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

by

Melissa Ann Liberatore

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This dissertation was presented

by

Melissa Ann Liberatore

It was defended on

July 23, 2012

and approved by

Professor Dennis Curran, Department of Chemistry

Professor Michael Trakselis, Department of Chemistry

Professor Judith Klein-Seetharaman, Department of Structural Biology

Dissertation Advisor: Professor Craig Wilcox, Department of Chemistry

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Melissa Ann Liberatore, PhD

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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 ^1H 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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LIST OF ABBREVIATIONS

Ac	acetyl
Ala	alanine
Bn	benzyl
Boc	*tert*-butyloxycarbonyl
BOP-Cl	*bis*(2-oxo-3-oxazolindinyl)phosphinic chloride
*t*Bu	*tert*-butyl
Cbz	carbobenzyloxy
CDI	1,1'-carbodiimidazole
COSY	correlation spectroscopy
DBF	dibenzofulvene
DBU	1,8-diazabicyclo[5.4.0]undec-7-ene
DCC	*N*,*N'*-dicyclohexylcarbodiimide
DCM	dichloromethane
DIEA	*N*,*N'*-diisopropylethylamine
DMAP	4-(dimethylamino)pyridine
DME	1,2-dimethoxyethane
DMF	*N*,*N'*-dimethylformamide
DMSO	dimethyl sulfoxide
EDCI	*N*-ethyl-*N'*-(3-dimethylaminopropyl)carbodiimide hydrochloride
ee	enantiomeric excess
EI	electron ionization
equiv	equivalents
ESI	electrospray ionization
Et	ethyl
EXSY	exchange spectroscopy
Fmoc	9-fluorenylmethyloxycarbonyl
Gly	glycine
HATU	*o*-(7-azabenzotriazol-1-yl)-*N*,*N*,*N´*,*N´*-tetramethyl-uronium-hexafluorophosphate
HBTU	*o*-benzotriazole-*N*,*N*,*N´*,*N´*-tetramethyl-uronium-hexafluorophosphate
HMBC	heteronuclear multiple bond coherence
HMQC	heteronuclear multiple quantum coherence

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
*p*NB	*para*-nitrobenzyl
*i*Pr	isopropyl
Py	pyridine
PyBOP	(benzotriazol-1-yloxy)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]

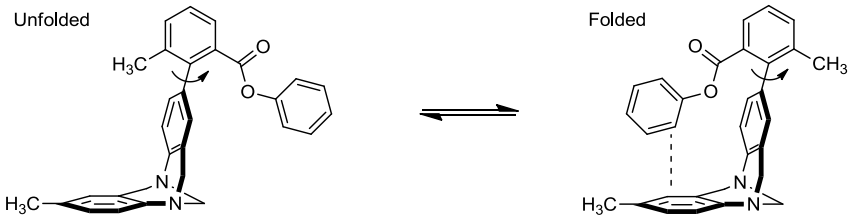


Figure 1. Two conformational states of a torsion balance designed to measure edge-to-face aromatic interactions.

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 $kJ \cdot mol^{-1}$) 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.

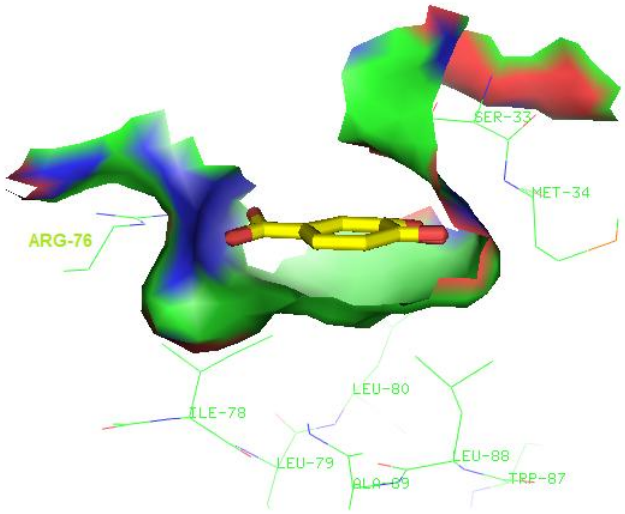


Figure 2. Computer modeling of the chorismate lyase binding site with vanillate inhibitor depicted; the inhibitor is nestled in the hydrophobic pocket (Ile-78, Leu-79, and Leu-80) but also forms a hydrogen bond with neighboring Arg-76.

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.[6] 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.[7] 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_2H chain (amino acid nos. 88 to 91). The aromatic backbone of the balance would be devised to take

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.

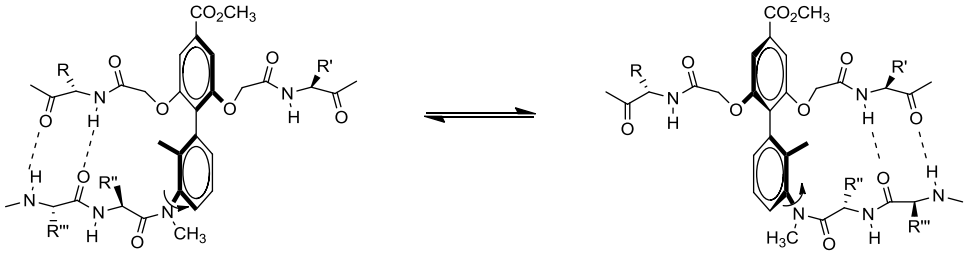
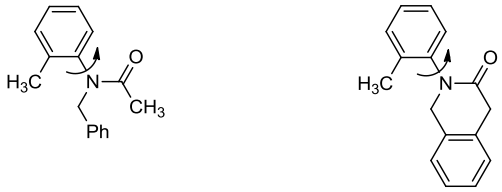


Figure 3. Example of two conformers of a β-turn mimetic torsion balance. The R groups are different and each represents an amino acid side chain.

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 kcal·mol^{-1} at 408 K for one acyclic amide example shown in Figure 4.[8] The rate constant for this rotation is k = 95 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 kcal·mol^{-1} at 346 K, with a rate constant of k = 51 s^{-1}.



(a) (b)

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;[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·mol^{-1} (without the additional *o*-methyl susbstituent, the barriers were less than 20 kcal·mol^{-1}). The additional *ortho*-group increased the stability of the atropisomers[10] at room temperature and allowed for easier separation.

This is the first time our group has incorporated a *o,o,o′*-trisubstituted biphenyl in a torsion balance, which is meant to structurally emulate a β-turn. The biphenyl unit contributes an additional site for restricted rotation.[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.[11a, b] Based on work by Kawano,[11c] we used computer simulations to estimate the barrier for rotation around the aryl-aryl bond to be $\Delta G^{\ddagger}$ = 27 kcal·mol^{-1} at 343 K. One biphenyl example of Kawano is shown in Figure 5,[11c] with a barrier of 28.2 kcal·mol^{-1}; this barrier was from the combination of optical rotation measurements and half-life times for racemization at various temperatures.

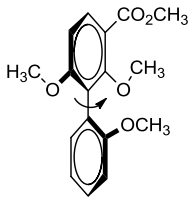
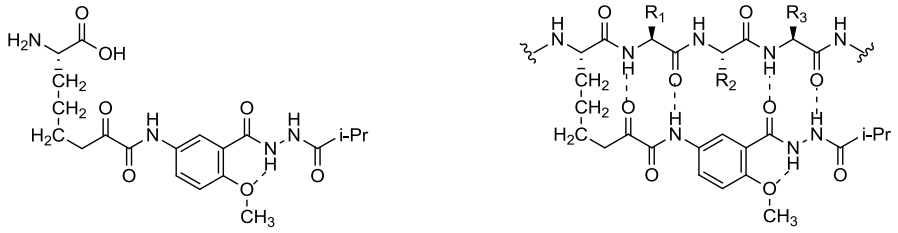


Figure 5. Kawano biphenyl compound with three *ortho*-substituents.

The combination of the *o,o,o′*-trisubstituted biphenyl with the N-aryl atropisomer gives a molecule that would serve as a β-turn mimic, by imitating the familiar bonds and dihedral angles necessary to connect and bring two antiparallel β-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 β-strand mimic[12] for protein folding studies. Gellman[13] and coworkers have reported the use of a β-hairpin motif containing a hydrophobic cluster of naturally occurring amino acids to probe the folding and stability of antiparallel β-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 β-hairpin conformation was generated by the nonpolar side chain cluster. Nowick[14] and coworkers describe several β-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 β-turn in a β-strand.



(a) (b)

Figure 6. (a) Nowick's unnatural amino acid "Orn(*i*-PrCO-Hao)". (b) Nowick's β-sheet mimic containing "Orn(*i*-PrCO-Hao)".

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[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,[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.

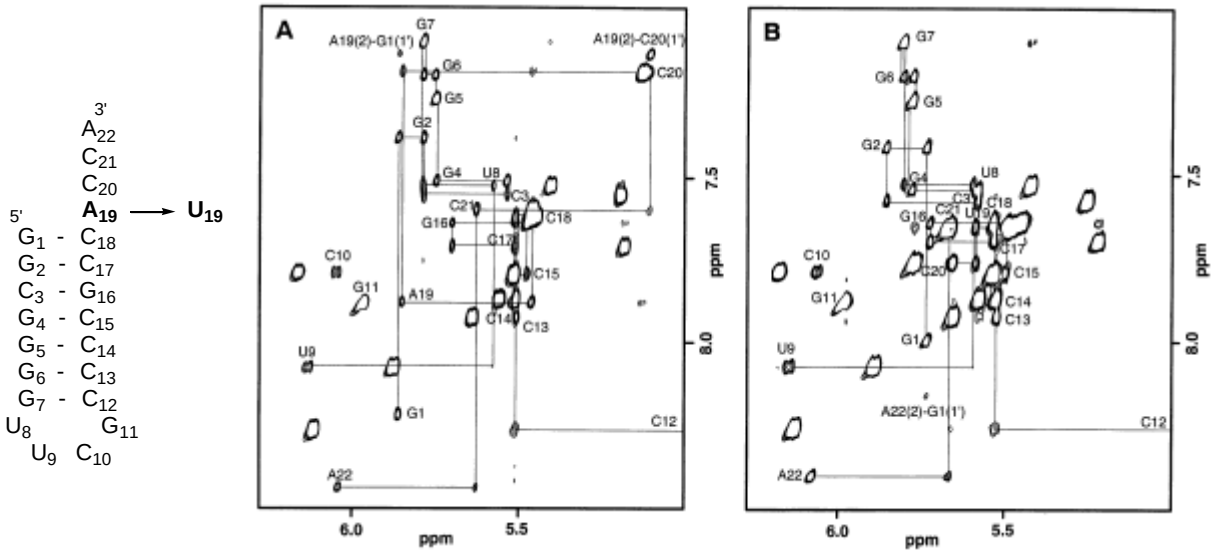


Figure 7. Secondary structure of Williamson's tRNA variants and the corresponding NOESY spectra (reprinted from the literature).[15a] NOESY spectra (A) and (B) show the NOEs of interest for variants A19 and U19, respectively. (Copyright (1994) National Academy of Sciences, U.S.A. Printed with permission.)

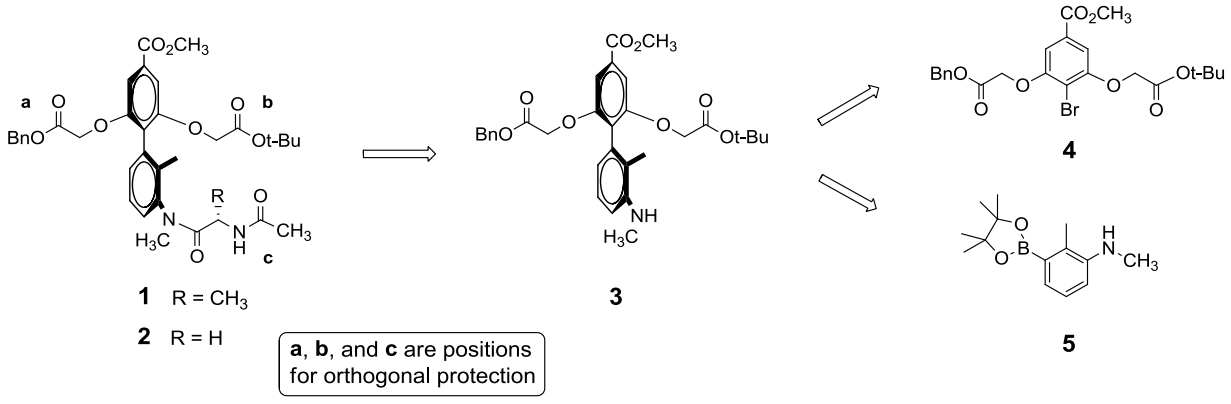
NMR assignments of other similar compounds, such as TAR RNA that is located in all viral mRNAs,[15b, c] were also performed by the Williamson group. The use of NOESY experiments, in addition to COSY, TOCSY, and various ^{13}C-detected experiments, aided in determining the conformation of the wild-type 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[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.

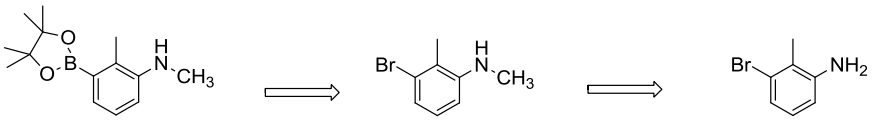
Scheme 1. Retrosynthetic pathway for β-turn mimetic torsion balance.



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[16] between asymmetric bromide **4** and boronic ester **5**. The preparation of **4** could be achieved efficiently from the Fischer esterification[17] of starting material 4-bromo-3,5-dihydroxybenzoic acid, followed by dual application of the Williamson aryl ether synthetic protocol.[18] The retrosynthetic plan for **5**, shown in Scheme 2, involves a Miyaura boration[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.



5 **6** R = CH_3 **7**

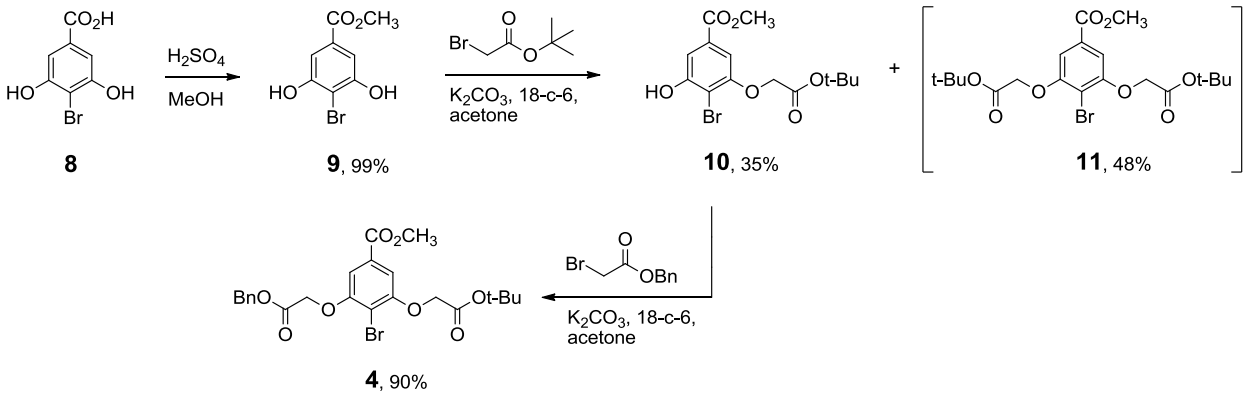
2.2 SYNTHESIS OF THE TORSION BALANCE

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[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[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.

Scheme 3. Synthesis of coupling fragment bromide 4.

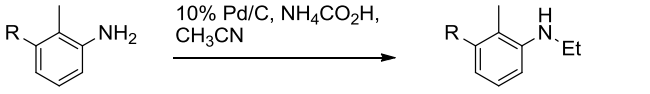


2.2.2 Synthesis of the Arylboronic Ester Coupling Fragment

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-*o*-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.

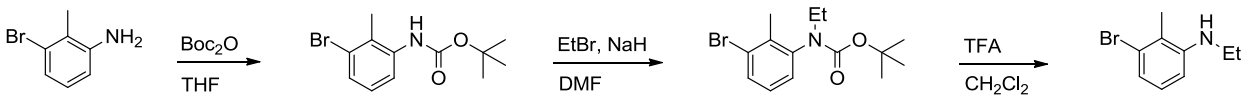


7, R = Br
12, R = H

13, R = Br, 0%
14, R = H, 100%

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_2O) 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.



7

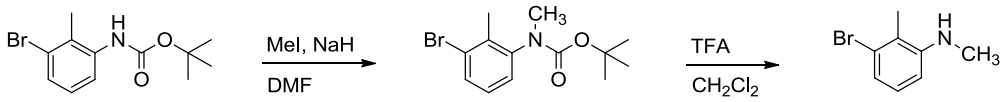
15, 96%

16, 97%

13, 100%

The synthesis of the *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 *N*-methylaniline 6.

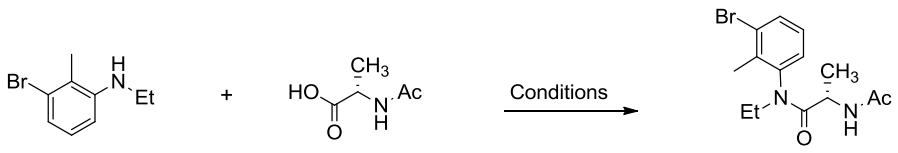


15 **17,** 100% **6,** 100%

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 *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 *bis*(2-oxo-3-oxazolindinyl)phosphinic chloride (BOP-Cl), triethylamine (NEt_3), in CH_2Cl_2[22] gave only 14% yield of expected amide **19** (Entry 1). Reaction of **13** and **18** with *N,N'*-dicyclohexylcarbodiimide (DCC) and 4-(dimethylamino)pyridine (DMAP) in CH_2Cl_2[23] resulted in a 61% recovery of the starting material (SM) **13** but with no product isolated (Entry 2). Employment of reagents *N*-ethyl-*N*′-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDCI), 1-hydroxybenzotriazole (HOBt) with **13** and **18** in CH_2Cl_2[24] 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 CH_2Cl_2 and DMF gave a similar 15% yield (Entry 5). Use of either of the phosphate salts *o*-benzotriazole-*N,N,N´,N´*-tetramethyl-uronium-hexafluorophosphate (HBTU) or *o*-(7-azabenzotriazol-1-yl)-*N,N,N´,N´*-tetramethyl-uronium-hexafluorophosphate (HATU) with the corresponding triazoles and *N,N*′-diisopropylethylamine

(DIEA) in CH_3CN[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.



13 **18** **19**

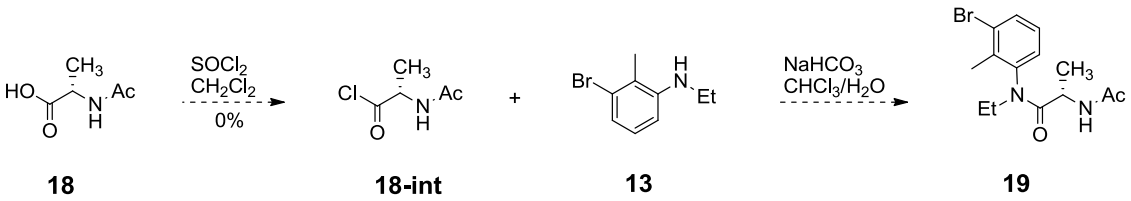
Entry	Conditions [a]	Yield
1	BOP-Cl, NEt_3, CH_2Cl_2, 2 d	14%[b]
2	DCC, DMAP, CH_2Cl_2, 12 h	SM
3	EDCI, HOBt, CH_2Cl_2, 3 d	34% [b]
4	EDCI, HOBt, DMF, 2 d	14% [b]
5	EDCI, HOBt, CH_2Cl_2/DMF (1:1), 3 d	15% [b]
6	HBTU, HOBt, DIEA, CH_3CN, 24 h	SM
7	HATU, HOAt, DIEA, CH_3CN, 24 h	SM

[a] 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.



18 **18-int** **13** **19**

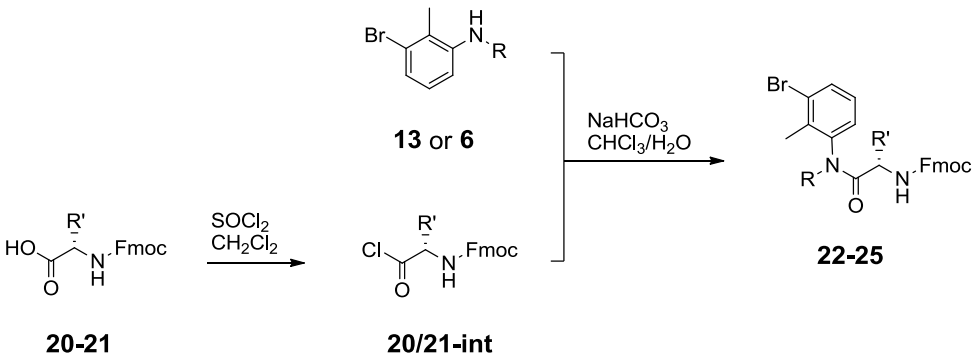
According to Montalbetti,[27] formation of amino acid halides can be difficult due to possible cyclization to a five-membered *N*-carboxyanhydride ring; however, this can be suppressed with other protecting groups, such as the 9-fluorenylmethyloxycarbonyl (Fmoc) groups. Therefore, *N*-acetyl-alanine 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_2Cl_2) gave amide **22** in a 24% yield. In another attempt, we utilized a Carpino procedure[28] involving initial conversion of the amino acid to its corresponding fluoride by treatment with cyanuric fluoride and DIEA in CH_2Cl_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,[26] 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_2Cl_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.

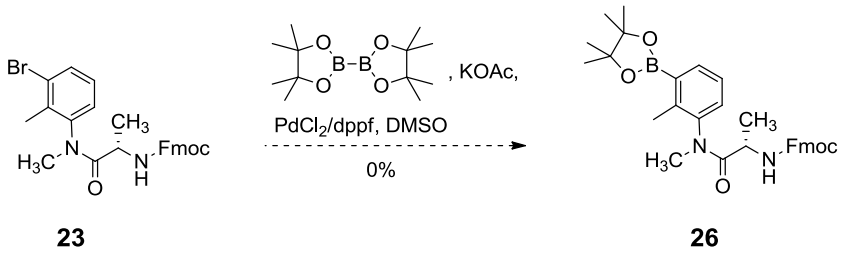
Table 2. Results for amidation reactions between anilines 13 and 6 and *N*-Fmoc amino acids 20 and 21.



Entry	Amine	Amino Acid	Product	R	R′	% Yield
1	**13**	**20**	**22**	CH_2CH_3	CH_3	68
2	**6**	**20**	**23**	CH_3	CH_3	96
3	**13**	**21**	**24**	CH_2CH_3	H	85
4	**6**	**21**	**25**	CH_3	H	94

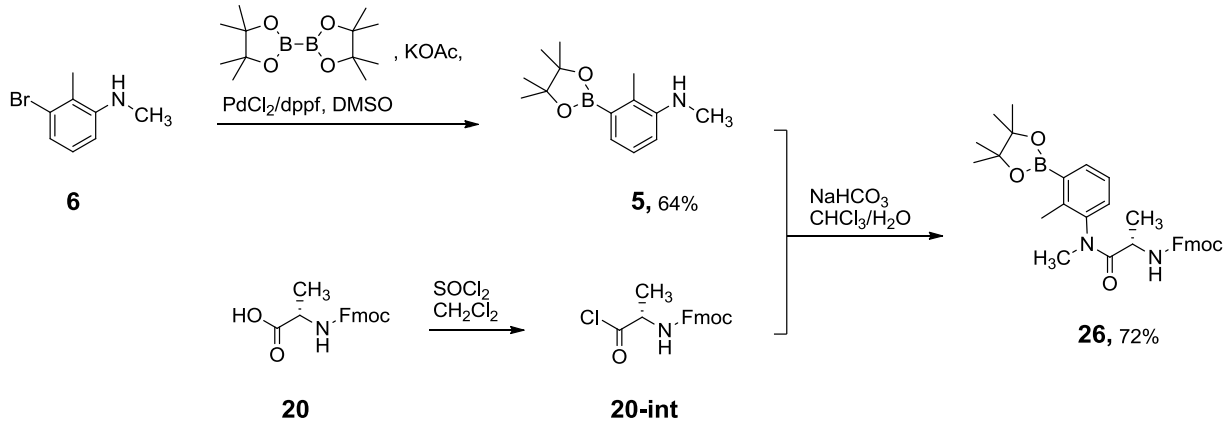
The next step enroute to the formation of the torsion balance was a Miyaura boration,[19] with bis(pinacolato)diboron used as the boronate reactant. The *N*-methylanilide **23** was first employed due to its simplicity in structure and ^{1}H NMR interpretation in comparison with its *N*-ethyl counterpart **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 δ = 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.

Scheme 8. Attempted boration of anilide 23.



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[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,[26] to produce amide **26** in 72% yield.

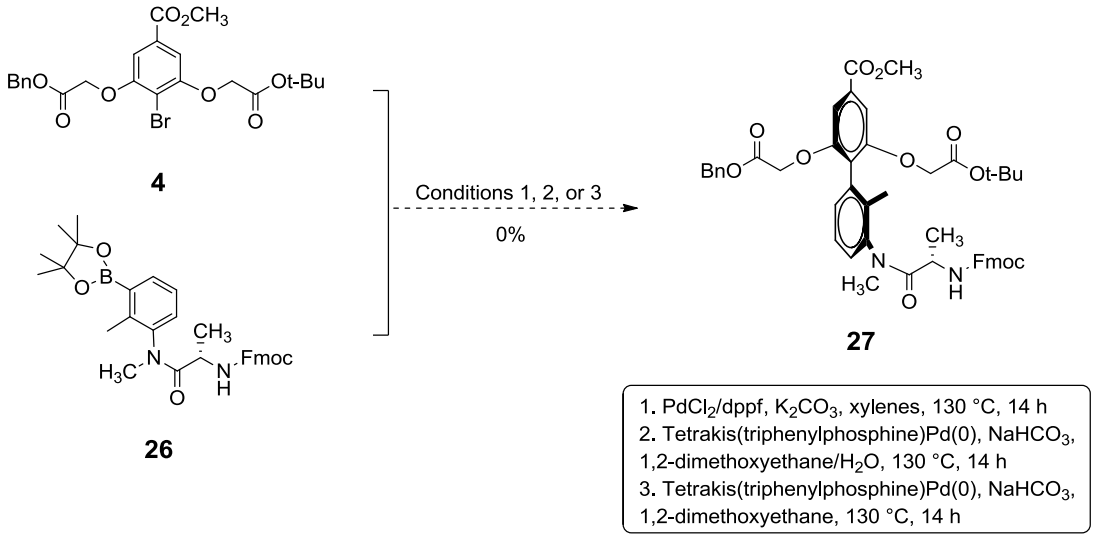
Scheme 9. Synthesis of boronic esters 5 and 26.



2.2.3 Final Steps to Synthesize the Torsion Balance

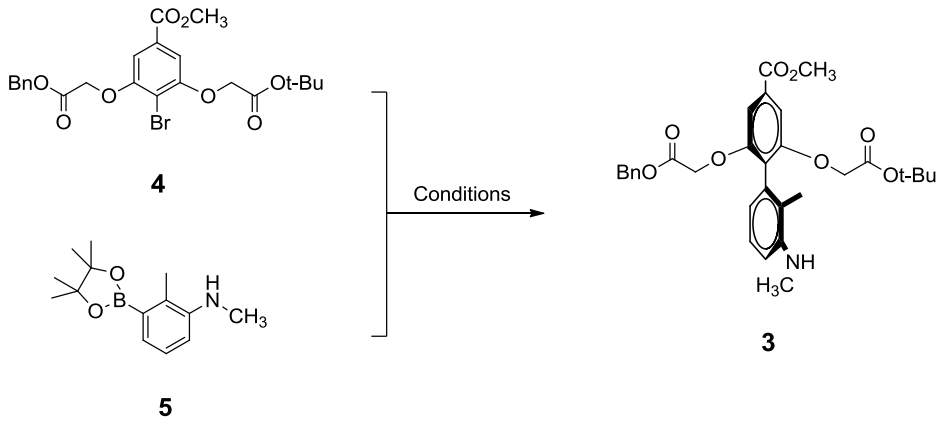
At this point we envisioned that a Suzuki–Miyaura cross-coupling reaction between bromide **4** and amide **26** would produce biaryl amide **27**, which could be Fmoc-deprotected and acylated to generate torsion balance **1**. However, there are few examples in the literature that show cross-coupling of two substrates with a total of three *ortho*-substituents[29] 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.

Scheme 10. Attempted Suzuki-Miyaura cross-coupling of fragments 4 and 26.



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_2O 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[dibenzylideneacetone]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 $K_3PO_4 \cdot H_2O$ 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.



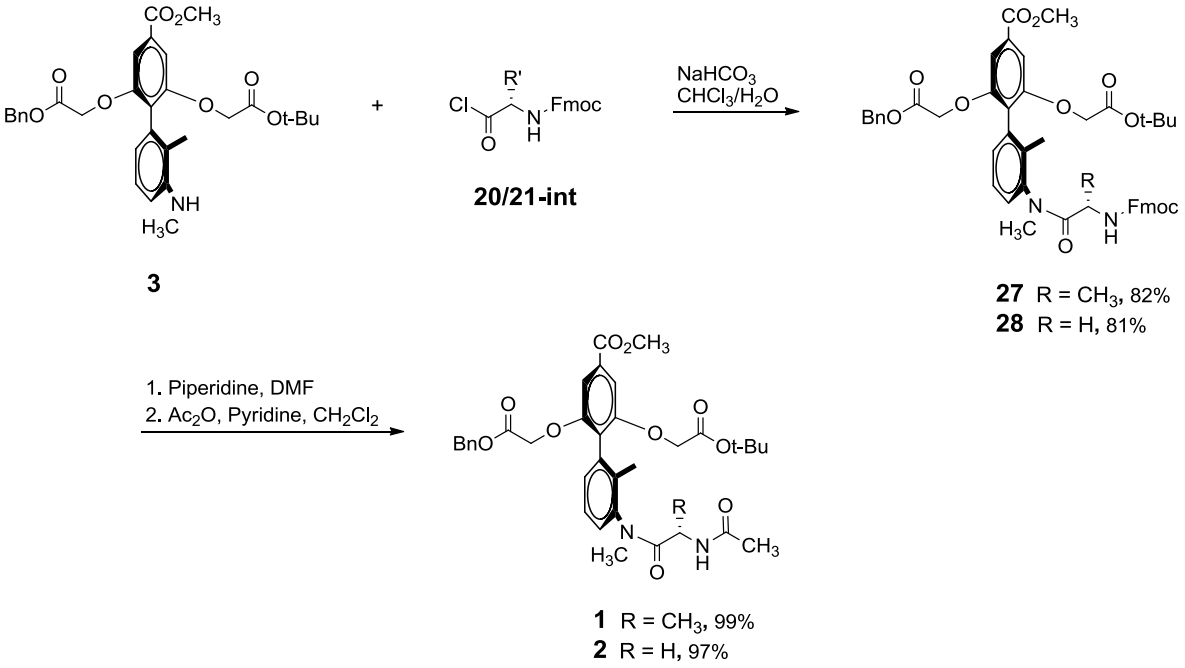
Entry	Conditions [a]	Yield [b]
1	$Pd(PPh_3)_4$, $NaHCO_3$, H_2O, DME	28%
2	$Pd(PPh_3)_4$, $NaHCO_3$, H_2O, DMF	0%
3	$Pd(OAc)_2$, JohnPHOS®, KF, DME	0%
4	$Pd_2(dba)_3$, JohnPHOS®, KF, DME	0%
5	$Pd_2(dba)_3$, JohnPHOS®, KF, DMF	10%
6	$Pd_2(dba)_3$, SPHOS®, $K_3PO_4 \cdot H_2O$, DME	31%
7	$Pd_2(dba)_3$, SPHOS®, $K_3PO_4 \cdot H_2O$, H_2O, DME	9%
8	$Pd_2(dba)_3$, SPHOS®, $K_3PO_4 \cdot H_2O$, isopropanol	0%
9	$Pd_2(dba)_3$, SPHOS®, $K_3PO_4 \cdot H_2O$, toluene	47%
10	$Pd_2(dba)_3$, SPHOS®, $K_3PO_4 \cdot H_2O$, toluene	37%

[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_2Cl_2[32] 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.

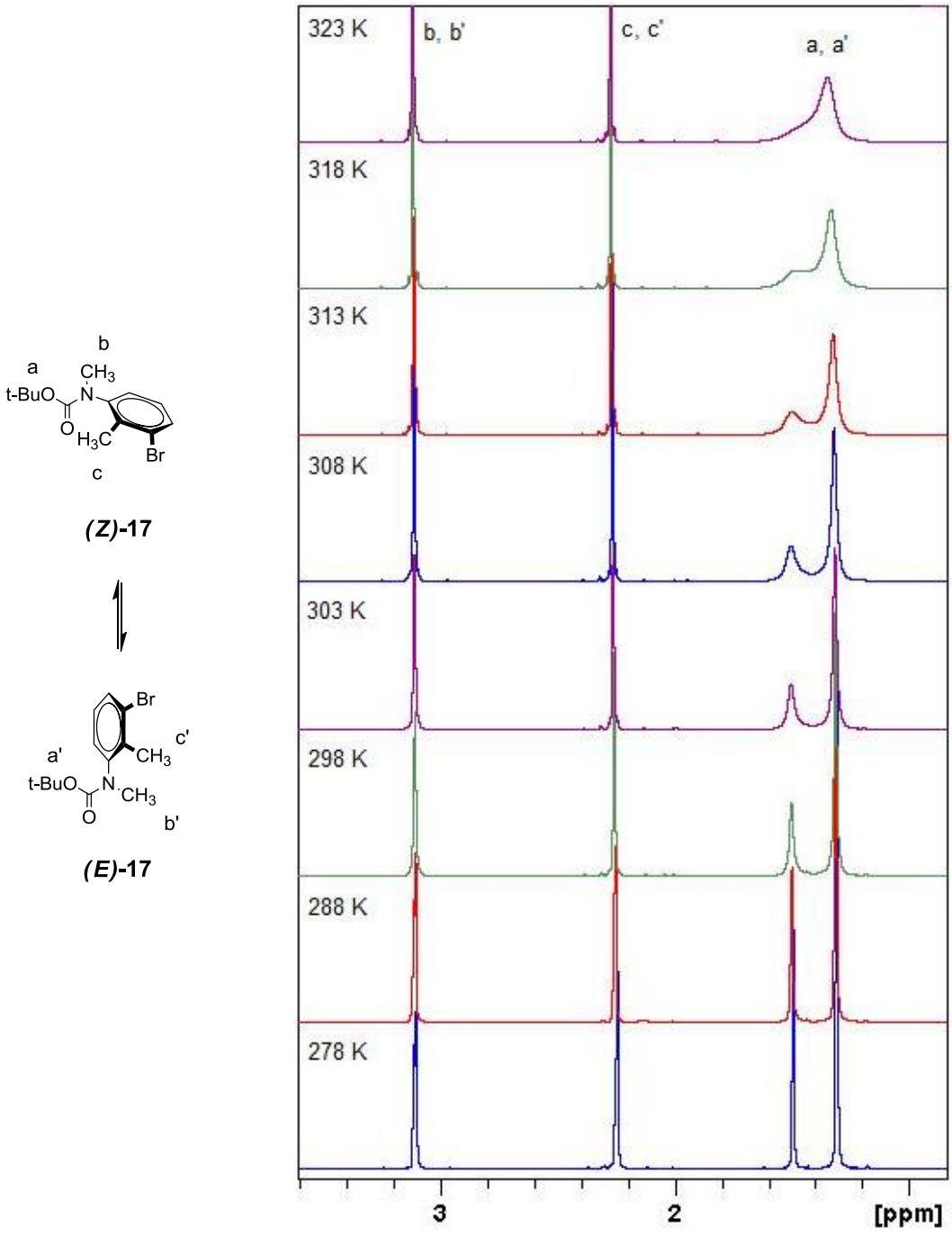


Figure 8. Carbamates *(Z)*-17 and *(E)*-17 and a selected area of the variable temperature ^{1}H NMR spectra from 278 K to 323 K (5 to 50 °C, chloroform-*d*, 500 MHz). Protons of interest are denoted.

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 δ = 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

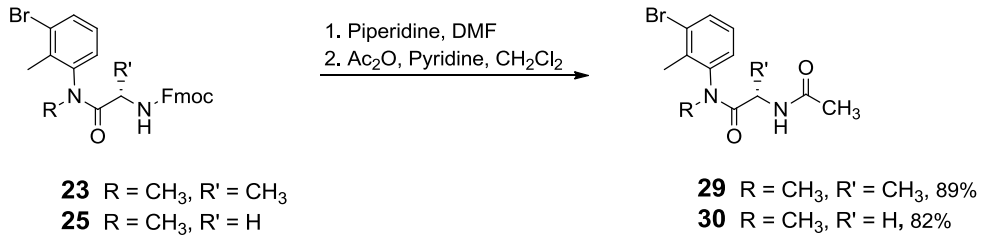
Although amides **23** and **25** could not be applied toward the synthesis of torsion balances **1** and **2**, they could potentially supply information about the rotational barrier of the N-aryl bond; rotation around this bond will provide the conformational switch necessary for our studies. Four 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,[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_2Cl_2[32] to give **29** and **30** in yields of 89% and 82%, respectively.

Scheme 12. Synthesis of torsion balance precursor amides 29 and 30.



23 R = CH_3, R' = CH_3
25 R = CH_3, R' = H

29 R = CH_3, R' = CH_3, 89%
30 R = CH_3, R' = H, 82%

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,[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

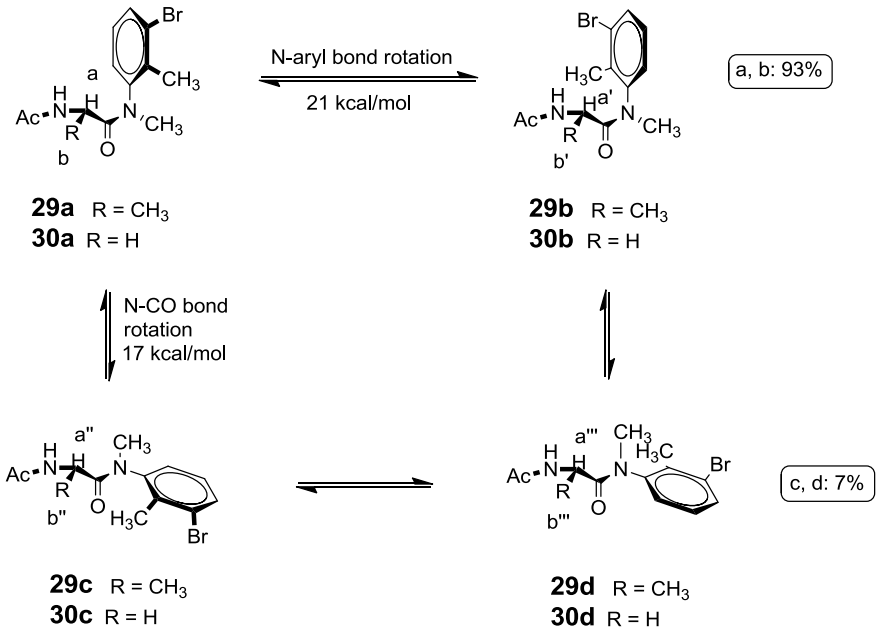
four doublets at δ = 0.90–1.30 ppm (representing the three α-methyl protons of alanine) as the temperature approached 70 °C. These results showed that the interconversion between the conformers was fast; however it was possible that rotation around both the N-aryl and the N-CO bonds contributed to the 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 δ = 5.10 and 1.30 ppm, representing the alanine α-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 α-methyl isomer peaks (at δ = 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, δ = 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.



29a R = CH_3
30a R = H

29b R = CH_3
30b R = H

29c R = CH_3
30c R = H

29d R = CH_3
30d R = H

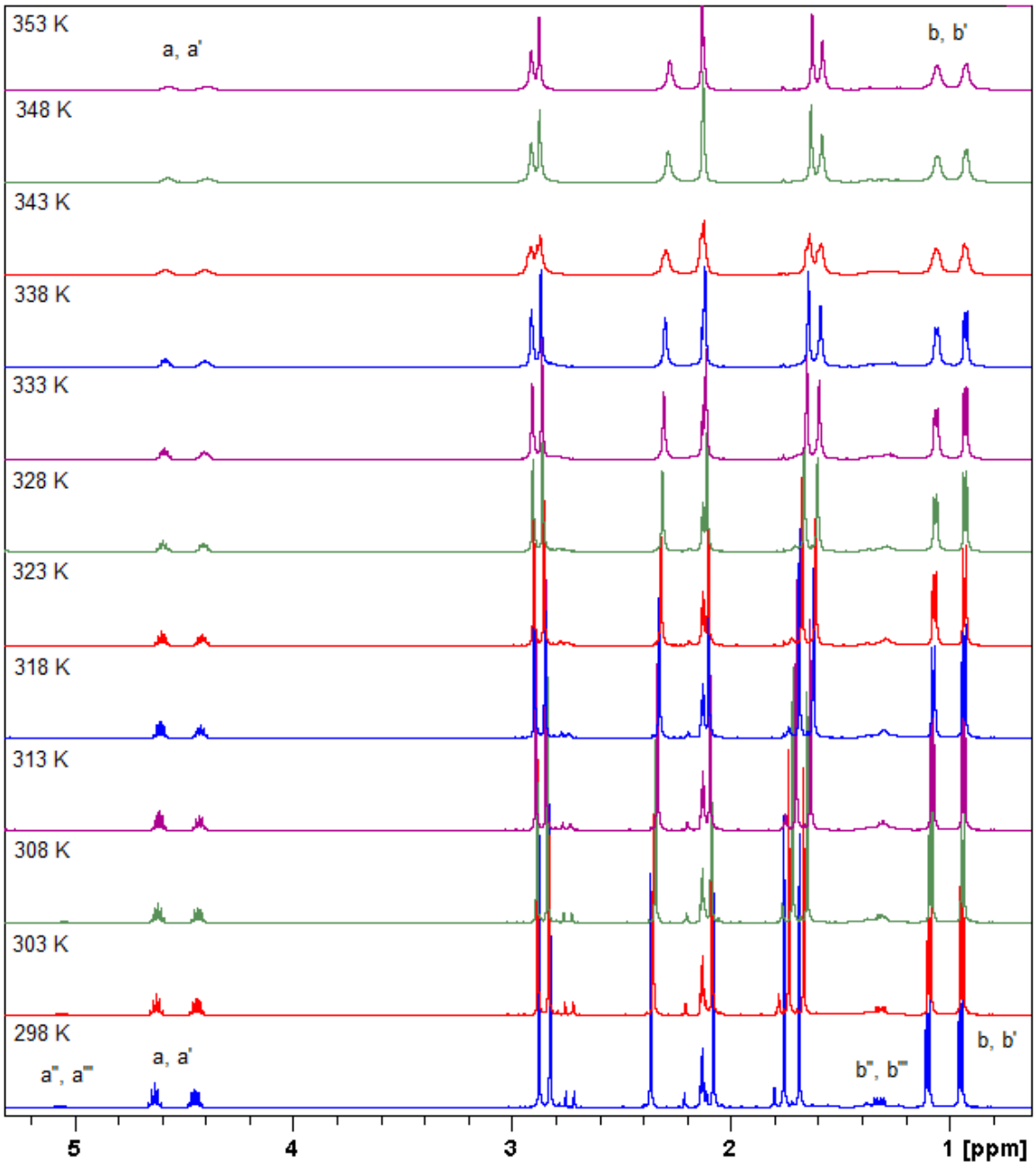
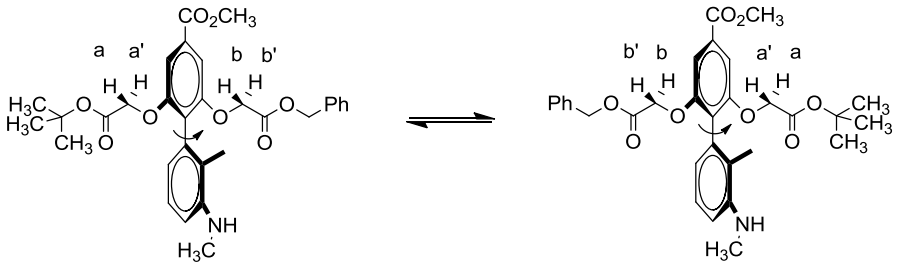


Figure 9. Variable temperature ^{1}H NMR spectra of amide 29 from 298 K to 353 K (25 to 80 °C, toluene-d_8, 500 MHz). Protons of interest are denoted as follows and coincide with Scheme 13: a and a´ correspond to the two major conformers that arise from N-aryl bond rotation; a´´ and a´´´ correspond to 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.[11] 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.



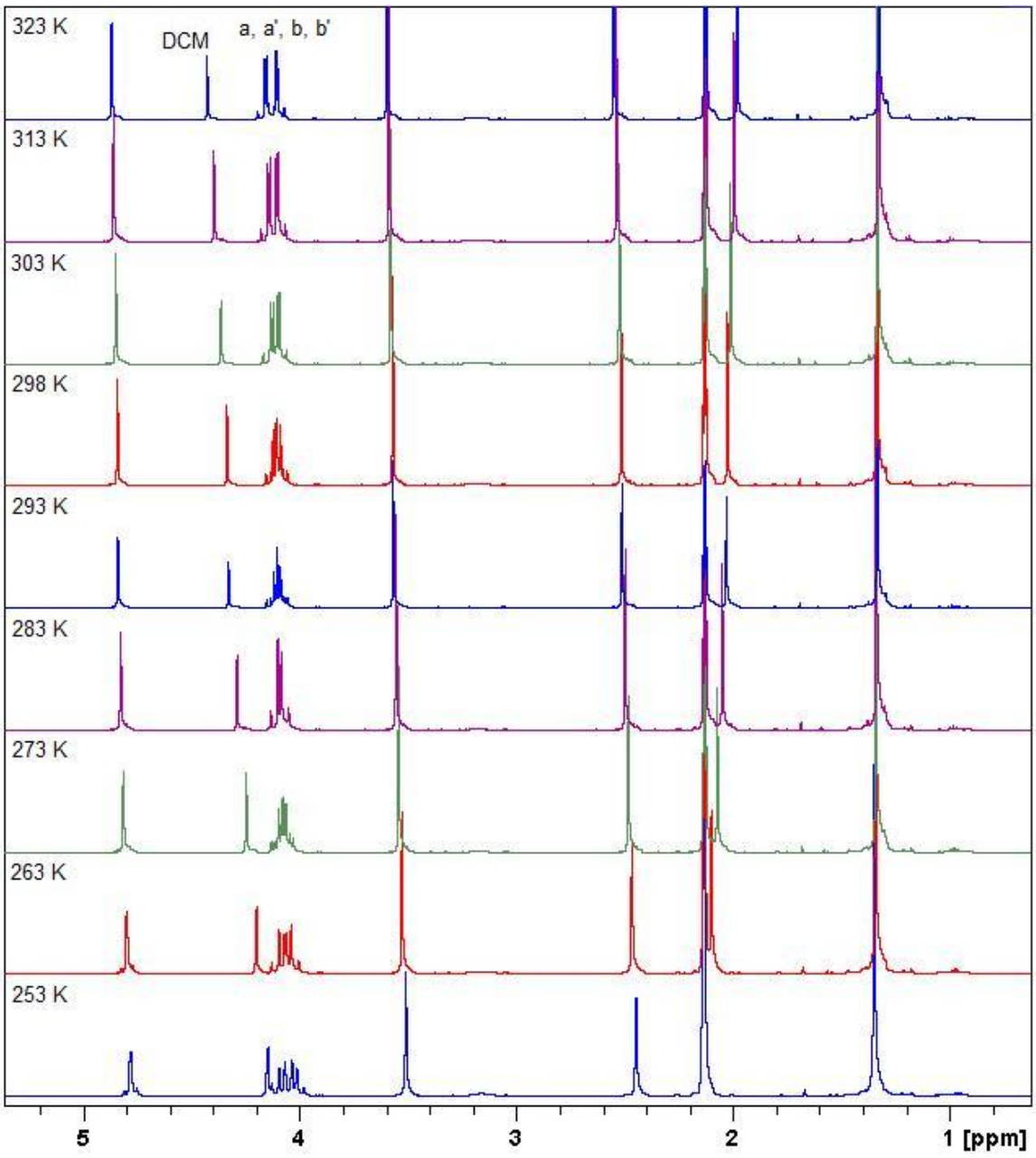


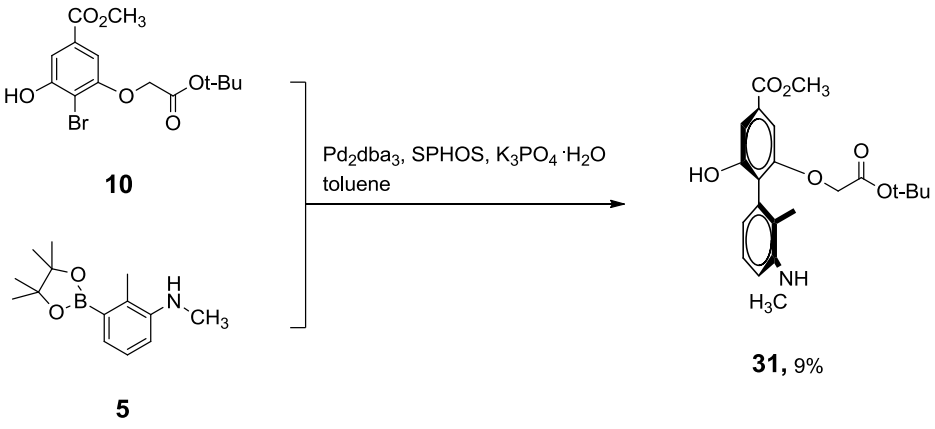
Figure 10. Variable temperature ^{1}H NMR spectra of amine 3 from 253 K to 323 K (-20 to 50 °C, toluene-d_8, 500 MHz). Dichloromethane (DCM) impurity is denoted. The diastereotopic protons of interest, coinciding with Scheme 14, are designated as a, a′, b and b′.

Only peak shifting was observed for the denoted diastereotopic protons and no peak broadening was detected. Kinetic equilibrium experiments were attempted via HPLC.[37] 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.

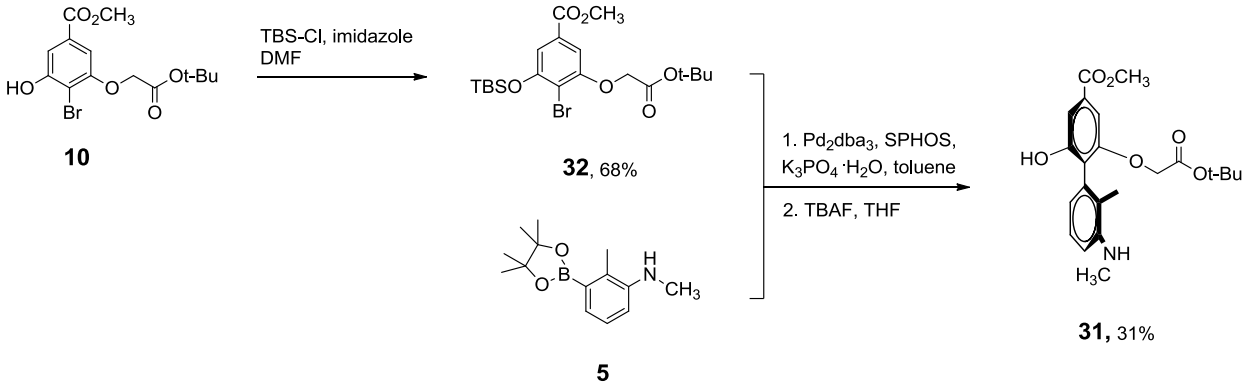
Scheme 15. Synthesis of biaryl amine 31.



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 *ortho*-methylene 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).

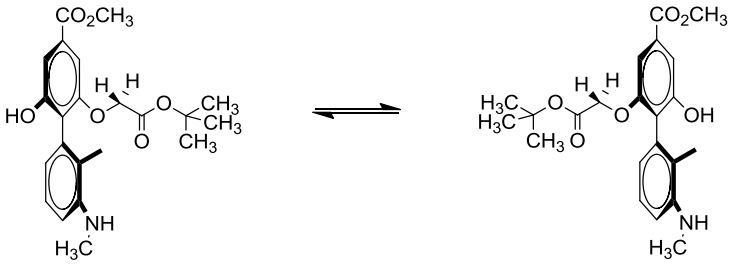
Scheme 16. Syntheses of TBS protected benzoate 32 and use toward biaryl amine 31.



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.[37] 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.



31-*S* **31-*R***

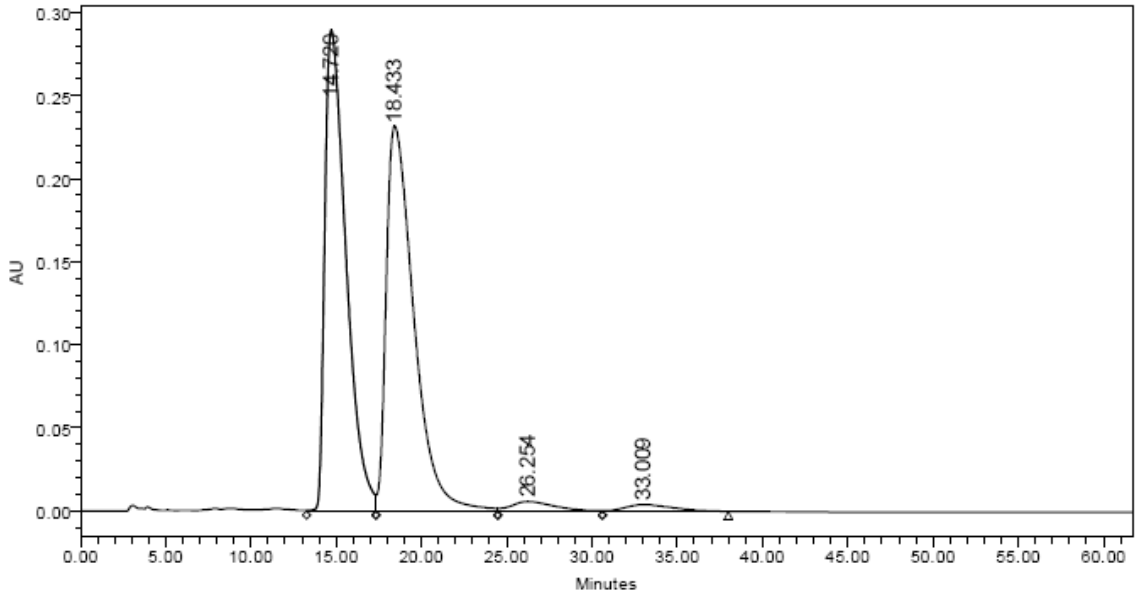


Figure 11. Analytical HPLC analysis of amine 31. The two peaks (enantiomers) were separated successfully by chiral preparatory HPLC, and enantiomer 31-SEE (18.4 min retention time) was used in the kinetic experiment.

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.[37] Using the Arrhenius kinetics, the rate of racemization was $k_{rac} = 0.363\ \mu s^{-1}$ 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) at 418 K in hexanes, with a racemization half life of 2.2 days at 418 K.

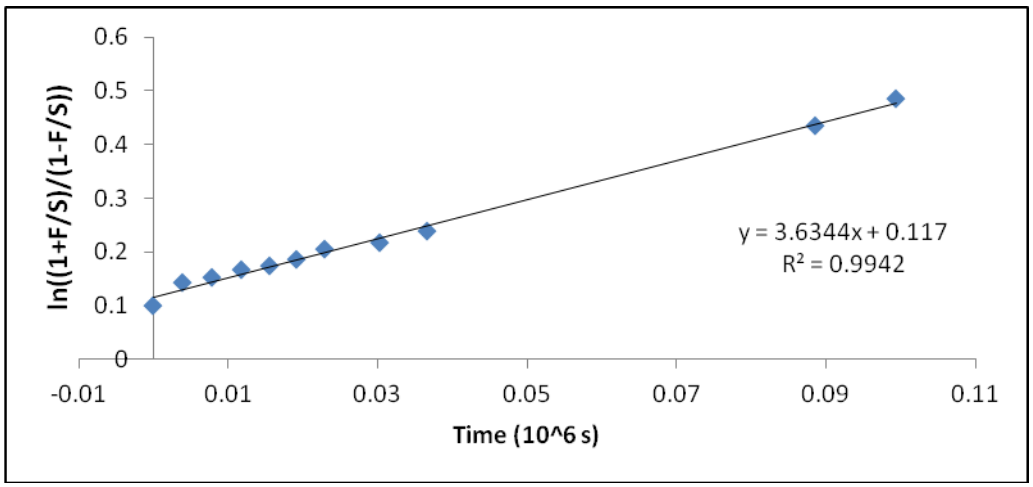


Figure 12. Equilibrium plot for amine 31 based upon the logarithmic decrease in ee from 31-SEE (denoted as S in the y-axis label) to 31-FEE (denoted as F in the y-axis label) over time.

3.2 EVALUATION OF HYDROGEN BOND SELECTIVITY OF INITIAL TORSION BALANCES

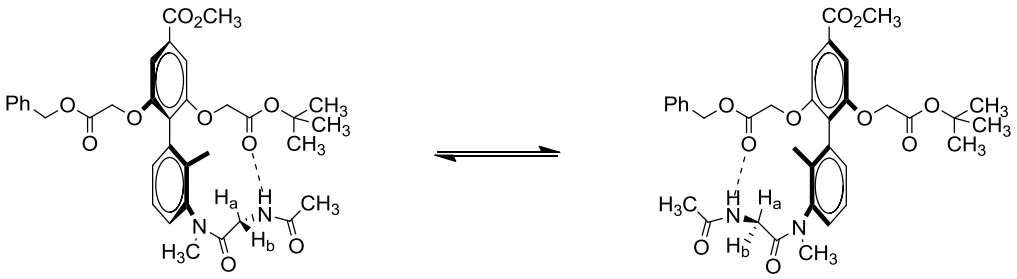
3.2.1 Hydrogen Bond Selectivity and Folding Preferences of Initial Torsion Balances

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.



2-S_a,R_a **2-S_a,S_a**

Before our examination into the folding preferences of **2**, we carried out a variable temperature ^{1}H NMR study,[35b] 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* (δ = 3.29 ppm) than in toluene-d_8 (δ = 3.06

ppm). In both solvents, the two singlets are seen in almost a 1:1 conformer ratio: in chloroform-*d* 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.

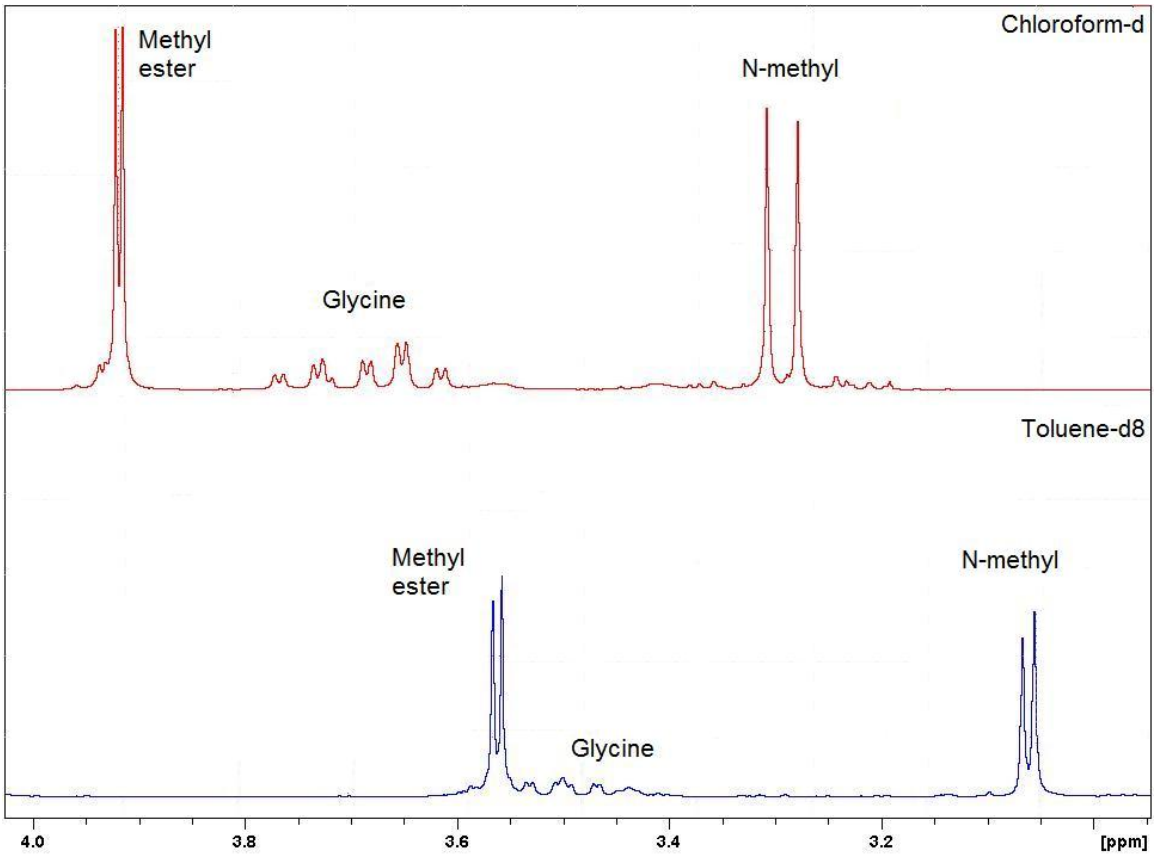


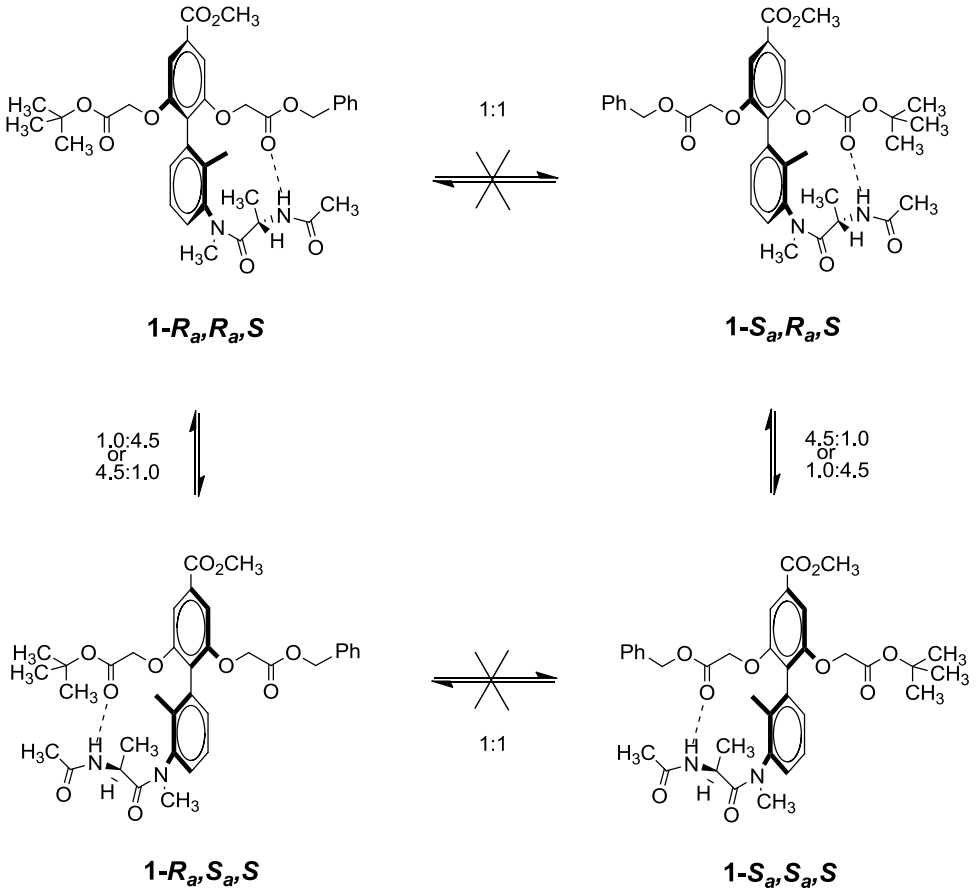
Figure 13. Ester hydrogen bonding selectivity of the glycine torsion balance 2 can be studied from the ratios of the denoted *N*-methyl protons in chloroform-*d* and toluene-d_8 (500 MHz). Both ratios were essentially 1:1, meaning that there was little selectivity.

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*, 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*$_a$*R*$_a$*S*** and **1-*R*$_a$*S*$_a$*S*** are in equilibrium with each other because N-aryl bond rotation is fast; likewise, **1-*S*$_a$*R*$_a$*S*** and **1-*S*$_a$*S*$_a$*S*** 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*$_a$*R*$_a$*S*** and **1-*R*$_a$*S*$_a$*S*** must equal the amount of **1-*S*$_a$*R*$_a$*S*** and **1-*S*$_a$*S*$_a$*S***. 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.

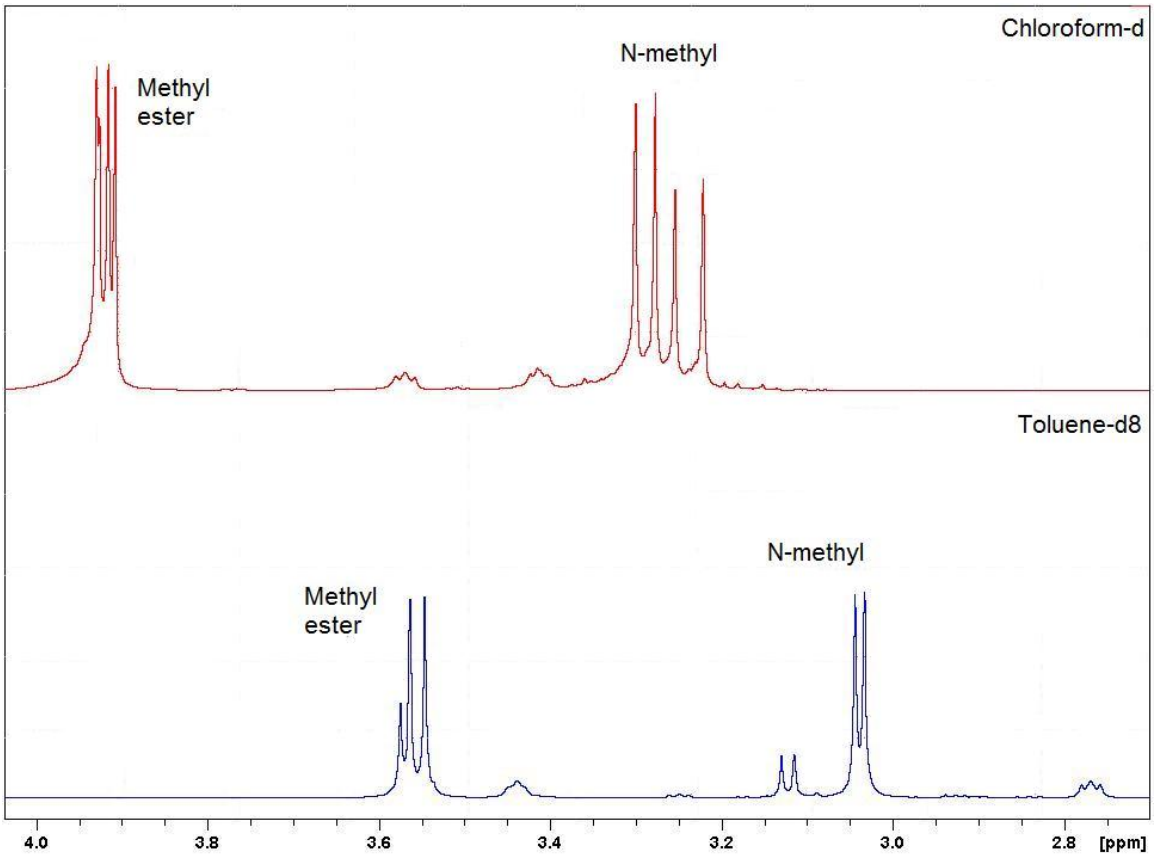


Figure 14. Differences in folding preference exhibited by alanine torsion balance 1 can be seen from the ratios of the *N*-methyl protons (denoted) and others in chloroform-*d* and toluene-d_8 (500 MHz).

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*$_a$*R*$_a$*S*** and **1-*S*$_a$*R*$_a$*S*** 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*$_a$*S*$_a$*S*** and **1-*S*$_a$*S*$_a$*S***.

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 α-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 α-methyl to the aryl group of the biphenyl core). An EXSY[36] 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 α-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

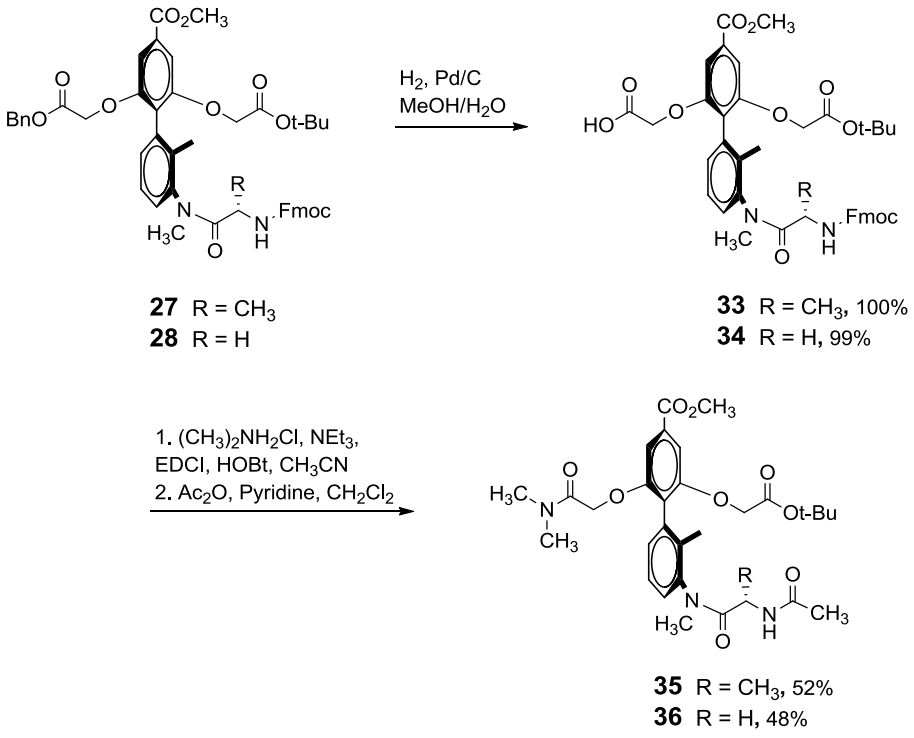
In our NMR analyses of the torsion balances **1** and **2**, we were not able to detect a higher selectivity of the amino acid proton towards the carbonyl oxygen of either the *tert*-butyl or the benzyl esters. Therefore, we next changed one ester carbonyl to an amide in order to test whether we could 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[40] 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

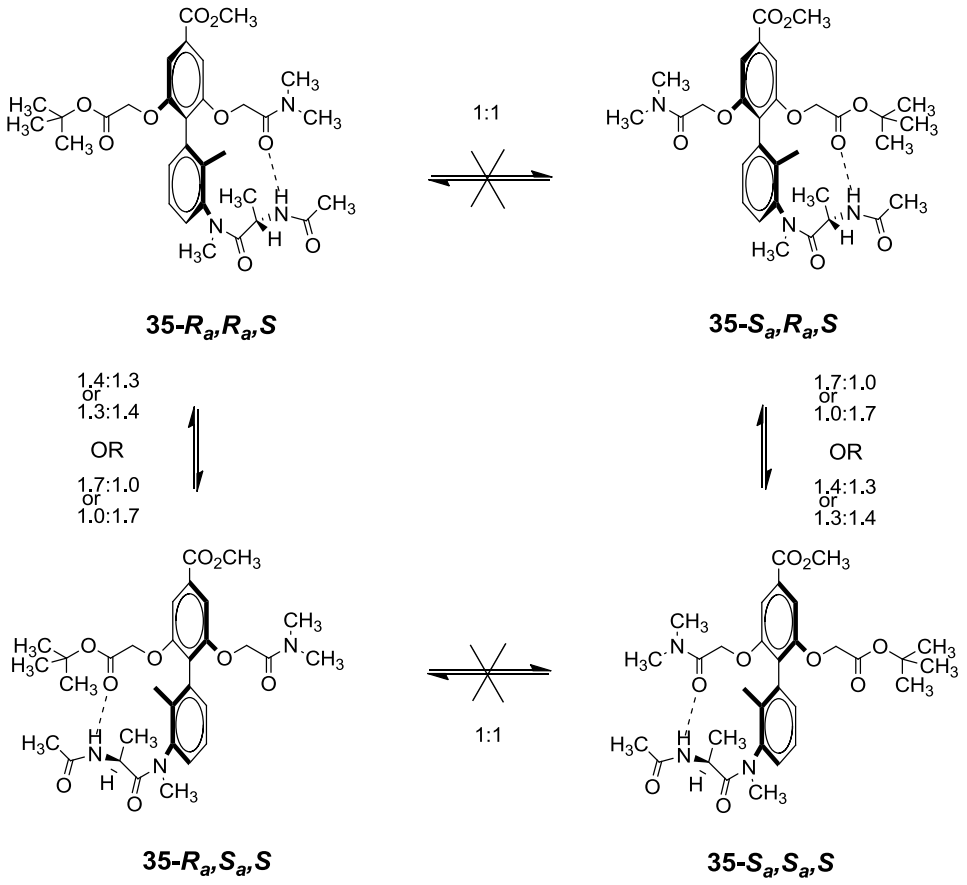
dimethylamine hydrochloride and standard coupling reagents EDCI, HOBt, and triethylamine (NEt_3) in acetonitrile for 80 h at room temperature;[41] this resulted in both the amide coupling and also the cleavage of the Fmoc protecting group in preparation for acylation. Although this shortened the synthetic 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.

Scheme 20. Synthesis of torsion balance derivatives 35 and 36.



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.



35-R_a,R_a,*S* **35-S_a,R_a,*S***

35-R_a,S_a,*S* **35-S_a,S_a,*S***

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.[42] 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 α-methyl and the aryl group.

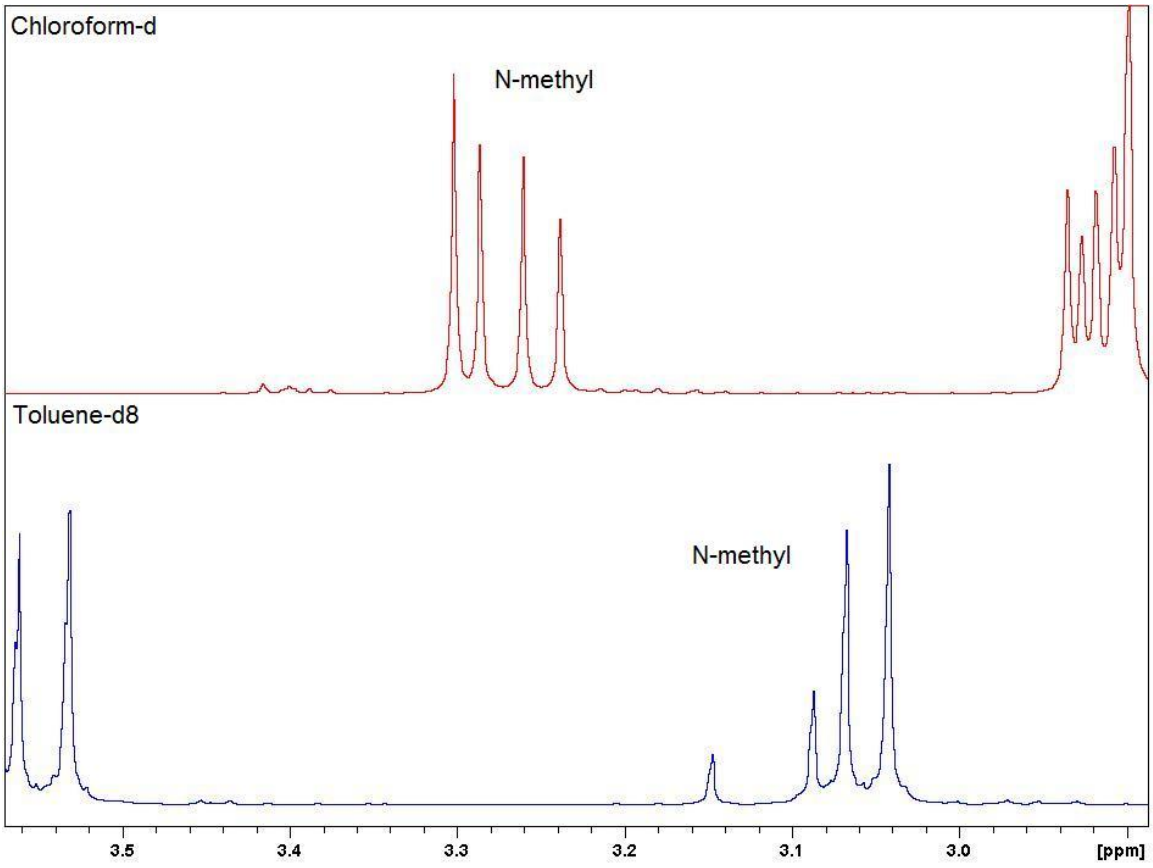


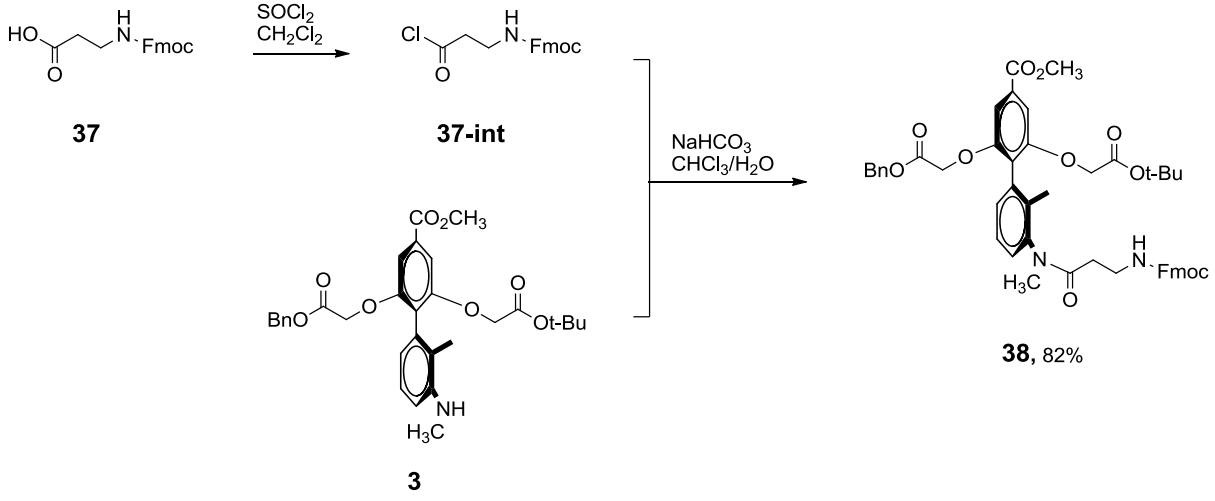
Figure 15. Differences in folding preference exhibited by alanine torsion balance derivative 35 can be seen from the ratios of the denoted *N*-methyl protons in chloroform-*d* and toluene-d_8 (500 MHz).

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 α-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

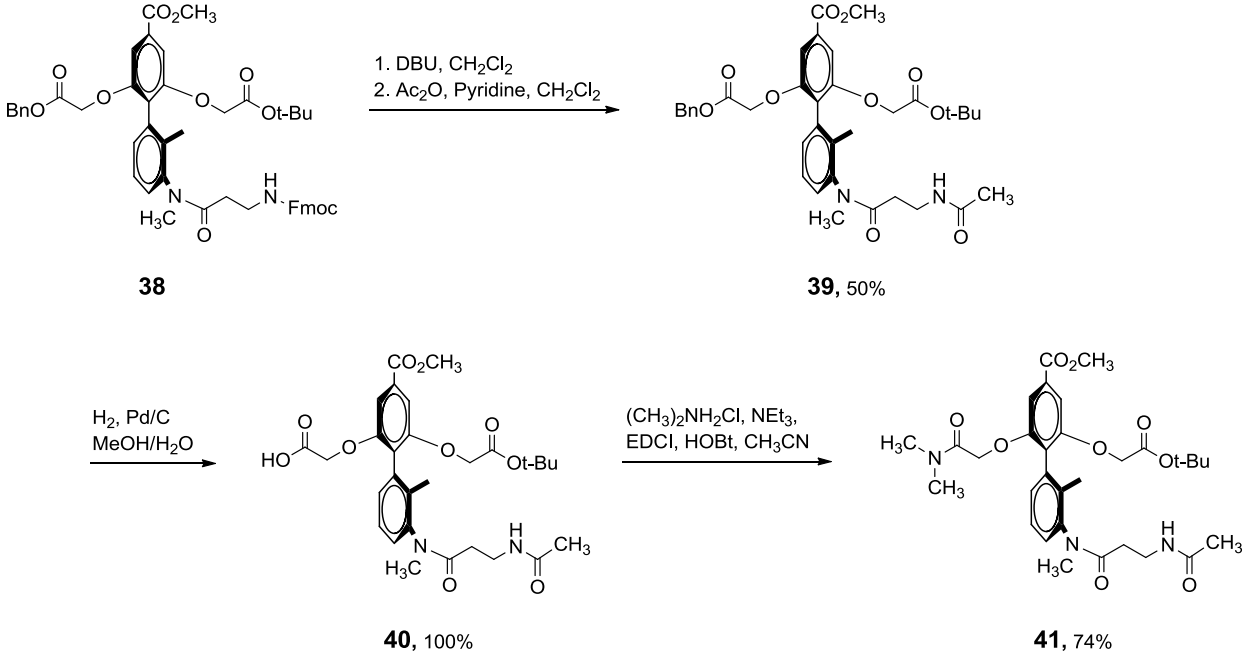
We also subjected derivative **36** to the same studies as **35**, and we noted a carbonyl preference of 1.4:1.0 in toluene-d_8 in comparison to the ratio of 1.0:1.2 for balance **2**. Further analyses involving **36** and other balances were done in comparison with β-alanine derivatives. It was important 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 β-alanine was made because it provided an additional methylene spacer to separate the nitrogen proton from the β-turn core. The syntheses of the β-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-β-alanine acid chloride was generated from *N*-Fmoc-β-alanine **37** and added to **3** to produce biaryl amide **38** in an 82% yield.

Scheme 22. Synthesis of β-alanine biaryl amide 38.



Amide **38** was then treated with DBU in CH_2Cl_2 to cleave the Fmoc group,[43] and the product was acylated using acetic anhydride and pyridine in CH_2Cl_2[32] 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[40] to afford carboxylic acid **40** in quantitative yield, after which **40** was treated with dimethylamine hydrochloride and standard coupling reagents EDCI, HOBt, and triethylamine (NEt_3) in acetonitrile for 80 h at room temperature[41] 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.

Scheme 23. Synthesis of β-alanine torsion balance analog 39 and its dimethylamide derivative 41.



38

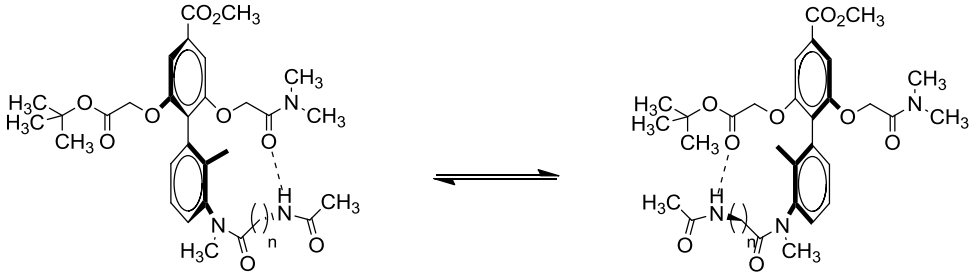
39, 50%

40, 100%

41, 74%

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.



36-R_a,R_a, n = 1
41-R_a,R_a, n = 2

36-R_a,S_a, n = 1
41-R_a,S_a, n = 2

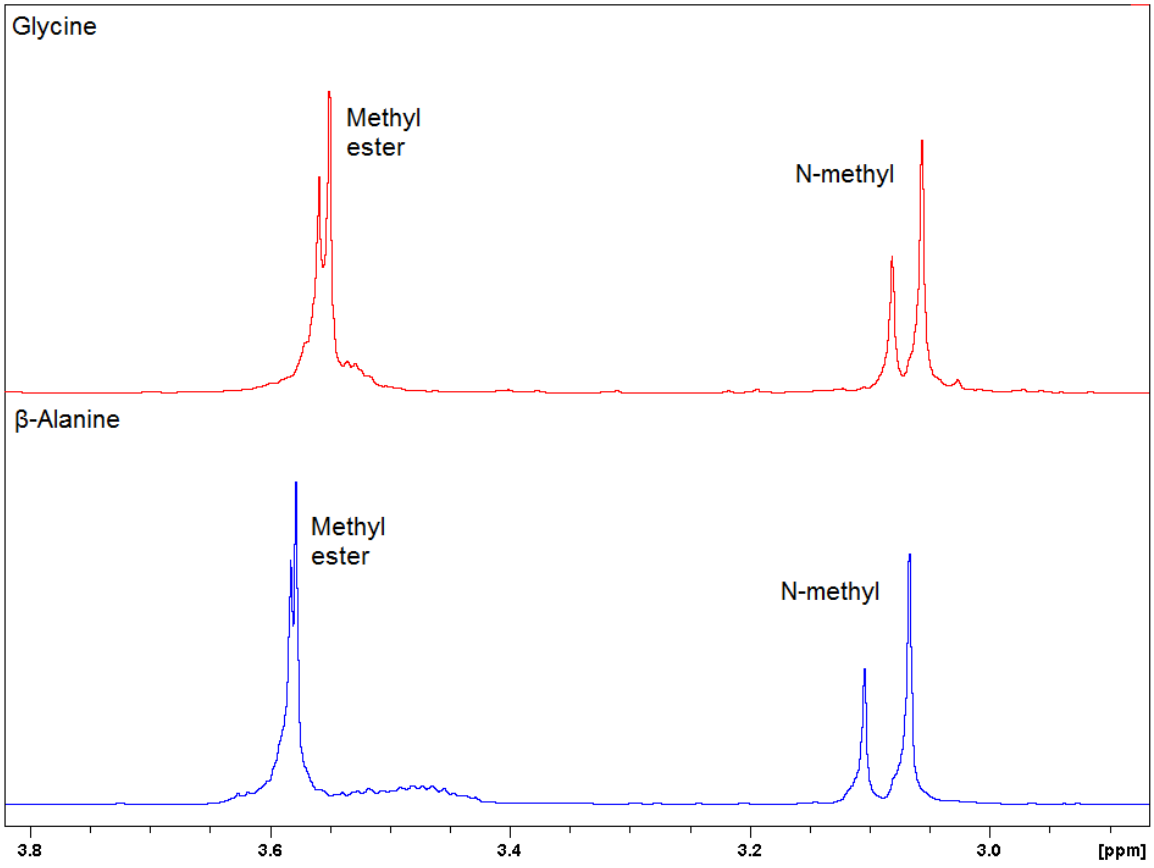
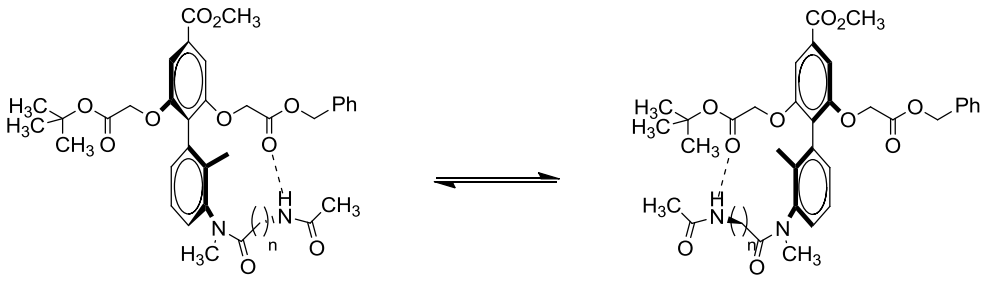


Figure 16. Folding preference exhibited by glycine derivative 36 and β-alanine derivative 41 in toluene-d_8 at 298 K (500 MHz). The *N*-methyl protons are denoted; both derivatives have conformer ratios of 1.0:1.9.

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.



2-R_a,R_a, n = 1
39-R_a,R_a, n = 2

2-R_a,S_a, n = 1
39-R_a,S_a, n = 2

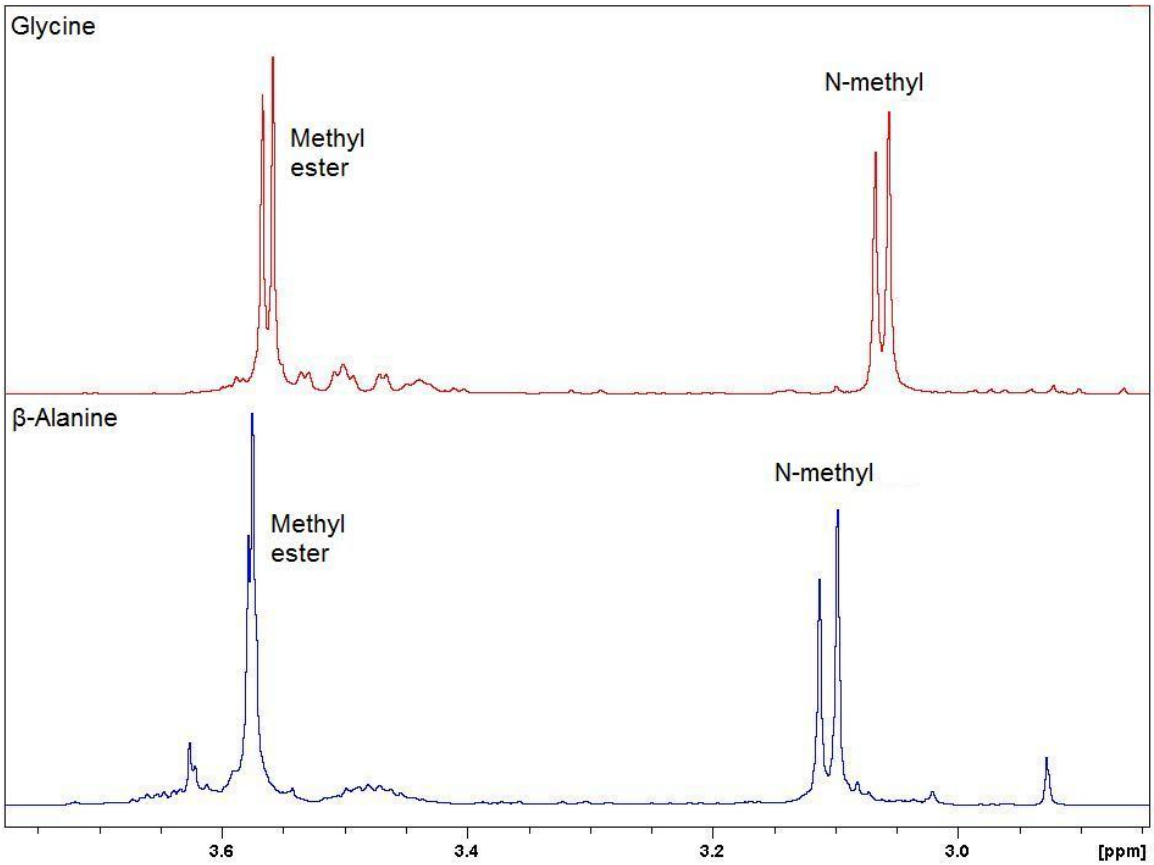


Figure 17. Folding preference exhibited by glycine balance 2 and β-alanine derivative 39 in toluene-d_8 at 298 K (500 MHz). The *N*-methyl protons are denoted.

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_aS_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

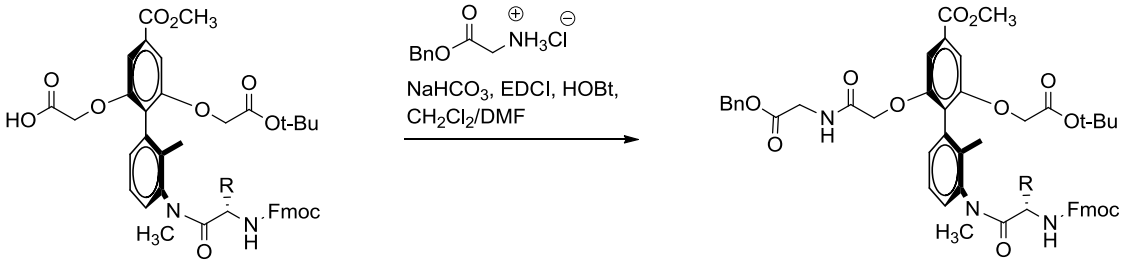
We have described the attainment of an initial set of new molecular torsion balance targets that can be applied toward the study of dynamics in the antiparallel β-sheet motif. This balance utilizes the restricted rotation around an aryl–aryl bond and an N-aryl bond to impose a two–state nature of folding. 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 β-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 HOBt[44] to yield amides **42** and **43** in 99% and 93%, respectively.

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



33 R = CH_3
34 R = H

42 R = CH_3, 99%
43 R = H, 93%

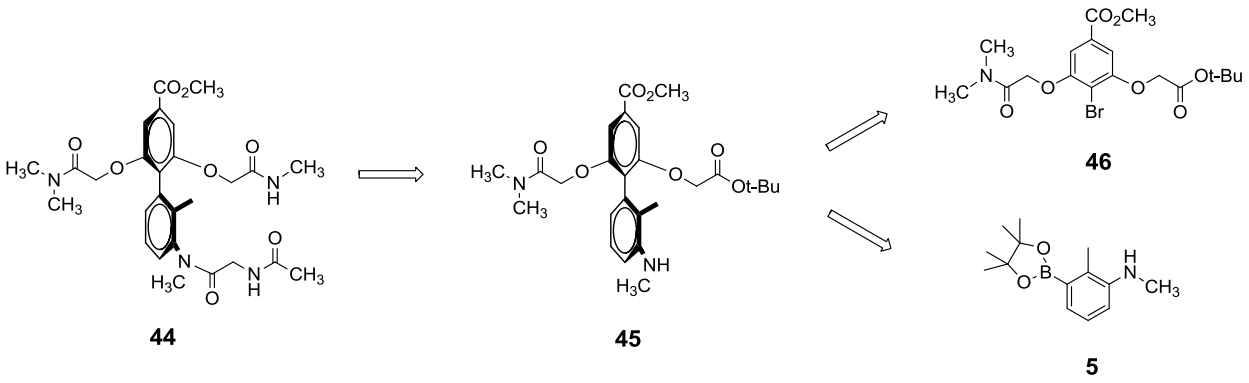
The ^{1}H NMR spectra of these bis(amino acid) torsion balances were examined to determine the amount of conformers present; similar to biaryl amides **27** and **28**, we observed four configurations for alanine-glycine amide **42** and two for glycine-glycine amide **43**. We hope to overcome the issue 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

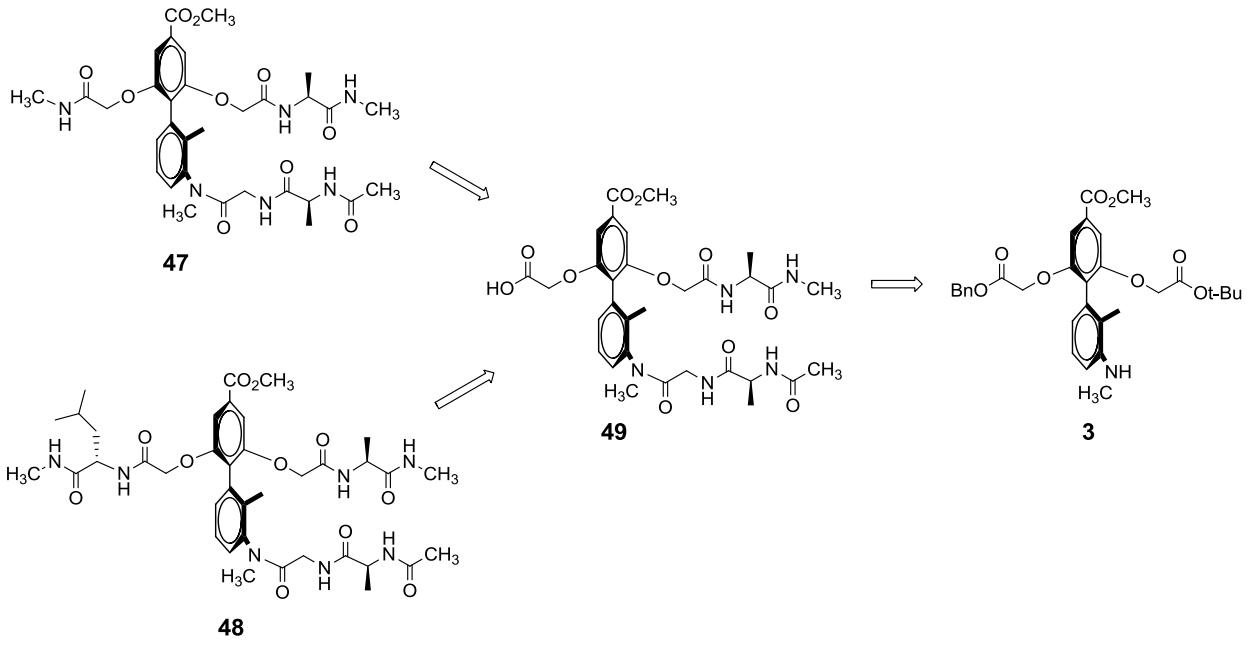
We next planned to expand upon the torsion balance and create target molecules to test how specific changes of amino acids or functional groups would affect peptide folding in antiparallel β-sheets. The first torsion balance design, amide **44**, would test the preference of an amino acid chain to form 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[41] or methylated leucine[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[44], *N*-acetyl-alanine[45], and Fmoc-protected glycine acid chloride derivative[26].

Scheme 28. Retrosynthetic pathway for target amides 47 and 48.



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[44] and *N*-acetyl-alanine[45]. Amide **56** would be synthesized by an amide coupling[26] of symmetrical biaryl amine **58** and Fmoc-protected alanine acid chloride derivative. Amine **58** would be generated by a Suzuki–Miyaura cross-coupling reaction[16] between symmetrical bromide **11** and boronic ester **5**.

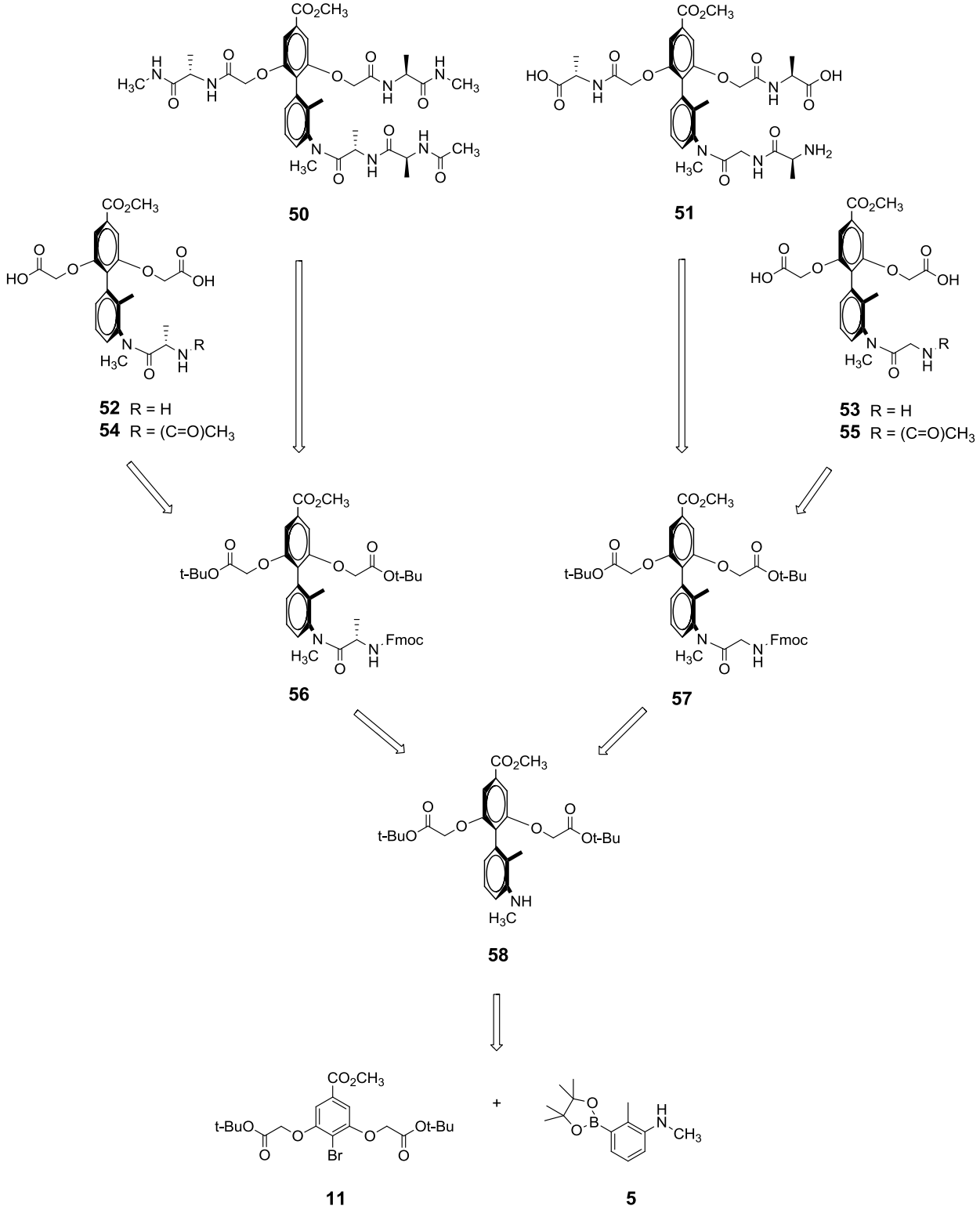
Amide **51** would test the effect of salt-bridging between ammonium and carboxylate moieties of amino acid chains on the rate of rotation around the N-aryl bond. Amide **51** would be

prepared from deprotections and amidation reactions with symmetrical glycine torsion balance **57**. The amidation reagents would include a benzyl protected alanine[45] and *N*-Cbz-alanine[45]. 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 deprotections[45,46] on symmetrical alanine **56** and glycine **57** torsion balances, respectively.

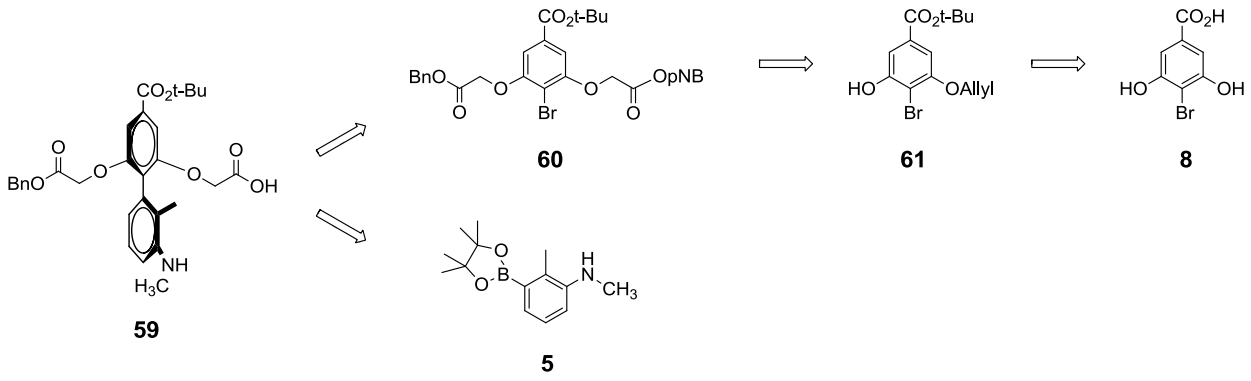
Control amides **54** and **55** would aid in the test for the effect of salt-bridging between an amino acid ammonium and a simple carboxylate moiety on the rate of rotation around the N-aryl bond. These controls would be produced by performing Fmoc-deprotection and acylation,[32] followed by *tert*-butyl ester deprotection[46] 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.



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[16] between asymmetric bromide **60** and boronic ester **5**. The generation of **60** would be realized from two Williamson aryl ether syntheses[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[17] of commercially available acid **8**, then protection of the phenolic groups, with allyl bromide,[47] followed by conversion of the methyl ester to a *tert*-butyl ester[48].

Scheme 30. Retrosynthetic pathway for core target 59.

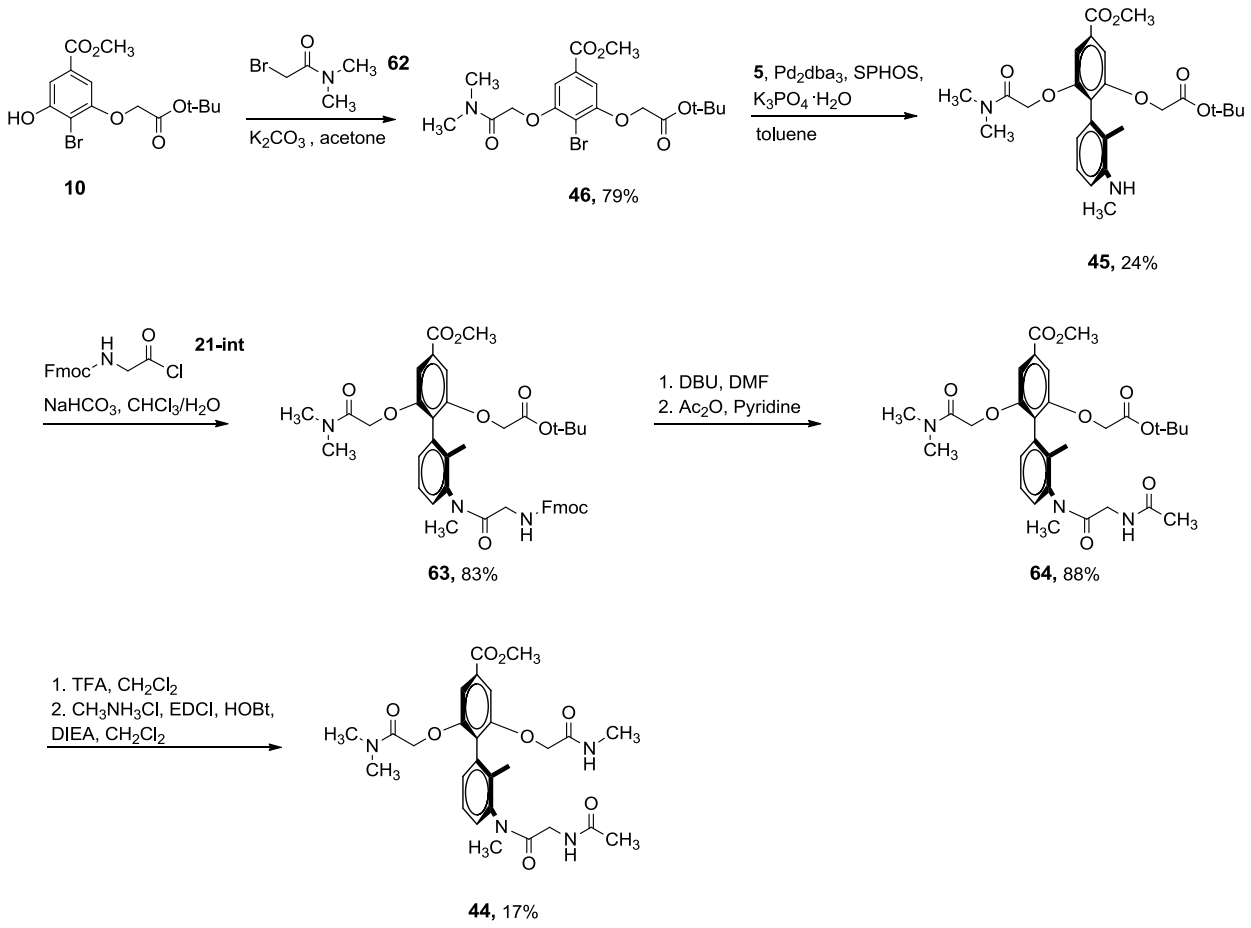


4.2 SYNTHESIS OF ADVANCED TORSION BALANCE TARGETS

4.2.1 Synthesis of the Priority Target 44

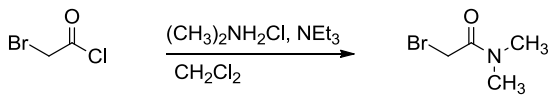
The synthesis of the priority target **44** is shown in Scheme 31. Reaction of ether **10** with dimethylacetamide **62** by a Williamson aryl ether protocol[18] afforded bromide **46** in a good 79% yield. A Suzuki–Miyaura cross-coupling reaction[16] between bromide **46** and boronic ester **5** produced biaryl amine **45** in 24% yield. Carpino's amino acid chloride procedure[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[43] in DMF followed by acetic anhydride and pyridine,[32] to supply amide **64** in an excellent 88% yield. Cleavage of the *tert*-butyl ester with TFA[46] and amidation[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,[50] and the moderate 39% yield of **62** is on par with that literature; the synthesis of **62** is shown in Scheme 32.

Scheme 32. Synthesis of dimethyl bromoacetamide 62.



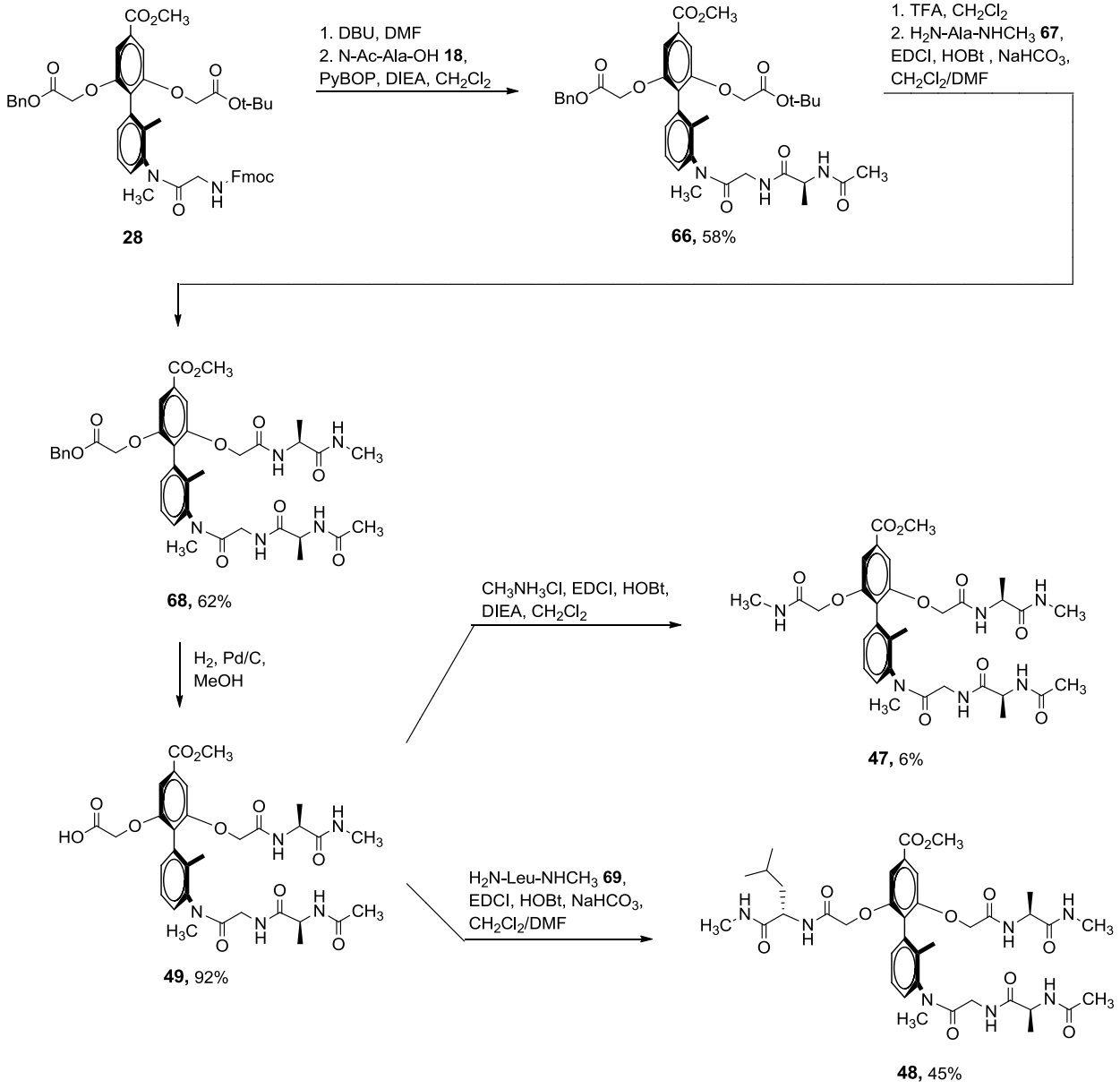
65 **62,** 39%

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[43] in DMF; the DBU was removed by flash chromatography, and the crude was then reacted with *N*-acetyl-alanine **18**, (benzotriazol-1-yloxy)tripyrrolidinophosphonium hexafluorophosphate (PyBOP)[45] 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 ^{1}H NMR spectrum. In another trial with tris (2-aminoethyl)amine (TAEA)[45] 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[44] 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[40] 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[49] produced target

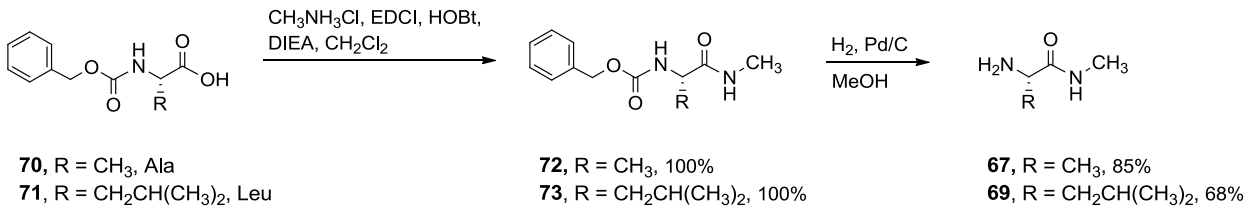
47 in 6% yield, and treatment with methylated leucine **69**, EDCI, HOBt and sodium bicarbonate[44] supplied target **48** in 45% yield.

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[49] 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[40] 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.



70, R = CH_3, Ala
71, R = $CH_2CH(CH_3)_2$, Leu

72, R = CH_3, 100%
73, R = $CH_2CH(CH_3)_2$, 100%

67, R = CH_3, 85%
69, R = $CH_2CH(CH_3)_2$, 68%

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.

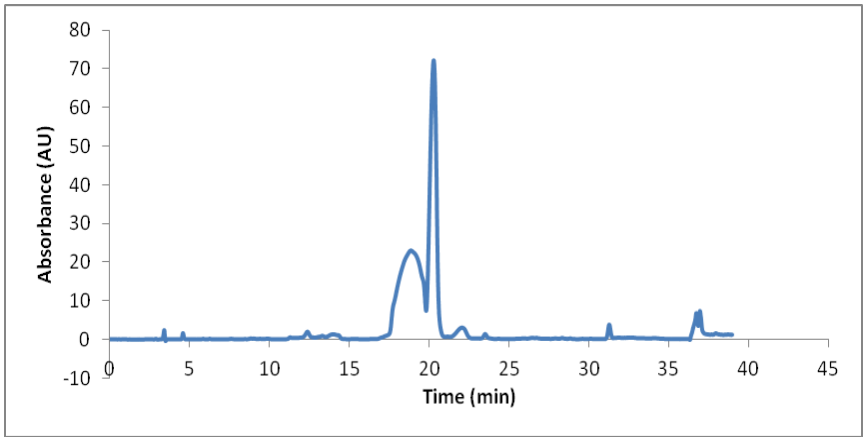


Figure 18. Best analytical HPLC trace of target 48 conformer peaks; target 47 was similar in appearance.

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.

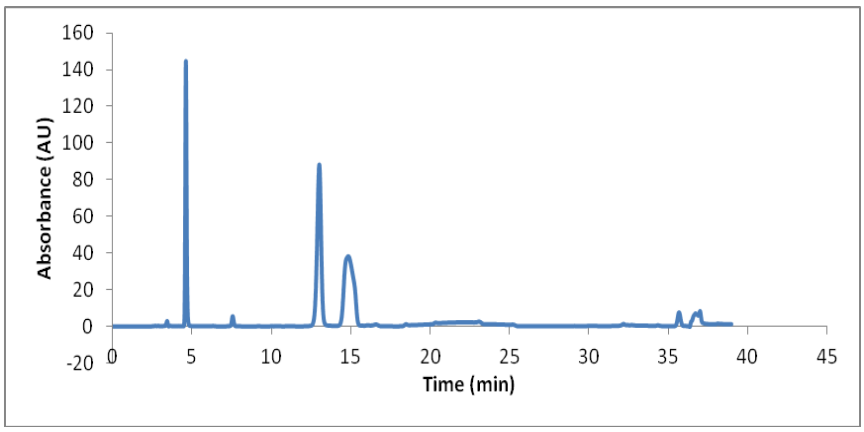
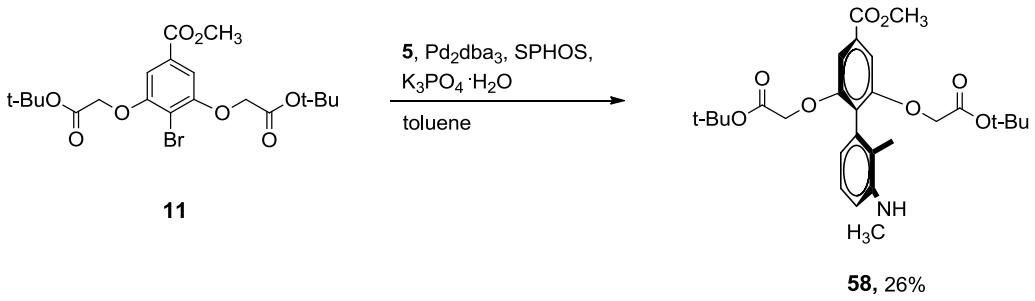


Figure 19. Analytical HPLC chromatogram of the precursor carboxylic acid 49; the two conformer peaks are at 13 and 15 minutes and eluted out with a 20-32% acetonitrile gradient.

4.2.3 Synthesis of the Symmetrical Targets 50-53 and Controls 54-55

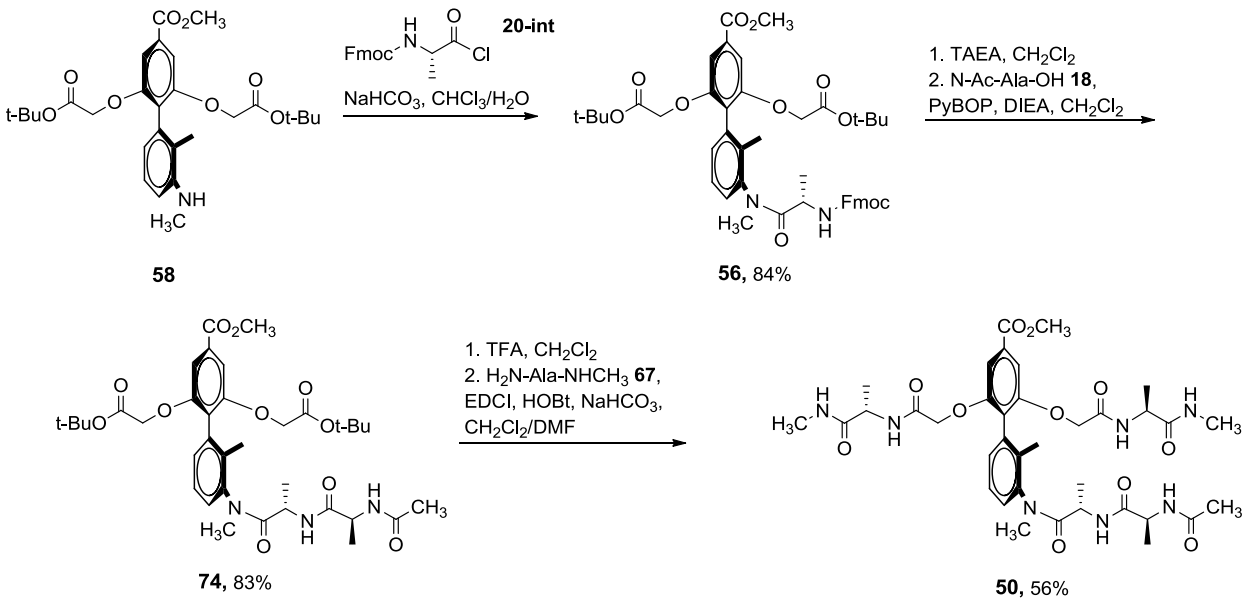
The symmetrical targets were our next aim, and were generated from a symmetrical biaryl amine. Biaryl amine **58**, shown in Scheme 35, was synthesized from symmetrical bromide **11** and boronic ester **5** in a modest 26% yield. The Suzuki–Miyaura cross-coupling conditions[16] used were identical to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

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



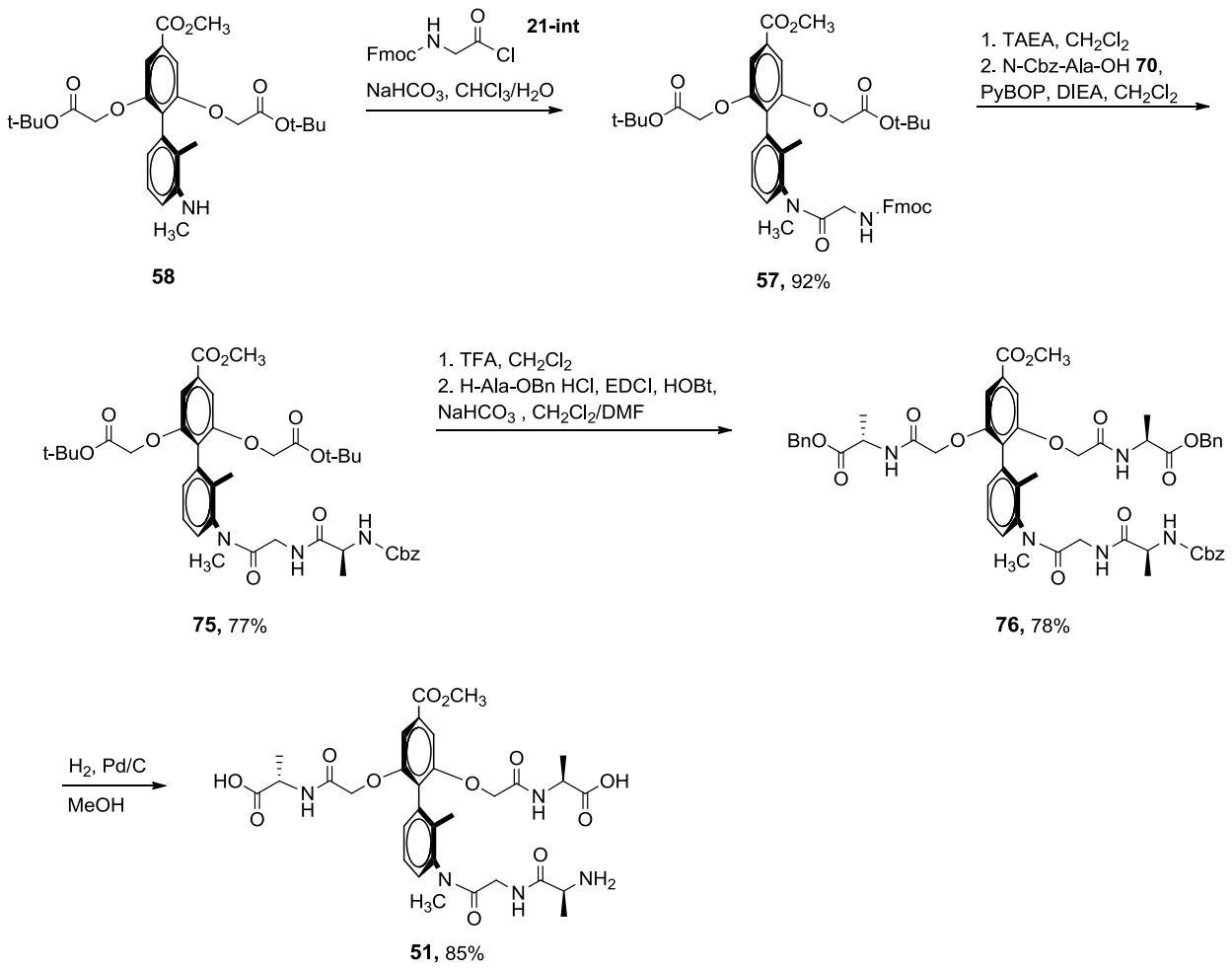
The synthesis of target **50** is shown in Scheme 36, and is similar to that of **47** and **48**. Carpino's amino acid chloride procedure[26] was used to generate *N*-Fmoc-alanine acid chloride **20-int**, which was combined with biaryl amine **58** to give amide **56** in a good 84% yield. Amide **56**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



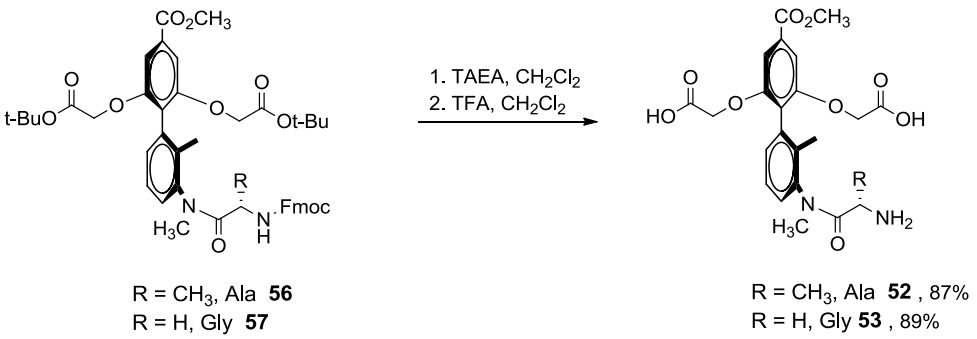
The synthesis of target **51**, which showcases a final global deprotection, is depicted in Scheme 37. Carpino's amino acid chloride procedure[26] was utilized to generate *N*-Fmoc-glycine acid chloride **21-int**, which was then combined with biaryl amine **58** to give amide **57** in an excellent 92% yield. Amide **57** was then treated with TAEA[45] to remove the Fmoc group, and then coupled with *N*-Cbz-alanine **70** using PyBOP[45] and DIEA to give amide **75** in 77% yield. Cleavage of the *tert*-butyl ester with TFA[46] and amidation[44] with commercially available L-alanine benzyl ester hydrochloride, EDCI, HOBt, and sodium bicarbonate produced amide **76** in a good one-pot yield of 78%. Global deprotection of **76**, using standard hydrogenation conditions,[40] gave target **51** in 85% yield.

Scheme 37. Synthesis of target 51.



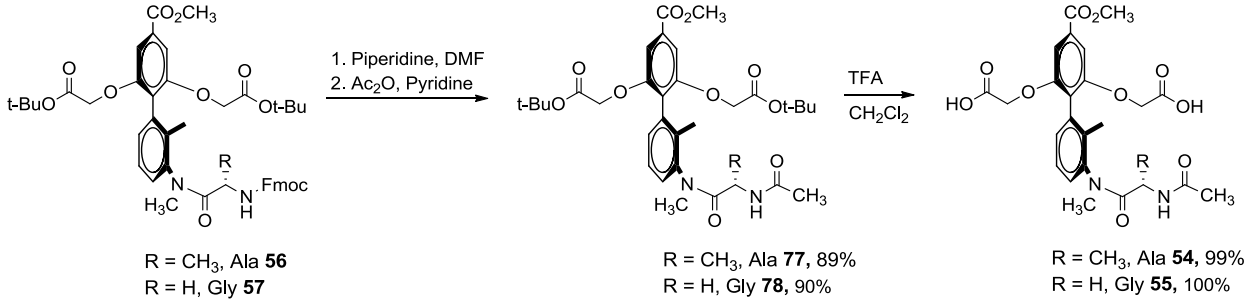
The synthesis of alanine and glycine zwitterion targets **52** and **53**, respectively, is depicted in Scheme 38. Symmetrical amides **56** and **57** were subjected to Fmoc deprotection using TAEA[45] to reveal the free amine; the crude was then treated with TFA[46] to cleave the *tert*-butyl groups 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[43] was substituted for TAEA in CH_2Cl_2, also with minimal effect.

Scheme 38. Synthesis of zwitterion targets 52 and 53.



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[43] followed by acetic anhydride and pyridine,[32] to supply amides **77** and **78** in excellent 90% and 89% yields, respectively. Cleavage of the *tert*-butyl ester with TFA[46] 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.

Scheme 39. Synthesis of control targets 54 and 55.



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[51]) 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.

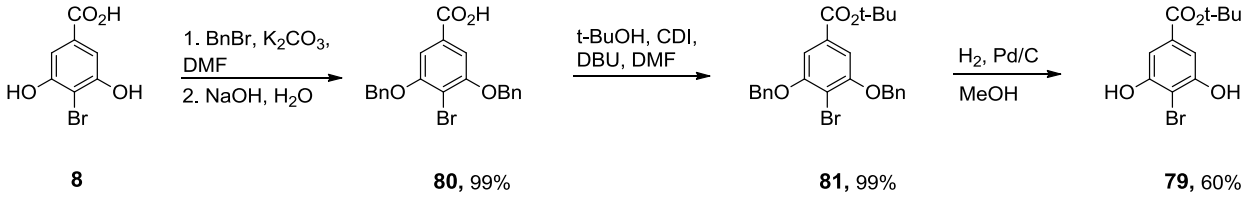
Our plan was to synthesize **59** by initially generating the *tert*-butyl ester analog **79** of methyl ester **9**, and subsequently generate an asymmetrical bromide similar to **4** via Williamson ether protocols. Several reaction conditions from the literature were tried, as depicted in Scheme 40 — di-*tert*-butyl dicarbonate and magnesium perchlorate ($Mg(ClO_4)_2$),[52] *tert*-butanol and DMAP,[46] *tert*-butanol, EDCI and DMAP,[53] *tert*-butanol, sulfuric acid and magnesium sulfate ($MgSO_4$),[54] *tert*-butanol, 1,1'-carbodiimidazole (CDI) and DBU,[55] and *N*,*N*-dimethylformamide di-*tert*-butyl acetal[56] — 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 $Mg(ClO_4)_2$;[52a] 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.

8 **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.[57] Acid **80** was then subjected to *tert*-butyl esterification with *tert*-butanol, CDI and DBU to furnish ester **81** in quantitative yield.[57] Deprotection of the benzyl ethers with standard hydrogenation conditions[40] led to an orange oil, and ^{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.[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[38] followed by acetic acid in a THF/water mixture;[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 $Mg(ClO_4)_2$[52] was unsuccessful.

Scheme 41. Second attempted synthesis of benzoate 79.



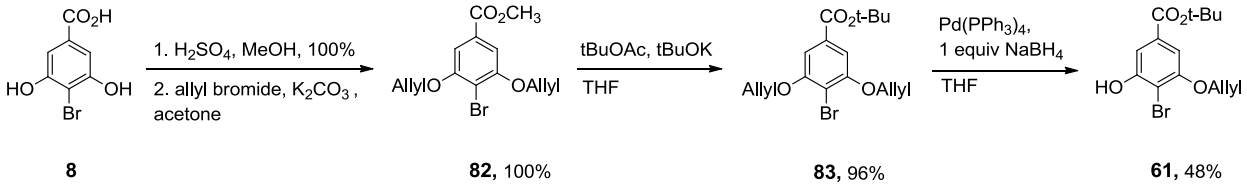
8 **80,** 99% **81,** 99% **79,** 60%

The purified benzoate **79** was then utilized in an attempt to perform a Williamson ether synthesis[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[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[17] to form the methyl aryl ester moiety, and then alkylation was performed with allyl bromide to protect the phenolic groups;[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.[48] The *tert*-butyl ester **83** was then treated with 1 equiv of sodium borohydride ($NaBH_4$) and tetrakis(triphenylphosphine)Pd(0) [$Pd(PPh_3)_4$] to cleave only one allyl ether group[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[60] were used to obtain **79** in 82% yield.

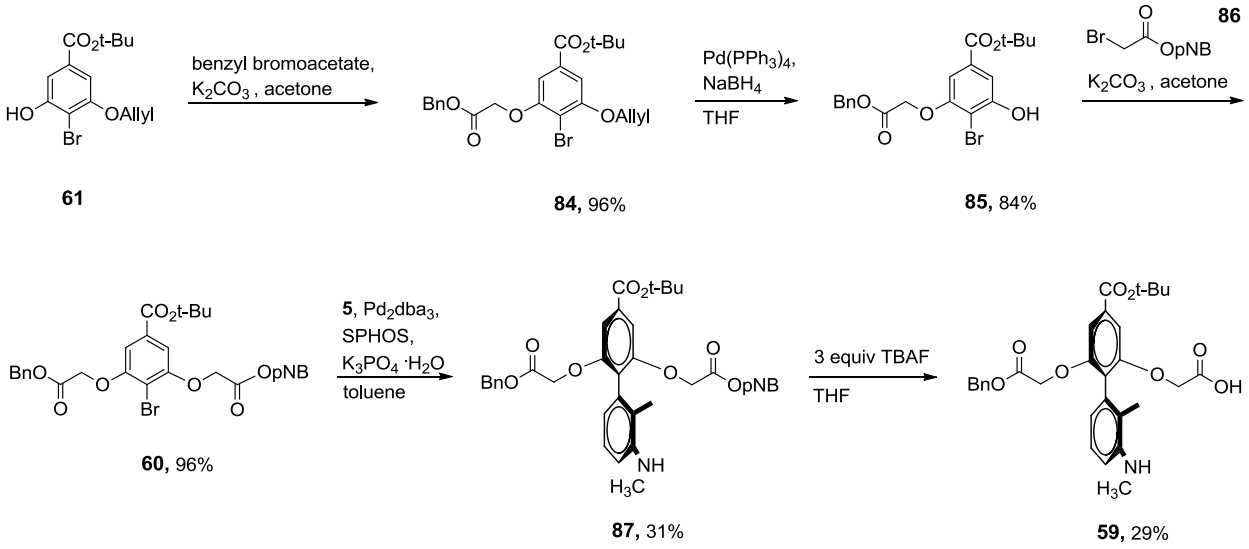
Scheme 42. Revised synthesis toward *tert*-butyl esterification.



8 **82,** 100% **83,** 96% **61,** 48%

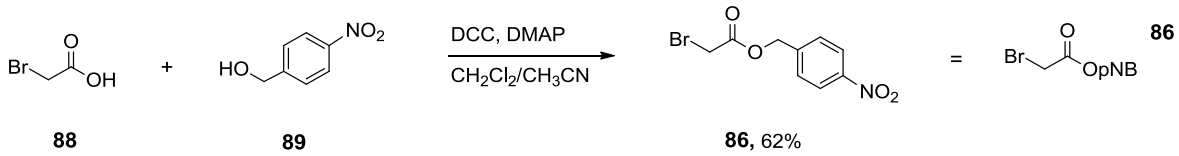
The remaining synthetic steps culminating with the core target **59** are shown in Scheme 43. Bromide **61** was subjected to Williamson ether conditions[18] with benzyl bromoacetate to produce bromide **84** in 96% yield. The remaining allyl group of bromide **84** was removed with $NaBH_4$ and $Pd(PPh_3)_4$ to afford bromide **85** in 84% yield.[60] Bromide **85** was treated with *p*NB bromoacetate **86** in a Williamson ether synthesis[18] to generate asymmetrical bromide **60** in 96% yield. A Suzuki–Miyaura cross-coupling reaction[16] 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 *p*NB ester[61] 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 ^1H NMR spectrum.

Scheme 43. Remaining synthesis toward core target 59.



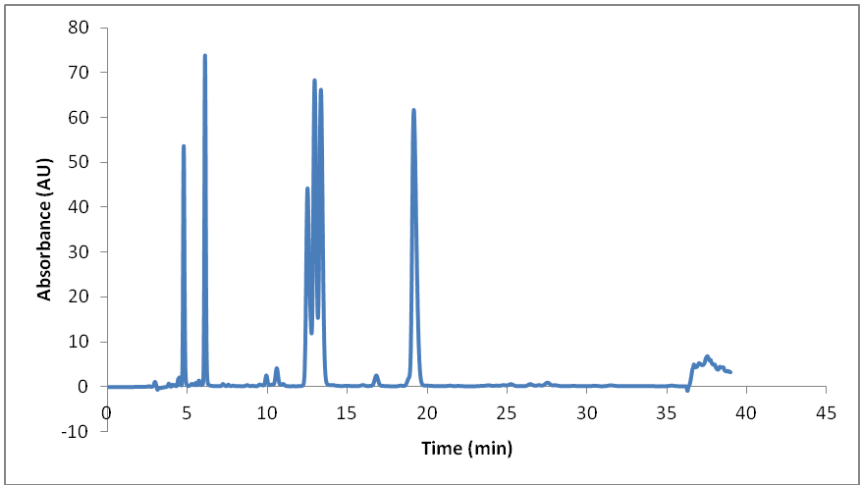
p-Nitrobenzyl (*p*NB) bromoacetate **86** was prepared by an esterification reaction[62] with bromoacetic acid **88** and *para*-nitrobenzyl alcohol **89**, using DCC and DMAP as coupling reagents. The *p*NB bromoacetate **86**, depicted in Scheme 44, was furnished in 62% yield. Commercially available 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] after Suzuki–Miyaura coupling to give **59** resulted in additional cleavage of the benzyl ester, and Suzuki–Miyaura cross coupling[16] of the allyl analog with boronic ester **5** resulted in no isolation of product by analysis of the ^{1}H NMR spectrum. Therefore, the *p*NB ester was the best choice for our orthogonal deprotection strategy toward **59**.

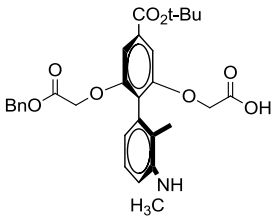
Scheme 44. Synthesis of *p*-nitrobenzyl bromoacetate 86.



88 **89** **86,** 62%

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[64] and (S)-(-)-α-methylbenzylamine[65], 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).





59

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.

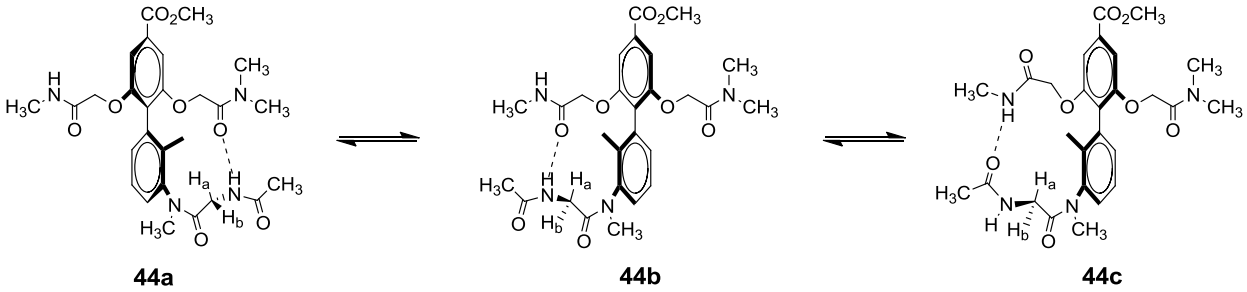
5.0 NMR STUDIES OF ADVANCED TORSION BALANCE TARGETS

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.



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.

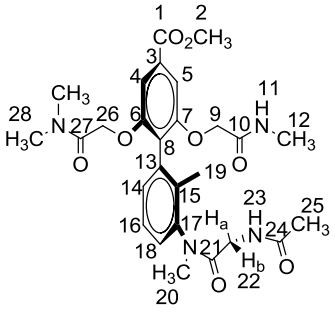


Figure 21. Representation of balance 44 with numbering of atom location (hydrogen, carbon, or nitrogen) for structure assignment.

Table 4. Target 44 assignments (700 MHz) in both chloroform-*d* (left) and toluene-d_8 (right).

No.	^{1}H, ppm, $CDCl_3$	^{13}C, ppm	^{1}H, ppm, Toluene-d8	^{13}C, ppm
1	--	166.0	--	nd
2	3.97 (s, 3H)	52.0	3.59 (s, 3H)	52.0
3	--	132.0	--	nd
4	7.32-7.29 (singlets, 1H)	108.0	7.53-7.34 (singlets, 2H)	107.0
5	7.32-7.29 (singlets, 1H)	108.0	7.53-7.34 (singlets, 2H)	107.0
6	--	123.0	--	nd
7	--	123.0	--	nd
8	--	157.0	--	nd
9	4.55-4.47 (m, 2H)	68.0	4.24, 4.14-4.08 (ABq, m, 2H)	68.0
10	--	168.0	--	nd
11	6.22, 6.07, 5.88 (three broad s, 1H)	--	6.21, 5.50 (two broad s, 1H)	--
12	2.75, 2.71 (two s, 3H)	26.0	2.72, 2.35 (two app s, 3H)	25.0
13	--	126.0	--	nd
14	7.39-7.16 (m, 1H)	127.0	nd	nd
15	--	136.0	--	nd
16	7.39-7.16 (m, 1H)	127.0	nd	nd
17	--	141.0	--	nd
18	7.39-7.16 (m, 1H)	131.0	nd	nd
19	2.07-1.92 (singlets, 3H)	15.0	2.13 (app s, 3H)	14.0
20	3.83-3.30 (singlets, 3H)	38.0	3.10, 3.06 (two s, 3H)	36.0
21	--	168.0	--	nd
22	3.82-3.54 (m, 2H)	42.0	3.86-3.49 (m, 2H)	42.0
23	6.49, 6.41 (two broad s, 1H)	--	6.38, 6.21 (two broad s, 1H)	--
24	--	170.0	--	nd
25	2.09-2.01 (singlets, 3H)	23.0	1.52, 1.49 (two s, 3H)	22.0
26	4.87-4.71 (m, 2H)	66.0	4.59, 4.14-4.08 (ABq, m, 2H)	66.0
27	--	167.0	--	nd
28	2.95-2.92 (singlets, 6H)	37.0	2.55, 2.44, 2.32, 1.95 (four s, 6H)	35.0

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

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.

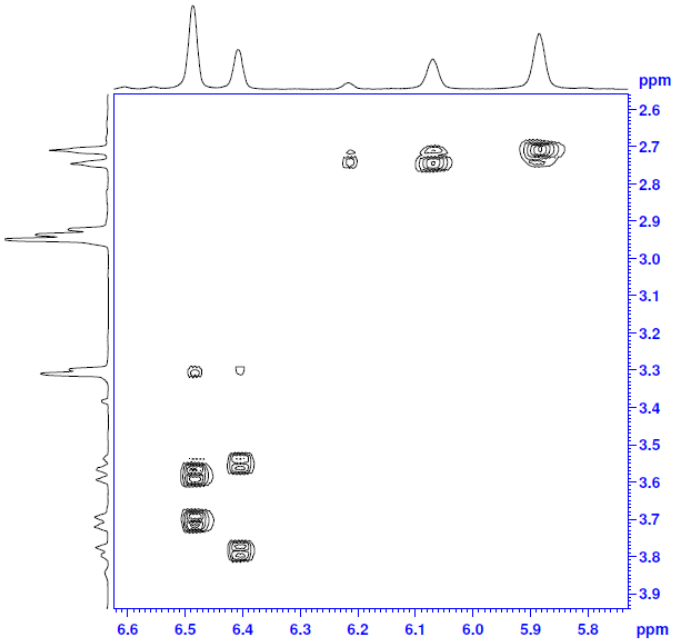


Figure 22. Expansion of TOCSY NMR spectrum in chloroform-*d* at room temperature (700 MHz). The expansion shows the correlation of the two amide protons with their respective neighbors, which was necessary for assignment.

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.

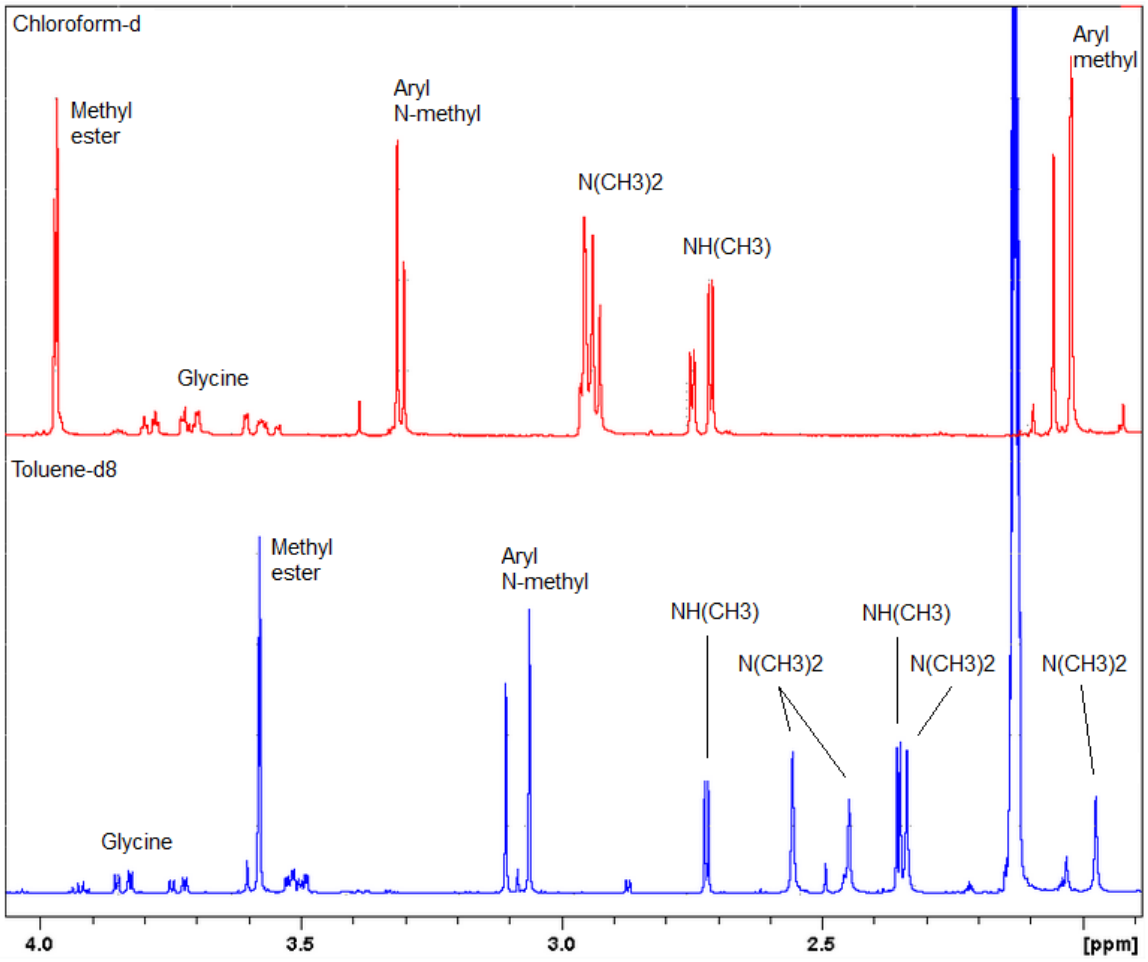


Figure 23. Expansion of ^{1}H NMR spectra comparison of target 44 in chloroform-*d* (top) and toluene-d_8 (bottom) (700 MHz). The N-methyl peaks display the largest changes in chemical shift over a range of 2 ppm.

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.

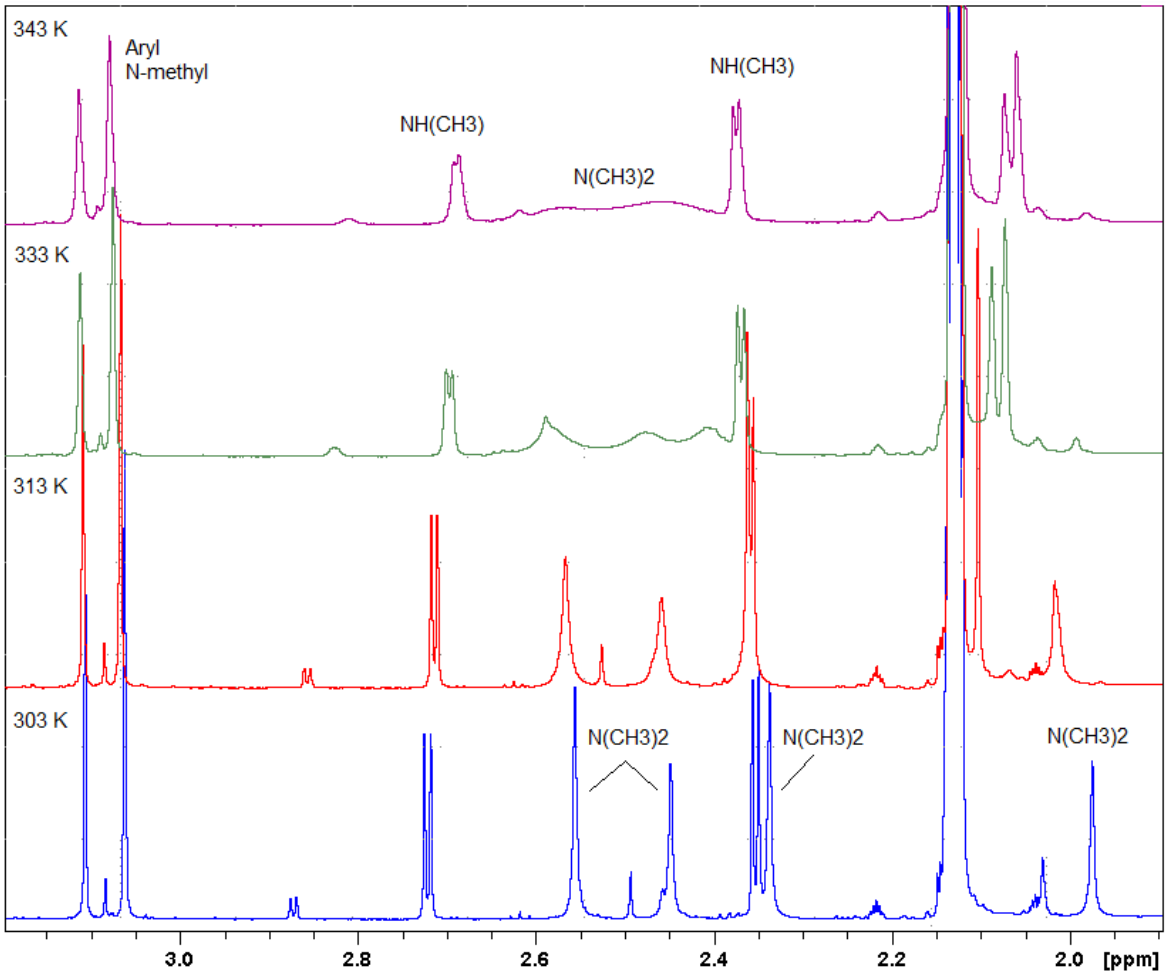


Figure 24. High temperature ^{1}H NMR studies performed in toluene-d_8 (700 MHz). The increase in temperature to 343 K shows a complete coalescence of the four N-dimethyl peaks, while the other N-methyl peaks showed less broadening and coalescence, if any.

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_2O). The sample was sonicated for five minutes at room temperature; the ^1H NMR spectrum acquired revealed no change. Then sonication was performed for 1 h at room temperature, after which a ^1H 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 β-sheet motif secondary structure.

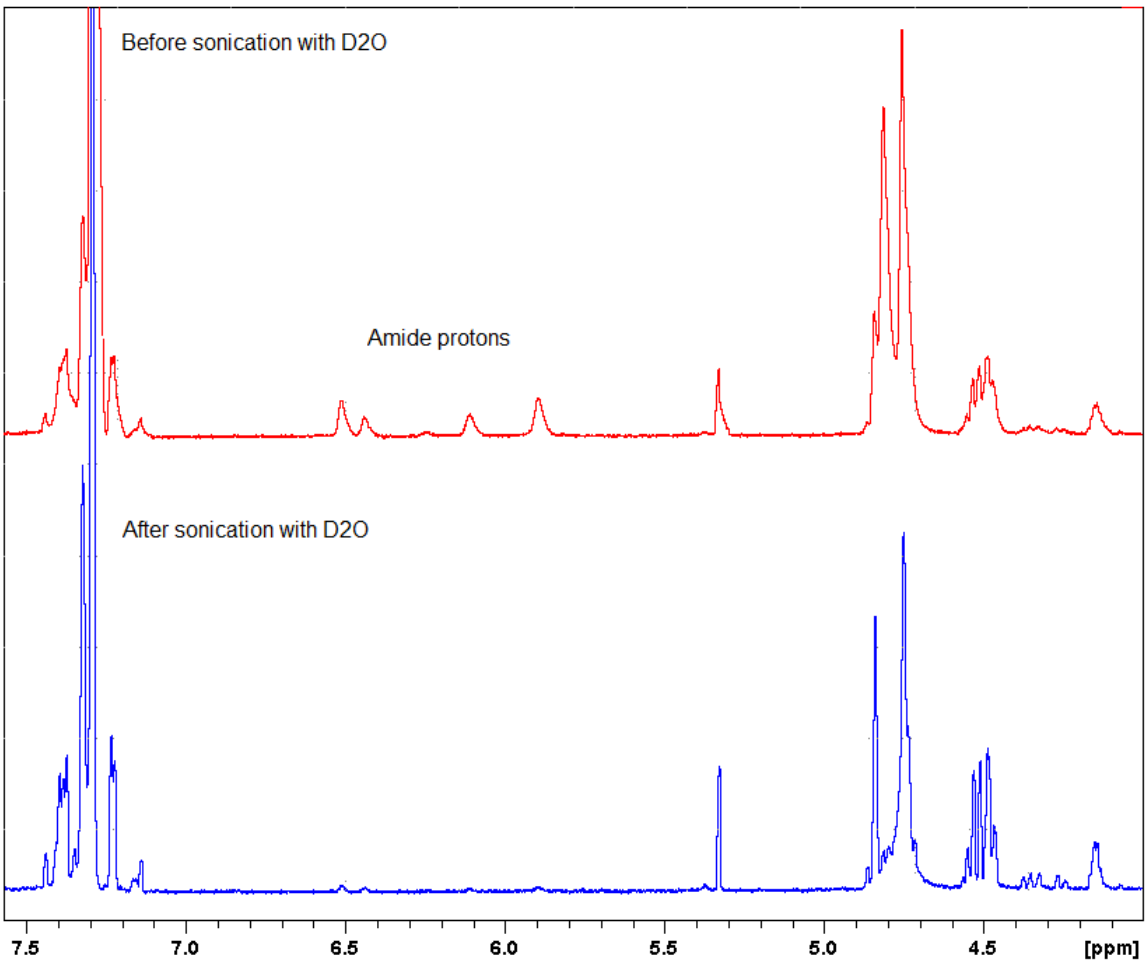


Figure 25. Expansion of ^{1}H NMR spectra (400 MHz) depicting "before and after" exchange with deuterated water at 298 K. After sonication of the NMR sample for 1 h with D_2O, all of the amide protons (between 6.6 and 5.9 ppm) had disappeared almost completely.

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_2Cl_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.

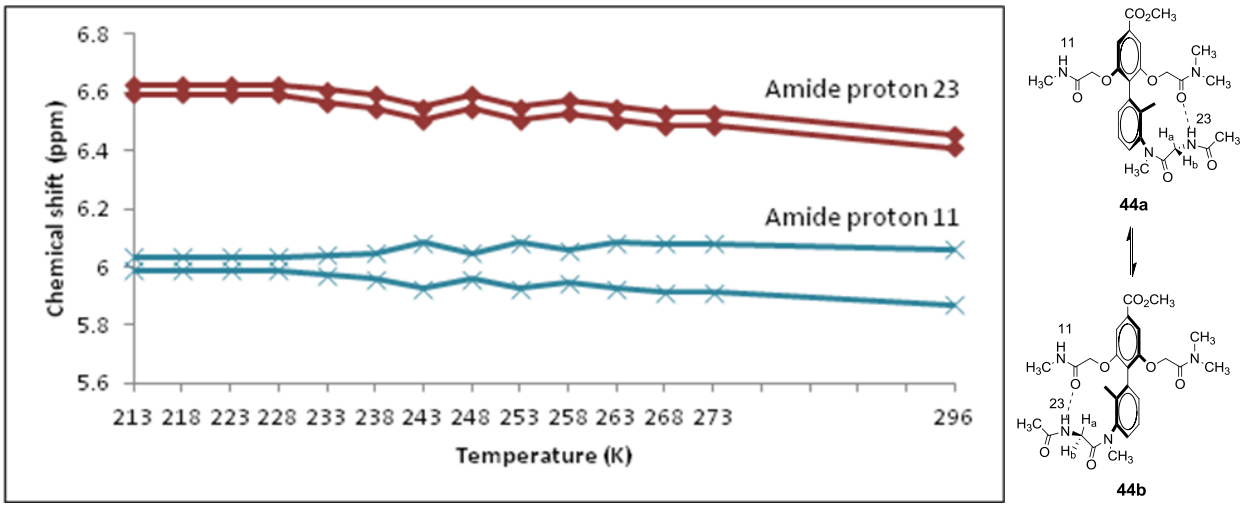


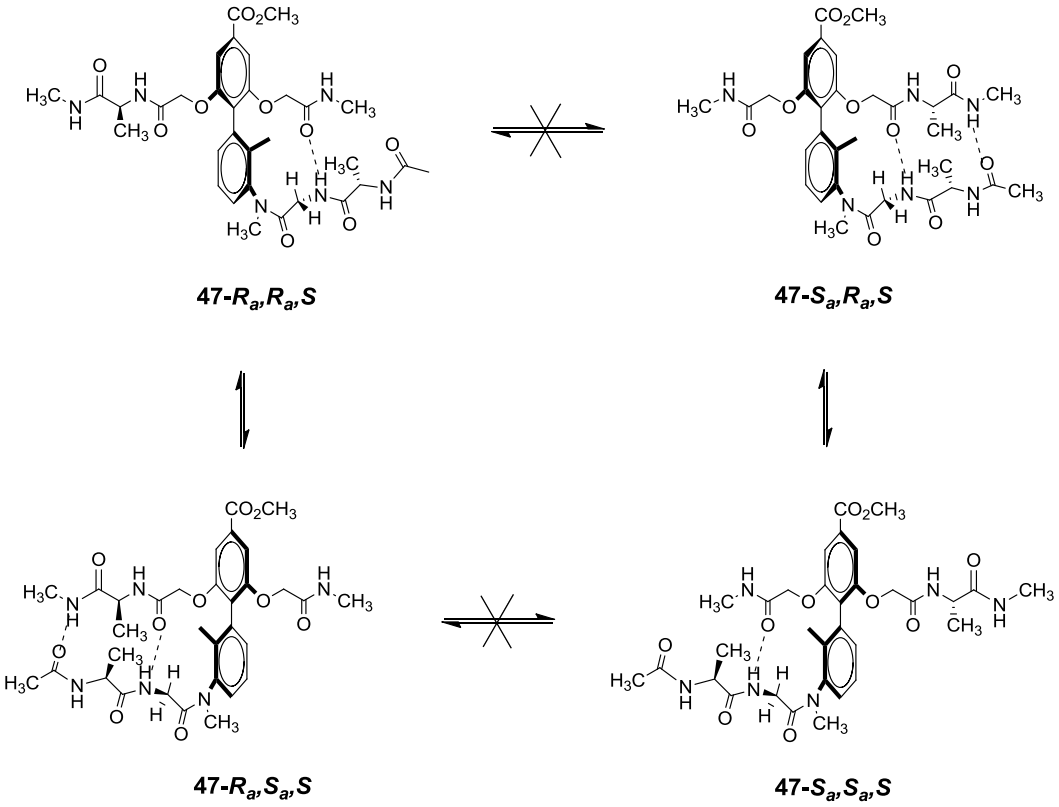
Figure 26. Low temperature ^{1}H NMR studies (methylene chloride-d_2, 400 MHz) were performed on target 44 to probe for intramolecular hydrogen bonding. The two amide protons were studied (location nos. 11 and 23; each amide proton has two ^{1}H NMR peaks, corresponding to the two major conformers 44a 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

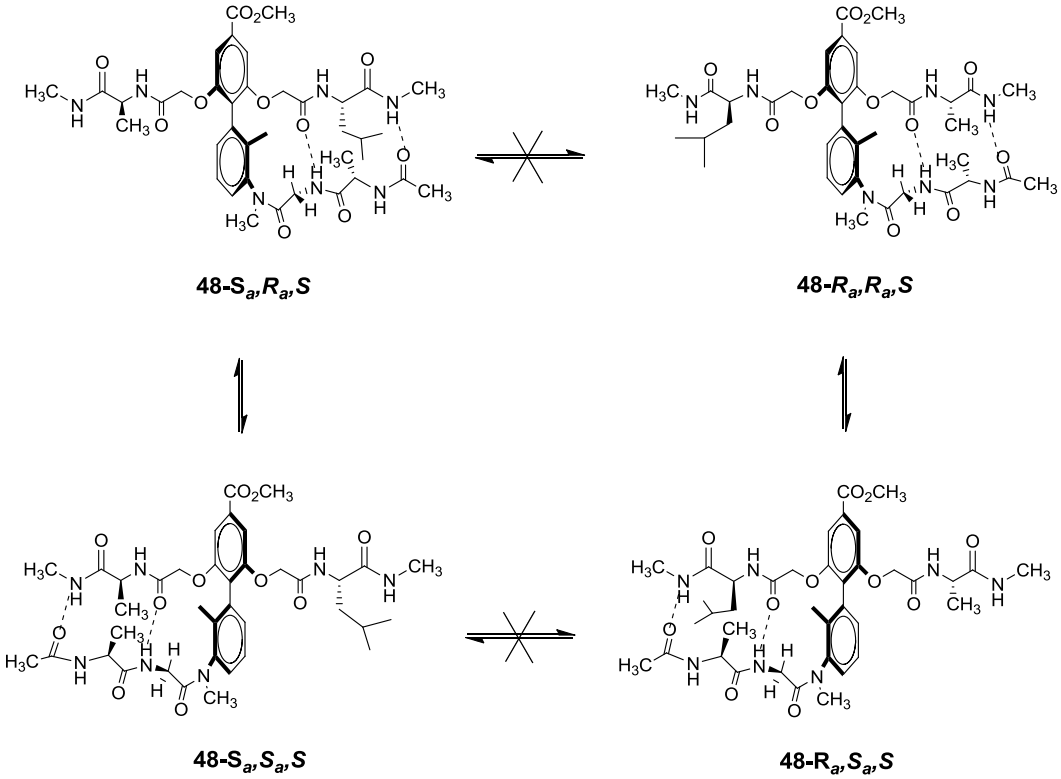
Amide **47** was designed to test the possibility that an amino acid chain may in our model hydrogen bond more strongly when there are one or two sites for hydrogen bonding in a chain; amide **48** would test the partiality of an amino acid chain to form hydrogen bonds more strongly with an alanine or 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.

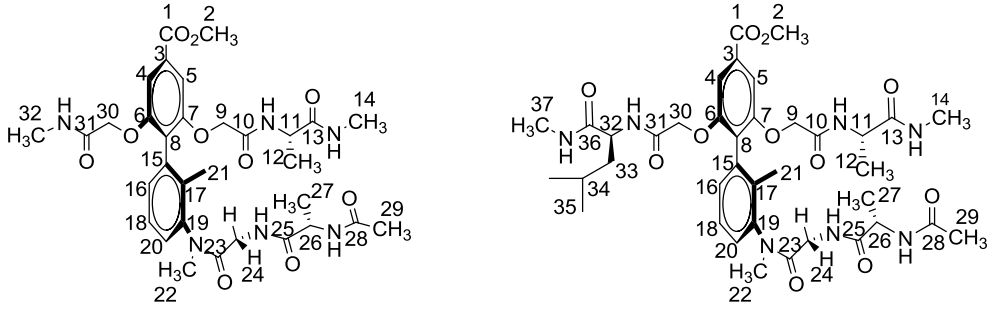


Figure 27. Representation of balances 47 (left) and 48 (right) with numbering of atom location (hydrogen or carbon) for structure assignment.

Table 5. Target 47 (5A, left) and 48 (5B, right) ^{1}H and ^{13}C NMR assignments (700 MHz) in MeOD-d_4.

No.	^{1}H, ppm, MeOD	^{13}C, ppm
1	--	166.0
2	3.97 (singlets, 3H)	51.0
3	--	131.0
4	7.44-7.45 (singlets, 1H)	107.0
5	7.44-7.45 (singlets, 1H)	107.0
6	--	124.0
7	--	124.0
8	--	156.0
9	4.66-4.58 (m, 2H)	67.0
10	--	168.0
11	4.42-4.36 (m, 1H)	49.0
12	1.38-1.36 (m, 3H)	17.0
13	--	173.0
14	2.74-2.69 (singlets, 3H)	25.0
15	--	127.0
16	7.47-7.35 (m, 1H)	127.0
17	--	135.0
18	7.47-7.35 (m, 1H)	127.0
19	--	141.0
20	7.47-7.35 (m, 1H)	131.0
21	2.06-2.05 (singlets, 3H)	13.0
22	3.29-3.26 (singlets, 3H)	35.0
23	--	169.0
24	3.68-3.60 (m, 2H)	41.0
25	--	173.0
26	4.35-4.28 (m, 1H)	48.0
27	1.24-1.21 (m, 3H)	17.0
28	--	171.0
29	2.00-1.99 (singlets, 3H)	21.0
30	4.57-4.50 (m, 2H)	68.0
31	--	168.0
32	2.71-2.66 (singlets, 3H)	25.0

No.	^{1}H, ppm, MeOD	^{13}C, ppm
1	--	167.0
2	3.96 (singlets, 3H)	52.0
3	--	131.0
4	7.43-7.43 (singlets, 1H)	107.0
5	7.43-7.43 (singlets, 1H)	107.0
6	--	123.0
7	--	123.0
8	--	156.0
9	4.69-4.52 (m, 2H)	67.0
10	--	168.0
11	4.39-4.37 (m, 1H)	48.0
12	1.38-1.37 (m, 3H)	17.0
13	--	172.0
14	2.74-2.69 (singlets, 3H)	25.0
15	--	127.0
16	7.48-7.36 (m, 1H)	127.0
17	--	136.0
18	7.48-7.36 (m, 1H)	127.0
19	--	141.0
20	7.48-7.36 (m, 1H)	130.0
21	2.07-2.05 (singlets, 3H)	14.0
22	3.29-3.27 (singlets, 3H)	34.0
23	--	169.0
24	3.70-3.55 (m, 2H)	42.0
25	--	172.0
26	4.36-4.28 (m, 1H)	48.0
27	1.24-1.20 (m, 3H)	18.0
28	--	172.0
29	2.06-2.00 (singlets, 3H)	22.0
30	4.69-4.52 (m, 2H)	67.0
31	--	167.0
32	4.39-4.37 (m, 1H)	52.0
33	1.55-1.46 (m, 2H)	42.0
34	1.41-1.37 (app m, 1H)	24.0
35	0.93-0.84 (m, 6H)	23.0
36	--	173.0
37	2.74-2.69 (singlets, 3H)	25.0

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_3CN 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_2Cl_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_2Cl_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.

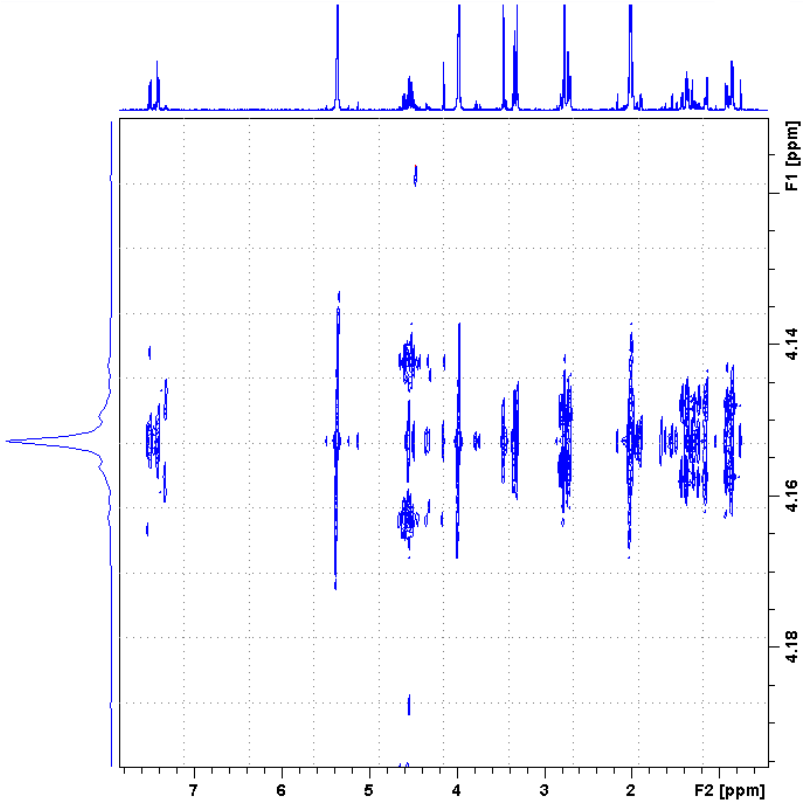
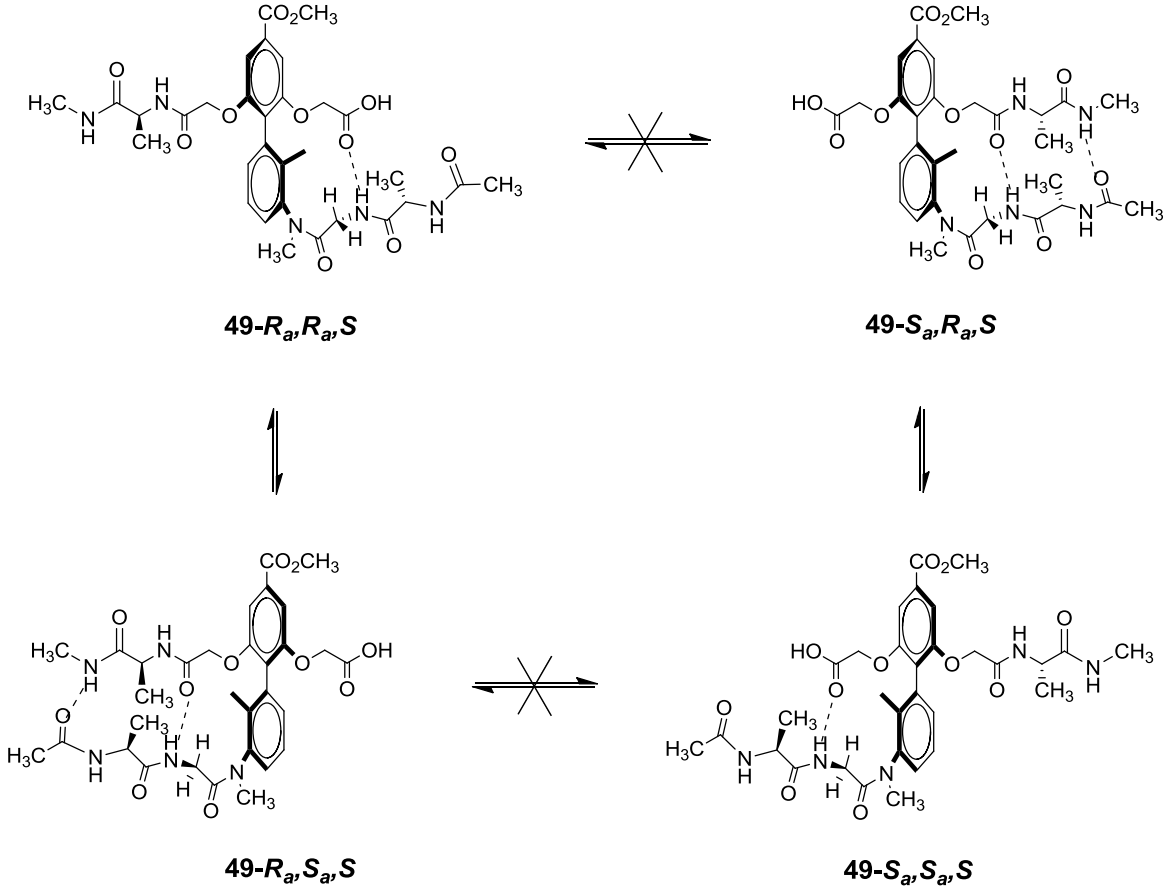


Figure 28. A J-resolution 2D NMR experiment (methylene chloride-d_2, 700 MHz) was performed on target 48; the projected 1D NMR can be seen at the top, illustrating the collapse (disappearance) of all amide protons, glycine methylene protons, and alanine methine peaks.

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.



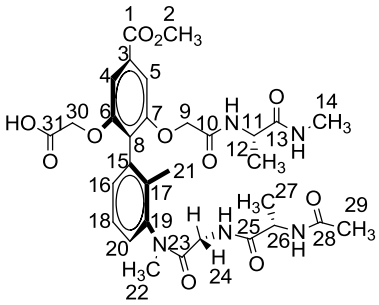


Figure 29. Representation of balance 49 with numbering of atom location (hydrogen or carbon) for structure assignment.

Table 6. Precursor acid 49 ^{1}H NMR assignments (400 MHz) in MeOD-d_4 of HPLC-separated conformer pairs.

No.	^{1}H, ppm, MeOD, 13 min elution	^{1}H, ppm, MeOD, 15 min elution
1	--	--
2	3.96 (s, 3H)	3.96 (s, 3H)
3	--	--
4	nd (aromatic 1H)	nd (aromatic 1H)
5	nd (aromatic 1H)	nd (aromatic 1H)
6	--	--
7	--	--
8	--	--
9	4.72-4.49 (singlets, ABq, 2H)	4.70-4.59 (singlets, 2H)
10	--	--
11	4.40 (quintet, 1H)	4.39 (q, 1H)
12	1.38 (dd, 3H)	1.37 (dd, 3H)
13	--	--
14	2.74, 2.73 (two s, 3H)	2.70, 2.68 (two s, 3H)
15	--	--
16	7.45-7.33 (m, 1H)	7.43-7.31 (m, 1H)
17	--	--
18	7.45-7.33 (m, 1H)	7.43-7.31 (m, 1H)
19	--	--
20	7.45-7.33 (m, 1H)	7.43-7.31 (m, 1H)
21	2.06 (s, 3H)	2.08, 2.06 (two s, 3H)
22	3.28, 3.26 (two s, 3H)	3.30, 3.26 (two s, 3H)
23	--	--
24	3.73-3.55 (m, 2H)	3.77-3.57 (m, 2H)
25	--	--
26	4.33-4.25 (m, 1H)	4.35-4.29 (m, 1H)
27	1.22, 1.20 (two d, 3H)	1.28, 1.23 (two d, 3H)
28	--	--
29	2.01, 2.00 (two s, 3H)	2.01, 2.00 (two s, 3H)
30	4.72-4.49 (singlets, ABq, 2H)	4.70-4.59 (singlets, 2H)
31	--	--

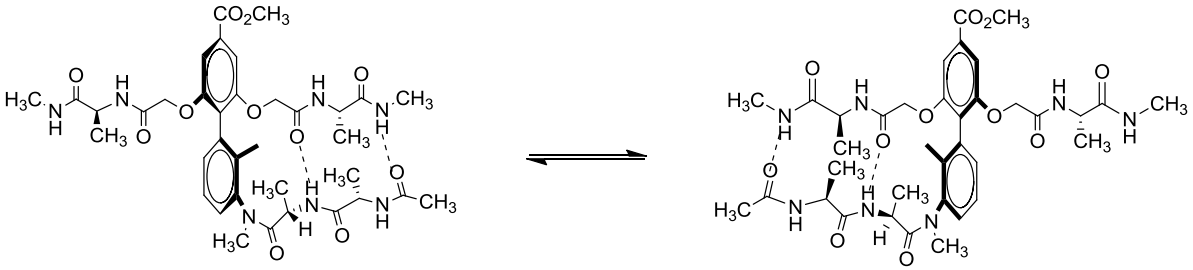
A ROESY NMR spectrum was obtained for precursor **49** in CD_3CN at 343 K; however, no EXSY crosspeaks were observed (two mixing times, 0.2 s and 0.1 s, were used to confirm this observation). ROESYs at room temperature in CD_3CN for both sets of diastereomers revealed

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.



50-R$_a$,S$_a$,S$_a$ **50-S$_a$,S$_a$,S$_a$**

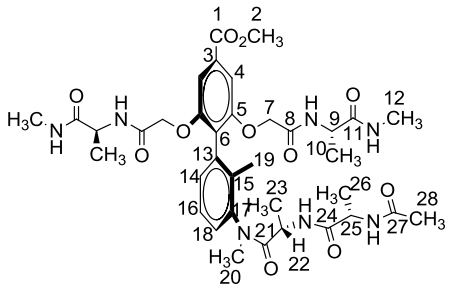


Figure 30. Representation of balance 50 with numbering of atom location (hydrogen or carbon) for structure assignment.

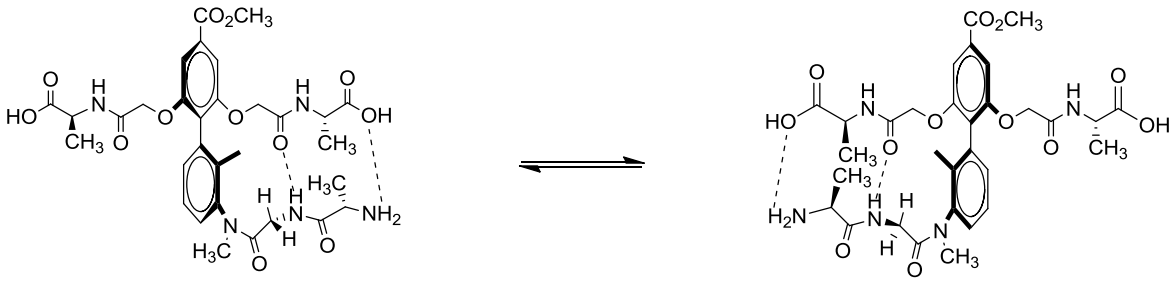
Table 7. Target 50 ^{1}H and ^{13}C NMR assignments (700 MHz) in MeOD-d_4.

No.	^{1}H, ppm, MeOD	^{13}C, ppm
1	--	166.0
2	3.97, 3.96 (singlets, 3H)	52.0
3	--	134.0
4	7.44-7.43 (singlets, 2H)	107.0
5	--	124.0
6	--	156.0
7	4.63-4.48 (m, 4H)	68.0
8	--	169.0
9	4.35-4.30 (m, 2H)	48.0
10	1.38-1.11 (doublets, m, 6H)	18.0
11	--	173.0
12	2.77-2.70 (singlets, 6H)	26.0
13	--	128.0
14	7.44-7.35 (m, 1H)	130.0
15	--	136.0
16	7.44-7.35 (m, 1H)	130.0
17	--	142.0
18	7.44-7.35 (m, 1H)	132.0
19	2.07-2.04 (singlets, 3H)	14.0
20	3.50-3.23 (singlets, 3H)	38.0
21	--	174.0
22	4.35-4.30 (m, 1H)	50.0
23	1.38-1.11 (doublets, m, 3H)	18.0
24	--	168.0
25	4.35-4.30 (m, 1H)	50.0
26	1.38-1.11 (doublets, m, 3H)	18.0
27	--	172.0
28	2.00-1.96 (singlets, 3H)	21.0

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.



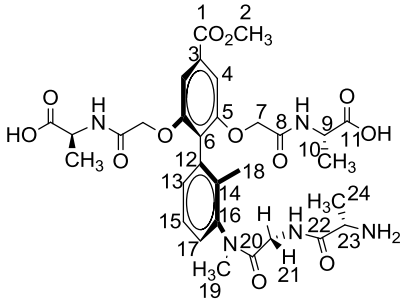


Figure 31. Representation of balance 51 with numbering of atom location (hydrogen or carbon) for structure assignment.

Table 8. Target 51 ^{1}H and ^{13}C NMR assignments (700 MHz) in MeOD-d_4.

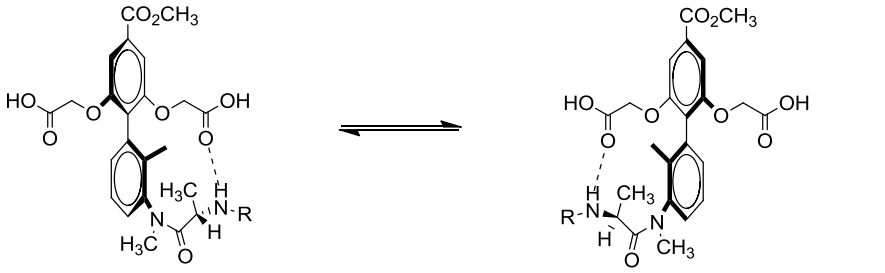
No.	^{1}H, ppm, MeOD	^{13}C, ppm
1	--	167.0
2	3.98-3.97 (two s, 3H)	51.0
3	--	133.0
4	7.47-7.46 (two s, 2H)	107.0
5	--	123.0
6	--	156.0
7	4.64-4.51 (m, 4H)	67.0
8	--	167.0
9	4.39, 4.27 (two quintets, 2H)	47.0, 48.0
10	1.33-1.28 (m, 6H)	18.0
11	--	175.0
12	--	127.0
13	7.46-7.44 (m, 1H)	129.0
14	--	135.0
15	7.39-7.35 (m, 1H)	129.0
16	--	142.0
17	7.39-7.35 (m, 1H)	132.0
18	2.07, 2.06 (two s, 3H)	13.0
19	3.29, 3.27 (two s, 3H)	36.0
20	--	168.0
21	3.73-3.66 (m, 2H)	42.0
22	--	169.0
23	3.98-3.97 (m, 1H)	52.0
24	1.53-1.52 (dd, 3H)	27.0

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,[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.

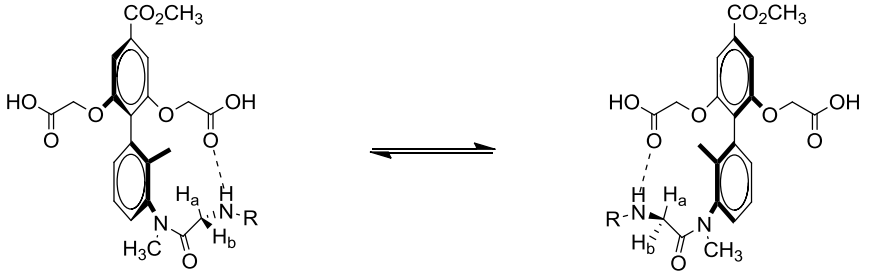
Scheme 51. Alanine zwitterion 52 and corresponding control 54.



52-R*a*,S*a*, R = H
54-R*a*,S*a*, R = (C=O)CH_3

52-S*a*,S*a*, R = H
54-S*a*,S*a*, R = (C=O)CH_3

Scheme 52. Glycine zwitterion 53 and corresponding control 55.



53-*R*, R = H
55-*R*, R = (C=O)CH_3

53-*S*, R = H
55-*S*, R = (C=O)CH_3

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.

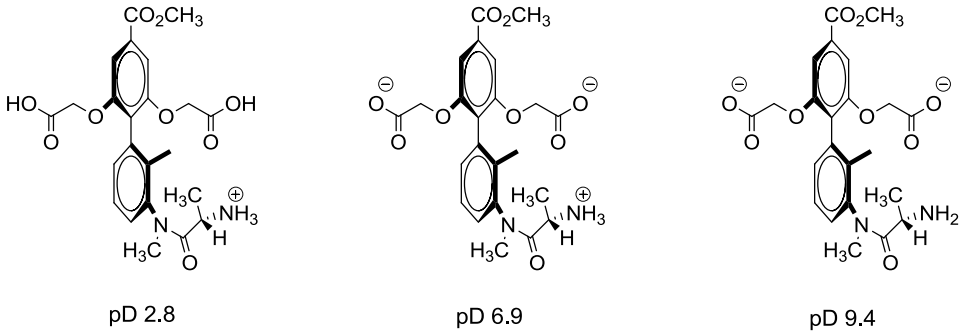


Figure 32. Structures of alanine zwitterion 52 in different pD buffers.

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).

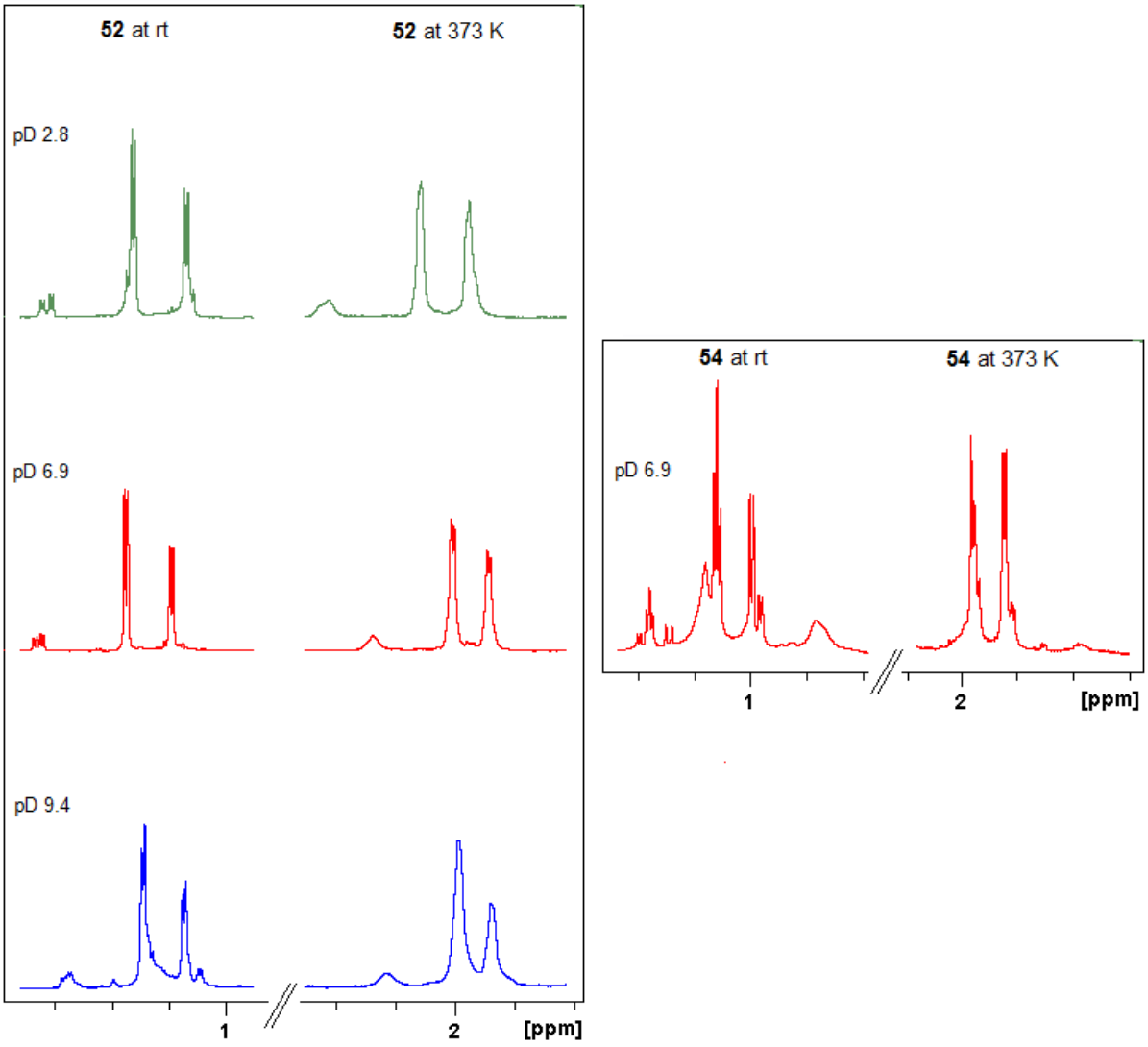


Figure 33. Variable temperature and buffer ^{1}H NMR spectra (600 MHz) of alanine α-methyl protons of zwitterion 52 in three pD buffers 2.8, 6.9, and 9.4 (left), and control 54 only in pD buffer 6.9 (right). Both compounds were subjected to either room temperature or 373 K and all showed chemical shift 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_2O 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 α-methyl major conformer peaks for zwitterion 52 and control 54, corresponding to Figure 32 (listed in "upfield/downfield" format).

	Zwitterion **52**		Control **54**	
pD	373K	rt	373K	rt
2.8	1.04/1.0	0.773/1.0		
6.9	0.862/1.0	0.785/1.0	0.924/1.0	0.78/1.0
9.4	0.661/1.0	0.588/1.0		

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_3CN, and high temperature ROESYs were obtained for **51** in either buffer pD 6.9 or CD_3CN; these data, focusing on crosspeaks of interest, are displayed in Figure 34.

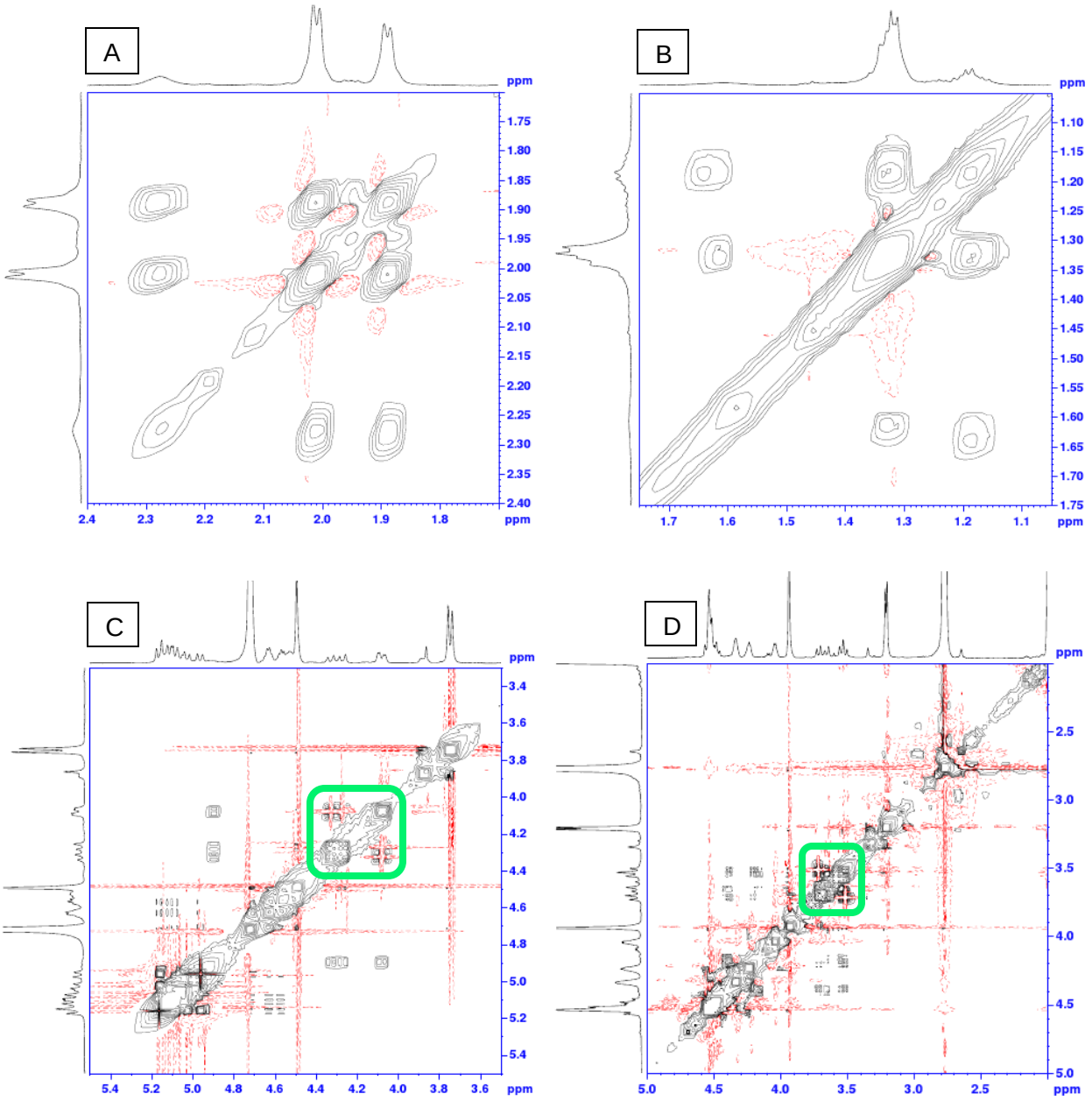


Figure 34. EXSY analyses were performed on alanine zwitterion 52 and amide zwitterion 51 to obtain rate calculations. The alanine α-methyl protons of 52 were examined using NOESYs (600 MHz, 1.4 s mixing time) in (A) D_2O buffer 6.9 at 373 K and (B) CD_3CN at 343 K. The glycine protons of 51 (highlighted in boxes) were examined using ROESYs (600 MHz, 0.2 s mixing time) in (C) D_2O buffer 6.9 at 343 K and (D) CD_3CN at 343 K.

Using EXSY analysis, the rates for **52** were calculated to be 22.6 kcal·mol^{-1} at 373 K in D_2O buffer pD 6.9 and 19.7 kcal·mol^{-1} at 343 K in CD_3CN, and the rate for **51** was calculated to

be 18.6 kcal·mol^{-1} at 343 K in CD_3CN. 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 α-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.

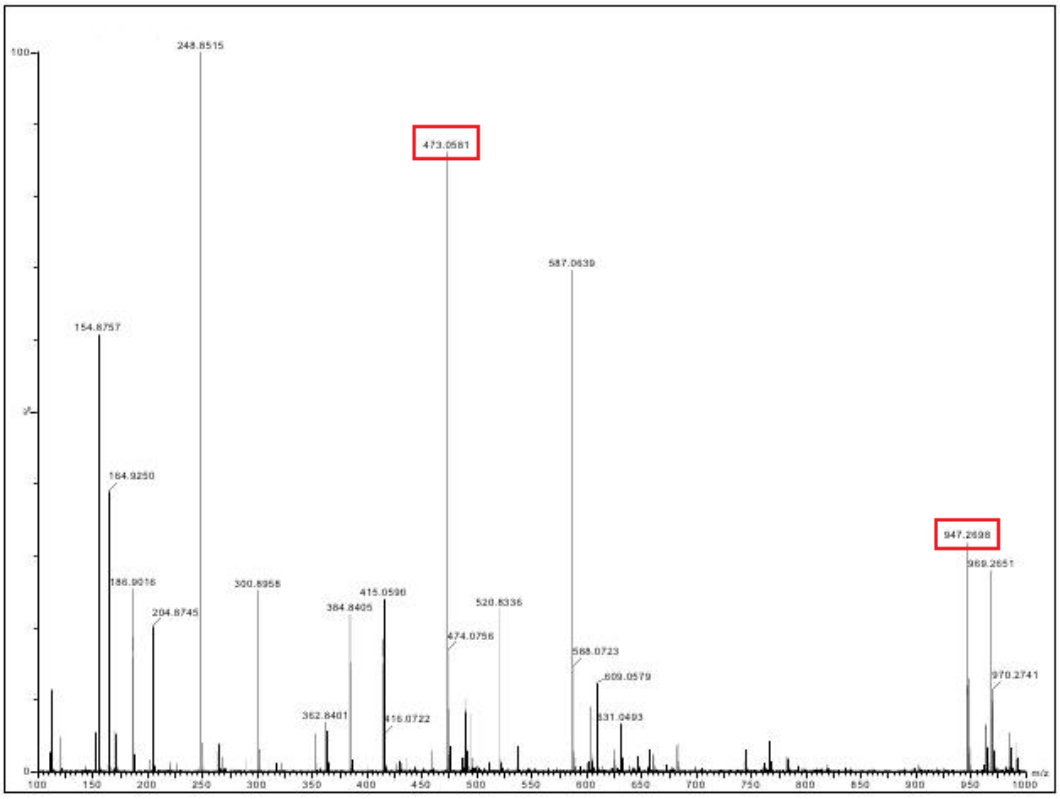


Figure 35. ESI negative ion mode LRMS of zwitterion 52 in methanol, directly injected into the source of the mass spectrometry instrument. The peaks of interest showing the monomer and dimer are highlighted at masses of 473.0581 and 947.2698, respectively.

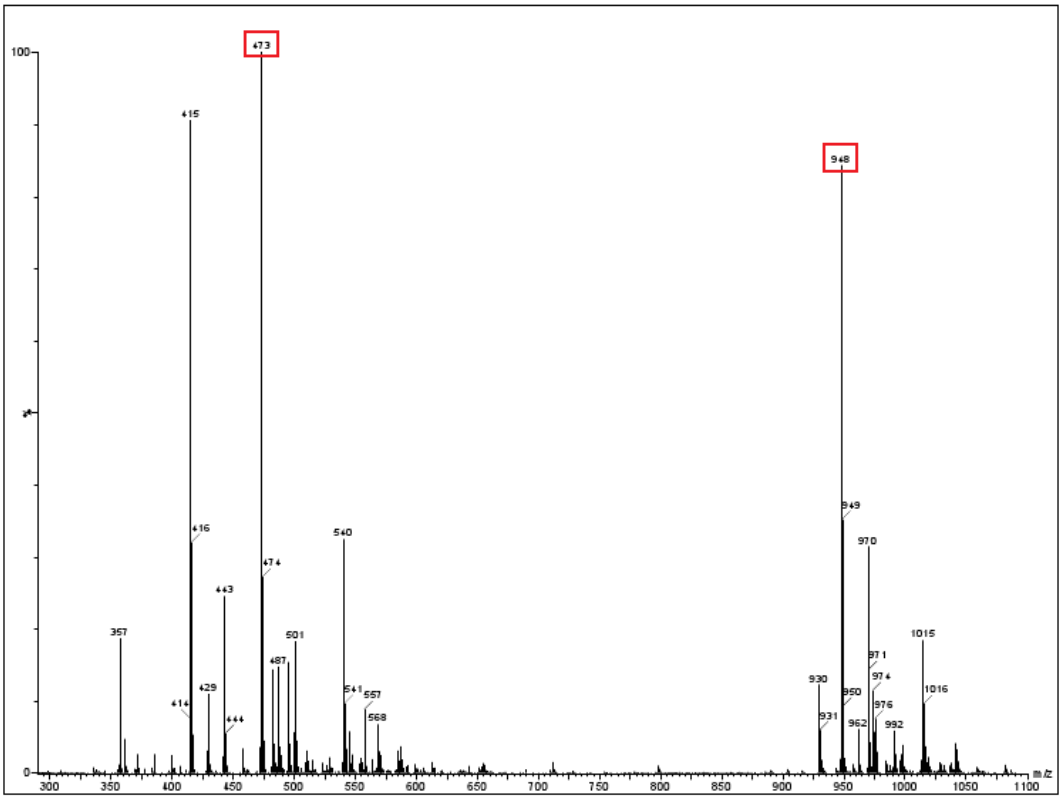


Figure 36. ESI negative ion mode LRMS of 52 in water. The peaks of interest showing the monomer and dimer are highlighed at masses of 473 and 948, respectively. The ESI positive ion mode LRMS, not shown, also displayed monomer and dimer peak masses.

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.

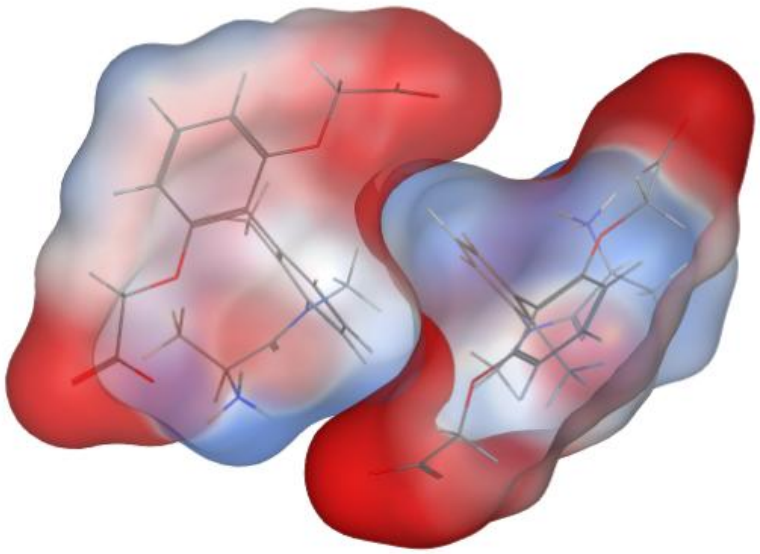
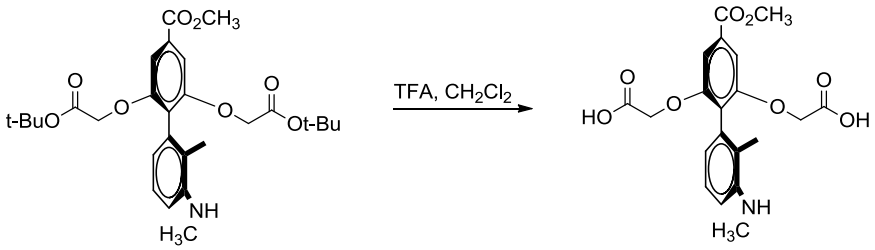


Figure 37. MOE illustration of the electrostatic map of alanine zwitterion 52, depicting a possible dimer based upon hydrophobic interactions.

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.

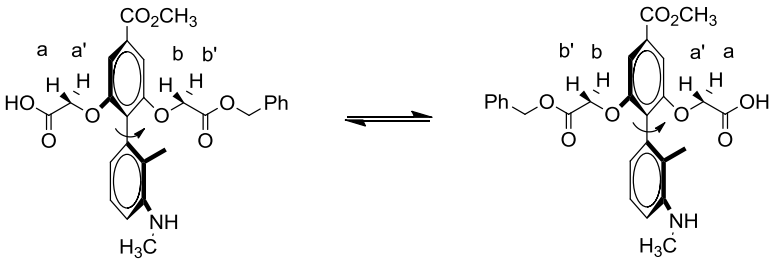


5.6 ANALYSIS OF CORE TARGET

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.



59-*S* **59-*R***

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'.

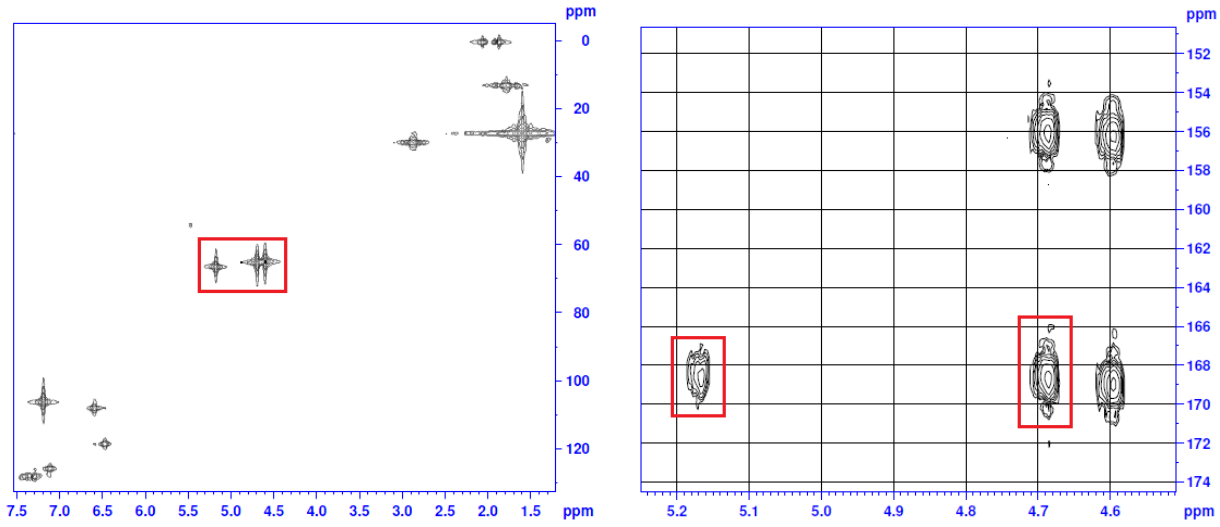


Figure 38. HMQC (left) and HMBC expansion (right) of target 59 (acetonitrile-d_3, 700 MHz), with focus on the o-methylenes and also the adjacent benzyl methylene. The HMBC shows the o-methylene protons b and b' at 4.68 ppm in alignment with the benzyl methylene protons at 5.16 ppm; both are highlighted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

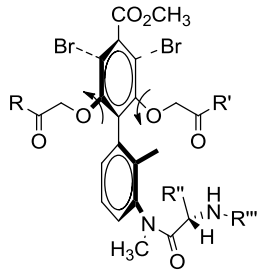
Torsion balances were used to study intramolecular hydrogen bond preference by analyzing ^{1}H NMR peak data. An amino acid chain (a single alanine or glycine residue) was found to preferably hydrogen bond with an amide versus an ester carbonyl (1.4:1.0 at 298 K in toluene-d_8), and with a secondary 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^{o}, relative to the plane of the aromatic ring). Modeling suggests that 90^{o} 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).

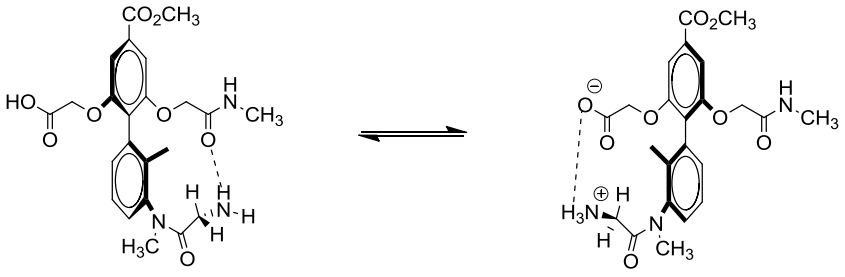
Scheme 55. Scaffold of torsion balance with proposed bromine incorporation.



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.



6.0 EXPERIMENTAL

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 (δ), 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[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_2Cl_2 and evaporating the CH_2Cl_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 (λ = 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 H_2O (0.1% TFA):CH_3CN (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 H_2O (0.1% TFA):CH_3CN (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 H_2O (0.1% TFA):CH_3CN (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[68] were prepared with silica gel 60 (Merck,

230-240 mesh ASTM). 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 (Et_2O) were distilled from sodium and benzophenone. CH_2Cl_2 was distilled from CaH_2. 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 sealable 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 10. Kinetic equilibrium data for 31-SEE.

Time (s)	% ee
0	90.4
3900	86.6
7800	85.9
11820	84.6
15540	84.0
19140	83.0
22860	81.4
30240	80.5
36660	78.8
88500	64.8
99420	61.5

6.2 PREPARATION OF BUFFER SOLUTIONS USED AS NMR SOLVENTS FOR TORSION BALANCE ANALYSES

Preparation of Stock Solutions

Deuterium chloride (35% wt in D_2O) was purchased from Aldrich. To a 5 mL volumetric flask was added 0.043 mL of the DCl solution, which was diluted to 5 mL with D_2O. This stock solution was 0.10 M in DCl and was used to adjust the pD of the buffer solutions.[69]

Sodium deuteroxide (40% wt in D_2O) 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 D_2O. This stock solution was 0.10 M in NaOD and used to adjust the pD of the buffer solutions.[69]

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 D_2O was dried by lyophilization. The solid was redissolved in another 20 mL portion of D_2O, 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 DCl stock solution was diluted to 10 mL with D_2O 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.[69]

Preparation of the 0.050 M Deuterated Phosphate Buffer

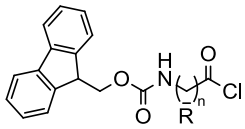
A solution of 1.4196 g (0.0100 mol) of Na_2HPO_4 and 1.3609 g (0.0100 mol) of KH_2PO_4 in 10 mL of D_2O was dried by lyophilization. The solid was redissolved in another portion of D_2O, and the solvent was again removed by lyophilization. The deuterated solid was transferred to a 100 mL volumetric flask and diluted to 100 mL with D_2O. 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.[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_2O 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.[69]

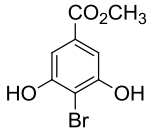
6.3 EXPERIMENTAL PROCEDURES



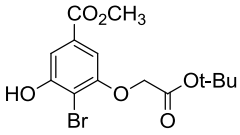
= Fmoc-Xaa-Cl

General procedure A for generating Fmoc-protected amino acid chlorides: To a solution of *N*-(9-fluorenylmethoxycarbonyl)-X_{aa} (1.0 equiv.) in CH_2Cl_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_2Cl_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_{aa}) used include L-alanine, glycine, and β-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.

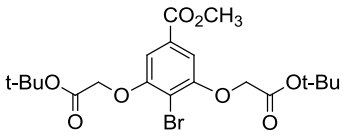


Methyl 4-bromo-3,5-dihydroxybenzoate (9) To 5.0 g (21.5 mmol) of carboxylic acid **8** in 33.1 mL of methanol was added 1.98 mL (37.1 mmol) of H_2SO_4 dropwise, and the reaction mixture was refluxed for 16 h. The reaction was quenched by addition of $NaHCO_3$ (6.24 g, 74.3 mmol), and the volatile components 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 $MgSO_4$ and filtered, and the volatile components were removed under reduced pressure to give 5.27 g (99.4%) of **9** as a white solid: R_f 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; ^{1}H NMR (300 MHz, DMSO) δ 10.52 (s, 2H), 7.00 (s, 2H), 3.79 (s, 3H); ^{13}C NMR (300 MHz, DMSO) δ 166.3, 156.0, 129.7, 107.4, 103.9, 52.7; HRMS (EI) *m/z* calcd for $C_8H_7O_4Br$ 245.9528, found 245.9519.



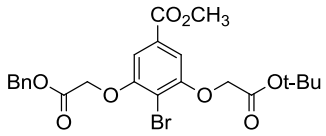
Methyl 4-bromo-3-(2-*tert*-butoxy-2-oxoethoxy)-5-hydroxybenzoate (10). In a round-bottom flask, 5.2 g (21.1 mmol) of benzoate **9**, 3.11 mL (21.1 mmol) of *tert*-butyl bromoacetate, 6.1 g (44.2 mmol) of K_2CO_3, and 0.27 g (1.1 mmol) of 18-crown-6 were dissolved in acetone (105.2 mL), and the reaction 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 $MgSO_4$ 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 5:1/3:2/0:1) to give 2.63 g (34.6%) of **10** as a white solid: R_f 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, $CDCl_3$) 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); ^{13}C NMR (500 MHz, $CDCl_3$) 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 $C_{14}H_{17}O_8Br$ 360.0209, found 360.0205.



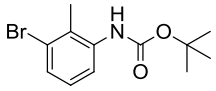
Methyl 4-bromo-3,5-bis(2-tert-butoxy-2-oxoethoxy)benzoate (11). In a round-bottom flask, 0.25 g (1.0 mmol) of benzoate **9**, 0.45 mL (3.0 mmol) of *tert*-butyl bromoacetate, 0.41 g (3.0 mmol) of K_2CO_3, and 0.26 g (1.0 mmol) of 18-crown-6 were dissolved in acetone (25.0 mL), and the reaction mixture was refluxed for 16 h. The volatile components were removed under reduced pressure, and the residue was diluted with CH_2Cl_2 (50 mL) and water (50 mL); the organic layer was extracted, and the aqueous layer was washed with additional CH_2Cl_2 (3 × 50 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 3:1) to give 0.39 g (89%) of **11** as a white solid: R_f 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 $[M+Na]^+$ $C_{20}H_{27}O_8BrNa$ 497.0787, found 497.0819. Benzoate **11** was also generated as the major product (48%) in the reaction used to furnish benzoate **10**.

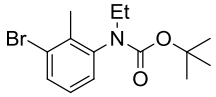


Methyl 3-(2-(benzyloxy)-2-oxoethoxy)-4-bromo-5-(2-*tert*-butoxy-2-oxoethoxy) benzoate (4). In a round-bottom flask, 2.56 g (7.09 mmol) of benzoate **10**, 1.35 mL (8.51 mmol) of benzyl bromoacetate, 1.17 g (8.51 mmol) of K_2CO_3, and 0.46 g (1.77 mmol) of 18-crown-6 were dissolved in acetone (35.4 mL), and the reaction mixture was refluxed for 24 h. The volatile components were removed under reduced pressure, and the residue was diluted with CH_2Cl_2 (120 mL) and water (120 mL); the organic layer was extracted, and the aqueous layer was washed with additional CH_2Cl_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.

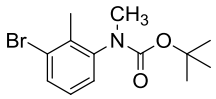


***tert*-Butyl 3-bromo-2-methylphenylcarbamate (15).** In a round-bottom flask, 4.0 mL (32.5 mmol) of amine **7** and 14.2 g (64.9 mmol) of Boc anhydride were dissolved in THF (48.4 mL), and the reaction mixture was refluxed for 4 h. The volatile components were removed under reduced pressure; the residue was diluted with ethyl acetate (100 mL), washed with 1.0 M HCl (2 × 100 mL) and water (100 mL), dried over $MgSO_4$ 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: R_f 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, $CDCl_3$) 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); ^{13}C NMR (300 MHz, $CDCl_3$) 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 $C_{12}H_{16}NO_2Br$ 285.0364, found 285.0368.



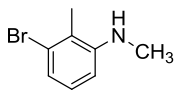
***tert*-Butyl 3-bromo-2-methylphenyl(ethyl)carbamate (16).** To a stirred solution of 0.80 g (2.8 mmol) of carbamate **15** in DMF (6.0 mL) at 0 °C was added 0.34 g (8.4 mmol, 60% in mineral oil) of NaH, previously washed with hexanes (3 × 10 mL) to remove the mineral oil, over a 15 min period. A solution 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 $MgSO_4$ 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, 20:1) to give 0.85 g (96.6%) of **16** as a colorless oil: R_f 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, $CDCl_3$) 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); ^{13}C NMR (300 MHz, 323 K, $CDCl_3$) 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 $C_{14}H_{20}NO_2Br$ 313.0677, found 313.0669.

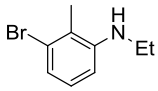


***tert*-Butyl 3-bromo-2-methylphenyl(methyl)carbamate (17).** To a stirred solution of 5.0 g (17.5 mmol) of carbamate **15** in DMF (45.0 mL) at 0 °C was added 2.1 g (52.5 mmol, 60% in mineral oil) of NaH, previously washed with hexanes (3 × 20 mL) to remove the mineral oil, over a 15 min period. A 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

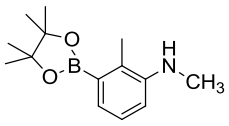
dried over $MgSO_4$ and filtered, and the volatile components were removed under reduced pressure to give a crude red oil. The oil was purified by flash chromatography (SiO_2, hexanes/ethyl acetate, 3:2) to give 5.23 g (99.7%) of **17** as a red oil: R_f 0.65 (hexanes/ethyl acetate, 3:2); IR (thin film, 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, $CDCl_3$) 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)]; ^{13}C NMR (300 MHz, 323 K, $CDCl_3$) 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 $C_{13}H_{18}NO_2Br$ 299.0521, found 299.0513.



3-Bromo-*N*,2-dimethylbenzenamine (6). To a stirred solution of 5.23 g (17.4 mmol) of carbamate **17** in CH_2Cl_2 (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 $NaHCO_3$ solution (100 mL) and CH_2Cl_2 (100 mL); the organic layer was extracted and washed with additional chilled saturated $NaHCO_3$ solution (100 mL). The aqueous layers were washed with CH_2Cl_2 (3 × 100 mL); all organic layers were dried over $MgSO_4$ and filtered, and the volatile components were removed under reduced pressure to give 3.48 g (99.8%) of **6** as a yellow oil: R_f 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, $CDCl_3$) 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); ^{13}C NMR (300 MHz, $CDCl_3$) δ 148.4, 127.8, 125.5, 121.2, 121.0, 108.2, 31.0, 16.7; HRMS (EI) *m/z* calcd for $C_8H_{10}NBr$ 198.9997, found 198.9999.

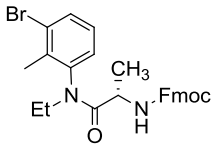


3-Bromo-*N*-ethyl-2-methylbenzenamine (13). To a stirred solution of 0.85 g (2.7 mmol) of carbamate **16** in CH_2Cl_2 (4.1 mL) at room temperature was added 4.1 mL of trifluoroacetic acid, and the reaction mixture was stirred for 1 h. The mixture was dissolved in chilled saturated $NaHCO_3$ solution (30 mL) and CH_2Cl_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_2Cl_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, $CDCl_3$) δ 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, $CDCl_3$) δ 147.5, 127.8, 125.6, 121.0, 120.9, 108.7, 38.7, 16.8, 14.9; HRMS (EI) *m/z* calcd for $C_9H_{12}NBr$ 213.0153, found 213.0150.



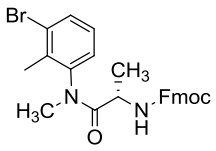
***N*,2-Dimethyl-3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl) benzenamine (5).** To a flame-dried round-bottom flask were added 3.48 g (17.4 mmol) of amine **6**, 4.86 g (19.1 mmol) of bis(pinacolato)diboron, 0.38 g (0.52 mmol) of [1,1′-bis(diphenylphosphino)ferrocene] dichloropalladium(II), and 5.12 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_2Cl_2 (150 mL) and water (150 mL); the organic layer was extracted, and the aqueous layer was washed with additional CH_2Cl_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; ^1H 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 $[M+H]^+$ $C_{14}H_{23}NO_2B$ 248.1822, found 248.1805.



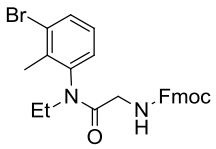
(*S*)-(9*H*-Fluoren-9-yl)methyl 1-((3-bromo-2-methylphenyl) (ethyl)amino)-1-oxopropan-2-ylcarbamate (22). To 0.09 g (0.41 mmol) of amine **13** in 4.0 mL of $CHCl_3$ was added 0.15 g (0.44 mmol) of Fmoc-alanine acid chloride **20-int** (generated according to General Procedure A and used immediately 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_2Cl_2 (30 mL) and saturated $NaHCO_3$ solution (30 mL), the organic layer was extracted, and the aqueous layer was washed with CH_2Cl_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; ^{1}H NMR (300 MHz, $CDCl_3$) 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)]; ^{13}C NMR (300 MHz, $CDCl_3$) 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, 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 $[M+Na]^+$ $C_{27}H_{27}N_2O_3NaBr$ 529.1103, found 529.1123. $[\alpha]_D^{25\,°C}$ = +45.4, c = 1.00, $CHCl_3$.



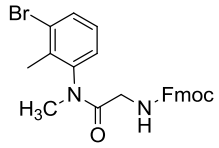
(*S*)-(9*H*-Fluoren-9-yl)methyl 1-((3-bromo-2-methylphenyl) (methyl)amino)-1-oxopropan-2-ylcarbamate (23). To 0.08 g (0.4 mmol) of amine **6** in 4.0 mL of $CHCl_3$ was added 0.15 g (0.44 mmol) of Fmoc-alanine acid chloride **20-int** (generated according to General Procedure A and used immediately thereafter) 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_2Cl_2 (30 mL) and saturated $NaHCO_3$ solution (30 mL), the organic layer was extracted, and the aqueous layer was washed with CH_2Cl_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 40:1/20:1/10:1/6:1/4:1) to give 0.19 g (96.3%) of **23** as a white foam: R_f 0.34 (hexanes/ethyl acetate, 3:2); IR (thin film, cm^{-1}) 3419, 3066, 2927, 1718, 1655, 1561, 1531, 1450, 1392, 1327, 1248, 1107, 1070, 1029, 996, 789, 759, 739; ^1H NMR (300 MHz, $CDCl_3$) 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)]; ^{13}C NMR (300 MHz, $CDCl_3$) 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 $C_{26}H_{25}N_2O_3Br$ 492.1049, found 492.1044. $[\alpha]_D^{25\,°C}$ = +46.9, c = 1.00, $CHCl_3$.



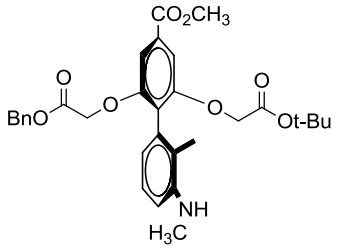
(9*H*-Fluoren-9-yl)methyl 2-((3-bromo-2-methylphenyl) (ethyl)amino)-2-oxoethylcarbamate (24). To 0.08 g (0.36 mmol) of amine **13** in 4.0 mL of $CHCl_3$ was added 0.14 g (0.5 mmol) of Fmoc-glycine acid chloride **21-int** (generated according to General Procedure A and used immediately thereafter) in 2.5 mL of $CHCl_3$ 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_2Cl_2 (30 mL) and saturated $NaHCO_3$ solution (30 mL), the organic layer was extracted,

and the aqueous layer was washed with CH_2Cl_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 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; ^1H 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 $C_{26}H_{25}N_2O_3Br$ 492.1049, found 492.1044.



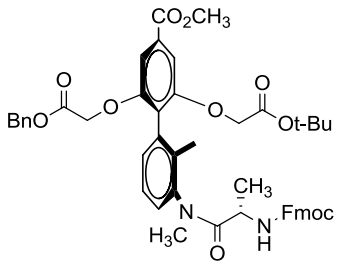
(9*H*-Fluoren-9-yl)methyl-2-((3-bromo-2-methylphenyl)(methyl)amino)-2-oxoethyl carbamate (25). To 0.08 g (0.437 mmol) of amine **6** in 4.0 mL of $CHCl_3$ was added 0.14 g (0.44 mmol) of Fmoc-glycine acid chloride **21-int** (generated according to General Procedure A and used immediately thereafter) 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_2Cl_2 (30 mL) and saturated $NaHCO_3$ solution (30 mL), the organic layer was extracted, and the aqueous layer was washed with CH_2Cl_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 20:1/10:1/5:1/3:1/1:1) to give 0.18 g (93.6%) of **25** as a white foam: R_f 0.20 (hexanes/ethyl acetate, 3:2); IR (thin film, cm^{-1}) 3330, 3065, 2928, 1717, 1664, 1590, 1562, 1516, 1449, 1392, 1327, 1249, 1168, 1113, 1032, 998, 760, 738, 721; ^1H NMR (300 MHz, $CDCl_3$) 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); ^{13}C NMR (300 MHz, $CDCl_3$) 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 $C_{25}H_{23}N_2O_3Br$ 478.0892, found 478.0890.



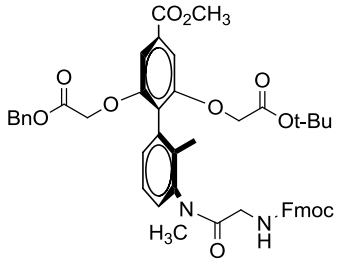
Methyl 2-(2-(benzyloxy)-2-oxoethoxy)-6-(2-(*tert*-butoxy)-2-oxoethoxy)-2'-methyl-3'-(methyl amino)-[1,1'-biphenyl]-4-carboxylate (3). In a resealable oven-dried Schlenk flask containing a magnetic stir bar, 1.27 g (2.50 mmol) of benzoate **4**, 0.93 g (3.75 mmol) of boronic ester **5**, 0.092 g (0.100 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_3PO_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 CH_2Cl_2 (225 mL) and water (225 mL); the organic layer was extracted, and the aqueous layer was washed with additional CH_2Cl_2 (3 × 225 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 5:1/4:1/3:1) to give 0.502 g (36.5%) of **3** as a brown oil/foam: R_f 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, $CDCl_3$) 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); ^{13}C NMR (300 MHz, $CDCl_3$) 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, 128.4, 126.0, 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 for $[M+Na]^+$ $C_{31}H_{35}NO_8Na$ 572.2260, found 572.2312.



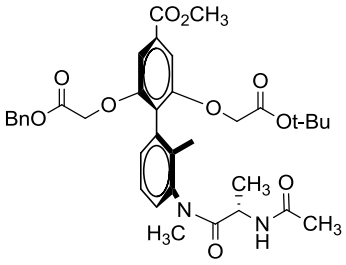
Methyl 3'-((S)-2-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)-*N*-methylpropanamido)-2-(2-(benzyloxy)-2-oxoethoxy)-6-(2-(*tert*-butoxy)-2-oxoethoxy)-2'-methyl-[1,1'-biphenyl]-4-carboxylate (27). To 0.24 g (0.437 mmol) of carboxylate **3** in 4.0 mL of $CHCl_3$ was added 0.17 g (0.6 mmol) of Fmoc-alanine acid chloride **20-int** (generated according to General Procedure A and used immediately thereafter) in 2.5 mL of $CHCl_3$ 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_2Cl_2 (60 mL) and saturated $NaHCO_3$ solution (60 mL), the organic layer was extracted, and the aqueous layer was washed with CH_2Cl_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 (SiO_2, hexanes/ethyl acetate, elution gradient 10:1/5:1/2:1/1:1:1) to give 0.30 g (82%) of **27** as a white foam: R_f 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, $CDCl_3$) 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)]; ^{13}C NMR (500 MHz, $CDCl_3$) 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 $[M+Na]^+$ $C_{49}H_{50}N_2O_{11}Na$ 865.3312, found 865.3275. $[\alpha]_D^{25\,°C}$ = +40.6, c = 1.00, $CHCl_3$.



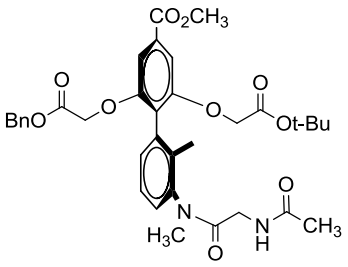
Methyl-3'-(2-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)-*N*-methylacetamido)-2-(2-(benzyloxy)-2-oxoethoxy)-6-(2-(*tert*-butoxy)-2-oxoethoxy)-2'-methyl-[1,1'-biphenyl]-4-carboxylate (28). To 0.24 g (0.44 mmol) of carboxylate **3** in 4.0 mL of $CHCl_3$ was added 0.18 g (0.60 mmol) of Fmoc-glycine acid chloride **21-int** (generated according to General Procedure A and used immediately thereafter) in 2.5 mL of $CHCl_3$ 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_2Cl_2 (60 mL) and saturated $NaHCO_3$ solution (60 mL), the organic layer was extracted, and the aqueous layer was washed with CH_2Cl_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 (SiO_2, 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, $CDCl_3$) 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, $CDCl_3$) δ 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 $[M+Na]^+$ $C_{48}H_{48}N_2O_{11}Na$ 851.3156, found 851.3071.



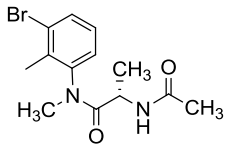
Methyl-3'-((S)-2-acetamido-*N*-methylpropanamido)-2-(2-(benzyloxy)-2-oxoethoxy)-6-(2-(*tert*-butoxy)-2-oxoethoxy)-2'-methyl-[1,1'-biphenyl]-4-carboxylate (1). To 0.042 g (0.05 mmol) of carboxylate **27** in a round-bottom flask was added 0.22 mL of a 20% piperidine/DMF solution, and the reaction mixture 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, $CDCl_3$) δ 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* =

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, $CDCl_3$) δ 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, 156.2, 156.1, 155.9, 155.9, 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, 128.6, 128.6, 128.5, 128.5, 128.5, 128.4, 128.3, 127.9, 127.6, 127.0, 126.9, 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 $[M+Na]^+$ $C_{36}H_{42}N_2O_{10}Na$ 685.2737, found 685.2675. $[\alpha]_D^{25\,°C}$ = +32.4 (c = 0.98, $CHCl_3$).



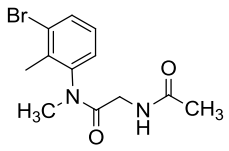
Methyl-3'-(2-acetamido-*N*-methylacetamido)-2-(2-(benzyloxy)-2-oxoethoxy)-6-(2-(*tert*-butoxy)-2-oxoethoxy)-2'-methyl-[1,1'-biphenyl]-4-carboxylate (2). To 0.041 g (0.05 mmol) of carboxylate **28** in a round-bottom flask was added 0.22 mL of a 20% piperidine/DMF solution, and the reaction mixture was 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 (SiO_2, 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 ^{1}H NMR, <5%) white foam: R_f 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; ^{1}H NMR (500 MHz, $CDCl_3$) 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)]; ^{13}C NMR (300 MHz, $CDCl_3$) 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 $C_{35}H_{40}N_2O_{10}$ 648.2683, found 648.2672.



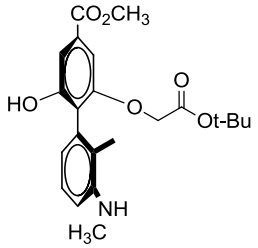
(*S*)-2-Acetamido-*N*-(3-bromo-2-methylphenyl)-*N*-methylpropanamide (29). To 0.049 g (0.10 mmol) of carbamate **23** in a round-bottom flask was added 0.44 mL (0.89 mmol) of a 20% piperidine/DMF solution, and the reaction mixture was stirred at room temperature for 20 min. Then 1.52 mL (16.1 mmol) of 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; ^1H 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 $C_{13}H_{17}N_2O_2Br$ 312.0473, found 312.0470. $[\alpha]_D^{25\,°C}$ = +27.1 (c = 1.00, $CHCl_3$).



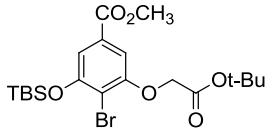
2-Acetamido-*N*-(3-bromo-2-methylphenyl)-*N*-methylacetamide (30). To 0.048 g (0.10 mmol) of carbamate **25** in a round-bottom flask was added 0.44 mL (0.89 mmol) of a 20% piperidine/DMF solution, and the reaction mixture was stirred at room temperature for 20 min. Then 1.52 mL (16.1 mmol) of acetic 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: R_f 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, $CDCl_3$) 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)]; ^{13}C NMR (500 MHz, $CDCl_3$) 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 $C_{12}H_{15}N_2O_2Br$ 298.0317, found 298.0321.



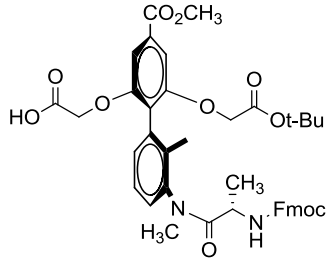
Methyl-2-(2-(*tert*-butoxy)-2-oxoethoxy)-6-hydroxy-2'-methyl-3'-(methylamino)-[1,1'-biphenyl]-4-carboxylate (31). In a resealable oven-dried Schlenk flask containing a magnetic stir bar, 0.25 g (0.68 mmol) of benzoate **10**, 0.25 g (1.03 mmol) of boronic ester **5**, 0.025 g (0.03 mmol) of tris[dibenzylideneacetone]dipalladium(0), 0.045 g (0.11 mmol) of 2-dicyclohexylphosphino-2′,6′-dimethoxybiphenyl (SPhos ligand), and 0.472 g (2.05 mmol) of K_3PO_4 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 CH_2Cl_2 (60 mL) and water (60 mL); the organic layer was extracted, and the aqueous layer was washed with additional CH_2Cl_2 (3 × 60 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 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, 1721, 1586, 1497, 1436, 1368, 1340, 1235, 1158, 1119, 1009, 843, 786, 770, 752, 724; ^{1}H NMR (300 MHz, $CDCl_3$) δ 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, $CDCl_3$) δ 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 (EI) *m/z* calcd for $C_{22}H_{27}NO_6$ 401.1838, found 401.1837.



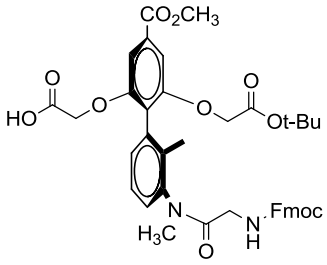
Methyl 4-bromo-3-(2-(*tert*-butoxy)-2-oxoethoxy)-5-((*tert*-butyldimethylsilyl)oxy) benzoate (32). To a solution of 0.18 g (0.5 mmol) of benzoate **10** in DMF (2.0 mL) was added 0.11 g (0.68 mmol) of *tert*-butyldimethylsilyl chloride and 0.09 g (1.25 mmol) of imidazole. The yellow solution was stirred for 24 h at room temperature. The residue was diluted with CH_2Cl_2 (15 mL) and water (15 mL); the organic layer was extracted, and the aqueous layer was washed with additional CH_2Cl_2 (3 × 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 °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, $CDCl_3$) δ 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, $CDCl_3$) δ

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 $[M+Na]^+$ $C_{20}H_{31}O_6NaSiBr$ 497.0971, found 497.0975.



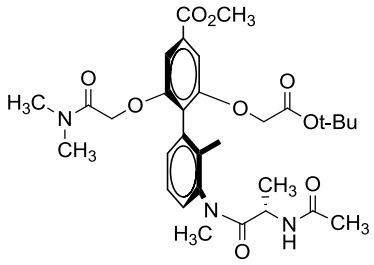
2-((3'-((S)-2-((((9*H*-Fluoren-9-yl)methoxy)carbonyl)amino)-*N*-methylpropanamido)-6-(2-(*tert*-butoxy)-2-oxoethoxy)-4-(methoxycarbonyl)-2'-methyl-[1,1'-biphenyl]-2-yl)oxy)acetic acid (33). To 0.118 g (0.14 mmol) of carboxylate **27** and 0.012 g of 10% Pd/C in a round-bottom flask was added 1.7 mL of 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; ^{1}H 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)]; ^{13}C 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, 106.7, 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 $C_{42}H_{44}N_2O_{11}$ 752.2945, found 752.2913. $[\alpha]_D^{25\,°C}$ = +34.6, c = 1.00, $CHCl_3$.



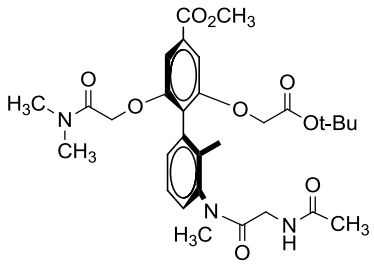
2-((3'-(2-((((9*H*-Fluoren-9-yl)methoxy)carbonyl)amino)-*N*-methylacetamido)-6-(2-(*tert*-butoxy)-2-oxoethoxy)-4-(methoxycarbonyl)-2'-methyl-[1,1'-biphenyl]-2-yl)oxy)acetic acid (34). To 0.125 g (0.15 mmol) of carboxylate **28** and 0.013 g of 10% Pd/C in a round-bottom flask was added 1.8 mL of a 9:1 MeOH/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 0.11 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; ^{1}H NMR (500 MHz, $CDCl_3$) 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 $[M+Na]^+$ $C_{41}H_{42}N_2O_{11}Na$ 761.2686, found 761.2667.



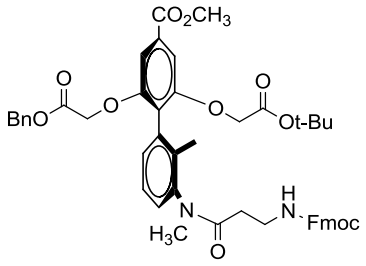
Methyl 3'-((S)-2-acetamido-*N*-methylpropanamido)-2-(2-(*tert*-butoxy)-2-oxoethoxy)-6-(2-(dimethylamino)-2-oxoethoxy)-2'-methyl-[1,1'-biphenyl]-4-carboxylate (35). To a round-bottom flask equipped with a condenser and nitrogen gas inlet were added 0.044 g (0.06 mmol) of acid **33**, 0.005 g (0.06 mmol) 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_2Cl_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_2CO_3 solution (pH 9) and extracted with CH_2Cl_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_2, hexanes/ethyl acetate/methanol, elution gradient 1:1:0/0:1:0/0:9:1) to give 0.018 g (51.6%) of **35** as a yellow oil: R_f 0.10 (100% ethyl acetate); IR (thin film, cm^{-1}) 3323, 2931, 1750, 1722, 1650, 1580, 1502, 1422, 1393, 1369, 1324, 1236, 1127, 1024, 996, 845, 807, 732; ^1H NMR (500 MHz, $CDCl_3$) δ 7.36-7.23 (m, 3H), 7.23-7.12 (m, 2H), one proton of conformers [6.59-6.51 (m, *J* = 8 Hz), 6.46 (d, *J* = 8 Hz)], five protons of conformers [4.96 (d, *J* = 15 Hz), 4.84 (d, *J* = 15 Hz), 4.78 (d, *J* = 17 Hz), 4.75 (d, *J* = 15 Hz), 4.71-4.61 (m), 4.59-4.47 (m), 4.39 (quintet, *J* = 7 Hz), 4.33 (quintet, *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, *J* = 6 Hz), 1.08 (d, *J* = 6 Hz), 1.05 (d, *J* = 6 Hz)]; ^{13}C NMR (500 MHz, $CDCl_3$) δ 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) *m/z* calcd for $C_{31}H_{41}N_3O_9$ 599.2843, found 599.2837. $[\alpha]_D^{25\,^\circ C}$ = +40.3, c = 1.00, $CHCl_3$.



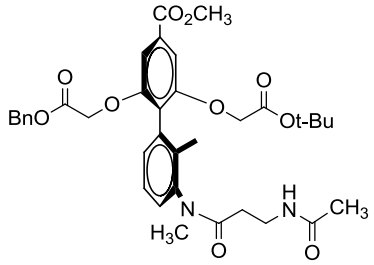
Methyl 3'-(2-acetamido-*N*-methylacetamido)-2-(2-(*tert*-butoxy)-2-oxoethoxy)-6-(2-(dimethyl amino)-2-oxoethoxy)-2'-methyl-[1,1'-biphenyl]-4-carboxylate (36). To a round-bottom flask equipped with a condenser and nitrogen gas inlet were added 0.049 g (0.07 mmol) of acid **34**, 0.005 g (0.07 mmol) of 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, 0.031 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 (SiO_2, 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 CH_2Cl_2 (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 K_2CO_3 solution (pH 9) and extracted with CH_2Cl_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_2, 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: R_f 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, $CDCl_3$) 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, 136.0, 135.8, 135.7, 131.4, 131.3, 131.0, 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 $C_{30}H_{39}N_3O_9$ 585.2686, found 585.2686.



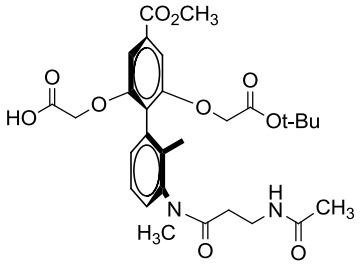
Methyl 3'-(3-((((9*H*-Fluoren-9-yl)methoxy)carbonyl)amino)-*N*-methylpropanamido)-2-(2-(benzyloxy)-2-oxoethoxy)-6-(2-(*tert*-butoxy)-2-oxoethoxy)-2'-methyl-[1,1'-biphenyl]-4-carboxylate (38). To 0.20 g (0.37 mmol) of carboxylate **3** in 4.0 mL of $CHCl_3$ was added 0.15 g (0.55 mmol) of Fmoc-β-alanine 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_2Cl_2 (60 mL) and saturated $NaHCO_3$ solution (60 mL), the organic layer was extracted, and the aqueous layer was washed with CH_2Cl_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 (SiO_2, hexanes/ethyl acetate, elution gradient 4:1/2:1/1:1) to give 0.25 g (81.9%) of **38** as a white foam: R_f 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, $CDCl_3$) 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)]; ^{13}C NMR (300 MHz, $CDCl_3$) 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, 128.5, 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 $[M+Na]^+$ $C_{49}H_{50}N_2O_{11}Na$ 865.3312, found 865.3262.

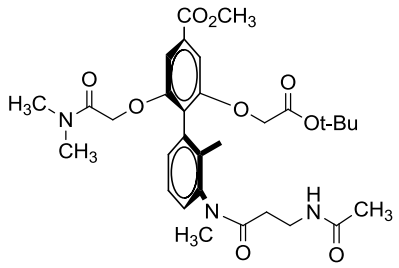


Methyl 3'-(3-acetamido-*N*-methylpropanamido)-2-(2-(benzyloxy)-2-oxoethoxy)-6-(2-(*tert*-butoxy)-2-oxoethoxy)-2'-methyl-[1,1'-biphenyl]-4-carboxylate (39). To 0.231 g (0.27 mmol) of carboxylate **38** dissolved in CH_2Cl_2 (0.83 mL) was added 0.37 mL (2.47 mmol) of 1,8-diazabicyclo[5.4.0]undec-7-ene, 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 CH_2Cl_2 (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 $MgSO_4$ and filtered, and 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 0.091 g (50%) of **39** as a white foam: R_f 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; ^{1}H NMR (500 MHz, $CDCl_3$) 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)]; ^{13}C NMR (300 MHz, $CDCl_3$) 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, 128.5, 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 $[M+Na]^+$ $C_{36}H_{42}N_2O_{10}Na$ 685.2737, found 685.2714.

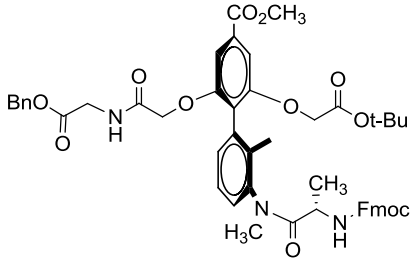


2-((3'-(3-Acetamido-*N*-methylpropanamido)-6-(2-(*tert*-butoxy)-2-oxoethoxy)-4-(methoxy carbonyl)-2'-methyl-[1,1'-biphenyl]-2-yl)oxy)acetic acid (40). To 0.07 g (0.11 mmol) of carboxylate **39** and 0.008 g of 10% Pd/C in a round-bottom flask was added 1.26 mL of a 9:1 MeOH/H_2O solution. The flask was 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 (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_f 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; ^1H NMR (500 MHz, $CDCl_3$) δ 7.37-7.20 (m, 4H), 7.12 (d, *J* = 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)]; ^{13}C NMR (300 MHz, $CDCl_3$) 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) *m/z* calcd for $[M+Na]^+$ $C_{29}H_{36}N_2O_{10}Na$ 595.2268, found 595.2229.



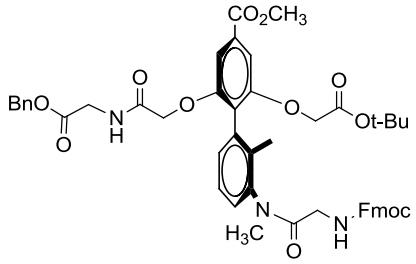
Methyl-3'-(3-acetamido-*N*-methylpropanamido)-2-(2-(*tert*-butoxy)-2-oxoethoxy)-6-(2-(dimethylamino)-2-oxoethoxy)-2'-methyl-[1,1'-biphenyl]-4-carboxylate (41). To a round-bottom flask equipped with a condenser and nitrogen gas inlet were added 0.040 g (0.07 mmol) of acid **40**, 0.008 g (0.07 mmol) of 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, $CDCl_3$) δ 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, $CDCl_3$) δ 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, 35.3, 33.7, 29.7, 28.0, 28.0, 23.3, 23.3, 14.5; HRMS (EI) *m/z* calcd for $C_{31}H_{41}N_3O_9$ 599.2843, found 599.2847.



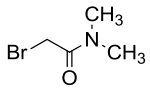
Methyl 3'-((S)-2-((((9*H*-Fluoren-9-yl)methoxy)carbonyl)amino)-*N*-methylpropanamido)-2-(2-((2-(benzyloxy)-2-oxoethyl)amino)-2-oxoethoxy)-6-(2-(*tert*-butoxy)-2-oxoethoxy)-2'-methyl-[1,1'-biphenyl]-4-carboxylate (42). A solution of 0.040 g (0.053 mmol) of acid **33** and 0.011 g (0.053 mmol) of glycine benzyl ester hydrochloride in CH_2Cl_2/DMF (3:1, 0.28 mL) was treated sequentially at 0 °C with 0.005 g (0.053 mmol) of $NaHCO_3$, 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 H_2O (1.0 mL). The aqueous layer was extracted with ethyl acetate (3 × 5 mL), and the combined organic layers were washed with H_2O (7 mL) and brine (7 mL), dried over $MgSO_4$ and filtered, and 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, 1:1/0:1) to give 0.047 g (99%) of **42** as a yellow oil/foam: R_f 0.74 (100% ethyl acetate); IR (thin film, cm^{-1}) 3408, 2929, 1749, 1721, 1657, 1580, 1531, 1450, 1419, 1392, 1369, 1328, 1238, 1193, 1130, 1029, 998, 738; ^1H NMR (300 MHz, $CDCl_3$) 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)]; ^{13}C NMR (500 MHz, $CDCl_3$) 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, 128.4, 128.3, 128.2, 128.1, 127.7, 127.3, 127.1, 126.9, 126.8, 126.6, 126.5, 125.3, 125.2, 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, 47.1, 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; $[\alpha]_D^{25\,°C}$ = +22.4, c = 1.00, $CHCl_3$.



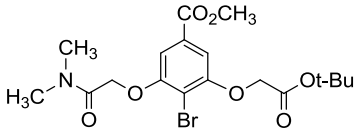
Methyl 3'-(2-((((9*H*-Fluoren-9-yl)methoxy)carbonyl)amino)-*N*-methylacetamido)-2-(2-((2-(benzyloxy)-2-oxoethyl)amino)-2-oxoethoxy)-6-(2-(*tert*-butoxy)-2-oxoethoxy)-2'-methyl-[1,1'-biphenyl]-4-carboxylate (43). A solution of 0.042 g (0.057 mmol) of acid **34** and 0.012 g (0.057 mmol) of glycine benzyl ester hydrochloride in CH_2Cl_2/DMF (3:1, 0.30 mL) was treated sequentially at 0 °C with 0.005 g (0.057 mmol) of $NaHCO_3$, 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 H_2O (1.0 mL). The aqueous layer was extracted

with ethyl acetate (3 × 5 mL), and the combined organic layers were washed with H_2O (7 mL) and brine (7 mL), dried over $MgSO_4$ and filtered, and 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, 1:1/0:1) to give 0.047 g (93.2%) of **43** as a yellow oil/foam: R_f 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, $CDCl_3$) 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)]; ^{13}C NMR (500 MHz, $CDCl_3$) δ 169.2, 169.0, 168.8, 167.8, 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, 128.5, 128.4, 128.4, 128.3, 127.8, 127.7, 127.6, 127.5, 127.4, 127.3, 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.



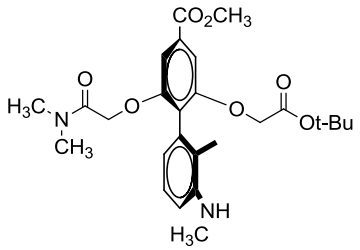
2-Bromo-*N*,*N*-dimethylacetamide (62). To 1.05 mL (12.7 mmol) of bromoacetyl chloride **65** and 1.25 g (15.2 mmol) of dimethylamine hydrochloride in a round-bottom flask equipped with a condenser and nitrogen gas inlet were added CH_2Cl_2 (72.2 mL) and 4.4 mL (33.3 mmol) of

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; ^1H 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 $[M+Na]^+$ $C_4H_8NOBrNa$ 187.9687, found 187.9660.



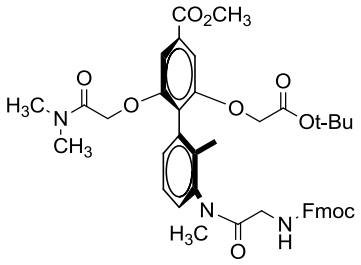
Methyl-4-bromo-3-(2-(tert-butoxy)-2-oxoethoxy)-5-(2-(dimethylamino)-2-oxoethoxy) benzoate (46). In a round-bottom flask, 1.08 g (3.0 mmol) of benzoate **10**, 0.55 g (3.3 mmol) of dimethylacetamide **62**, and 0.5 g (3.6 mmol) of K_2CO_3 were dissolved in acetone (15.0 mL), and the reaction mixture was 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; ^1H 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 $[M+Na]^+$ $C_{18}H_{24}NO_7BrNa$ 468.0634, found 468.0623.



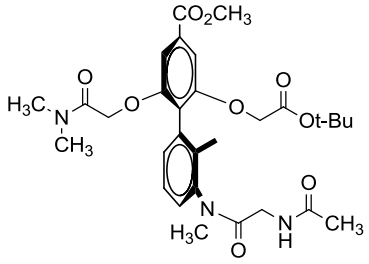
Methyl-2-(2-(*tert*-butoxy)-2-oxoethoxy)-6-(2-(dimethylamino)-2-oxoethoxy)-2'-methyl-3'-(methylamino)-[1,1'-biphenyl]-4-carboxylate (45). In a resealable oven-dried Schlenk flask containing a magnetic stir bar, 1.45 g (3.25 mmol) of benzoate **46**, 1.21 g (4.88 mmol) of boronic ester **5**, 0.12 g (0.13 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_3PO_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_2Cl_2 (20 mL) and water (20 mL); the organic layer was extracted, and the aqueous layer was washed with additional CH_2Cl_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; ^1H 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 $[M+H]^+$ $C_{26}H_{35}N_2O_7$ 487.2444, found 487.2446.



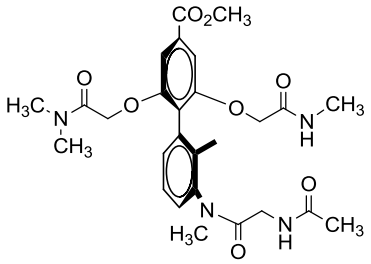
Methyl 3'-(2-((((9*H*-fluoren-9-yl)methoxy)carbonyl)amino)-*N*-methylacetamido)-2-(2-(*tert*-butoxy)-2-oxoethoxy)-6-(2-(dimethylamino)-2-oxoethoxy)-2'-methyl-[1,1'-biphenyl]-4-carboxylate (63). To 0.38 g (0.79 mmol) of carboxylate **45** in 5.0 mL of $CHCl_3$ was added 0.27 g (0.87 mmol) of Fmoc-glycine 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_2Cl_2 (20 mL) and saturated $NaHCO_3$ solution (20 mL), the organic layer was extracted, and the aqueous layer was washed with CH_2Cl_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 $[M+H]^+$ $C_{43}H_{48}N_3O_{10}$ 766.3340, found 766.3338.



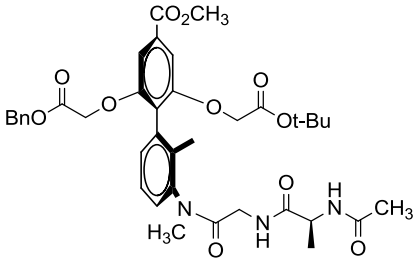
Methyl-3'-(2-acetamido-*N*-methylacetamido)-2-(2-(*tert*-butoxy)-2-oxoethoxy)-6-(2-(dimethylamino)-2-oxoethoxy)-2'-methyl-[1,1'-biphenyl]-4-carboxylate (64). To 0.27 g (0.36 mmol) of carboxylate **63** in a round-bottom flask were added 0.48 mL (3.22 mmol) of 1,8-diazabicyclo[5.4.0]undec-7-ene and DMF (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 5:1:0/1:1:0/1:10:0/0:1:0/0:9:1) 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, $CDCl_3$) δ 169.8, 169.7, 168.9, 168.6, 167.7, 167.4, 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 $[M+H]^+$ $C_{30}H_{40}N_3O_9$ 586.2765, found 586.2770.



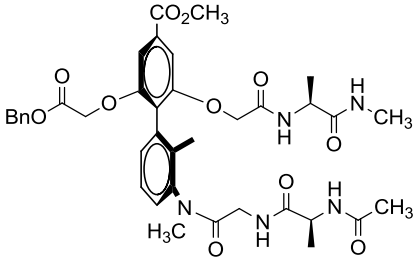
Methyl-3'-(2-acetamido-*N*-methylacetamido)-2-(2-(dimethylamino)-2-oxoethoxy)-2'-methyl-6-(2-(methylamino)-2-oxoethoxy)-[1,1'-biphenyl]-4-carboxylate (44). To a solution of 0.15 g (0.25 mmol) of carboxylate **64** in CH_2Cl_2 (0.38 mL) at room temperature was added 0.38 mL of trifluoroacetic acid, and 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_2Cl_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 $NaHCO_3$ 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 reddish brown crude oil. The oil was purified by flash chromatography (SiO_2, hexanes/ethyl acetate/methanol elution gradient 1:9:0/0:1:0/0:9:1/0:4:1/ 0:0:1) to give 0.023 g (17.2%) of **44** as an off-white foam/gel: R_f 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, $CDCl_3$) 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)]; ^{13}C NMR (400 MHz, $CDCl_3$) 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 $[M+H]^+$ $C_{27}H_{35}N_4O_8$ 543.2455, found 543.2450.



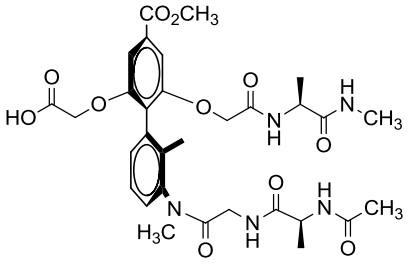
Methyl-3'-(2-((S)-2-acetamidopropanamido)-*N*-methylacetamido)-2-(2-(benzyloxy)-2-oxoethoxy)-6-(2-(*tert*-butoxy)-2-oxoethoxy)-2'-methyl-[1,1'-biphenyl]-4-carboxylate (66). To 1.33 g (1.61 mmol) of carboxylate **28** in a round-bottom flask were added 2.17 mL (14.5 mmol) of 1,8-diazabicyclo[5.4.0]undec-7-ene 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 (SiO_2, ethyl acetate/methanol elution gradient 9:1/4:1/1:1) to give a crude oil of the free amine that was confirmed by ^{1}H NMR (300 MHz, $CDCl_3$) 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 CH_2Cl_2 (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 (SiO_2, 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 NH_4Cl 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: R_f 0.49 (ethyl acetate/methanol, 9:1); IR

(thin film, cm^{-1}) 3316, 2980, 2933, 1751, 1723, 1656, 1580, 1525, 1423, 1395, 1370, 1329, 1299, 1236, 1135, 1029, 997, 845, 805, 753; ^{1}H NMR (400 MHz, $CDCl_3$) 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); ^{13}C NMR (400 MHz, $CDCl_3$) 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 $[M+H]^+$ $C_{38}H_{46}N_3O_{11}$ 720.3132, found 720.3186. $[\alpha]_D^{25\,°C}$ = -10.9, c = 1.00, $CHCl_3$.

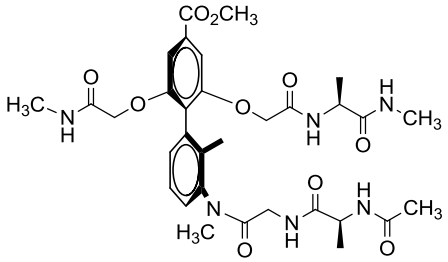


Methyl-3'-(2-((S)-2-acetamidopropanamido)-*N*-methylacetamido)-2-(2-(benzyloxy)-2-oxoethoxy)-2'-methyl-6-(2-(((S)-1-(methylamino)-1-oxopropan-2-yl)amino)-2-oxoethoxy)-[1,1'-biphenyl]-4-carboxylate (68). To a solution of 0.44 g (0.61 mmol) of carboxylate **66** in CH_2Cl_2 (0.93 mL) at room temperature 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 ^{1}H NMR (400 MHz, $CDCl_3$) 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 CH_2Cl_2/DMF (1:1, 3.22 mL) was treated sequentially at 0 °C with 0.062 g (0.73 mmoL) of

$NaHCO_3$, 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 H_2O (2.0 mL). The aqueous layer was extracted with ethyl acetate (3 × 10 mL), and the combined organic layers were washed with H_2O (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, hexanes/ethyl acetate/methanol elution gradient 1:1:0/1:9:0/0:1:0/0:9:1/0:8:1) to give 0.29 g (62%) of **68** as an off-white gel/foam: R_f 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)]; ^{13}C 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 $[M+Na]^+$ $C_{38}H_{45}N_5O_{11}Na$ 770.3013, found 770.3008. $[\alpha]_D^{25\,°C}$ = -9.97, c = 1.00, MeOH.

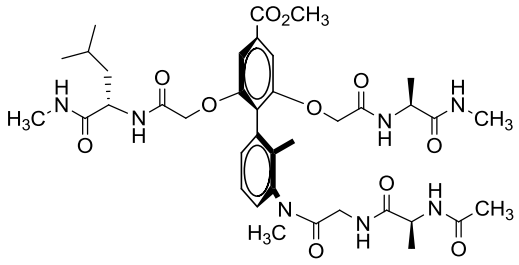


2-((3'-(2-((S)-2-Acetamidopropanamido)-*N*-methylacetamido)-4-(methoxycarbonyl)-2'-methyl-6-(2-(((S)-1-(methylamino)-1-oxopropan-2-yl)amino)-2-oxoethoxy)-[1,1'-biphenyl]-2-yl)oxy)acetic acid (49). To 0.28 g (0.37 mmol) of carboxylate **68** and 0.028 g of 10% Pd/C in a round-bottom flask was added 4.4 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_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_f 0.01 (ethyl acetate/methanol, 3:1); IR (thin film, cm^{-1}) 3314, 3090, 2938, 1656, 1544, 1419, 1329, 1248, 1205, 1134, 1000, 840, 802, 753, 722; ^{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); ^{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, 135.5, 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 $[M+H]^+$ $C_{31}H_{40}N_5O_{11}$ 658.2724, found 658.2747. $[\alpha]_D^{25\,°C}$ = -11.0, c = 1.00, MeOH.



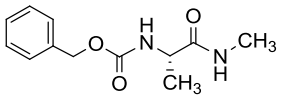
Methyl-3'-(2-((S)-2-acetamidopropanamido)-*N*-methylacetamido)-2'-methyl-2-(2-(((S)-1-(methylamino)-1-oxopropan-2-yl)amino)-2-oxoethoxy)-6-(2-(methylamino)-2-oxoethoxy)-[1,1'-biphenyl]-4-carboxylate (47). To a round-bottom flask equipped with a condenser and nitrogen gas inlet were added 0.12 g (0.18 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_2Cl_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) δ 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) δ 173.8, 173.5, 173.2, 173.1, 171.7, 169.5, 169.4, 169.2,

169.1, 168.4, 168.3, 168.3, 166.1, 156.0, 156.0, 155.8, 155.5, 141.2, 141.2, 135.6, 135.4, 131.6, 131.5, 131.0, 130.9, 127.4, 127.0, 123.9, 110.0, 107.9, 107.7, 107.4, 107.3, 70.2, 68.0, 67.3, 67.1, 54.8, 53.4, 51.6, 48.9, 43.5, 41.9, 41.4, 41.1, 40.7, 38.4, 36.4, 35.4, 33.7, 31.6, 29.3, 29.0, 28.9, 27.0, 24.9, 24.6, 22.3, 21.1, 17.3, 17.3, 16.6, 15.6, 13.4, 13.0; HRMS (Q-TOF) *m/z* calcd for $[M+H]^+$ $C_{32}H_{43}N_6O_{10}$ 671.3041, found 671.3046. $[\alpha]_D^{25\,°C}$ = -10.0, c = 1.00, MeOH.



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_2Cl_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_2O (2.0 mL). The aqueous layer was extracted with ethyl acetate (3 × 10 mL), and the combined organic layers were washed with H_2O (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,

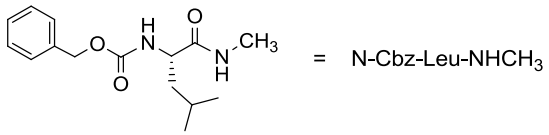
1242, 1131, 805, 754; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.48-6.89 (m, 9H), 6.60-6.46 (m, 1H), one proton of conformers [6.43 (d, *J* = 8 Hz), 6.26 (d, *J* = 8 Hz)], 4.57-4.36 (m, 7H), three protons of conformers [3.96 (s), 3.94 (s)], 3.69-3.62 (m, 2H), three protons of conformers [3.34 (s), 3.28 (s)], 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, $CDCl_3$) δ 172.9, 172.8, 178.8, 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, 167.3, 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, 127.6, 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, 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 $[M+H]^+$ $C_{38}H_{54}N_7O_{11}$ 784.3881, found 784.3872. $[\alpha]_D^{25\,°C}$ = +3.53, c = 1.00, MeOH.



= N-Cbz-Ala-NHCH$_3$

(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_2Cl_2 (67.2

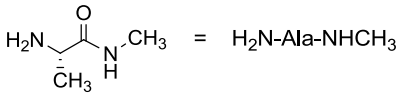
mL). The reaction mixture was stirred at room temperature for 16 h, and then the reaction mixture was diluted with ethyl acetate (20 mL) and washed with 1.0 M HCl (20 mL), saturated $NaHCO_3$ solution (20 mL) and brine (20 mL). The organic layers were combined, dried over $MgSO_4$ and filtered, and the volatile components were removed under reduced pressure to give 3.16 g (99.5%) of **72** as a white solid: R_f 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; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.29 (broad s, 1H), 7.22 (s, 5H), 6.43 (d, J = 7 Hz, 1H), 5.02,4.95 (ABq, J_{AB}= 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); ^{13}C NMR (400 MHz, $CDCl_3$) 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 $[M+H]^+$ $C_{12}H_{17}N_2O_3$ 237.1239, found 237.1224. $[\alpha]_D^{25\,°C}$ = -19.7, c = 1.00, $CHCl_3$.



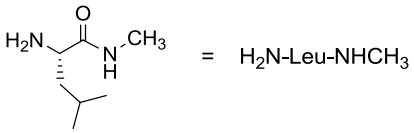
= N-Cbz-Leu-NHCH$_3$

(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 CH_2Cl_2 (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 $NaHCO_3$ solution (20 mL) and brine (20 mL). The organic layers were combined, dried over $MgSO_4$ and filtered, and the volatile components were removed under reduced pressure to give 1.58 g (100%) of **73** as a white solid: R_f 0.09 (hexanes/ethyl acetate, 7:3); mp 124-126 °C; IR

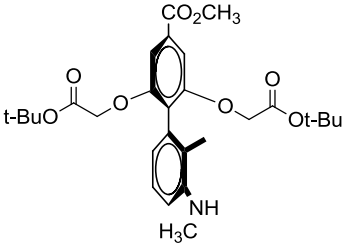
(thin film, cm^{-1}) 3305, 3033, 2960, 2874, 1685, 1652, 1564, 1537, 1468, 1415, 1390, 1324, 1283, 1241, 1162, 1133, 1108, 1047, 969, 835, 756, 733; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.30 (s, 5H), 6.98, 6.62 (2 broad s, 1H), 5.96 (broad s, 1H), 5.10,5.01 (ABq, *J* = 12 Hz, 2H), 4.29-4.18 (broad q, *J* = 9 Hz, 1H), 2.73 (d, *J* = 4 Hz, 3H), 1.69-1.52 (m, 3H), 0.92 (d, *J* = 3 Hz, 6H); ^{13}C NMR (400 MHz, $CDCl_3$) δ 173.3, 156.5, 136.3, 128.5, 128.1, 127.9, 66.9, 53.6, 41.6, 26.2, 24.7, 22.9, 22.0; HRMS (Q-TOF) *m/z* calcd for $[M+H]^+$ $C_{15}H_{23}N_2O_3$ 279.1709, found 279.1704. $[\alpha]_D^{25\,°C}$ = -25.2, c = 1.00, $CHCl_3$.



(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, $CDCl_3$) δ 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, $CDCl_3$) δ 176.3, 128.3, 50.4, 25.6, 21.3; HRMS (EI) *m/z* calcd for $[M]^+$ $C_4H_{10}N_2O$ 102.0793, found 102.0844. $[\alpha]_D^{25\,°C}$ = +2.12, c = 1.00, MeOH.

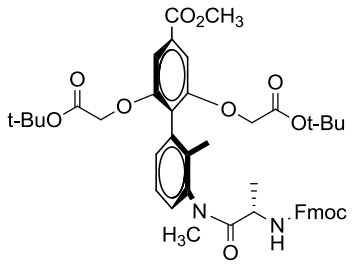


(S)-2-Amino-*N*,4-dimethylpentanamide (69). To 1.5 g (5.4 mmol) of carboxylate **73** and 0.15 g of 10% Pd/C in a round-bottom flask was added 64.2 mL of MeOH. The flask was sealed with a septum; a balloon attached to a syringe needle and filled with hydrogen gas was inserted. The reaction mixture was 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 1:1:0/0:1:0/0:9:1/0:1:1/0:0:1) 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; ^1H 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 $[M+H]^+$ $C_7H_{17}N_2O$ 145.1341, found 145.1299. $[\alpha]_D^{25\,°C}$ = +9.59, c = 1.00, MeOH.



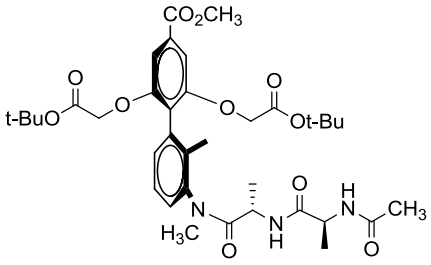
Di-*tert*-butyl-2,2'-((4-(methoxycarbonyl)-2'-methyl-3'-(methylamino)-[1,1'-biphenyl]-2,6-diyl)bis(oxy))diacetate (58). In a resealable oven-dried Schlenk flask containing a magnetic stir bar, 5.11 g (10.7 mmol) of benzoate **11**, 4.0 g (16.1 mmol) of boronic ester **5**, 0.35 g (0.38 mmol)

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 K_3PO_4 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 CH_2Cl_2 (40 mL) and water (40 mL); the organic layer was extracted, and the aqueous layer was washed with additional CH_2Cl_2 (3 × 40 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 9:1/6:1/5:1) to give 1.25 g (25.8%) of **58** as a brown oil: R_f 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; ^{1}H NMR (400 MHz, $CDCl_3$) 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); ^{13}C NMR (400 MHz, $CDCl_3$) 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 $[M+H]^+$ $C_{28}H_{38}NO_8$ 516.2597, found 516.2597.



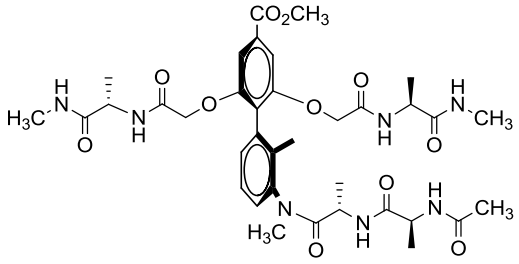
Di-*tert*-butyl-2,2'-((3'-((S)-2-((((9*H*-fluoren-9-yl)methoxy)carbonyl)amino)-*N*-methyl propanamido)-4-(methoxycarbonyl)-2'-methyl-[1,1'-biphenyl]-2,6-diyl)bis(oxy))diacetate (56). To 0.50 g (0.97 mmol) of carboxylate **58** in 11.0 mL of $CHCl_3$ was added 0.35 g (1.07 mmol) of Fmoc-alanine acid chloride **20-int** (generated according to General Procedure A and used immediately thereafter) in 7.0 mL of $CHCl_3$ 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_2Cl_2 (50 mL) and saturated $NaHCO_3$ solution (50 mL), the organic layer was extracted, and the aqueous layer was washed with CH_2Cl_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 5:1/3:1) to give 0.66 g (84.1%) of **56** as a brown gel/foam: R_f 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; ^{1}H NMR (400 MHz, $CDCl_3$) 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)]; ^{13}C NMR (400 MHz, $CDCl_3$) 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, 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. $[\alpha]_D^{25\ °C}$ = +35.5, c = 1.00, $CHCl_3$.



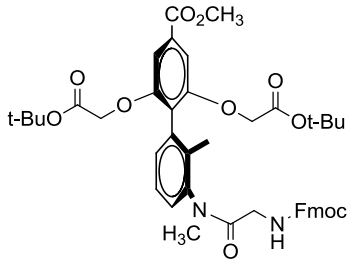
Di-*tert*-butyl-2,2'-((3'-((S)-2-((S)-2-acetamidopropanamido)-*N*-methylpropanamido)-4-(methoxycarbonyl)-2'-methyl-[1,1'-biphenyl]-2,6-diyl)bis(oxy))diacetate (74). To 0.67 g (0.83 mmol) of carboxylate **56** in a round-bottom flask were added 5.0 mL (33.1 mmol) of tris(2-aminoethyl)amine and CH_2Cl_2 (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 $MgSO_4$ 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 (300 MHz, $CDCl_3$) 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 CH_2Cl_2 (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)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 (SiO_2, hexanes/ethyl acetate/methanol elution gradient 1:9:0/0:1:0/0:9:1) to furnish a crude oil, which was dissolved in ethyl acetate (30 mL) and washed with saturated NH_4Cl solution (4 × 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; ^1H NMR (400 MHz, $CDCl_3$) δ 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, $CDCl_3$) δ 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 $[M+H]^+$ $C_{36}H_{50}N_3O_{11}$ 700.3445, found 700.3447. $[\alpha]_D^{25\,°C}$ = +29.4, c = 1.00, $CHCl_3$.



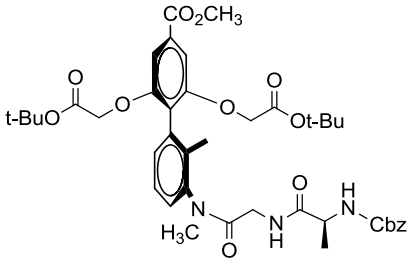
Methyl-3'-((S)-2-((S)-2-acetamidopropanamido)-*N*-methylpropanamido)-2'-methyl-2,6-bis(2-(((S)-1-(methylamino)-1-oxopropan-2-yl)amino)-2-oxoethoxy)-[1,1'-biphenyl]-4-carboxylate (50). To a solution of 0.29 g (0.41 mmol) of carboxylate **74** in CH_2Cl_2 (0.63 mL) at room temperature was added 0.63 mL 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 ^{1}H NMR (300 MHz, $CDCl_3$) 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 CH_2Cl_2/DMF (1:1, 2.18 mL) was treated sequentially at 0 °C with 0.084 g (0.99 mmoL) of $NaHCO_3$, 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 H_2O (4.0 mL). The aqueous layer was extracted with ethyl acetate (3 × 20 mL), and the combined organic layers were washed with H_2O (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, 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; ^1H NMR (300 MHz, MeOD) δ 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) δ 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, 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. $[\alpha]_D^{25\,°C}$ = -2.48, c = 1.00, MeOH.



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

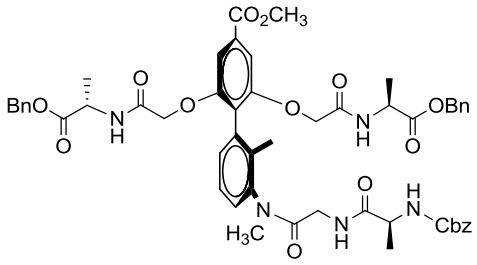
with CH_2Cl_2 (50 mL) and saturated $NaHCO_3$ solution (50 mL), the organic layer was extracted, and the aqueous layer was washed with CH_2Cl_2 (3 × 50 mL). The organics were dried over $MgSO_4$ and filtered, and the volatile components were removed under reduced pressure to give a light brown crude 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; ^1H NMR (300 MHz, $CDCl_3$) δ 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, $CDCl_3$) δ 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 $[M+Na]^+$ $C_{45}H_{50}N_2O_{11}Na$ 817.3312, found 817.3322.



Di-*tert*-butyl-2,2'-((3'-(2-((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_2Cl_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

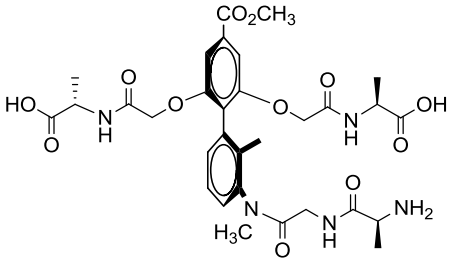
ethyl acetate (15 mL) was added, and the organic layer was extracted with brine (10 mL). The organic layer was further extracted with phosphate buffer (pH = 5.5, 50 mM, 3 × 10 mL), then dried over $MgSO_4$ and filtered. The volatile components were removed under reduced pressure to give a crude oil of 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)]. Then 0.32 g (0.56 mmol) of crude free amine and 0.15 g (0.67 mmol) of acid **70** were dissolved in CH_2Cl_2 (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 (SiO_2, hexanes/ethyl acetate/methanol elution gradient 1:1:0/1:9:0/0:1:0/0:9:1) to furnish a crude oil, which was dissolved in ethyl acetate (30 mL) and washed with saturated NH_4Cl 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: R_f 0.45 (ethyl acetate/methanol 9:1); IR (thin film, cm^{-1}) 3324, 2979, 1750, 1723, 1655, 1580, 1523, 1423, 1395, 1370, 1331, 1301, 1235, 1133, 1029, 998, 845, 753; ^{1}H NMR (400 MHz, $CDCl_3$) 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)]; ^{13}C NMR (400 MHz, $CDCl_3$) δ 173.0, 172.9, 171.2, 169.1, 167.7, 167.6, 167.4,

166.2, 156.4, 156.2, 155.9, 155.9, 155.7, 155.7, 140.2, 136.2, 135.7, 135.6, 131.4, 130.7, 128.5, 128.1, 128.1, 127.5, 126.9, 123.1, 106.3, 106.2, 82.5, 82.3, 67.0, 67.0, 65.9, 65.7, 60.4, 52.4, 50.7, 42.0, 36.4, 36.3, 28.0, 28.0, 27.8, 21.0, 18.7, 18.5, 14.6, 14.5, 14.2; HRMS (Q-TOF) *m/z* calcd for $[M+H]^+$ $C_{41}H_{52}N_3O_{12}$ 778.3551, found 778.3533. $[\alpha]_D^{25\,°C}$ = -3.90, c = 1.00, MeOH.

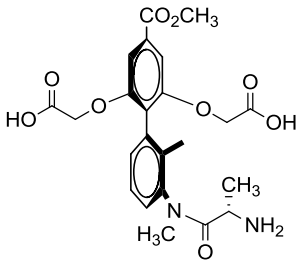


(2S,2'S)-Dibenzyl 2,2'-((2,2'-((3'-(2-((S)-2-(((benzyloxy)carbonyl)amino)propanamido)-*N*-methylacetamido)-4-(methoxycarbonyl)-2'-methyl-[1,1'-biphenyl]-2,6-diyl)bis(oxy)) bis(acetyl))bis(azanediyl))dipropanoate (76). To a solution of 0.30 g (0.39 mmol) of carboxylate **75** in CH_2Cl_2 (0.58 mL) at 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 ^{1}H NMR (400 MHz, $CDCl_3$) 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 CH_2Cl_2/DMF (1:1, 2.03 mL) was treated sequentially at 0 °C with 0.07 g (0.85 mmoL) of $NaHCO_3$, 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 H_2O (2.0 mL). The aqueous layer was extracted with ethyl acetate (3 × 10 mL), and the combined organic layers were washed with H_2O (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, 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: R_f 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, $CDCl_3$) 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),]; ^{13}C NMR (400 MHz, $CDCl_3$) 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, 135.4, 135.3, 135.3, 134.8, 132.0, 131.9, 131.8, 130.6, 130.5, 129.4, 128.6, 128.6, 128.5, 128.5, 128.5, 128.4, 128.3, 128.1, 128.0, 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, 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 $[M+Na]^+$ $C_{53}H_{57}N_5O_{14}Na$ 1010.3800, found 1010.3802. $[\alpha]_D^{25\,°C}$ = -4.23, c = 1.00, $CHCl_3$.

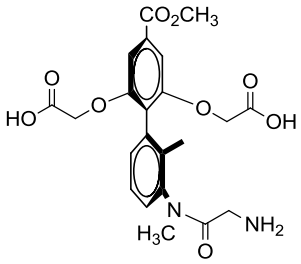


(2S,2'S)-2,2'-((2,2'-((3'-(2-((S)-2-Aminopropanamido)-*N*-methylacetamido)-4-(methoxy carbonyl)-2'-methyl-[1,1'-biphenyl]-2,6-diyl)bis(oxy))bis(acetyl))bis(azanediyl))dipropanoic acid (51). To 0.26 g (0.26 mmol) of carboxylate **76** and 0.078 g of 10% Pd/C in a round-bottom flask was added 3.13 mL of 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 (SiO_2) 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: R_f 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; ^{1}H 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); ^{13}C 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 $[M+H]^+$ $C_{31}H_{40}N_5O_{12}$ 674.2673, found 674.2725. $[\alpha]_D^{25\,°C}$ = +11.7, c = 1.00, MeOH.



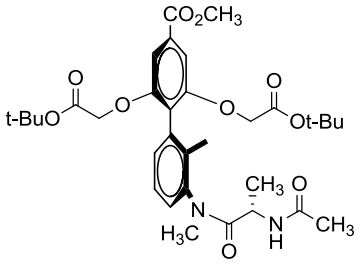
2,2'-((3'-((S)-2-Amino-*N*-methylpropanamido)-4-(methoxycarbonyl)-2'-methyl-[1,1'-biphenyl]-2,6-diyl)bis(oxy))diacetic acid (52). To 0.67 g (0.83 mmol) of carboxylate **56** in a round-bottom flask were added 5.0 mL (33.1 mmol) of tris(2-aminoethyl)amine and CH_2Cl_2 (5.0 mL); the reaction mixture was 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 $MgSO_4$ 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 (300 MHz, $CDCl_3$) 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 CH_2Cl_2 (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 (SiO_2, ethyl acetate/methanol elution gradient 9:1/4:1/2:1/1:1/1:4/0:1) to give 0.07 g (87%) of **52** as a light brown gel/foam: R_f 0.18 (ethyl acetate/methanol, 1:1); IR (thin film, cm^{-1}) 3381, 2922, 1662, 1412, 1202, 1125, 800; ^{1}H 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), 3.33 (s), 3.31 (s), 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, 132.0, 131.8, 131.7, 131.6, 131.0, 130.9, 130.8, 130.7, 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, 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 $[M+H]^+$ $C_{23}H_{27}N_2O_9$ 475.1717, found 475.1719. $[\alpha]_D^{25\,°C}$ = +21.8, c = 1.00, MeOH.



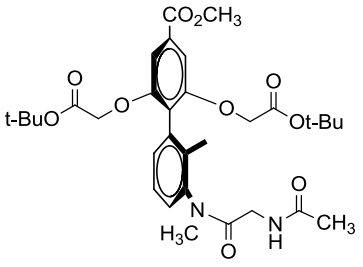
2,2'-((3'-(2-Amino-*N*-methylacetamido)-4-(methoxycarbonyl)-2'-methyl-[1,1'-biphenyl]-2,6-diyl)bis(oxy))diacetic acid (53). To 0.60 g (0.75 mmol) of carboxylate **57** in a round-bottom flask were added 4.52 mL (29.9 mmol) of tris(2-aminoethyl)amine and CH_2Cl_2 (4.52 mL); the reaction mixture was stirred 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 CH_2Cl_2 (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 (SiO_2, ethyl acetate/methanol elution gradient 9:1/4:1/2:1/1:1/1:1/1:4/0:1) 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, 1415, 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), 3.33 (s), 3.31 (s)], three protons of conformers [2.05 (s), 2.04 (s), 1.99 (s)]; ^{13}C 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 $[M+H]^+$ $C_{22}H_{25}N_2O_9$ 461.1560, found 461.1569.



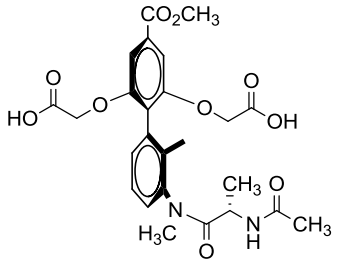
Di-*tert*-butyl-2,2'-((3'-((S)-2-acetamido-*N*-methylpropanamido)-4-(methoxycarbonyl)-2'-methyl-[1,1'-biphenyl]-2,6-diyl)bis(oxy))diacetate (77). To 0.050 g (0.062 mmol) of carboxylate **56** in a round-bottom flask was added 0.28 mL (0.56 mmol) of a 20% piperidine/DMF solution, and the reaction mixture 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, $CDCl_3$) δ 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, $CDCl_3$) δ 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 $[M+Na]^+$ $C_{33}H_{42}N_2O_{10}Na$ 651.2894, found 651.2953. $[\alpha]_D^{25\,°C}$ = +23.9, c = 1.00, $CHCl_3$.



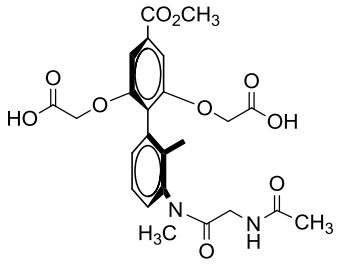
Di-*tert*-butyl-2,2'-((3'-(2-acetamido-*N*-methylacetamido)-4-(methoxycarbonyl)-2'-methyl-[1,1'-biphenyl]-2,6-diyl)bis(oxy))diacetate (78). To 0.04 g (0.05 mmol) of carboxylate **57** in a round-bottom flask was added 0.22 mL (0.45 mmol) of a 20% piperidine/DMF solution, and the

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 (SiO_2, 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, 3380, 2925, 2359, 1724, 1657, 1581, 1424, 1369, 1330, 1235, 1133, 845; ^1H NMR (400 MHz, $CDCl_3$) δ 7.35-7.14 (m, 5H), 6.51 (broad s, 1H), four protons of conformers [4.64,4.53 (ABq, J_{AB} = 16 Hz), 4.55,4.50 (ABq, J_{AB} = 16 Hz)], 3.93 (s, 3H), 3.70 (qd, J = 18, 4 Hz, 2H), 3.32 (s, 3H), 2.05 (s, 3H), 2.00 (s, 3H), 1.47 (s, 18H); ^{13}C NMR (400 MHz, $CDCl_3$) δ 169.7, 168.9, 167.7, 167.4, 166.3, 155.9, 155.7, 140.1, 135.8, 135.7, 131.3, 130.7, 127.0, 126.8, 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 $[M+Na]^+$ $C_{32}H_{42}N_2O_{10}Na$ 637.2737, found 637.2771.



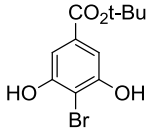
2,2'-((3'-((S)-2-Acetamido-*N*-methylpropanamido)-4-(methoxycarbonyl)-2'-methyl-[1,1'-biphenyl]-2,6-diyl)bis(oxy))diacetic acid (54). To a solution of 0.03 g (0.05 mmol) of carboxylate **77** in CH_2Cl_2 (0.07 mL) at room temperature was added 0.07 mL of trifluoroacetic acid, and the reaction mixture 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, 1142, 1028, 997, 756; ^{1}H NMR (400 MHz, MeOD) δ 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) δ 173.9, 173.7, 171.3, 170.8, 170.7, 170.6, 170.4, 170.4, 170.3, 169.3, 169.2, 166.5, 166.4, 156.3, 156.1, 156.0, 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 $[M+H]^+$ $C_{25}H_{29}N_2O_{10}$ 517.1822, found 517.1823. $[\alpha]_D^{25\,°C}$ = +31.1, c = 1.00, MeOH.

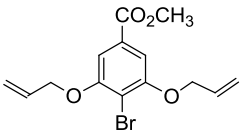


2,2'-((3'-(2-Acetamido-*N*-methylacetamido)-4-(methoxycarbonyl)-2'-methyl-[1,1'-biphenyl]-2,6-diyl)bis(oxy))diacetic acid (55). To a solution of 0.03 g (0.05 mmol) of carboxylate **78** in CH_2Cl_2 (0.08 mL) at room temperature was added 0.08 mL of trifluoroacetic acid, and the reaction mixture was stirred 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, $CDCl_3$) δ 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, $CDCl_3$) δ 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 $[M-H]^+$ $C_{24}H_{25}N_2O_{10}$ 501.1509, found 501.1508.

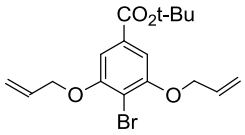


***tert*-Butyl 4-bromo-3,5-dihydroxybenzoate (79).** To a solution of 5.25 g (14.2 mmol) benzoate **83** in THF (132.0 mL) was added 0.66 g (0.57 mmol) of catalytic tetrakis(triphenylphosphine)Pd(0). The yellow solution was stirred for 5 min at room temperature, and then 1.61 g (42.7 mmol) of sodium 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 CH_2Cl_2 (110 mL) and water (100 mL); the organic layer was extracted, and the aqueous layer was washed with additional CH_2Cl_2 (3 × 100 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 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, cm^{-1}) 3391, 2980, 1680, 1593, 1503, 1426, 1369, 1279, 1159, 1122, 1040, 973, 869, 844, 758; ^1H NMR (400 MHz, $CDCl_3$) δ 7.29 (s, 2H), 6.32 (broad s, 2H), 1.60 (s, 9H); ^{13}C NMR (400 MHz, $CDCl_3$) δ 165.4, 153.3, 132.5, 108.8, 103.9, 82.2, 28.2; HRMS (Q-TOF) *m/z* calcd for $[M+]^+$ $C_{11}H_{13}O_4Br$ 287.9997, found 287.9966.



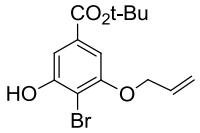
Methyl 3,5-bis(allyloxy)-4-bromobenzoate (82). In a round-bottom flask, 4.21 g (17.0 mmol) of benzoate **9**, 3.26 mL (37.8 mmol) of allyl bromide, and 5.22 g (37.8 mmol) of K_2CO_3 were

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 $MgSO_4$ and filtered, and the volatile components were removed under reduced pressure to give 5.84 g (100%) of **82** as a white solid: R_f 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, $CDCl_3$) 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); ^{13}C NMR (300 MHz, $CDCl_3$) 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 $[M+Na]^+$ $C_{14}H_{15}O_4BrNa$ 349.0051, found 349.0024.



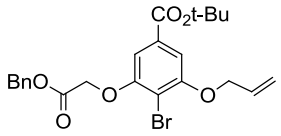
***tert*-Butyl 3,5-bis(allyloxy)-4-bromobenzoate (83).** In a round-bottom flask sealed with a septum, 0.30 g (0.87 mmol) of benzoate **82** and 0.12 mL (0.87 mmol) of *tert*-butyl acetate were combined. To the flask was added 0.009 mL (0.009 mmol) *tert*-butoxide (1M solution in THF) via syringe, and 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; ^1H NMR (300 MHz, $CDCl_3$) δ 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, $CDCl_3$) δ 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 $[M-C_4H_8]^+$ $C_{13}H_{14}O_4Br$ 313.0075, found 313.0045.



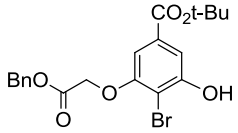
***tert*-Butyl 3-(allyloxy)-4-bromo-5-hydroxybenzoate (61).** To a solution of 0.073 g (0.20 mmol) of benzoate **83** in THF (2.81 mL) was added 0.005 g (0.004 mmol) of catalytic tetrakis(triphenylphosphine)Pd(0). The yellow solution was stirred for 5 min at room temperature, and then 0.008 g (0.20 mmol) 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_2Cl_2 (15 mL) and water (10 mL); the organic layer was extracted, and the aqueous layer was washed with additional CH_2Cl_2 (3 × 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; ^1H NMR

(300 MHz, $CDCl_3$) 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); ^{13}C NMR (400 MHz, $CDCl_3$) 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 $[M-C_4H_8]^+$ $C_{10}H_9O_4Br$ 271.9684, found 271.9659.

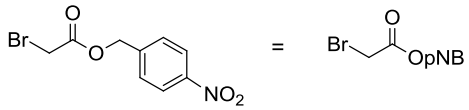


***tert*-Butyl 3-(allyloxy)-5-(2-(benzyloxy)-2-oxoethoxy)-4-bromobenzoate (84).** In a round-bottom flask, 0.13 g (0.39 mmol) of benzoate **61**, 0.08 mL (0.47 mmol) of benzyl bromoacetate, and 0.065 g (0.47 mmol) of K_2CO_3 were dissolved in acetone (2.0 mL), and the reaction mixture was refluxed for 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 $MgSO_4$ 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 20:1/15:1) to give 0.18 g (96.3%) of **84** as a colorless oil: R_f 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; ^{1}H NMR (400 MHz, $CDCl_3$) 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); ^{13}C NMR (400 MHz, $CDCl_3$) 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,

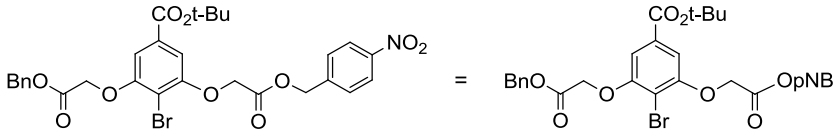
81.7, 70.0, 67.1, 66.2, 28.1; HRMS (Q-TOF) *m/z* calcd for $[M+Na]^+$ $C_{23}H_{25}O_6BrNa$ 499.0732, found 499.0745.



***tert*-Butyl 3-(2-(benzyloxy)-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 × 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; ^1H 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.

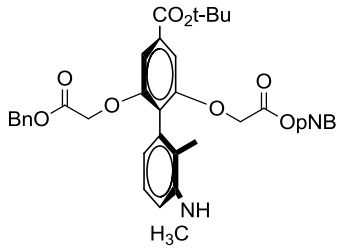


4-Nitrobenzyl 2-bromoacetate (86). To 0.33 g (2.4 mmol) of bromoacetic acid **88** and 0.31 g (2.0 mmol) of 4-nitrobenzyl alcohol **89** in a round-bottom flask were added 8.0 mL of a 1:1 CH_2Cl_2/CH_3CN solution and 0.01 g (0.03 mmol) of 4-(dimethylamino)pyridine. Then 0.50 g (2.4 mmol) of *N*,*N'*-dicyclohexylcarbodiimide 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 $MgSO_4$ and filtered, and 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 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, $CDCl_3$) δ 8.14 (d, *J* = 8 Hz, 2H), 7.52 (d, *J* = 8 Hz, 2H), 5.27 (s, 2H), 3.92 (s, 2H); ^{13}C NMR (400 MHz, $CDCl_3$) δ 166.9, 147.7, 142.4, 128.4, 123.8, 66.2, 25.7; HRMS (Q-TOF) *m/z* calcd for $[M+H]^+$ $C_9H_9NO_4Br$ 273.9715, found 273.9724.



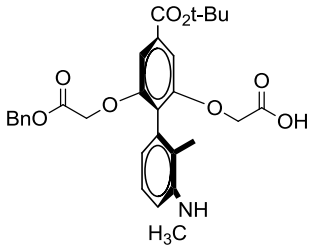
***tert*-Butyl 3-(2-(benzyloxy)-2-oxoethoxy)-4-bromo-5-(2-((4-nitrobenzyl)oxy)-2-oxoethoxy) benzoate (60).** In a round-bottom flask, 0.40 g (0.91 mmol) of benzoate **85**, 0.30 g (1.09 mmol) of 4-nitrobenzyl 2-bromoacetate **86**, and 0.15 g (1.09 mmol) of K_2CO_3 were dissolved in acetone (4.57 mL), 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 $MgSO_4$ 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, 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: R_f 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, $CDCl_3$) 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); ^{13}C NMR (400 MHz, $CDCl_3$) 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 $[M+Na]^+$ $C_{29}H_{28}NO_{10}BrNa$ 652.0794, found 652.0785.



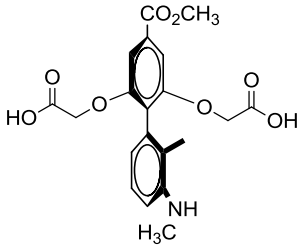
***tert*-Butyl 2-(2-(benzyloxy)-2-oxoethoxy)-2'-methyl-3'-(methylamino)-6-(2-((4-nitrobenzyl) oxy)-2-oxoethoxy)-[1,1'-biphenyl]-4-carboxylate (87).** In a resealable oven-dried Schlenk flask containing a magnetic stir bar, 1.41 g (2.24 mmol) of benzoate **60**, 1.30 g (5.26 mmol) of boronic ester **5**, 0.082 g (0.09 mmol) of tris[dibenzylideneacetone]dipalladium(0), 0.15 g (0.36 mmol) of 2-dicyclohexylphosphino-2′,6′-dimethoxybiphenyl (SPhos ligand), and 1.55 g (6.72 mmol) of K_3PO_4 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 CH_2Cl_2 (50 mL) and water (50 mL); the organic layer was extracted, and the aqueous layer was washed with additional CH_2Cl_2 (3 × 50 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 9:1/4:1/1:1) to give 0.47 g (31.4%) of **87** as a brown oil: R_f 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, $CDCl_3$) 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); ^{13}C NMR (400 MHz, $CDCl_3$) 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, 65.5, 65.2, 31.0, 28.2, 13.9; HRMS (Q-TOF) *m/z* calcd for $[M+Na]^+$ $C_{37}H_{38}N_2O_{10}Na$ 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 NH_4Cl solution (5.0 mL) and ethyl acetate (20 mL); the aqueous layer was extracted, and the organic layer was washed with 5% $NaHCO_3$ solution (3 x 10 mL). The aqueous $NaHCO_3$ 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 $MgSO_4$ and filtered, and 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 elution gradient 2:1/1:1/1:9/0:1) to give 0.11 g (29%) of **59** as a yellow oil: R_f 0.35 (ethyl acetate/methanol, 9:1); IR (thin film, cm^{-1}) 3583, 3435, 2928, 1758, 1710, 1580, 1520, 1420, 1368, 1328, 1247, 1192, 1135, 850, 789, 753; ^1H NMR (400 MHz, CD_3CN) 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); ^{13}C NMR (400 MHz, CD_3CN) 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 $[M-H]^+$ $C_{30}H_{32}NO_8$ 534.2128, found 534.2148.



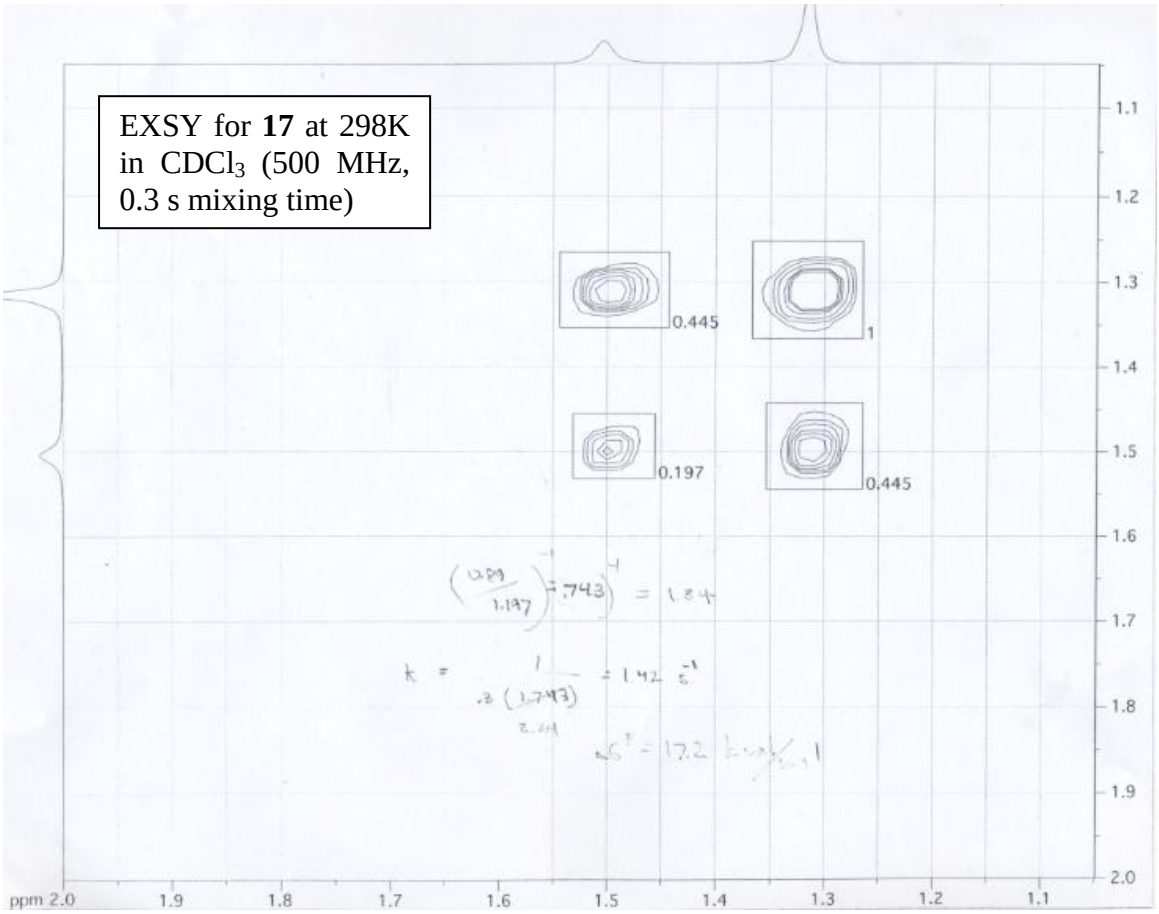
2,2'-((4-(Methoxycarbonyl)-2'-methyl-3'-(methylamino)-[1,1'-biphenyl]-2,6-diyl)bis(oxy)) diacetic acid (90). To a solution of 0.03 g (0.06 mmol) of carboxylate **58** in CH_2Cl_2 (0.09 mL) at room temperature was added 0.09 mL of trifluoroacetic acid, and the reaction mixture was stirred for 2 h. The volatile components were removed under reduced pressure to give 0.023 g (100%) of **90** as a brown gel/foam: R_f 0.01 (ethyl acetate/methanol, 3:1); IR (thin film, cm^{-1}) 3409,

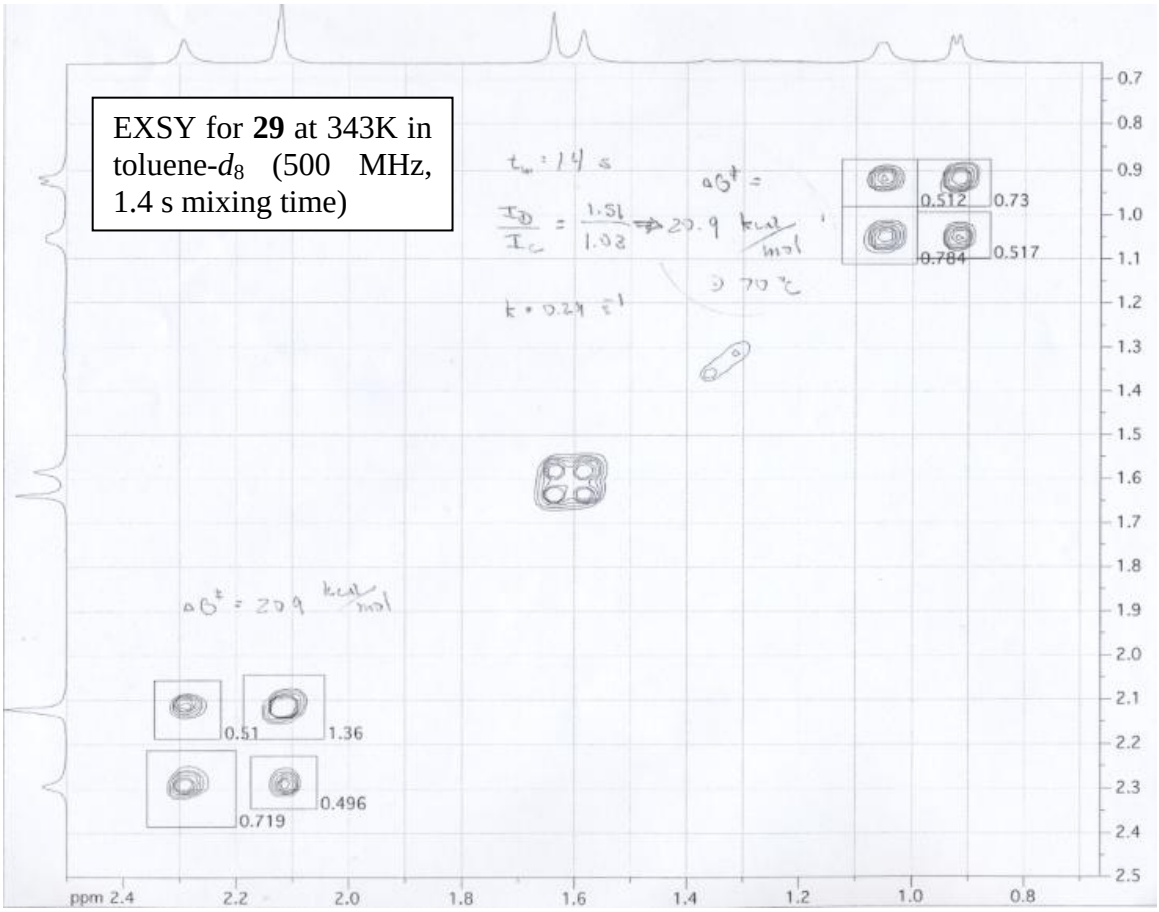
2924, 1718, 1669, 1580, 1420, 1327, 1241, 1200, 1137, 1028, 998, 864, 838, 798, 751, 721; ^{1}H NMR (400 MHz, MeOD) δ 7.42-7.37 (m, 2H), 7.33-7.32 (m, 3H), 4.69,4.64 (ABq, J_{AB} = 17 Hz, 4H), 3.95 (s, 3H), 3.10 (s, 3H), 2.23 (s, 3H); ^{13}C NMR (400 MHz, MeOD) δ 170.4, 166.3, 156.1, 136.4, 136.3, 131.1, 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 $[M-H]^+$ $C_{20}H_{20}NO_8$ 402.1189, found 402.1173.

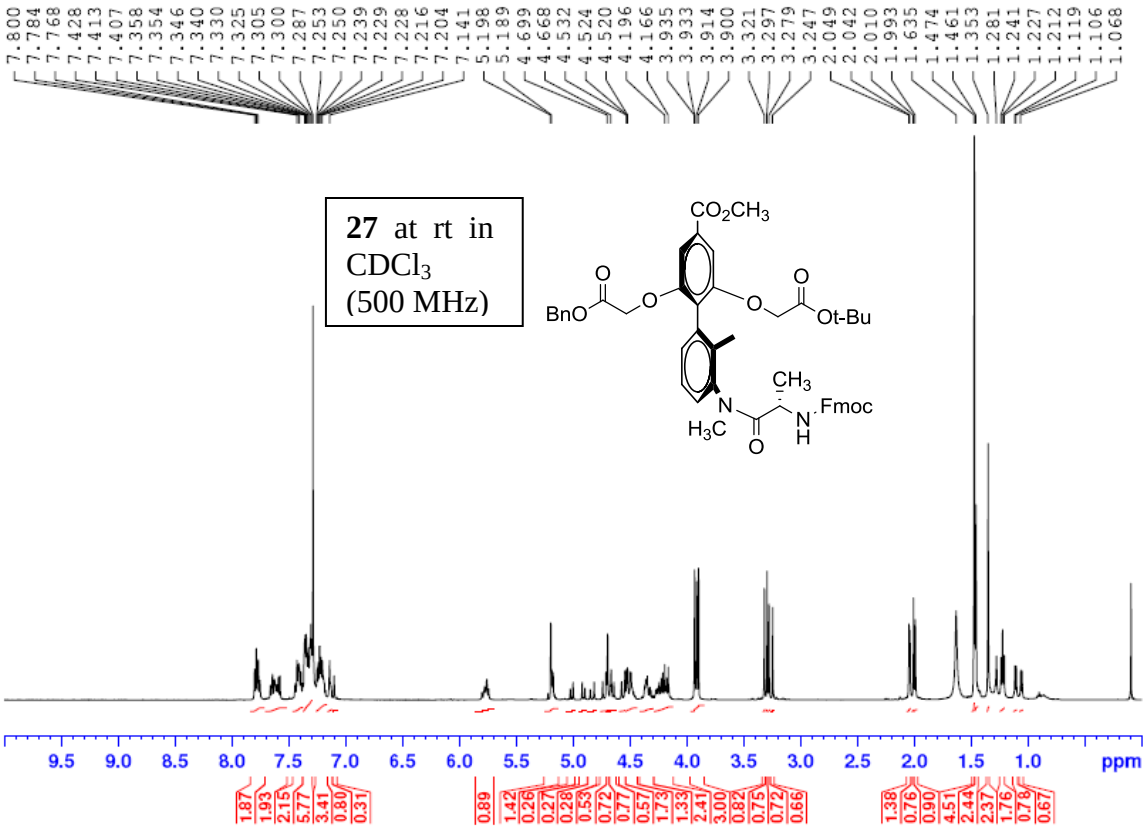
APPENDIX

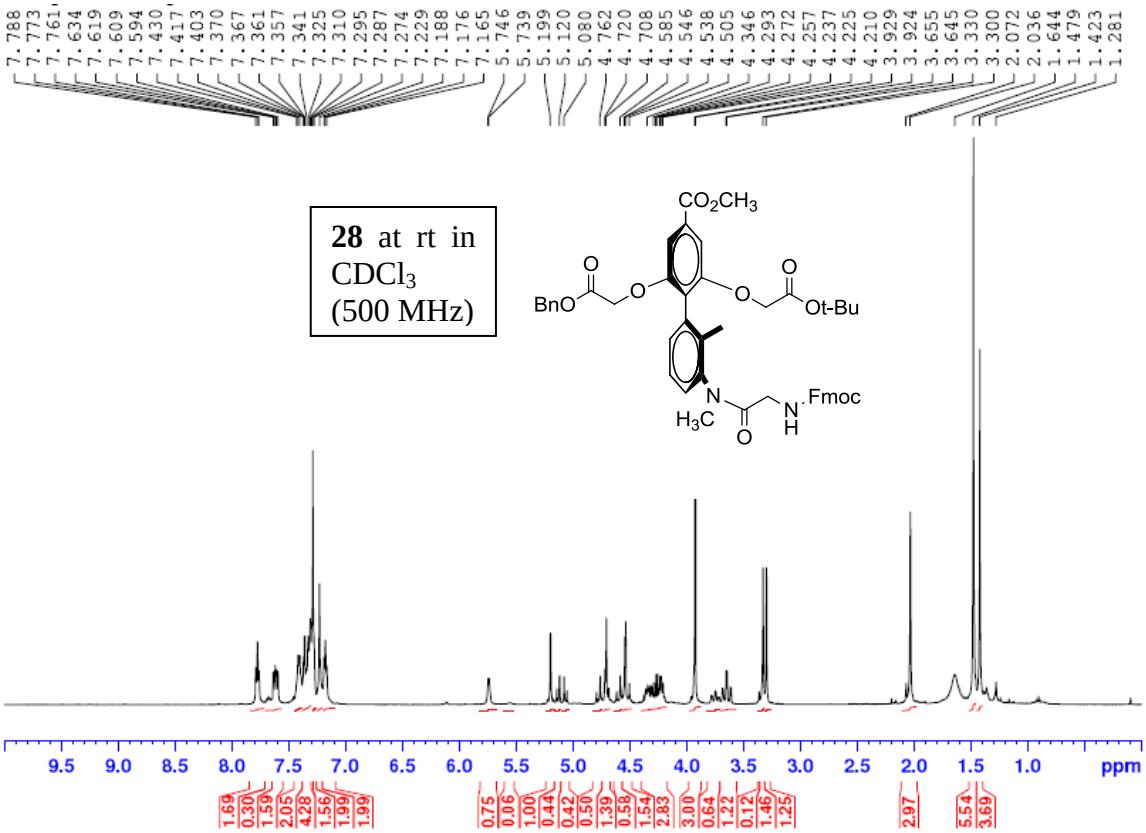
1. Select EXSY and ^{1}H NMR data from original torsion balances.

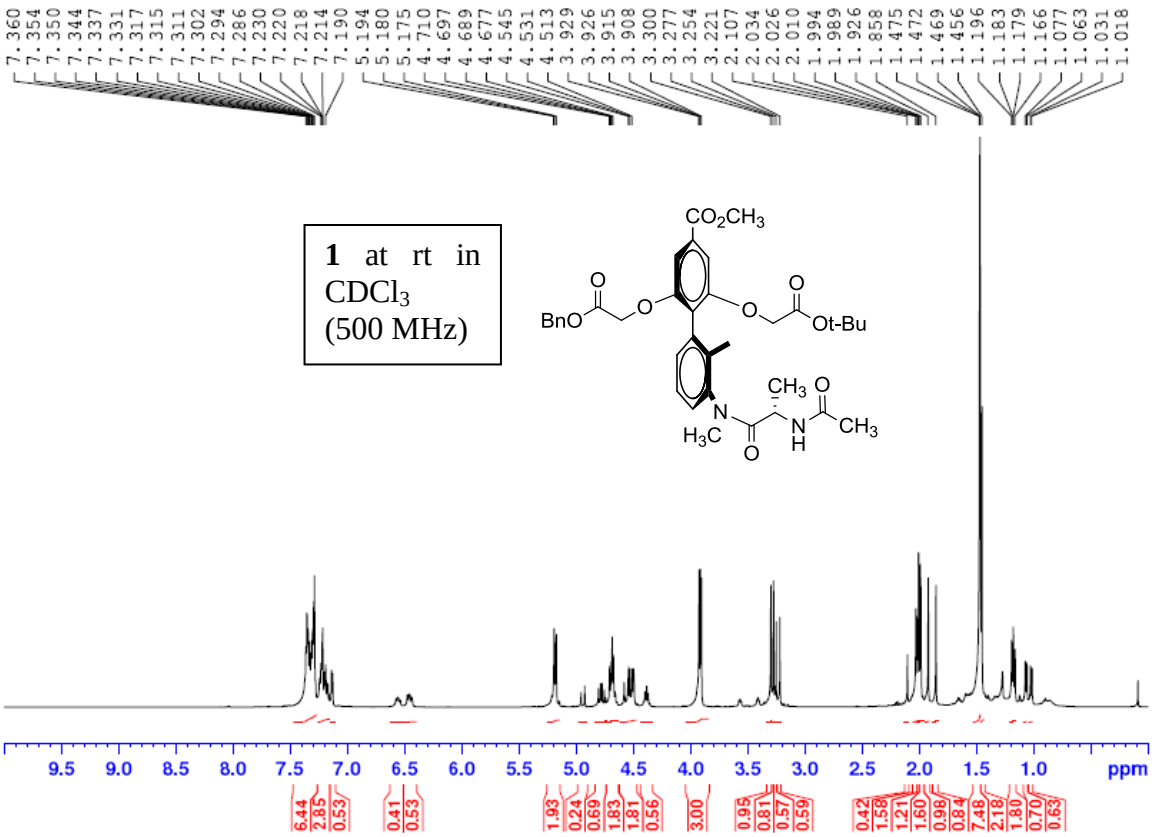
2. Select ^{1}H and ROESY NMR data from advanced torsion balances.

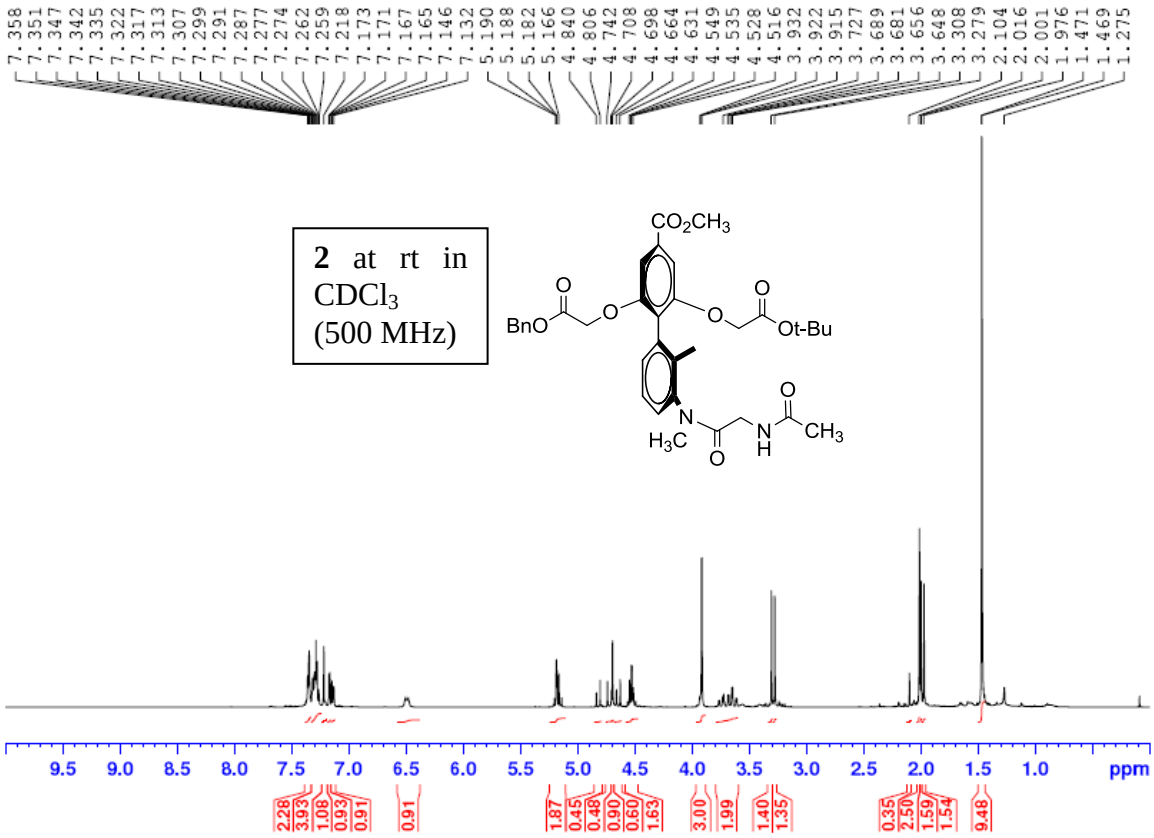

EXSY for **17** at 298K in $CDCl_3$ (500 MHz, 0.3 s mixing time)
0.445
1
0.197
0.445
ppm 2.0
1.9
1.8
1.7
1.6
1.5
1.4
1.3
1.2
1.1
1.1
1.2
1.3
1.4
1.5
1.6
1.7
1.8
1.9
2.0

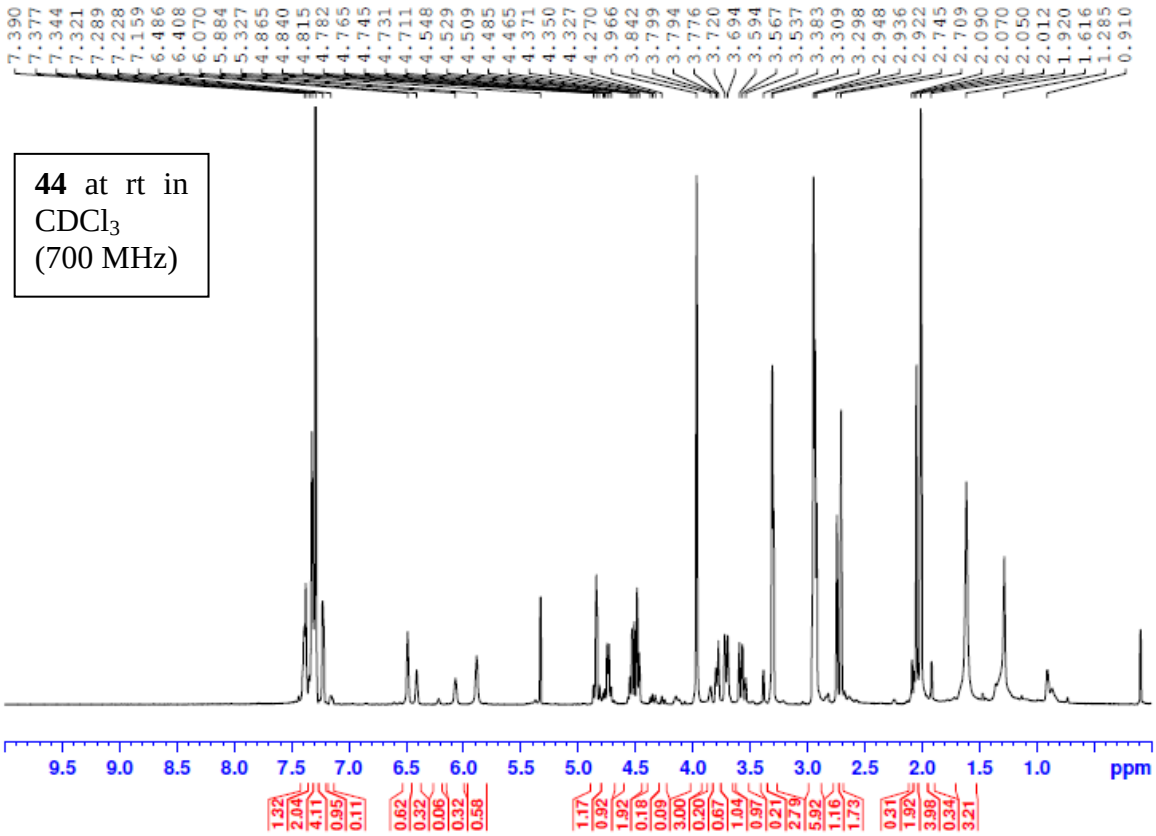

EXSY for **29** at 343K in toluene-d_8 (500 MHz, 1.4 s mixing time)
0.512
0.73
0.784
0.517
0.51
1.36
0.719
0.496
ppm 2.4
2.2
2.0
1.8
1.6
1.4
1.2
1.0
0.8

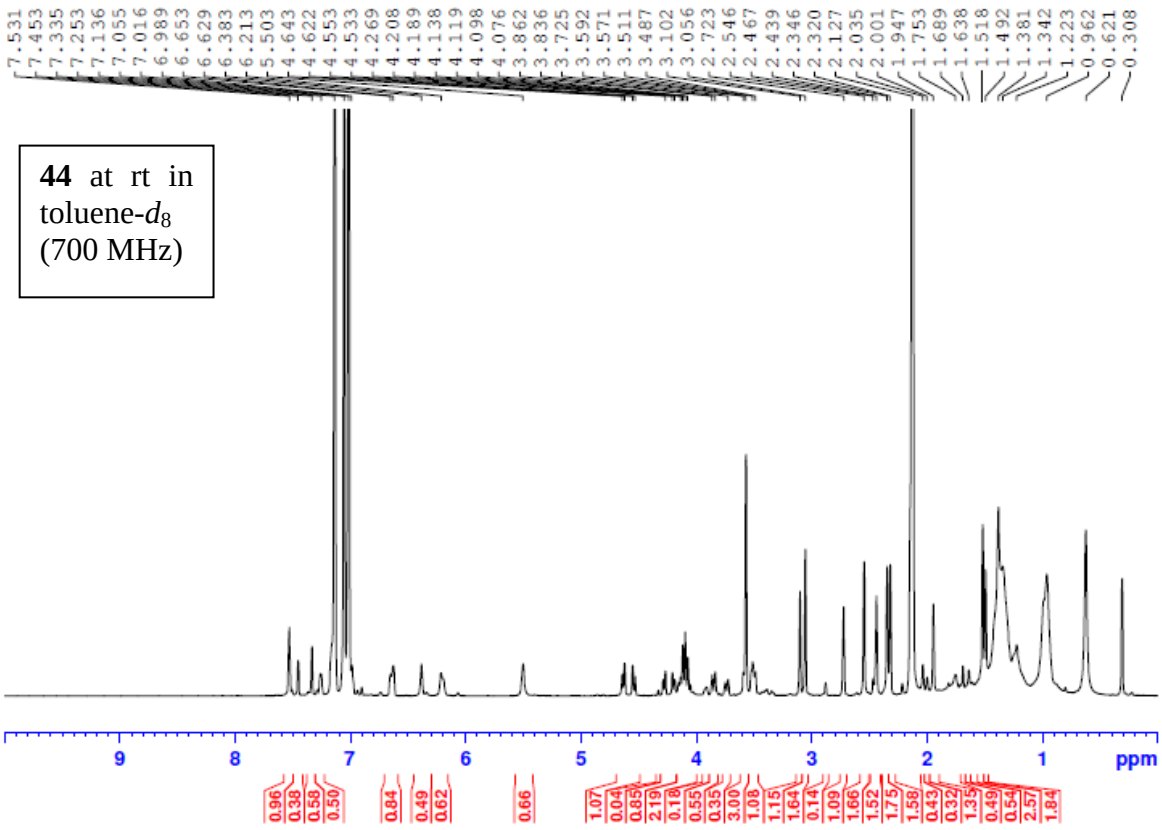

7.800 7.784 7.768 7.428 7.413 7.407 7.358 7.354 7.346 7.340 7.330 7.325 7.305 7.300 7.287 7.253 7.250 7.239 7.229 7.228 7.216 7.204 7.141 5.198 5.189 4.699 4.668 4.532 4.524 4.520 4.196 4.166 3.935 3.933 3.914 3.900 3.321 3.297 3.279 3.247 2.049 2.042 2.010 1.993 1.635 1.474 1.461 1.353 1.281 1.241 1.227 1.212 1.119 1.106 1.068
27 at rt in CDCl3 (500 MHz)
CO2CH3
BnO
Ot-Bu
CH3
Fmoc
H3C
9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 ppm
1.87 1.93 2.15 5.77 3.41 0.80 0.31 0.89 1.42 0.26 0.27 0.28 0.53 0.72 0.77 0.57 1.73 1.33 2.41 3.00 0.82 0.75 0.72 0.66 1.38 0.76 0.90 4.51 2.44 2.37 1.76 0.78 0.67


7.788 7.773 7.761 7.634 7.619 7.609 7.594 7.430 7.417 7.403 7.370 7.367 7.361 7.357 7.341 7.325 7.310 7.295 7.287 7.274 7.229 7.188 7.176 7.165 5.746 5.739 5.199 5.120 5.080 4.762 4.720 4.708 4.585 4.546 4.538 4.505 4.346 4.293 4.272 4.257 4.237 4.225 4.210 3.929 3.924 3.655 3.645 3.330 3.300 2.072 2.036 1.644 1.479 1.423 1.281
28 at rt in CDCl3 (500 MHz)
CO2CH3
BnO
Ot-Bu
Fmoc
H3C
9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 ppm
1.69 0.30 1.59 2.05 4.28 1.56 1.99 1.99 0.75 0.06 1.00 0.44 0.42 0.50 1.39 0.58 1.54 2.83 3.00 0.64 1.22 0.12 1.46 1.25 2.97 5.54 3.69

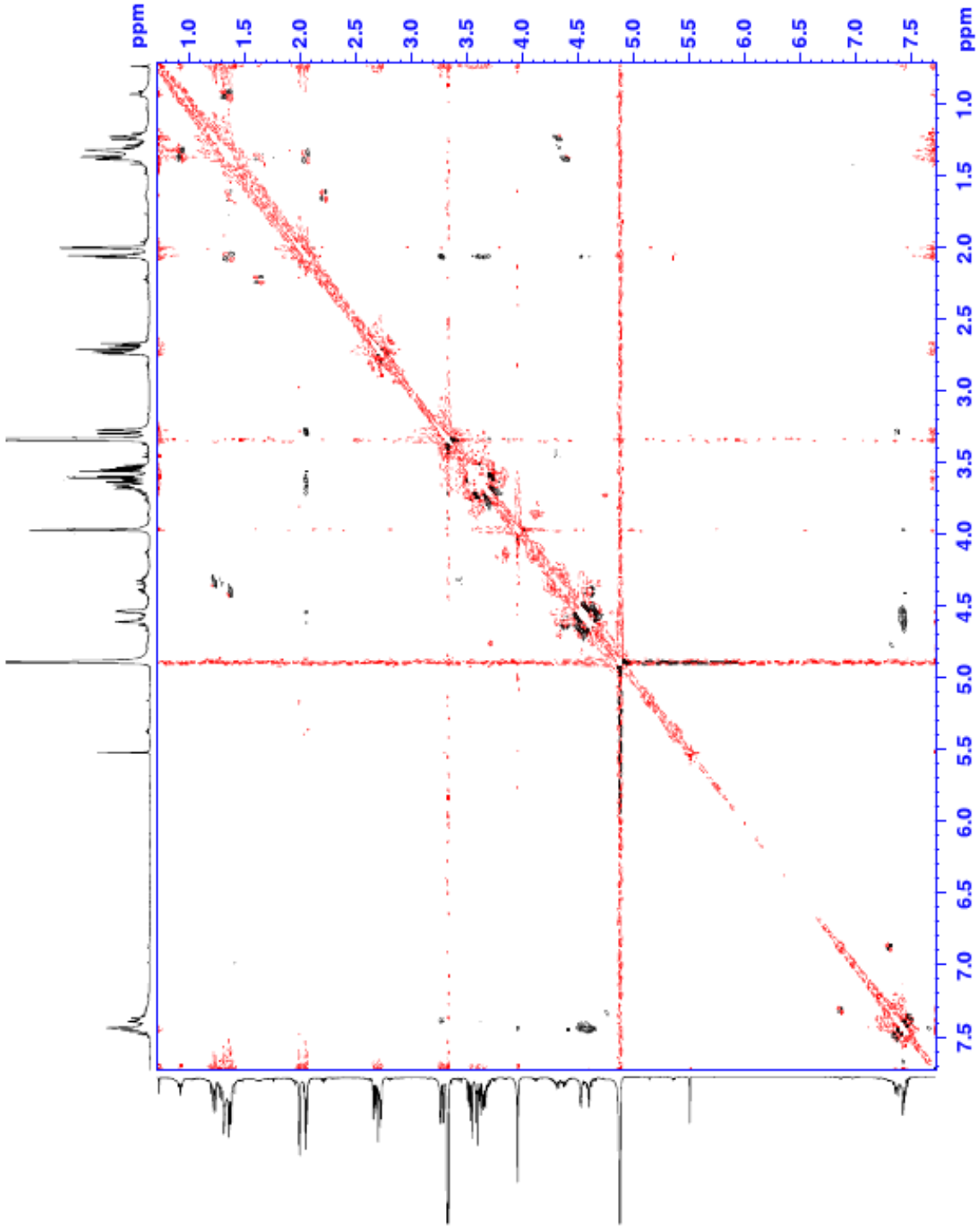

7.360 7.354 7.350 7.344 7.337 7.331 7.317 7.315 7.311 7.302 7.294 7.286 7.230 7.220 7.218 7.214 7.190 5.194 5.180 5.175 4.710 4.697 4.689 4.677 4.545 4.531 4.513 3.929 3.926 3.915 3.908 3.300 3.277 3.254 3.221 2.107 2.034 2.026 2.010 1.994 1.989 1.926 1.858 1.475 1.472 1.469 1.456 1.196 1.183 1.179 1.166 1.077 1.063 1.031 1.018
1 at rt in CDCl3 (500 MHz)
CO2CH3
BnO
Ot-Bu
CH3
H3C
CH3
9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 ppm
6.44 2.85 0.53 0.41 0.53 1.93 0.24 0.69 1.83 1.81 0.56 3.00 0.95 0.81 0.57 0.59 0.42 1.58 1.21 1.60 0.98 0.84 7.48 2.18 1.80 0.70 0.63


7.358 7.351 7.347 7.342 7.335 7.322 7.317 7.313 7.307 7.299 7.291 7.287 7.277 7.274 7.262 7.259 7.218 7.173 7.171 7.167 7.165 7.146 7.132 5.190 5.188 5.182 5.166 4.840 4.806 4.742 4.708 4.698 4.664 4.631 4.549 4.535 4.528 4.516 3.932 3.922 3.915 3.727 3.689 3.681 3.656 3.648 3.308 3.279 2.104 2.016 2.001 1.976 1.471 1.469 1.275
2 at rt in CDCl3 (500 MHz)
CO2CH3
BnO
Ot-Bu
CH3
H3C
9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 ppm
2.28 3.93 1.08 0.93 0.91 0.91 1.87 0.45 0.48 0.90 0.60 1.63 3.00 1.99 1.40 1.35 0.35 2.50 1.59 1.54 9.48

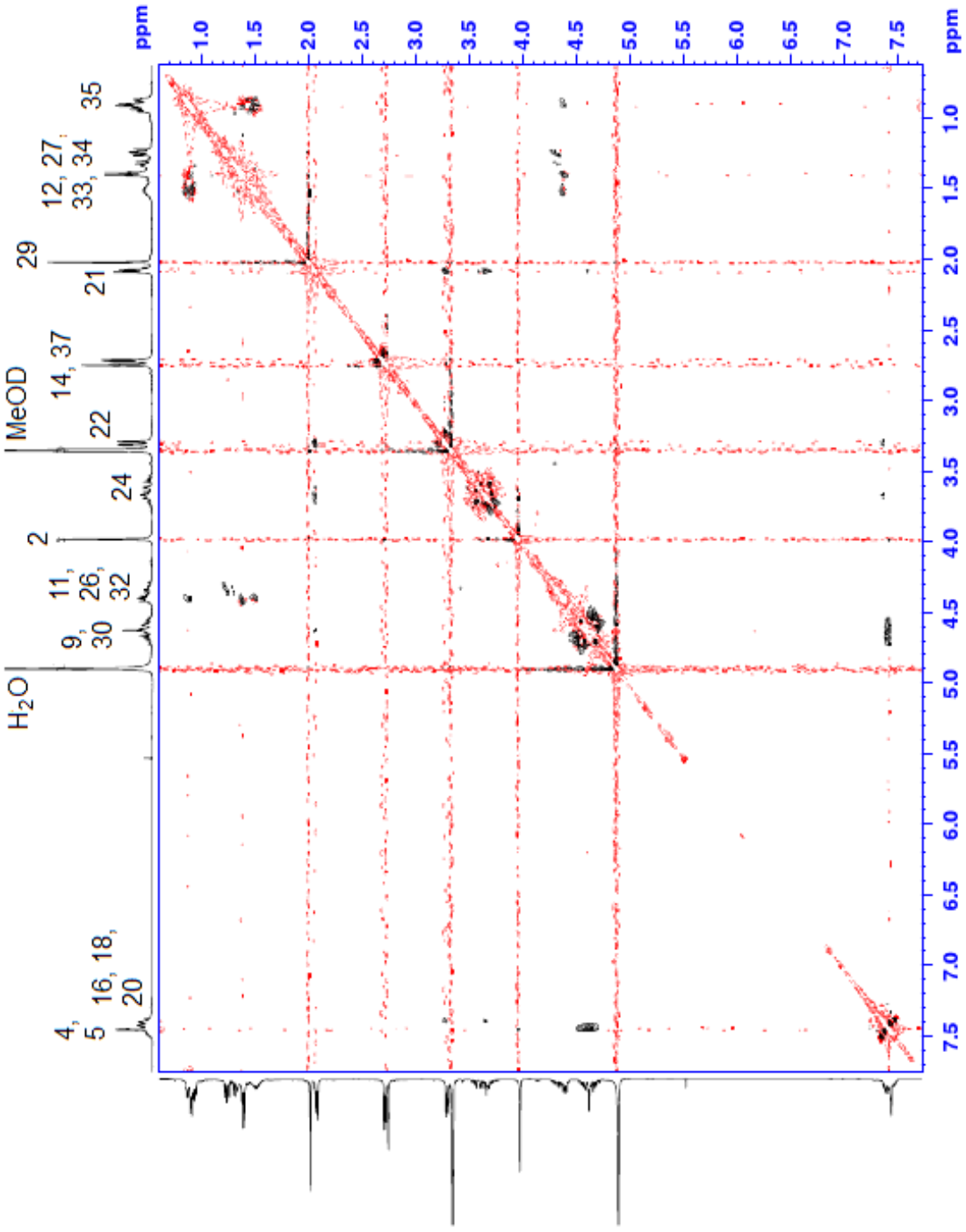

7.390
7.377
7.344
7.321
7.289
7.228
7.159
6.486
6.408
6.070
5.884
5.327
4.865
4.840
4.815
4.782
4.765
4.745
4.731
4.711
4.548
4.529
4.509
4.485
4.465
4.371
4.350
4.327
4.270
3.966
3.842
3.799
3.794
3.776
3.720
3.694
3.594
3.567
3.537
3.383
3.309
3.298
2.948
2.936
2.922
2.745
2.709
2.090
2.070
2.050
2.012
1.920
1.616
1.285
0.910
44 at rt in CDCl3 (700 MHz)
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
ppm
1.32
2.04
4.11
0.95
0.11
0.62
0.32
0.06
0.32
0.58
1.17
0.92
1.92
0.18
0.09
3.00
0.20
0.67
1.04
0.97
0.21
2.79
5.92
1.16
1.73
0.31
1.92
3.98
0.34
3.21


7.531
7.453
7.335
7.253
7.136
7.055
7.016
6.989
6.653
6.629
6.383
6.213
5.503
4.643
4.622
4.553
4.533
4.269
4.208
4.189
4.138
4.119
4.098
4.076
3.862
3.836
3.725
3.592
3.571
3.511
3.487
3.102
3.056
2.723
2.546
2.467
2.439
2.346
2.320
2.127
2.035
2.001
1.947
1.753
1.689
1.638
1.518
1.492
1.381
1.342
1.223
0.962
0.621
0.308
44 at rt in toluene-d8 (700 MHz)
9
8
7
6
5
4
3
2
1
ppm
0.96
0.38
0.58
0.50
0.84
0.49
0.62
0.66
1.07
0.04
0.85
2.19
0.18
0.55
0.35
3.00
1.08
1.15
1.64
0.14
1.09
1.66
1.52
1.75
1.58
0.43
0.32
1.35
0.49
0.54
2.57
1.84

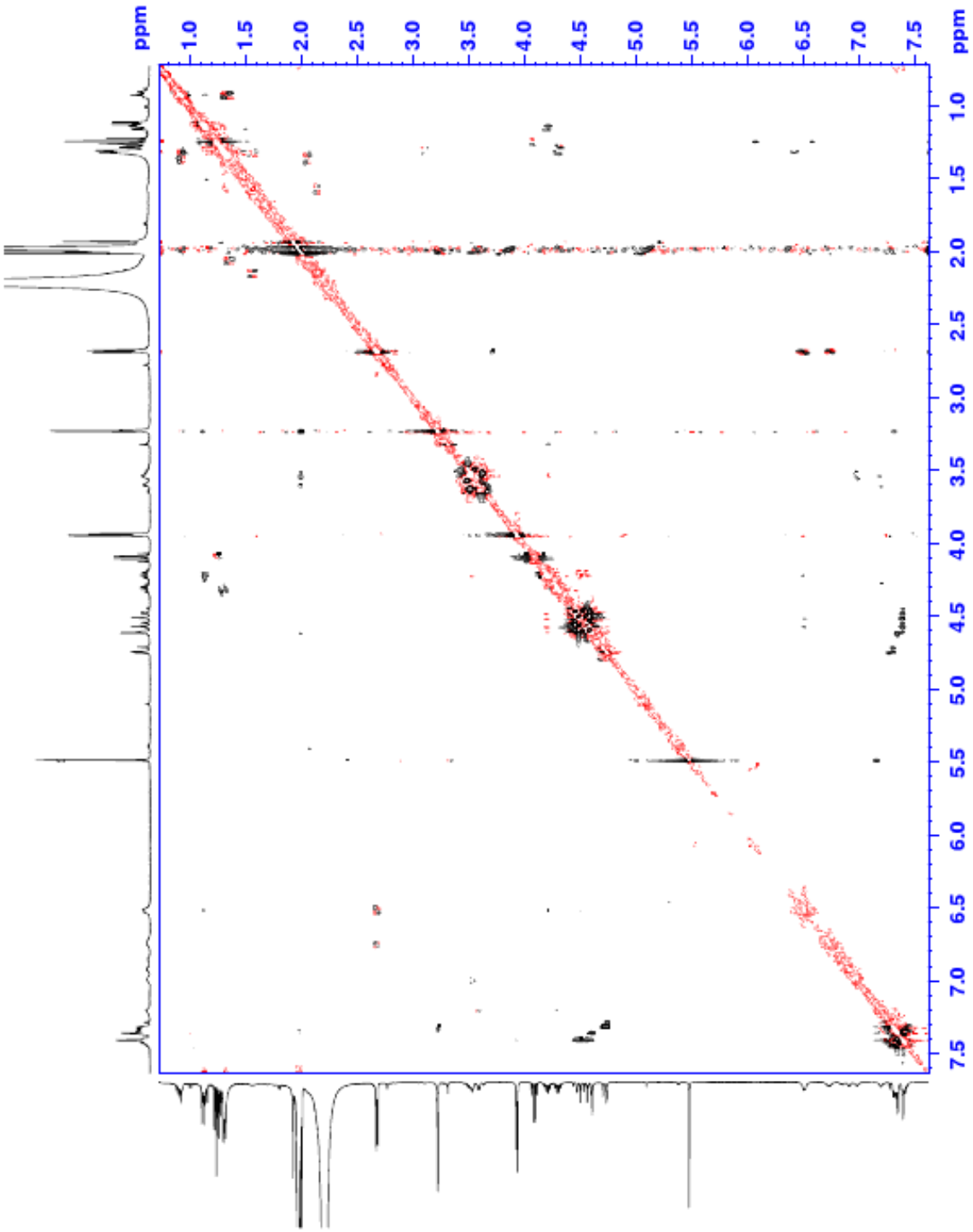
ROESY for **47** at rt in MeOD (400 MHz, 0.2 s mixing time)



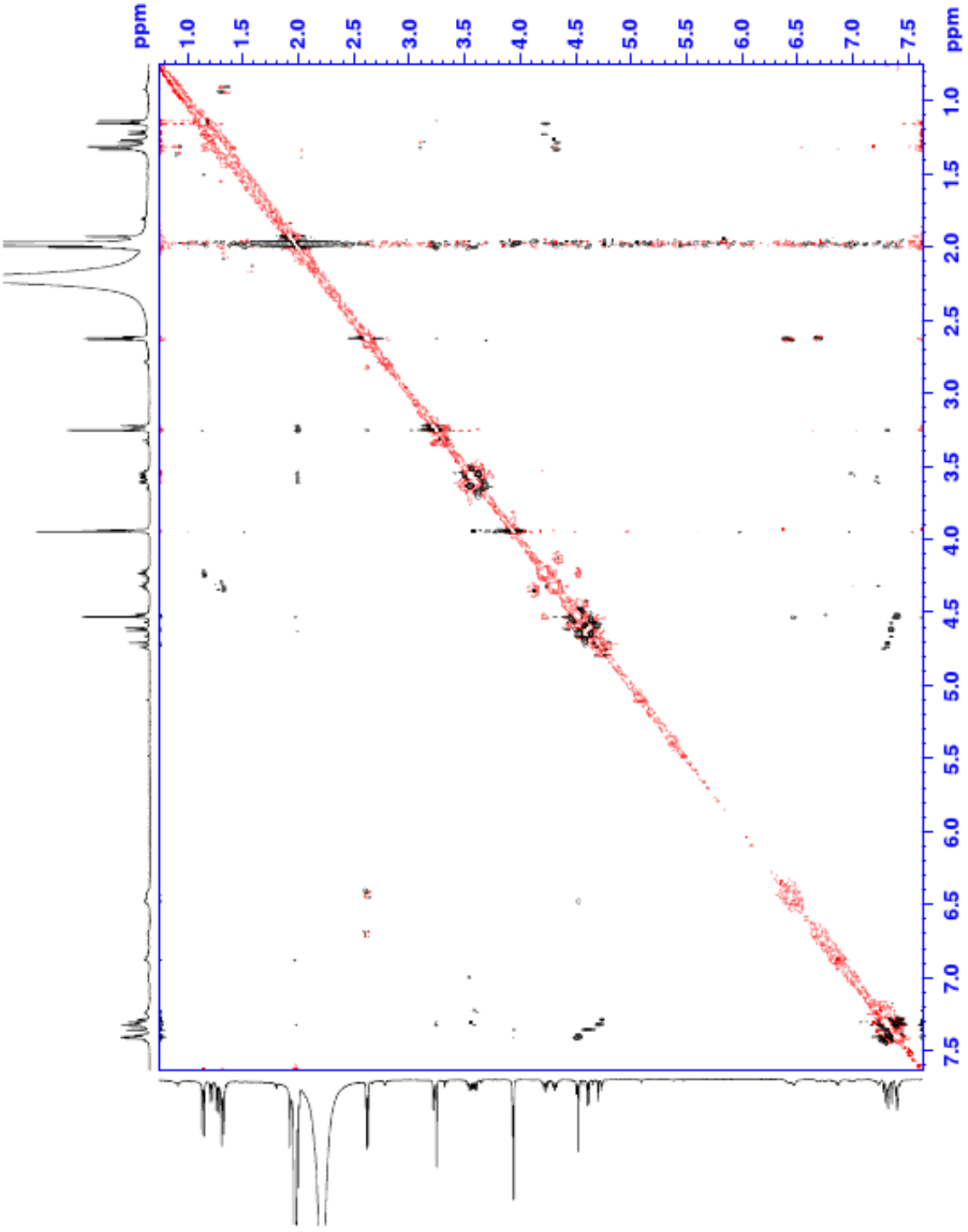
ROESY for **48** at rt in MeOD (400 MHz, 0.2 s mixing time)



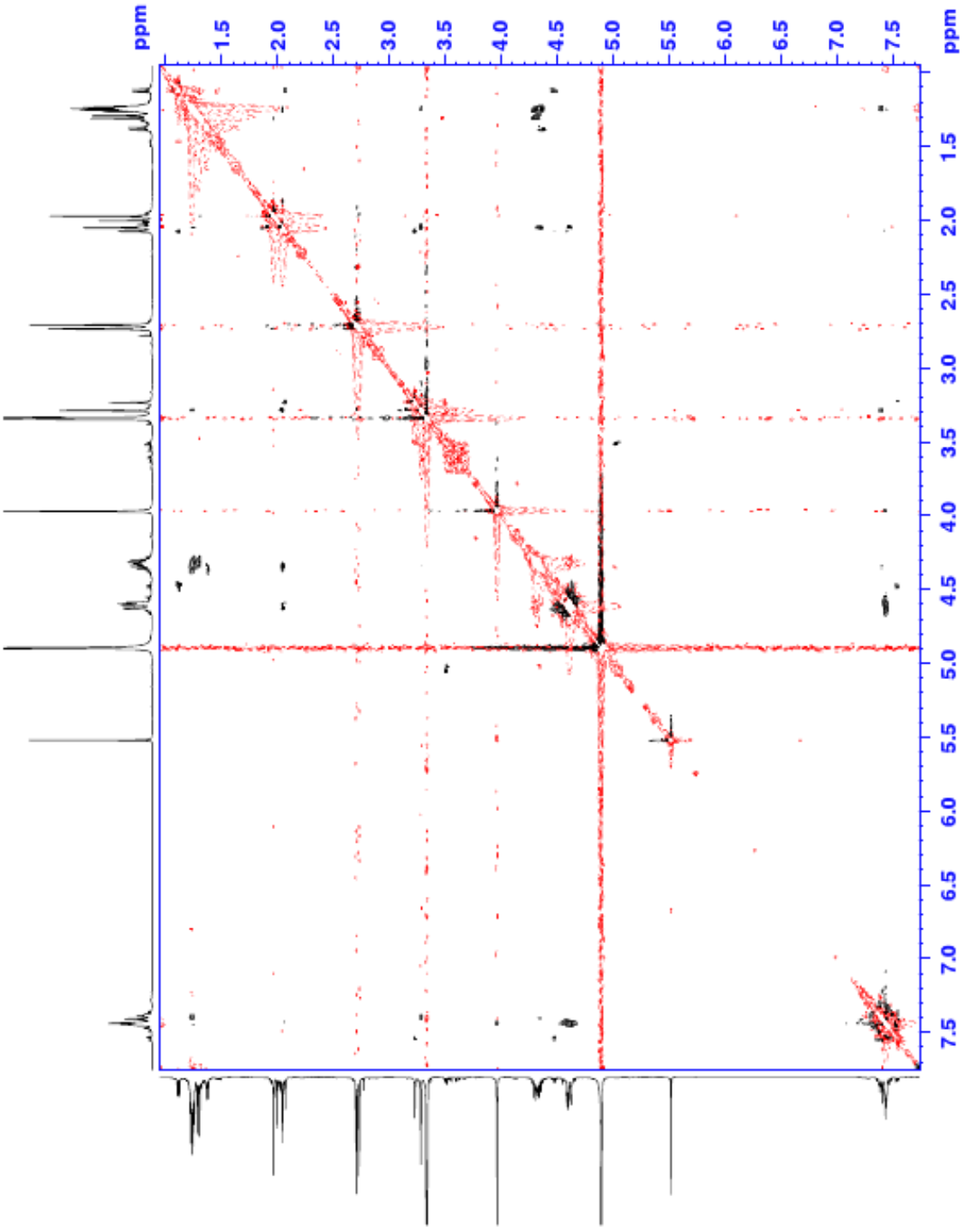
ROESY for **49**, peak at 13 min, at rt in MeOD (400 MHz, 0.2 s mixing time)



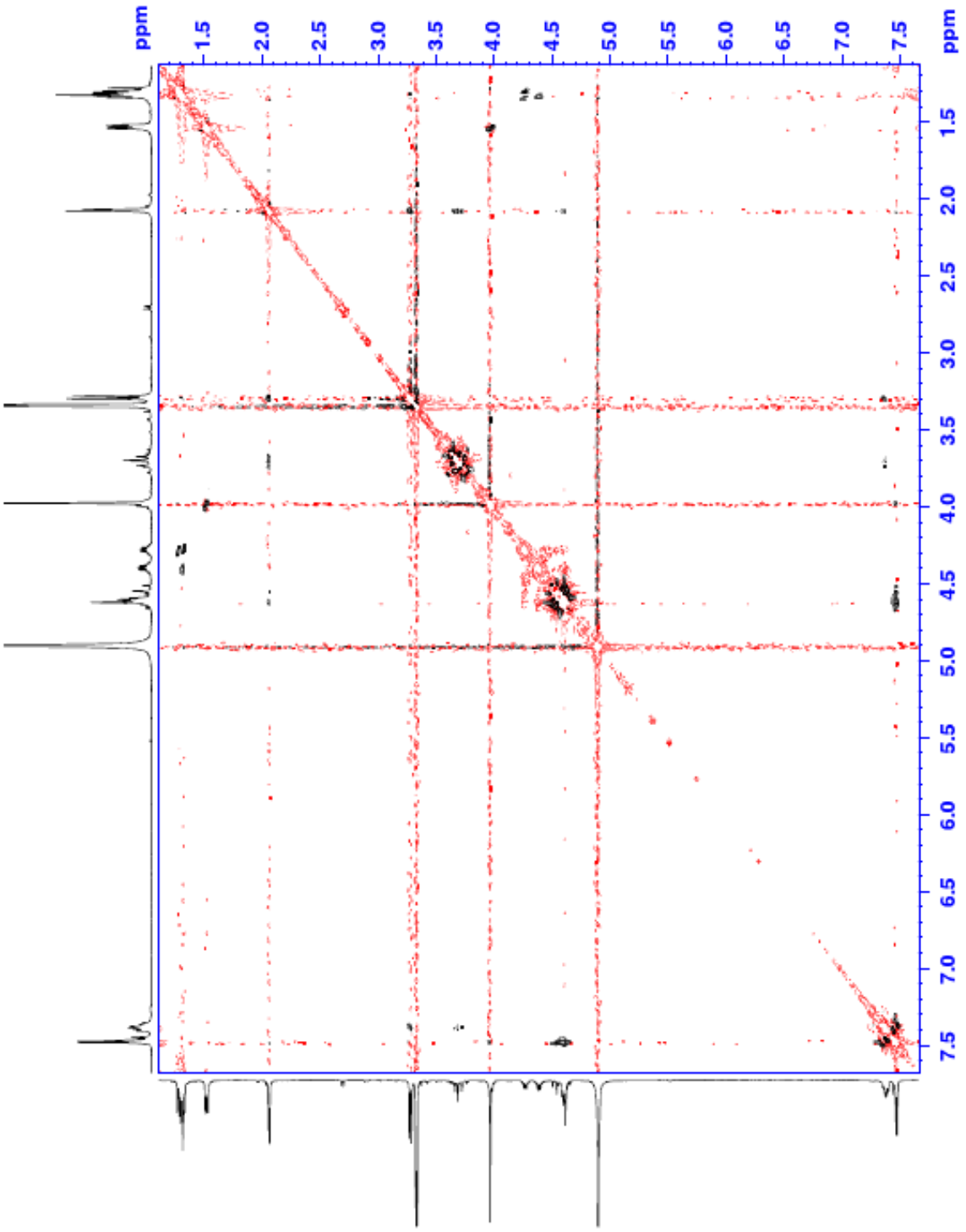
ROESY for **49**, peak at 15 min, at rt in MeOD (400 MHz, 0.2 s mixing time)



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)



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