1, 126.9, 125.2, 125.2, 123.1, 119.9, 119.6, 106.2, 106.1, 82.4, 82.2, 67.0, 65.9, 65.7, 52.3, 47.2, 43.4, 36.2, 28.0, 28.0, 14.6; HRMS (Q-TOF) $m/z$ calcd for [M+Na]$^+$ C$_{45}$H$_{50}$O$_{11}$Na 817.3312, found 817.3322.

![Chemical Structure](image)

**Di-tert-butyl-2,2'-(3'-((3'-((S)-2-(((benzyloxy)carbonyl)amino)propanamido)-N-methyl acetamido)-4-(methoxycarbonyl))-2'-methyl-[1,1'-biphenyl]-2,6-diyl)bis(oxy))diacetate** (75).

To 0.60 g (0.75 mmol) of carboxylate 57 in a round-bottom flask were added 4.52 mL (29.9 mmol) of tris(2-aminoethyl)amine and CH$_2$Cl$_2$ (4.52 mL); the reaction mixture was stirred at room temperature for 30 min and was monitored by TLC for verification of deprotection. Then
ethyl acetate (15 mL) was added, and the organic layer was extracted with brine (10 mL). The organic layer was further extracted with phosphate buffer (pH = 5.5, 50 mM, 3 × 10 mL), then dried over MgSO₄ and filtered. The volatile components were removed under reduced pressure to give a crude oil of the free amine that was confirmed by ¹H NMR (400 MHz, MeOD) δ 7.40-7.22 (m, 5H), 4.59-4.54 (m, 4H), 3.94 (s, 3H), two protons of conformers [3.96-3.84 (m), 3.24-3.04 (m)], 3.26 (s, 3H), three protons of conformers [2.17 (s), 2.07 (s)], 18 protons of conformers [1.47 (s), 1.46 (s)]. Then 0.32 g (0.56 mmol) of crude free amine and 0.15 g (0.67 mmol) of acid 70 were dissolved in CH₂Cl₂ (3.35 mL), and then 0.15 mL (0.84 mmol) of N,N-diisopropylethylamine was added via syringe. To avoid epimerization, 0.35 g (0.67 mmol) of (benzotriazol-1-yloxy)tripyrrolidinophosphonium hexafluorophosphate was immediately added to the reaction mixture, and the solution was stirred at room temperature for 1 h. The volatile components were removed under reduced pressure to give a brown crude oil. The oil was purified by flash chromatography (SiO₂, hexanes/ethyl acetate/methanol elution gradient 1:1:0/1:9:0/0:1:0/9:1) to furnish a crude oil, which was dissolved in ethyl acetate (30 mL) and washed with saturated NH₄Cl solution (4 × 10 mL) to remove excess N,N-diisopropylethylamine and give 0.34 g (77.2%) of 75 as a light brown gel/foam: Rf 0.45 (ethyl acetate/methanol 9:1); IR (thin film, cm⁻¹) 3324, 2979, 1750, 1655, 1580, 1523, 1423, 1395, 1370, 1331, 1235, 1133, 1029, 998, 845, 753; ¹H NMR (400 MHz, CDCl₃) δ 7.29 (broad s, 6H), 7.20-7.13 (m, 4H), one proton of conformers [5.88 (broad d, J = 6 Hz), 5.84 (broad d, J = 6 Hz)], 5.14-5.01 (m, 2H), 4.58-4.47 (m, 4H), 4.35-4.26 (m, 1H), three protons of conformers [3.91 (s), 3.91 (s)], 3.76-3.55 (m, 2H), three protons of conformers [3.24 (s), 3.24 (s)], 2.02 (s, 3H), 18 protons of conformers [1.45 (s), 1.43 (s), 1.42 (s)], three protons of conformers [1.37 (d, J = 6 Hz), 1.34 (d, J = 6 Hz)]; ¹³C NMR (400 MHz, CDCl₃) δ 173.0, 172.9, 171.2, 169.1, 167.7, 167.6, 167.4,
(2S,2'S)-Dibenzyll 2,2'-(2,2'-(3'-2'-(2'-(benzyloxy)carbonyl)amino)propanamido)-N-methylacetamido)-4-(methoxycarbonyl)-2'-methyl-[1,1'-biphenyl]-2,6-diyl)bis(oxy)

bis(acetyl)bis(azanediyl)dipropanoate (76). To a solution of 0.30 g (0.39 mmol) of carboxylate 75 in CH₂Cl₂ (0.58 mL) at room temperature was added 0.58 mL of trifluoroacetic acid, and the reaction mixture was stirred for 2 h. The volatile components were removed under reduced pressure to give a crude oil of the free acid that was confirmed by ^1H NMR (400 MHz, CDCl₃) δ 10.30 (broad s, 2H), 7.74 (broad s, 1H), 7.43-7.15 (broad m, 9 H), 6.10 (broad s, 1H), 5.12-5.03 (broad m, 2H), 4.70-4.58 (broad m, 4H), 4.33 (broad s, 1H), 3.93 (s, 3H), 3.78 (broad m, 2H), 3.27 (s, 3H), 2.01 (s, 3H), 1.37 (s, 3H). A solution of this crude oil and 0.18 g (0.85 mmol) of L-alanine benzyl ester hydrochloride in CH₂Cl₂/DMF (1:1, 2.03 mL) was treated sequentially at 0 °C with 0.07 g (0.85 mmol) of NaHCO₃, 0.11 g (0.85 mmol) of N-hydroxybenzotriazole, and 0.16 g (0.85 mmol) of N-ethyl-N''-(3-dimethylaminopropyl)-carbodiimide hydrochloride. The reaction mixture was stirred at 0 °C for 2 h and at room temperature for 16 h, and then quenched with H₂O (2.0 mL). The aqueous layer was extracted with ethyl acetate (3 × 10 mL), and the combined organic layers were washed with H₂O (10 mL)
and brine (10 mL), dried over MgSO₄ and filtered, and the volatile components were removed under reduced pressure to give a brown crude oil. The oil was purified by flash chromatography (SiO₂, hexanes/ethyl acetate elution gradient 1:1/1:3/1:6) to give 0.29 g (78.4%) of 76 as an off-white gel/foam: Rf 0.53 (ethyl acetate/methanol, 9:1); IR (thin film, cm⁻¹) 3404, 3317, 2935, 2360, 1722, 1677, 1580, 1525, 1453, 1418, 1388, 1326, 1242, 1151, 1128, 1067, 1001, 807, 751; ¹H NMR (400 MHz, CDCl₃) δ 7.44, (d, J = 9 Hz), 7.37-7.19 (m, 20H), one proton of conformers [6.92 (d, J = 7 Hz), 6.72 (d, J = 7 Hz)], one proton of conformers [6.47 (d, J = 8 Hz), 6.37 (d, J = 8 Hz)], 5.92 (broad s, 1H), 5.16-4.98 (m, 6H), 4.54-4.51 (m, 6H), 4.31 (broad s, 1H), 3.91 (s, 3H), 3.74-3.55 (m, 2H), 2.82 (s, 3H), 1.98 (s, 3H), nine protons of conformers [1.33 (d, J = 7 Hz), 1.27 (d, J = 7 Hz), 1.22 (d, J = 7 Hz)], ³¹C NMR (400 MHz, CDCl₃) δ 172.5, 172.5, 172.2, 172.1, 172.0, 171.9, 171.9, 168.7, 168.4, 167.2, 167.1, 167.0, 166.9, 165.9, 165.8, 162.6, 155.9, 155.8, 155.6, 155.4, 155.2, 155.1, 154.9, 154.9, 141.2, 141.1, 136.4, 135.6, 135.5, 135.4, 135.3, 135.3, 134.8, 132.0, 131.9, 131.8, 130.6, 130.5, 129.4, 128.6, 128.6, 128.6, 128.5, 128.5, 128.5, 128.4, 128.3, 128.1, 128.0, 127.8, 127.6, 127.5, 126.7, 126.5, 123.6, 123.3, 123.2, 108.1, 107.8, 107.4, 68.1, 67.9, 67.5, 67.1, 67.0, 66.7, 53.6, 52.6, 50.4, 47.9, 47.7, 47.4, 47.3, 41.8, 41.7, 37.0, 36.8, 36.5, 36.3, 36.2, 31.4, 19.0, 18.9, 18.3, 18.2, 18.1, 18.1, 18.0, 17.2, 15.0, 14.9, 14.6, 14.5; HRMS (Q-TOF) m/z calcd for [M+Na]⁺ C₅₅H₅₇N₅O₁₄Na 1010.3800, found 1010.3802. [α]D²⁵°C = -4.23, c = 1.00, CHCl₃.
(2S,2′S)-2,2′-(((2,2′-((3′-((2-(2-((S)-N-2-Aminopropanamido))-N-methylacetamido))-4-(methoxy carbonyl))-2′-methyl-[1,1′-biphenyl]-2,6-diyl)bis(oxy))bis(acetyl))bis(azanediyl))dipropanoic acid (51). To 0.26 g (0.26 mmol) of carboxylate 76 and 0.078 g of 10% Pd/C in a round-bottom flask was added 3.13 mL of MeOH. The flask was sealed with a septum; a balloon attached to a syringe needle and filled with hydrogen gas was inserted. The reaction mixture was stirred at room temperature for 4 h, after which the solution was filtered through a pad of silica gel (SiO₂) and further eluted with ethyl acetate (250 mL). The volatile components were removed under reduced pressure to give 0.15 g (84.6%) of 51 as a light brown gel/foam: Rf 0.04 (ethyl acetate/methanol, 3:1); IR (thin film, cm⁻¹) 3400, 2925, 1996, 1721, 1656, 1578, 1416, 1326, 1253, 1125, 1005, 868, 844, 807, 746; ¹H NMR (400 MHz, MeOD) δ 7.45-7.36 (m, 5H), 4.65-4.52 (m, 4H), 4.38 (m, J = 4 Hz, 1H), 4.30 (m, J = 4 Hz, 1H), 4.04 (q, J = 7 Hz, 1H), 3.95 (s, 3H), 3.78-3.63 (m, 2H), three protons of conformers [3.28 (s), 3.26 (s)], three protons of conformers [2.06 (s), 2.05 (s)], 1.54 (dd, J = 7, 3 Hz, 3H), 1.34-1.28 (m, 6H); ¹³C NMR (400 MHz, MeOD) δ 175.0, 174.4, 174.2, 170.1, 170.0, 169.2, 169.0, 168.3, 168.2, 168.2, 168.1, 166.1, 166.1, 155.7, 155.5, 155.4, 141.1, 141.1, 135.5, 135.4, 135.3, 135.2, 131.6, 131.5, 130.9, 127.4, 123.7, 123.5, 107.4, 107.3, 67.5, 67.2, 67.2, 51.8, 48.9, 41.5, 41.4, 35.5, 17.1, 17.0, 16.2, 16.2, 13.6; HRMS (Q-TOF) m/z calcd for [M+H]+ C₃₁H₄₀N₅O₁₂ 674.2673, found 674.2725. 

[α]D²⁵°C = +11.7, c = 1.00, MeOH.
2,2'-(3'-(S)-2-Amino-N-methylpropanamido)-4-(methoxycarbonyl)-2'-methyl-[1,1'-biphenyl]-2,6-diylbis(oxy)diacetic acid (52). To 0.67 g (0.83 mmol) of carboxylate 56 in a round-bottom flask were added 5.0 mL (33.1 mmol) of tris(2-aminoethyl)amine and CH₂Cl₂ (5.0 mL); the reaction mixture was stirred at room temperature for 30 min and was monitored by TLC for verification of deprotection. Then ethyl acetate (15 mL) was added, and the organic layer was extracted with brine (10 mL). The organic layer was further extracted with phosphate buffer (pH = 5.5, 50 mM, 3 × 15 mL), then dried over MgSO₄ and filtered. The volatile components were removed under reduced pressure to give a crude oil of the free amine that was confirmed by ¹H NMR (300 MHz, CDCl₃) δ 7.32-7.13 (m, 7H), 4.50-4.43 (m, 4H), 3.91 (s, 3H), one proton of conformers [3.49 (m), 3.32 (m)], three protons of conformers [3.14 (s), 3.12 (s)], 2.02 (s, 3H), 18 protons of conformers [1.44 (s), 1.43 (s)], three protons of conformers [1.12 (d, J = 7 Hz), 1.06 (d, J = 7 Hz)]. To a solution of 0.10 g (0.17 mmol) of the crude free amine in CH₂Cl₂ (0.26 mL) at room temperature was added 0.26 mL of trifluoroacetic acid, and the reaction mixture was stirred for 2 h. The volatile components were removed under reduced pressure to give a brown crude oil. The oil was purified by flash chromatography (SiO₂, ethyl acetate/methanol elution gradient 9:1/4:1/2:1:1:1:4/0:1) to give 0.07 g (87%) of 52 as a light brown gel/foam: Rf 0.18 (ethyl acetate/methanol, 1:1); IR (thin film, cm⁻¹) 3381, 2922, 1662, 1412, 1202, 1125, 800; ¹H NMR (400 MHz, MeOD) δ 7.41-7.27 (m, 5H), 4.76-4.43 (m, 4H), 4.04-3.95 (m, 1H), three protons of conformers [3.94 (s), 3.92 (s), 3.91 (s), 3.90 (s)], three protons of conformers [3.34
(s), 3.34 (s), 3.34 (s), 3.33 (s), 3.3 (d), 3.30 (s), 3.26 (s)], three protons of conformers [2.09 (s), 2.05 (s)], three protons of conformers [1.38 (d, J = 7 Hz), 1.34 (d, J = 7 Hz), 1.22 (d, J = 7 Hz)]; $^{13}$C NMR (400 MHz, MeOD) δ 172.8, 172.6, 170.1, 169.9, 169.7, 169.4, 169.3, 169.2, 166.6, 166.6, 166.5, 156.7, 156.6, 156.5, 156.4, 156.3, 156.2, 155.8, 155.8, 155.5, 140.0, 140.0, 140.0, 139.9, 136.8, 136.6, 136.2, 136.0, 135.5, 135.4, 135.3, 135.3, 132.0, 131.8, 131.7, 131.6, 131.0, 130.9, 130.8, 127.3, 127.2, 127.0, 126.8, 126.7, 126.4, 123.2, 123.2, 123.1, 123.0, 107.0, 106.6, 106.5, 106.4, 106.2, 106.0, 105.9, 105.3, 64.7, 64.7, 64.6, 64.3, 53.5, 51.6, 51.6, 36.0, 35.8, 35.7, 16.1, 16.0, 14.5, 14.4, 14.1, 14.0, 13.7, 13.6, 13.1; HRMS (Q-TOF) m/z calcd for [M+H]$^+$ C$_{23}$H$_{27}$N$_2$O$_9$ 475.1717, found 475.1719. [$\alpha$]$^D_{25}$ = +21.8, c = 1.00, MeOH.

2,2'-(3'- (2-Amino-N-methylacetamido)-4-(methoxycarbonyl)-2'-methyl-[1,1'-biphenyl]-2,6-diyldiis(oxy))diacetic acid (53). To 0.60 g (0.75 mmol) of carboxylate 57 in a round-bottom flask were added 4.52 mL (29.9 mmol) of tris(2-aminoethyl)amine and CH$_2$Cl$_2$ (4.52 mL); the reaction mixture was stirred at room temperature for 30 min and was monitored by TLC for verification of deprotection. Then ethyl acetate (15 mL) was added, and the organic layer was extracted with brine (10 mL). The organic layer was further extracted with phosphate buffer (pH = 5.5, 50 mM, 3 × 10 mL), then dried over MgSO$_4$ and filtered. The volatile components were removed under reduced pressure to give a crude oil of the free amine that was confirmed by $^1$H NMR (400 MHz, MeOD) δ 7.40-7.22 (m, 5H), 4.59-4.54 (m, 4H), 3.94 (s, 3H), two protons of conformers [3.96-3.84 (m), 3.24-3.04 (m)], 3.26 (s, 3H), three protons of conformers [2.17 (s),
2.07 (s), 18 protons of conformers [1.47 (s), 1.46 (s)]. To a solution of 0.10 g (0.17 mmol) of the crude free amine in CH₂Cl₂ (0.27 mL) at room temperature was added 0.27 mL of trifluoroacetic acid, and the reaction mixture was stirred for 2 h. The volatile components were removed under reduced pressure to give a brown crude oil. The oil was purified by flash chromatography (SiO₂, ethyl acetate/methanol elution gradient 9:1/4:1/2:1/1/1/4:0:1) to give 0.072 g (89%) of 53 as a brown gel/foam: R_f 0.11 (ethyl acetate/methanol, 1:1); IR (thin film, cm⁻¹) 3583, 3458, 2921, 2851, 1664, 1199, 1125; ¹H (400 MHz, MeOD) δ 7.37-7.26 (m, 5H), 4.77-4.48 (m, 4H), three protons of conformers [3.94 (s), 3.93 (s)], 3.64-3.48 (m, 2H), three protons of conformers [3.38 (s), 3.34 (s), 3.34 (s), 3.34 (s), 3.33 (s), 3.31 (s), 3.31 (s)], three protons of conformers [2.05 (s), 2.04 (s), 1.99 (s)], ¹³C NMR (400 MHz, MeOD) δ 166.5, 166.4, 166.1, 156.3, 156.2, 156.1, 139.6, 136.2, 135.5, 132.0, 131.9, 131.0, 130.9, 126.7, 126.6, 123.1, 106.3, 106.0, 64.7, 51.6, 50.9, 40.5, 36.7, 35.1, 13.4, 13.1; HRMS (Q-TOF) m/z calcd for [M+H]⁺ C₂₂H₂₅N₂O₉ 461.1560, found 461.1569.

![Chemical structure of Di-tert-butyl-2,2'-(3'-(S)-2-acetamido-N-methylpropanamido)-4-(methoxycarbonyl)-2'-methyl-[1,1'-biphenyl]-2,6-diylbis(oxy))diacetate (77).]

To 0.050 g (0.062 mmol) of carboxylate 56 in a round-bottom flask was added 0.28 mL (0.56 mmol) of a 20% piperidine/DMF solution, and the reaction mixture was stirred at room temperature for 30 min. Then 0.94 mL (9.99 mmol) of acetic anhydride and 2.86 mL (35.4 mmol) of pyridine were added, and the solution was stirred at room temperature for 30 min. The volatile components
were removed under reduced pressure to give a brown crude oil. The oil was purified by flash chromatography (SiO$_2$, hexanes/ethyl acetate elution gradient 1:1/1:2/1:5/1:10) to give 0.035 g (89.9%) of 77 as a light brown gel/foam: $R_f$ 0.02 (hexanes/ethyl acetate, 7:3); IR (thin film, cm$^{-1}$) 3582, 3325, 2926, 1751, 1725, 1645, 1581, 1438, 1392, 1369, 1330, 1234, 1133, 1029, 998, 845, 754; $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 7.34-7.12 (m, 5H), one proton of conformers [6.62 (d, $J = 7$ Hz), 6.51 (d, $J = 8$ Hz), four protons of conformers [4.78,4.55 (ABq, $J_{AB} = 16$ Hz), 4.53-4.48 (m)], one proton of conformers [4.68 (quintet, $J = 7$ Hz), 4.40 (quintet, $J = 7$ Hz)], three protons of conformers [3.93 (s), 3.92 (s)], three protons of conformers [3.30 (s), 3.26 (s)], three protons of conformers [2.06 (s), 2.01 (s)], three protons of conformers [2.00 (s), 1.92 (s)], 18 protons of conformers [1.46 (s), 1.45 (s)], three protons of conformers [1.19 (d, $J = 6$ Hz), 1.07 (d, $J = 7$ Hz)]; $^{13}$C NMR (400 MHz, CDCl$_3$) $\delta$ 173.5, 173.1, 169.1, 168.4, 168.1, 167.5, 167.5, 167.4, 166.3, 166.3, 156.1, 155.8, 155.8, 155.6, 140.8, 140.7, 136.0, 135.8, 135.7, 135.2, 131.3, 130.9, 130.7, 130.6, 128.2, 127.6, 127.0, 126.8, 126.5, 123.3, 122.9, 106.2, 106.0, 82.5, 81.9, 65.9, 65.9, 65.8, 65.4, 52.4, 52.3, 46.6, 46.3, 36.9, 36.7, 29.7, 29.4, 28.0, 28.0, 24.5, 23.4, 23.3, 22.7, 19.4, 17.5, 15.0, 14.7, 14.2; HRMS (Q-TOF) m/z calcd for [M+Na]$^+$ C$_{33}$H$_{42}$N$_2$O$_{10}$Na 651.2894, found 651.2953. $[^{25}C]_D$ = +23.9, c = 1.00, CHCl$_3$.

**Di-tert-butyl-2,2'-(3'-(2-acetamido-N-methylacetamido)-4-(methoxycarbonyl)-2'-methyl-[1,1'-biphenyl]-2,6-diyl)bis(oxy))diacetate (78).** To 0.04 g (0.05 mmol) of carboxylate 57 in a round-bottom flask was added 0.22 mL (0.45 mmol) of a 20% piperidine/DMF solution, and the
reaction mixture was stirred at room temperature for 30 min. Then 0.77 mL (8.12 mmol) of acetic anhydride and 2.33 mL (28.8 mmol) of pyridine were added, and the solution was stirred at room temperature for 30 min. The volatile components were removed under reduced pressure to give a brown crude oil. The oil was purified by flash chromatography (SiO₂, hexanes/ethyl acetate elution gradient 1:5/1:20) to give 0.028 g (90.1%) of 78 as a light brown gel/foam: R_f 0.22 (hexanes/ethyl acetate, 1:1); IR (thin film, cm⁻¹) 3583, 3335, 2925, 2854, 1723, 1620, 1580, 1437, 127.0, 123.1, 106.1, 105.9, 82.5, 82.2, 65.9, 65.6, 52.3, 42.2, 36.3, 28.0, 23.1, 14.6; HRMS (Q-TOF) m/z calcd for [M+Na]+ C₃₂H₄₂N₂O₁₀Na 637.2737, found 637.2771.

2,2'-(3'-(S)-2-Acetamido-N-methylpropanamido)-4-(methoxycarbonyl)-2'-methyl-[1,1'-biphenyl]-2,6-diylbis(oxy))diacetic acid (54). To a solution of 0.03 g (0.05 mmol) of carboxylate 77 in CH₂Cl₂ (0.07 mL) at room temperature was added 0.07 mL of trifluoroacetic acid, and the reaction mixture was stirred for 2 h. The volatile components were removed under reduced pressure to give 0.032 g (99%) of 54 as a brown gel/foam: R_f 0.04 (ethyl acetate/methanol, 9:1); IR (thin film, cm⁻¹) 3583, 3335, 2925, 2854, 1723, 1620, 1580, 1437,
1326, 1237, 1214, 1028, 997, 756; $^1$H NMR (400 MHz, MeOD) $\delta$ 7.46 (m, 5H), 4.75-4.64 (m, 4H), one proton of conformers [4.45 (q, $J = 7$ Hz), 4.35 (quintet, $J = 7$ Hz)], 3.95 (s, 3H), three protons of conformers [3.25 (s), 3.22 (s)], three protons of conformers [2.07 (s), 2.04 (s)], three protons of conformers [1.97 (s), 1.91 (s)], three protons of conformers [1.23 (d, $J = 7$ Hz), 1.10 (d, $J = 7$ Hz)]; $^{13}$C NMR (400 MHz, MeOD) $\delta$ 173.9, 173.7, 171.3, 170.8, 170.7, 170.6, 170.4, 170.3, 169.3, 169.2, 166.5, 166.4, 156.3, 156.1, 156.0, 156.0, 141.1, 141.0, 135.9, 135.8, 134.9, 131.1, 130.8, 130.7, 130.7, 127.4, 126.8, 126.2, 126.1, 123.7, 106.0, 105.9, 64.8, 64.7, 64.6, 51.6, 51.2, 46.6, 46.5, 35.9, 35.8, 21.0, 20.8, 17.2, 15.3, 13.9, 13.7; HRMS (Q-TOF) $m/z$ calcd for [M+H]$^+$ C$_{25}$H$_{29}$N$_2$O$_{10}$ 517.1822, found 517.1823. $[^{13}$C$_{25}$]$^D$ = +31.1, $c$ = 1.00, MeOH.

![Chemical structure](attachment:chemical_structure.png)

2,2'-(3'-{(2-Acetamido-N-methylacetamido)-4-(methoxycarbonyl)-2'-methyl-[1,1'-biphenyl]-2,6-diyl)bis(oxy))diacetic acid (55). To a solution of 0.03 g (0.05 mmol) of carboxylate 78 in CH$_2$Cl$_2$ (0.08 mL) at room temperature was added 0.08 mL of trifluoroacetic acid, and the reaction mixture was stirred for 2 h. The volatile components were removed under reduced pressure to give 0.025 g (100%) of 55 as a brown gel/foam: $R_f$ 0.02 (ethyl acetate/methanol, 9:1); IR (thin film, cm$^{-1}$) 3326, 2928, 2361, 1722, 1626, 1580, 1435, 1325, 1240, 1141, 1029, 998, 865, 805, 757; $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 7.35-7.13 (m, 6H), four protons of conformers [4.71 (s), 4.56 (s)], 3.95 (s, 3H), 3.73 (q, $J = 17$ Hz, 2H), 3.28 (s, 3H), 2.04 (s, 3H), 1.98 (s, 3H); $^{13}$C NMR (400 MHz, CDCl$_3$) $\delta$ 172.4, 171.7, 169.9, 168.4, 166.3, 156.0, 155.5, 139.7, 136.0, 135.2, 131.6, 131.3, 127.2, 126.9, 123.8, 107.1, 106.7, 66.4, 64.8,
52.6, 42.4, 36.8, 29.7, 22.7, 14.2; HRMS (Q-TOF) \( m/z \) calcd for [M-H]+ \( \text{C}_{24}\text{H}_{25}\text{N}_{10} \) 501.1509, found 501.1508.

**tert-Butyl 4-bromo-3,5-dihydroxybenzoate (79).** To a solution of 5.25 g (14.2 mmol) benzoate 83 in THF (132.0 mL) was added 0.66 g (0.57 mmol) of catalytic tetrakis(triphenylphosphine)Pd(0). The yellow solution was stirred for 5 min at room temperature, and then 1.61 g (42.7 mmol) of sodium borohydride was added. After stirring at room temperature for 1 h, the reaction was quenched and acidified to pH 3-4 with dropwise addition of 1.0 M HCl; the solvent was then removed under reduced pressure. The residue was diluted with \( \text{CH}_2\text{Cl}_2 \) (110 mL) and water (100 mL); the organic layer was extracted, and the aqueous layer was washed with additional \( \text{CH}_2\text{Cl}_2 \) (3 × 100 mL). The organics were dried over \( \text{MgSO}_4 \) and filtered, and the volatile components were removed under reduced pressure to give a brown crude oil. The oil was purified by flash chromatography (SiO\(_2\), hexanes/ethyl acetate elution gradient 10:1/8:1) to give 3.37 g (81.9%) of 79 as a white solid: \( R_f \) 0.31 (hexanes/ethyl acetate 7:3); mp 160-162 °C; IR (thin film, \text{cm}^{-1}) 3391, 2980, 1680, 1593, 1503, 1426, 1369, 1279, 1159, 1122, 1040, 973, 869, 844, 758; \(^1\)H NMR (400 MHz, CDCl\(_3\)) \( \delta \) 7.29 (s, 2H), 6.32 (broad s, 2H), 1.60 (s, 9H); \(^13\)C NMR (400 MHz, CDCl\(_3\)) \( \delta \) 165.4, 153.3, 132.5, 108.8, 103.9, 82.2, 28.2; HRMS (Q-TOF) \( m/z \) calcd for [M+]\(^+\) \( \text{C}_{11}\text{H}_{13}\text{O}_6\text{Br} \) 287.9997, found 287.9966.

![Methyl 3,5-bis(allyloxy)-4-bromobenzoate (82).](image)

**Methyl 3,5-bis(allyloxy)-4-bromobenzoate (82).** In a round-bottom flask, 4.21 g (17.0 mmol) of benzoate 9, 3.26 mL (37.8 mmol) of allyl bromide, and 5.22 g (37.8 mmol) of K\(_2\)CO\(_3\) were
dissolved in acetone (85.8 mL), and the reaction mixture was refluxed for 18 h. The volatile components were removed under reduced pressure, and the residue was diluted with ethyl acetate (110 mL) and water (100 mL); the organic layer was extracted, and the aqueous layer was washed with additional ethyl acetate (3 × 100 mL). The organic layers were dried over MgSO₄ and filtered, and the volatile components were removed under reduced pressure to give 5.84 g (100%) of 82 as a white solid: Rf 0.53 (hexanes/ethyl acetate 7:3); mp 57-59 °C; IR (thin film, cm⁻¹) 3089, 2990, 2951, 1722, 1649, 1582, 1421, 1364, 1331, 1243, 1125, 1022, 997, 929, 860, 763; ¹H NMR (300 MHz, CDCl₃) δ 7.29 (s, 2H), 6.11 (doublet of quintets, J = 17, 5 Hz, 2H), 5.54 (doublet of ABq, J = 17, 2 Hz, 2H), 5.35 (app dd, J = 11, 2 Hz, 2H), 4.70 (dt, J = 5, 2 Hz, 4H), 3.94 (s, 3H); ¹³C NMR (300 MHz, CDCl₃) δ 166.2, 155.9, 132.3, 129.8, 117.8, 107.6, 106.7, 69.8, 52.4; HRMS (Q-TOF) m/z calcd for [M+Na]+ C₁₄H₁₅O₄BrNa 349.0051, found 349.0024.

**tert-Butyl 3,5-bis(allyloxy)-4-bromobenzoate (83).** In a round-bottom flask sealed with a septum, 0.30 g (0.87 mmol) of benzoate 82 and 0.12 mL (0.87 mmol) of tert-butyl acetate were combined. To the flask was added 0.009 mL (0.009 mmol) tert-butoxide (1M solution in THF) via syringe, and the reaction mixture was then stirred under reduced pressure (vacuum) for 5 min at room temperature. The flask was then backfilled with nitrogen and an additional equivalent each of tert-butyl acetate (0.12 mL) and tert-butoxide (0.009 mL) were added, and the reaction mixture was stirred under reduced pressure (vacuum) for 5 min at room temperature. The above procedure of additions was repeated ten times until the conversion of the methyl ester to the tert-butyl ester was complete by TLC. The reaction mixture was then passed through a pad of silica
gel (SiO$_2$) and washed with ethyl acetate (150 mL). The volatile components were removed under reduced pressure to give 0.31 g (96.4%) of 83 as a yellow oil: $R_f$ 0.64 (hexanes/ethyl acetate, 7:3); IR (thin film, cm$^{-1}$) 3399, 3088, 2979, 2932, 1712, 1650, 1582, 1456, 1421, 1368, 1331, 1252, 1163, 1125, 1037, 996, 929, 849, 766; $^1$H NMR (300 MHz, CDCl$_3$) $\delta$ 7.21 (s, 2H), 6.10 (doublet of quintets, $J = 17$, 5 Hz, 2H), 5.54 (doublet of ABq, $J_{AB} = 17$, 2 Hz, 1H), 5.35 (doublet of ABq, $J_{AB} = 11$, 1 Hz, 1H), 4.69 (dt, $J = 5$, 2 Hz, 4H), 1.62 (s, 9H); $^{13}$C NMR (300 MHz, CDCl$_3$) $\delta$ 164.9, 155.9, 132.5, 131.8, 117.8, 107.3, 106.8, 81.5, 69.9, 28.1; HRMS (Q-TOF) $m/z$ calcd for [M-C$_4$H$_8$]$^+$/C$_{13}$H$_{14}$O$_4$Br 313.0075, found 313.0045.

*tert*-Butyl 3-(allyloxy)-4-bromo-5-hydroxybenzoate (61). To a solution of 0.073 g (0.20 mmol) of benzoate 83 in THF (2.81 mL) was added 0.005 g (0.004 mmol) of catalytic tetrakis(triphenylphosphine)Pd(0). The yellow solution was stirred for 5 min at room temperature, and then 0.008 g (0.20 mmol) of sodium borohydride was added. After stirring at room temperature for 1 h, the reaction was quenched and acidified to pH 3-4 with dropwise addition of 1.0 M HCl; the solvent was then removed under reduced pressure. The residue was diluted with CH$_2$Cl$_2$ (15 mL) and water (10 mL); the organic layer was extracted, and the aqueous layer was washed with additional CH$_2$Cl$_2$ ($3 \times 15$ mL). The organics were dried over MgSO$_4$ and filtered, and the volatile components were removed under reduced pressure to give a brown crude oil. The oil was purified by flash chromatography (SiO$_2$, hexanes/ethyl acetate elution gradient 20:1/15:1) to give 0.031 g (48%) of 61 as a white solid: $R_f$ 0.54 (hexanes/ethyl acetate, 7:3); mp 100-102 °C; IR (thin film, cm$^{-1}$) 3388, 2979, 2932, 1711, 1687, 1587, 1496, 1457, 1425, 1369, 1352, 1255, 1161, 1118, 1084, 1037, 982, 928, 868, 847, 804, 768; $^1$H NMR
(300 MHz, CDCl₃) δ 7.31 (d, J = 1 Hz, 1H), 7.14 (d, J = 1 Hz, 1H), 6.09 (doublet of quintets, J = 17, 5 Hz, 1H), 5.85 (broad s, 1H), 5.52 (app dd, J = 16, 2 Hz, 1H), 5.37 (app dd, J = 11, 2 Hz, 1H), 4.67 (app d, J = 5 Hz, 2H), 1.61 (s, 9H); ¹³C NMR (400 MHz, CDCl₃) δ 165.1, 155.4, 153.4, 132.4, 132.3, 118.0, 109.7, 105.5, 105.3, 81.8, 69.9, 28.1; HRMS (Q-TOF) m/z calcd for [M-C₄H₈]⁺ C₁₀H₉O₄Br 271.9684, found 271.9659.

**tert-Butyl 3-(allyloxy)-5-(2-(benzyloxy)-2-oxoethoxy)-4-bromobenzoate (84).** In a round-bottom flask, 0.13 g (0.39 mmol) of benzoate 61, 0.08 mL (0.47 mmol) of benzyl bromoacetate, and 0.065 g (0.47 mmol) of K₂CO₃ were dissolved in acetone (2.0 mL), and the reaction mixture was refluxed for 18 h. The volatile components were removed under reduced pressure, and the residue was diluted with ethyl acetate (20 mL) and water (15 mL); the organic layer was extracted, and the aqueous layer was washed with additional ethyl acetate (3 × 20 mL). The organic layers were dried over MgSO₄ and filtered, and the volatile components were removed under reduced pressure to give a crude yellow oil. The oil was purified by flash chromatography (SiO₂, hexanes/ethyl acetate elution gradient 20:1/15:1) to give 0.18 g (96.3%) of 84 as a colorless oil: Rₚ 0.47 (hexanes/ethyl acetate, 7:3); IR (thin film, cm⁻¹) 3404, 3089, 2977, 2932, 1762, 1711, 1584, 1497, 1454, 1420, 1391, 1368, 1334, 1252, 1192, 1162, 1129, 1037, 963, 849, 764, 737; ¹H NMR (400 MHz, CDCl₃) δ 7.37 (s, 5H), 7.25 (d, J = 1 Hz, 1H), 7.11 (d, J = 1 Hz, 1H), 6.09 (doublet of quintets, J = 17, 5 Hz, 1H), 5.55 (dd, J = 17, 1 Hz, 1H), 5.36 (d, J = 10 Hz, 1H), 5.27 (s, 2H), 4.82 (s, 2H), 4.68 (d, J = 5 Hz, 2H), 1.60 (s, 9H); ¹³C NMR (400 MHz, CDCl₃) δ 167.9, 164.7, 156.2, 155.4, 135.1, 132.4, 132.0, 128.6, 128.5, 128.4, 118.0, 107.7, 107.5, 106.5,
81.7, 70.0, 67.1, 66.2, 28.1; HRMS (Q-TOF) m/z calcd for [M+Na]+ C_{23}H_{25}O_6BrNa 499.0732, found 499.0745.

*tert*-Butyl 3-(2-(benzylxy)-2-oxoethoxy)-4-bromo-5-hydroxybenzoate (85). To a solution of 0.16 g (0.33 mmol) of benzoate 84 in THF (4.7 mL) was added 0.011 g (0.01 mmol) of catalytic tetrakis(triphenylphosphine)Pd(0). The yellow solution was stirred for 5 min at room temperature, and then 0.019 g (0.49 mmol) of sodium borohydride was added. After stirring at room temperature for 2 h, the reaction was quenched and acidified to pH 3-4 with dropwise addition of 1.0 M HCl; the solvent was then removed under reduced pressure. The residue was diluted with CH_2Cl_2 (30 mL) and water (20 mL); the organic layer was extracted, and the aqueous layer was washed with additional CH_2Cl_2 (3 x 30 mL). The organics were dried over MgSO_4 and filtered, and the volatile components were removed under reduced pressure to give a brown crude oil. The oil was purified by flash chromatography (SiO_2, hexanes/ethyl acetate elution gradient 15:1/10:1) to give 0.12 g (84%) of 85 as a yellow solid: R_f 0.40 (hexanes/ethyl acetate, 7:3); mp 77-79 °C; IR (thin film, cm\(^{-1}\)) 3401, 3066, 3033, 2978, 2933, 1759, 1711, 1588, 1497, 1425, 1392, 1369, 1256, 1186, 1117, 1037, 999, 968, 872, 847, 803, 767; \(^1\)H NMR (400 MHz, CDCl_3) δ 7.38 (s, 5H), 7.36 (d, J = 2 Hz, 1H), 7.04 (d, J = 2 Hz, 1H), 6.11 (broad s, 1H), 5.27 (s, 2H), 4.82 (s, 2H), 1.60 (s, 9H); \(^{13}\)C NMR (400 MHz, CDCl_3) δ 167.9, 164.7, 154.7, 153.7, 135.0, 132.6, 128.7, 128.6, 128.4, 110.6, 105.4, 105.1, 81.8, 67.2, 66.1, 28.1; HRMS (Q-TOF) m/z calcd for [M+Na]+ C_{20}H_{21}O_6BrNa 459.0419, found 459.0413.
4-Nitrobenzyl 2-bromoacetate (86). To 0.33 g (2.4 mmol) of bromoacetic acid 88 and 0.31 g (2.0 mmol) of 4-nitrobenzyl alcohol 89 in a round-bottom flask were added 8.0 mL of a 1:1 CH₂Cl₂/CH₃CN solution and 0.01 g (0.03 mmol) of 4-(dimethylamino)pyridine. Then 0.50 g (2.4 mmol) of N,N'-dicyclohexylcarbodiimide was added, and the reaction was stirred at room temperature for 2 h. The reaction mixture was filtered to remove the white precipitate (dicyclohexyl urea), and the filtrate was washed with water (8.0 mL). The organic layers were separated and dried over MgSO₄ and filtered, and the volatile components were removed under reduced pressure to give a yellow crude oil. The oil was purified by flash chromatography (SiO₂, hexanes/ethyl acetate elution gradient 15:1/10:1/8:1) to give 0.34 g (62%) of 86 as a yellow oil: R_f 0.41 (hexanes/ethyl acetate, 7:3); IR (thin film, cm⁻¹) 3469, 3113, 3081, 2962, 2857, 2454, 2294, 1932, 1744, 1607, 1521, 1404, 1348, 1279, 1211, 1157, 1111, 1009, 887, 846, 807, 738; ¹H NMR (400 MHz, CDCl₃) δ 8.14 (d, J = 8 Hz, 2H), 7.52 (d, J = 8 Hz, 2H), 5.27 (s, 2H), 3.92 (s, 2H); ¹³C NMR (400 MHz, CDCl₃) δ 166.9, 147.7, 142.4, 128.4, 123.8, 66.2, 25.7; HRMS (Q-TOF) m/z calcd for [M+H]+ C₉H₉NO₄Br 273.9715, found 273.9724.

**tert-Butyl 3-(2-(benzyloxy)-2-oxoethoxy)-4-bromo-5-(2-((4-nitrobenzyl)oxy)-2-oxoethoxy) benzoate (60).** In a round-bottom flask, 0.40 g (0.91 mmol) of benzoate 85, 0.30 g (1.09 mmol) of 4-nitrobenzyl 2-bromoacetate 86, and 0.15 g (1.09 mmol) of K₂CO₃ were dissolved in acetone (4.57 mL), and the reaction mixture was refluxed for 20 h. The volatile components were removed under reduced pressure, and the residue was diluted with ethyl acetate (30 mL) and
water (20 mL); the organic layer was extracted, and the aqueous layer was washed with additional ethyl acetate (3 × 30 mL). The organic layers were dried over MgSO₄ and filtered, and the volatile components were removed under reduced pressure to give a crude yellow oil. The oil was purified by flash chromatography (SiO₂, two columns, hexanes/ethyl acetate elution gradient both 6:1/5:1) to give 0.55 g (96%) of 60 as a pale yellow oil: Rf 0.31 (hexanes/ethyl acetate, 7:3); IR (thin film, cm⁻¹) 3583, 3086, 2978, 1761, 1711, 1585, 1523, 1497, 1442, 1421, 1391, 1368, 1345, 1279, 1253, 1194, 1136, 1038, 849, 802, 763; ¹H NMR (400 MHz, CDCl₃) δ 8.18 (d, J = 7 Hz, 2H), 7.49 (d, J = 7 Hz, 2H), 7.36 (s, 5H), 7.12 (s, 2H), 5.34 (s, 2H), 5.26 (s, 2H), 4.87 (s, 2H), 4.82 (s, 2H), 1.56 (s, 9H); ¹³C NMR (400 MHz, CDCl₃) δ 167.8, 167.7, 164.2, 155.6, 155.4, 147.8, 142.3, 135.0, 132.2, 128.7, 128.6, 128.5, 128.4, 123.8, 107.6, 107.3, 107.1, 82.0, 67.2, 66.2, 66.1, 65.5, 28.1; HRMS (Q-TOF) m/z calcd for [M+Na]+ C₂₉H₂₈NO₁₀BrNa 652.0794, found 652.0785.

**tert-Butyl 2-(2-(benzylxy)-2-oxoethoxy)-2'-methyl-3'-(methylamino)-6-(2-((4-nitrobenzyl)oxy)-2-oxoethoxy)-[1,1'-biphenyl]-4-carboxylate (87).** In a resealable oven-dried Schlenk flask containing a magnetic stir bar, 1.41 g (2.24 mmol) of benzoate 60, 1.30 g (5.26 mmol) of boronic ester 5, 0.082 g (0.09 mmol) of tris[dibenzyldeneacetone]dipalladium(0), 0.15 g (0.36 mmol) of 2-dicyclohexylphosphino-2',6'-dimethoxybiphenyl (SPhos ligand), and 1.55 g (6.72 mmol) of K₃PO₄ monohydrate were added and dried under reduced pressure for 2 min. Toluene (7.47 mL) was added, and the solution was degassed using the freeze-pump-thaw method three times under
a nitrogen atmosphere. The Schlenk flask was sealed; the reaction mixture was vigorously stirred
at 90 °C for 16 h, and cooled to room temperature. The mixture was diluted with CH₂Cl₂ (50
mL) and water (50 mL); the organic layer was extracted, and the aqueous layer was washed with
additional CH₂Cl₂ (3 × 50 mL). The organic layers were dried over MgSO₄ and filtered, and the
volatile components were removed under reduced pressure to give a brown crude oil. The oil was
purified by flash chromatography (SiO₂, hexanes/ethyl acetate elution gradient 9:1/4:1/1:1) to
give 0.47 g (31.4%) of 87 as a brown oil: Rf 0.20 (hexanes/ethyl acetate, 7:3); IR (thin film, cm⁻¹)
3443, 2976, 2930, 2816, 1759, 1710, 1584, 1523, 1493, 1421, 1368, 1347, 1328, 1280, 1190,
1163, 1136, 1029, 993, 848, 788, 754; ¹H NMR (400 MHz, CDCl₃) δ 8.22 (d, J = 9 Hz, 2H),
7.40 (d, J = 9 Hz, 2H), 7.37-7.35 (m, 3H), 7.32-7.30 (m, 2H), 7.23-7.19 (m, 3H), 6.69 (d, J = 8
Hz, 1H), 6.64 (d, J = 8 Hz, 1H), 5.28 (s, 2H), 5.21 (s, 2H), 4.70 (s, 2H), 4.66 (s, 2H), 2.94 (s,
3H), 1.89 (s, 3H), 1.60 (s, 9H); ¹³C NMR (400 MHz, CDCl₃) δ 168.5, 168.4, 164.9, 156.1, 155.9,
147.7, 147.1, 142.4, 135.1, 132.6, 132.3, 128.6, 128.5, 128.3, 128.3, 126.1, 125.4, 123.9, 121.4,
119.3, 108.8, 106.9, 106.6, 81.5, 66.9, 65.6, 65.2, 65.5, 51.0, 18.2, 13.9; HRMS (Q-TOF) m/z
calcd for [M+Na]⁺ C₃₇H₃₈N₂O₁₀Na 693.2424, found 693.2432.

2-((6-(2-(Benzyloxy)-2-oxoethoxy)-4-(tert-butoxycarbonyl)-2'-methyl-3'-(methylamino)-
[1,1'-biphenyl]-2-yl)oxy)acetic acid (59). To a solution of 0.47 g (0.70 mmol) of biaryl 87 in
THF (1.9 mL) was added a total of 3 equiv of tetrabutylammonium fluoride (1.0 M in THF) at
room temperature at intervals of 1 equiv (0.84 mL) every 10-15 min. At every interval, the
reaction was monitored by TLC for the disappearance of the biaryl starting material. After the
third equiv, the reaction developed a burgundy-purple color, and the starting material had disappeared via TLC. The reaction mixture was then diluted with saturated NH₄Cl solution (5.0 mL) and ethyl acetate (20 mL); the aqueous layer was extracted, and the organic layer was washed with 5% NaHCO₃ solution (3 x 10 mL). The aqueous NaHCO₃ layers were acidified to pH 3-4 with dropwise addition of 1.0 M HCl, and extracted with ethyl acetate (3 x 20 mL). The organic layers were dried over MgSO₄ and filtered, and the volatile components were removed under reduced pressure to give a yellow crude oil. The oil was purified by flash chromatography (SiO₂, hexanes/ethyl acetate elution gradient 2:1/1:1:9/0:1) to give 0.11 g (29%) of 59 as a yellow oil: Rf 0.35 (ethyl acetate/methanol, 9:1); IR (thin film, cm⁻¹) 3583, 3435, 2928, 1758, 1710, 1580, 1520, 1368, 1247, 1192, 1135, 850, 789, 753; ¹H NMR (400 MHz, CD₃CN) δ 7.40-7.32 (m, 5H), 7.20 (s, 2H), 7.12 (s, J = 8 Hz, 1H), 6.60 (d, J = 8 Hz, 1H), 6.49 (d, J = 8 Hz, 1H), 5.18 (s, 2H), 4.70 (s, 2H), 4.61 (s, 2H), 2.88 (s, 3H), 1.80 (s, 3H), 1.60 (s, 9H); ¹³C NMR (400 MHz, CD₃CN) δ 169.2, 168.6, 164.7, 156.1, 156.0, 147.7, 135.8, 133.0, 132.3, 128.6, 128.3, 128.1, 125.9, 124.8, 121.3, 118.7, 108.2, 106.3, 106.1, 81.3, 66.5, 65.3, 64.9, 30.0, 27.3, 13.3; HRMS (Q-TOF) m/z calcd for [M-H]⁺ C₃₀H₃₂NO₈ 534.2128, found 534.2148.

2,2'-(4-(Methoxycarbonyl)-2'-methyl-3'-(methylamino)-[1,1'-biphenyl]-2,6-diyl)bis(oxy)) diacetic acid (90). To a solution of 0.03 g (0.06 mmol) of carboxylate 58 in CH₂Cl₂ (0.09 mL) at room temperature was added 0.09 mL of trifluoroacetic acid, and the reaction mixture was stirred for 2 h. The volatile components were removed under reduced pressure to give 0.023 g (100%) of 90 as a brown gel/foam: Rf 0.01 (ethyl acetate/methanol, 3:1); IR (thin film, cm⁻¹) 3409,
2924, 1718, 1669, 1580, 1420, 1327, 1241, 1200, 1137, 1028, 998, 864, 838, 798, 751, 721; $^1$H NMR (400 MHz, MeOD) δ 7.42-7.37 (m, 2H), 7.33-7.32 (m, 3H), 4.69, 4.64 (ABq, $J_{AB} = 17$ Hz, 4H), 3.95 (s, 3H), 3.10 (s, 3H), 2.23 (s, 3H); $^{13}$C NMR (400 MHz, MeOD) δ 170.4, 166.3, 156.1, 136.4, 136.3, 131.1, 130.2, 126.6, 122.7, 120.0, 106.0, 64.6, 53.4, 51.6, 35.3, 12.8; HRMS (Q-TOF) m/z calcd for [M-H]$^+$ C$_{29}$H$_{20}$NO$_8$ 402.1189, found 402.1173.
APPENDIX

1. Select EXSY and $^1$H NMR data from original torsion balances.

2. Select $^1$H and ROESY NMR data from advanced torsion balances.
EXSY for 17 at 298K in CDCl$_3$ (500 MHz, 0.3 s mixing time)
EXSY for 29 at 343K in toluene-$d_8$ (500 MHz, 1.4 s mixing time)
27 at rt in CDCl₃ (500 MHz)

28 at rt in CDCl₃ (500 MHz)
1 at rt in CDCl₃ (500 MHz)

2 at rt in CDCl₃ (500 MHz)
$44$ at rt in CDCl$_3$
(700 MHz)

$44$ at rt in toluene-$d_8$
(700 MHz)
ROESY for 47 at rt in MeOD (400 MHz, 0.2 s mixing time)
ROESY for 48 at rt in MeOD (400 MHz, 0.2 s mixing time)
ROESY for 49, peak at 13 min, at rt in MeOD (400 MHz, 0.2 s mixing time)
ROESY for 49, peak at 15 min, at rt in MeOD (400 MHz, 0.2 s mixing time)
ROESY for 50 at rt in MeOD (400 MHz, 0.2 s mixing time)
ROESY for 51 at rt in MeOD (400 MHz, 0.2 s mixing time)


35. (a) Oki, M., Applications of dynamic NMR spectroscopy of organic compounds. VCH: Deerfield Beach. **1985**, Chapters 1, 2 and 4; (b) Variable temperature NMR spectra were acquired using a 500 MHz instrument. Probe temperatures (+/-0.5 K) were measured with a calibrated, digital thermocouple. The iNMR lineshape analysis program was used to model the observed spectra, and coalescence temperatures were determined for the conformer signals when possible.


55. (a) Ohta, S.; Shimabayashi, A.; Aono, M.; Okamoto, M., A general convenient one-pot procedure for the conversion of carboxylic acids into their t-butyl esters which is also applicable to aliphatic carboxylic acids. *Synthesis* **1982**, *10*, 833-834; (b) Sunasee, R.; Clive, D. L. J., A route to 1,4-disubstituted aromatics and its application to the synthesis of the antibiotic culpin. *J. Org. Chem.* **2008**, *73* (20), 8016-8020.


64. Keyser, S. K., Ph.D. dissertation, University of Pittsburgh. **2010**.