SYNTHESIS OF PROTEIN FOLDING MODELS AND A MOLECULAR TORSION BALANCE STUDY OF NEIGHBORING GROUP EFFECTS ON HYDROPHOBICALLY DRIVEN FOLDING

by

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SYNTHESIS OF PROTEIN FOLDING MODELS AND

A MOLECULAR TORSION BALANCE STUDY OF NEIGHBORING GROUP

EFFECTS ON HYDROPHOBICALLY DRIVEN FOLDING

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University of Pittsburgh, 2014

This project investigated how alkyl group size, functionality, and polarity may affect hydrophobic binding. To complete this investigation, we prepared eleven new torsion balances. The syntheses were convergent, and the synthesis of the most complex torsion balance required 12 steps in the longest continuous path, and 28 steps overall. In this project I developed experimental conditions for 16 new chemical transformations, and characterized 62 new compounds. This project included detailed physical organic investigations. I used NMR spectroscopy to measure the rates and equilibrium constants associated with the folding.

Torsion balances bearing a bicyclo[2.2.2]octyl moiety have higher magnitude (more favorable) folding energies than those that have a *trans*-cyclohexyl moiety. Modeling studies indicate that this is due to better contacts of the bicyclo[2.2.2]octyl unit compared with the *trans*-cyclohexyl unit. We observed that the polarity of functional groups nearby the contact surfaces has negligible effect on folding energy. In MeOD, branching functionality resulted in more folding compared to linear functionality. The folding energy in water was higher in water than in MeOD. The difference in folding energy between torsion balances with different contact areas was diminished in D_2O. We conclude that London attraction is enhanced more in water for poorly bound surfaces (less contact area) than for tightly bound surfaces (more contact area). In

other words, a torsion balance with less contact surface has more room for improvement in the London dispersion energy.

We evaluated the effect of the length of the alkyl group on folding. After a certain point the length of the alkyl group had no effect on folding. Thus, for these small models, excess surface free energy (γ) of the interacting surfaces is not significantly changed as the surface is extended. Lum-Chandler-Weeks theory predicts γ is different among hydrophobic spheres of changing size.

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PREFACE

First I would like to thank my research advisor Professor Craig Wilcox, for his guidance and support during these past five years. Dr. Wilcox is a nice and very knowledgeable advisor. He provided very insightful discussions about my research. I would also like to thank the past and present Wilcox group members, in addition to my friends and classmates in this department for their scientific advice and friendship. I also wish to thank Professor Dennis Curran, Professor Michael Trakselis and Professor Jeffry Madura for being members of my dissertation committee, and Professor Seth Horne for serving as my proposal mentor. In addition, I will be always thankful for my Master degree advisor Professor Zhenlin Zhong in Wuhan University, for providing me with an inspiring and first insightful research experience in the field of biomaterials.

I specially thank my parents for their unconditional support, love and care. They always give me faith and encouragement to face difficulties. I would not have made it this far without their love and support. I would also like to thank my sister and brother for being my best and reliable friends in my life.

The sweetest and happiest thing during the past five years is marrying my husband and my soul-mate Desheng. Thanks for the numerous rides to take me home from lab, no matter how late it was at night and how bad the weather was. I truly thank Desheng for sticking by my side even when I was depressed and irritable. Without him, this work would not have come this far.

1.0 INTRODUCTION

1.1 INTRODUCTION OF LCW THEORY

The chemical events that enable every living entity to survive and reproduce are based on interactions between biomolecules.[1] Biomolecules, including (but not limited to) receptors, enzymes, nucleic acids, lipids, hormones, and drugs, have different shapes and functions determined by a balance among many small (less than a few kcal/mol) energetic interactions.[1,2] These forces of interaction and equilibria are also influenced by solvation and cosolute effects. Revealing the details of these interactions will contribute to drug design, biomolecule computer simulations, and molecular recognition. In the majority of experiments important to health and technology, the contributions of these component forces are difficult to measure accurately due to the myriad thermodynamic substates that represent bound and unbound states, folded and unfolded states, and solvation states.[3] For example, the evaluation of the interactions of amino acid side chains in a single protein are confounded by the freedom of motion within the protein. These motions are seldom qualitatively understandable and are as yet incalculable. So it would be interesting to design biomimetic molecular model systems to analyze these interactions quantitatively.

The hydrophobic effect is the most significant example of the general phenomenon of solvophobicity, which arises from the interaction of the medium and the solute. It occupies a

dominant place in controlling protein stability and drug receptor binding, and has been historically measured by hydrocarbon-water partition experiments. Lum, Chandler, and Weeks developed a theory (LCW theory) to describe the solute size and temperature dependence of the hydrophobic effect.[4] As described by **Figure 1.1**, for spherical non-polar particles, when the radius is smaller than 12 Å, the excess free potential energy associated with non-polar surfaces in contact with water will increase steeply as the radius rises with a slope of approximately 7 cal/mol·Å^2. However, when the radius of the particle is more than 12 Å, at 298 K the excess free potential would reach a plateau at about 110 cal/mol·Å^2.[5]

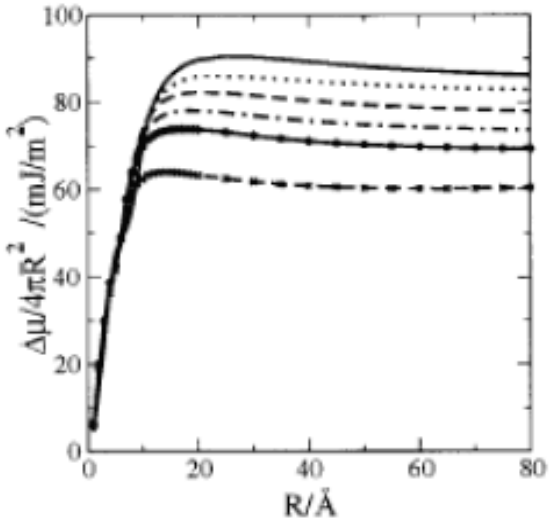

Figure 1.1 Excess surface free energy for a hard sphere of radius R in water.

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1.2 TRÖGER'S BASE AND THE MOLECULAR TORSION BALANCE

1.2.1 TRÖGER'S BASE AND THE MOLECULAR TORSION BALANCE CONCEPT

2,8-Dimethyldibenzodiazocine (Tröger's base, **Figure 1.2**), which has C2 symmetry, was chosen as the structural base in our system.[6] It was first synthesized in 1887 by Julius Tröger,[7] but the structure was not defined until 1935.[8] X-ray diffraction studies showed that the angle between the two phenyl rings in Tröger's base dibenzodiazocines varies from 89º-104º, and the distance between two methyl group is 6.6-7.1 Å.[6, 9] Because of its relatively rigid structure and chirality, Tröger's base analog would be a good choice to be the molecular core of a biomimetic molecule.[10]

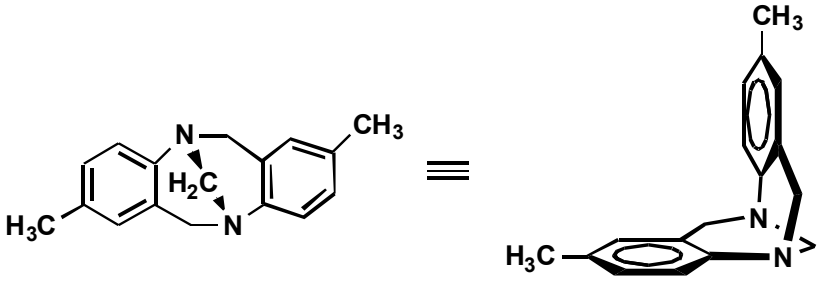


Figure 1.2 2,8-Dimethyldibenzodiazocine.

The concept of the molecular torsion balance was introduced by our group 20 years ago when a torsion balance based on Tröger's base was used to quantify substituent effects on the CH-π interaction.[6, 9-10] Tröger's base analogs have become widely used in supramolecular chemistry.[11,12] The torsion balances in this project are based on the use of Tröger's base as the scaffold. Molecular torsion balances can provide less ambiguous and more precise data to

evaluate individual molecular interactions of any kind, including hydrophobically assisted molecular folding.[2c, 11a, 13]

1.2.2 MEASURING THE EDGE-TO-FACE INTERACTION WITH THE FIRST GENERATION TORSION BALANCE

Edge-to-face interactions were first (and are still often) explained as an electrostatic phenomenon arising from the interactions of electric fields surrounding the aromatic rings.[14] Fischer et al. suggested recently that the total interaction enthalpy of face-to-edge interactions should be composed of both dispersion interactions and the counterbalancing effects of electrostatic and exchange repulsion forces, after they investigated the substituent effects on the aromatic edge-to-face interaction.[11c] Paliwal prepared the ester illustrated in **Figure 1.3** and found through X-ray crystallography that rings b and c of the folded isomer have an edge-to-face orientation in solid state.[13a] However, through studies in different solvents, the dielectric effect of solvent was found to not play an important role in modulating the intramolecular interactions. Furthermore, electron withdrawing or donating substituents had only a small effect on the strength of the interaction. It was concluded that the edge-to-face aromatic interaction was mainly driven by the London dispersion force.[13a]

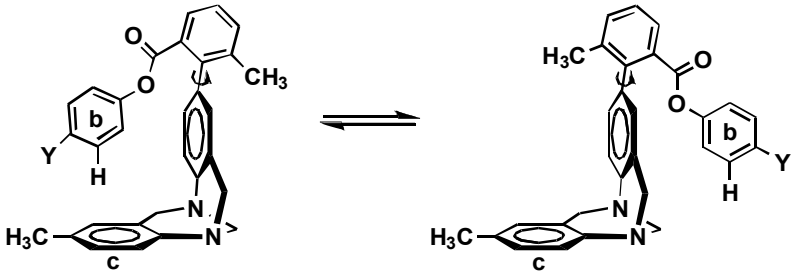


Figure 1.3 Folded (left) and unfolded (right) states of Paliwal's aryl ester.[13a]

1.2.3 MEASURING THE ALKYL CH-π INTERACTION WITH SECOND GENERATION TORSION BALANCES

Bhayana studied the value of a more symmetrical diester-based torsion balance.[13b, 13c] Our first generation of torsion balance featured ester and methyl groups as the two ortho substituents on the top phenyl ring.[13a, 15] We chose to replace the original methyl group with a second ester in the second generation torsion balances. This made the system more symmetrical. Thus we hoped to s avoid the influence on folding caused by the difference in polarity between the two ortho groups. Because the hydrophobic effect needs to be evaluated in aqueous solution, the second generation of torsion balance (**Figure 1.4**), was endowed with a hydrophilic moiety (R_2). The hydrophilic group (R_2) was introduced on the axis of rotation on the top of the upper phenyl ring. In this location, R_2 should have no effect on the folding equilibrium.

The folding equilibrium for a second generation molecular torsion balance is illustrated in **Figure 1.5** (R_1 = *tert*-butyl). The torsion balance is designed to evaluate the difference in energy between the conformation with *tert*-butyl on the inside (defined as the concave side of the dibenzodiazocine) versus the conformation with the methyl on the inside. A difference in conformer energy is expected because there will be differences in the solvation of the two groups and because CH-π interactions will be possible with the *tert*-butyl inside, but not possible when it is outside. This expectation was consistent with the experimental outcome. Several different esters were examined.

As revealed in **Table 1.1**, the folding energy rises with the increase of the size of ester alkyl group; the larger alkyl group has better contact with the arene and this enhances the CH-π interactions (such interactions are due principally to London dispersion). In water (D_2O), the folding energies increase by 0.22~0.45 kcal/mol compared to chloroform ($CDCl_3$). This increase in folding in water was attributed to the microscopic hydrophobic effects arising from solvent cohesiveness.

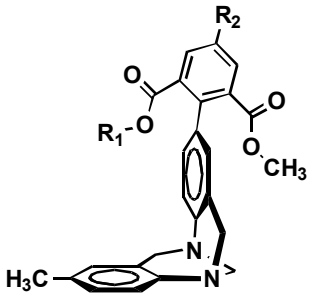


Figure 1.4 The second generation of torsion balance 1.

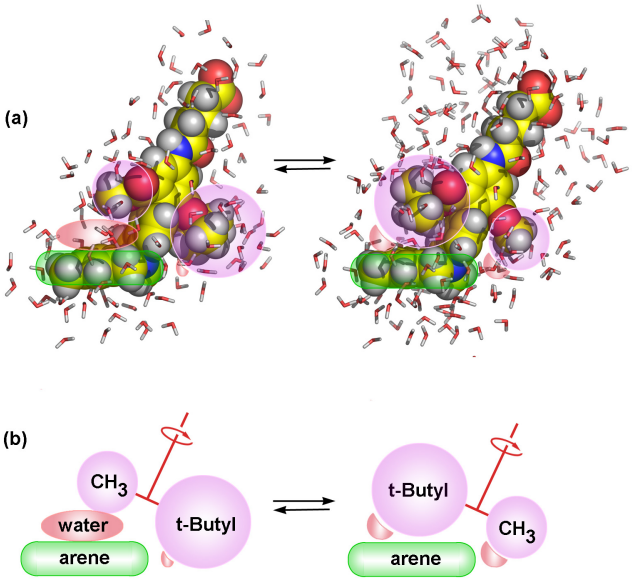


Figure 1.5 Folding equilibria for a second generation molecular torsion balance. (a) Solvated molecular structure and (b) schematic representation.

Table 1.1 Folding energies in CH-π effects.[a, d, e]

Esters	R_1	R_2	$-\Delta G_{fold}$ $CDCl_3$	$-\Delta G_{fold}$ D_2O [c]
1a	$(CH_3)_2$	NO_2	0.50	/ [b]
1b	$(CH_3)_2$	NH_2	0.50	/
1c	$(CH_3)_2$	$NHCO(CH_2)_3CO_2H$	0.50	0.72
1d	$(CH_3)_3$	NO_2	0.65	/
1e	$(CH_3)_3$	NH_2	0.65	/
1f	$(CH_3)_3$	$NHCO(CH_2)_3CO_2H$	0.65	0.92
1g	Cycloh	NO_2	0.36	/
1h	Cycloh	NH_2	0.36	/
1i	Cycloh	$NHCO(CH_2)_3CO_2H$	0.36	0.67
1j	1-adamantyl	NO_2	0.36	/
1k	1-adamantyl	NH_2	0.36	/
1l	1-adamantyl	$NHCO(CH_2)_3CO_2H$	0.36	0.68
1m	2-adamantyl	NO_2	0.55	/
1n	2-adamantyl	NH_2	0.55	/
1o	2-adamantyl	$NHCO(CH_2)_3CO_2H$	0.55	0.9
1p	CH_3-	NO_2	0.0	/
1q	CH_3-	NH_2	0.0	/
1r	CH_3-	$NHCO(CH_2)_3CO_2H$	0.0	0.0

[a] ±10% error. [b] Not sufficiently soluble. [c] 25 mM K_2CO_3 in D_2O. [d] kcal/mol. [e] The data were from Brijesh Bhayana's dissertation.

1.2.4 MEASURING HALOGEN BONDING WITH THE MOLECULAR TORSION BALANCE

The torsion balance has also been used to study halogen bonding.[16] Keyser studied the halogen bond effect on folding of torsion balances **2a-2l** (**Figure 1.6)**. The halogens studied were Cl, Br, and I, while the halogen bond acceptors were BocNH, $HOCH_2$, Me_2NOCCH_2 and BocNMe (**Table 1.2**). It was found that the temperature had little effect on the folding energies in the range from -5 to 15 ˚C. The data also show that the species with a hydroxyl group (enabling hydrogen bonding) have a higher folding ratio and folding free energy compared to those with a halogen (enabling a halogen bond). It is also seen that the carbamate and hydroxymethyl group have similar acceptor ability, though carbamate, which has sp^2 hybridized oxygen is predicted by theory to have better acceptor ability than that of the sp^3 hybridized oxygen in the hydroxylmethyl group.[5] This result may be due to the different geometric requirements for binding to these two groups. The tertiary carbamates **2k** and **2l**, were designed to increase the propensity for orthogonality and thus improve the halogen bond interactions. We observed that the folding ratios of **2k** and **2l** are indeed higher than those of **2a** and **2b**. Among **2g-j**, the iodinated species has the lowest folding free energy, while the Cl and Br species have the similar one. This is contrary to the bond strength sequence I > Br > Cl > F predicted by theory.[17] Keyser also examined the folding free energies in different solvents. She found a strong correlation between log K_{eq} and the solvent parameters (like dielectric constant, Taft and Kamlet's π and β scales, and Reichardt's normalized parameters), except for the case of CD_3OD. This was expected because CD_3OD is a good hydrogen bond donor, which can compete with the halogen and hydrogen bonds and alter the folding of the balances.

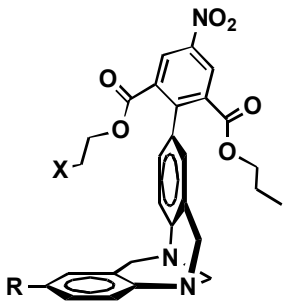


Figure 1.6 The torsion balance 2 for halogenated bond effect evaluation.

Table 1.2 Folding energies in XH-π effects at 5 °C in $CDCl_3$. [a, e]

Esters	R	X	Folding% [b]	$-\Delta G_{fold}$ $[CDCl_3]$ [c,d]
2a	BocNH	Br	58	0.18
2b		Cl	59	0.20
2c		OH	66	0.37
2d	$HOCH_2$	Br	60	0.21
2e		Cl	58	0.18
2f		OH	70	0.48
2g	Me_2NOCCH_2	Br	61	0.25
2h		Cl	62	0.26
2i		OH	78	0.71
2j		I	56	0.13
2k	BocNMe	Br	66	0.36
2l		Cl	66	0.37

[a] Determined by the analyses of ^1H NMR on 500 MHz NMR. [b] ±1.5% error. [c] ±15% error. [d] kcal/mol. [e] The data were from Sandra Keyser's dissertation.

1.2.5 MEASURING SALT BRIDGE STABILITY WITH A MOLECULAR TOSION BALANCE

It is accepted that buried salt bridges will stabilize the folded state of proteins, but many salt bridges are on the protein surface, and are partially or fully solvent exposed. Whether these solvent-exposed salt bridge interactions are stabilizing is still an open question.[18] Therefore, Keyser synthesized two series of torsion balances (**Figure 1.7**) with salt bridges exposed to solvent. With these in hand the stabilizing energy of the salt bridge can be evaluated from calculation of folding free energy of the torsion balances.[16] Series **3** contained an ammonium-carboxylate salt bridge, while the series **4** contained a guanidinium-carboxylate salt bridge. The folding free energy of these torsion balances was evaluated at different temperatures and pD. It was found that the salt bridge was a stabilizing interaction whether or not exposed to solvent. The difference of salt bridge stabilizing energy between ammonium and guanidinium-carboxylate is small based on experimental data, though the guanidinium-carboxylate series has stronger interactions in molecular modeling using force field, MMFF94s. The author attributed this to the leveling effect of solvent. The stabilizing energy for both ammonium-carboxylate and granidinium-carboxylate interactions varies from -0.3 to -0.5 kcal/mol. This stabilizing energy changes negligibly when the temperature varies from 5˚C to 25 ˚C, and the pD varies from 3.1 to 10.0.

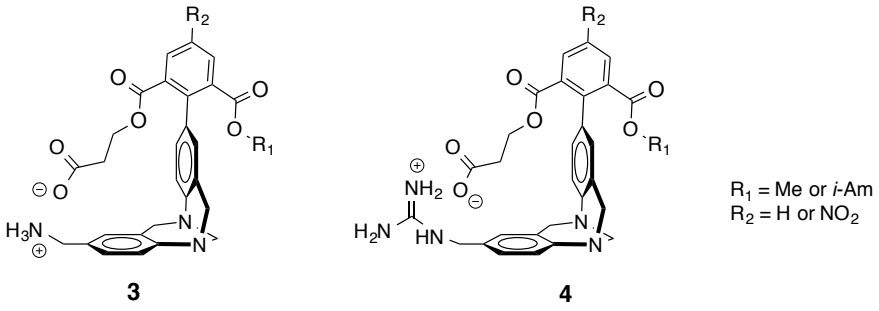


Figure 1.7 Molecular structures of torsion balances for salt bridge effect investigation.

2.0 EVALUATION OF NEIGHBORING GROUP EFFECTS VIA MOLECULAR TORSION BALANCE STUDIES

2.1 NEIGHBORING GROUP EFFECTS IN DRUG DISCOVERY

Drug discovery frequently relies on computational methods. Generally, small molecules will be docked into the structures of macromolecular targets and their potential complementarity to binding sites will be evaluated by computational approaches.[19] Automated analogue design and evaluation helps chemists to quickly eliminate molecules that cannot fit the binding site and to discover potential lead structures. For example, optimization of a series of caspase-3 inhibitors was begun starting from a co-crystal structure with salicylic acid. Molecular modeling eventually resulted in a compound with 20 nM potency.[19-20] Structure-based drug design thus provides a promising method for lead optimization.

Despite the significant advances in molecular simulation methodologies over the past decades and the increasing calculation performance of computers, the prediction of binding affinities is still difficult and inexact due especially to the difficulty of predicting solvation energies.[21] In this project, in order to measure the neighboring group effect on solvation and molecular folding, a number of molecular torsion balances were designed and synthesized.

2.2 EVALUATION OF NEIGHBORING GROUP EFFECTS WITH TORSION BALANCE

The function and the shapes of receptors, enzymes, nucleic acids, lipids, drugs, and other biologically active molecules are ultimately determined by a balance among many small (less than a few kcal/mol) energetic interactions.[1-2] These forces of interaction and equilibria are also influenced by solvation and cosolute effects. In the majority of experiments important to health and technology the contributions of these component forces are difficult to measure accurately due to the myriad thermodynamic substates that represent bound and unbound states, folded and unfolded states, and solvation states.[3] For example, the evaluation of the interactions of amino acid side chains in a single protein are confounded by the freedom of motion within the protein. These motions are seldom qualitatively understandable and are as yet incalculable. Molecular torsion balances can provide less ambiguous and more precise data to evaluate these individual interactions, including hydrophobically assisted molecular folding.

The concept of the molecular torsion balance was introduced 20 years ago when a torsion balance based on Tröger's base was used to quantify substituent effects on the CH-π interaction.[6-10, 13a, 15] Tröger's base analogs have become widely used in supramolecular chemistry.[11a-h] A second generation of torsion balances based on Tröger's base featured a more symmetrical core structure and a hydrophilic glutaric acid side chain to improve water solubility.[13c] This advance enabled the measurement of alkyl CH-π intereactions in water, and also made it possible to investigate the effect of water on molecular folding when the associating surface were less than one square nanometer. This was the first study to establish that a molecular torsion balance can be sensitive to nanoscale hydrophobic effects.

In this project we sought to investigate whether or not polar groups nearby hydrophobic surfaces would have an effect on the magnitude of hydrophobically assisted folding. We refer to this as a "neighboring group effect" in hydrophobic surface association. We reasoned that this effect might arise from the influence of the polar group on the structure and orderliness of water at the hydrophobic surface. If the polar group influenced the excess free energy of the water at the nearby hydrophobic surface, a change in the contribution of desolvation to binding or folding energy might be observed.

Several different torsion balances were prepared to investigate these proposed neighboring group effects in hydrophobic binding. The water-soluble torsion balances in this paper incorporate a series of bicyclo[2.2.2]octane (Bco) and *trans*-cyclohexane (Cy) groups. The bicyclo[2.2.2]octyl alcohols used in the Bco series are symmetrical and rigid, and therefore reduce the number of thermodynamic substates that compose the folded and unfolded conformations, while the *trans*-cyclohexane (Cy) series provide an interesting comparison. In **Figure 2.2**, the upper two molecular structures represent truncated analogs of **5g** and **5k**. The reason of using truncated molecular structures here is because the effect of the top hydrophilic moiety is negligible on folding. Removing this linear part can simplify the calculation while not affecting the result. The bottom two figures are the solvated molecular structure for a truncated analog of **5l**. As we can see, the water structure of the folded state is significantly different from the unfolded state. Therefore, it was investigated in this project whether the polarity of the neighboring groups will affect the water structure of the molecule surface

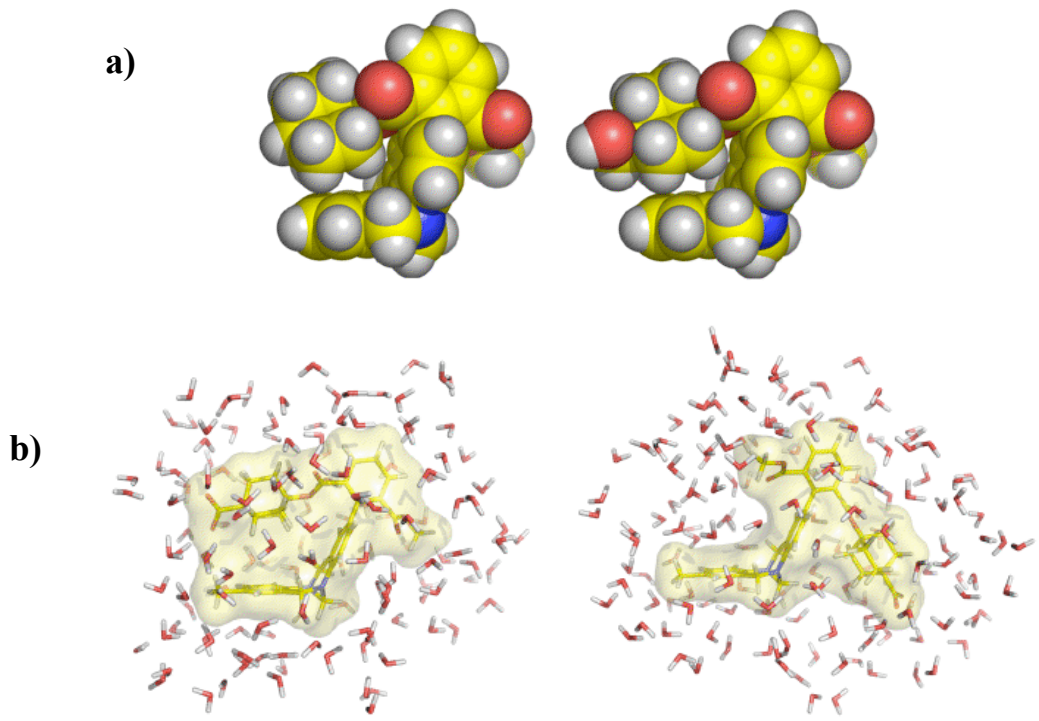
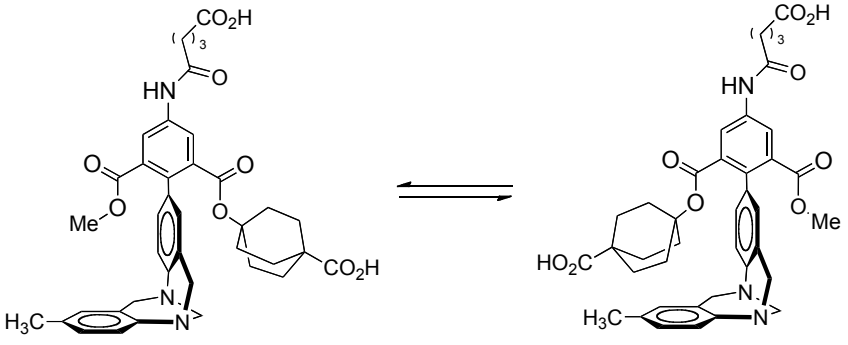


Figure 2.1 a) Minimized structures for truncated analogs of 5g and 5k; b) solvated molecular structure for a truncated analog of 5l.

In the torsion balances with hydroxyl or carboxylic acid group (**5e, f, k, l**), we used benzyl protecting groups to allow clean hydrogenolysis of torsion balances (**7c, d, i, j**) to generate the hydroxyl and carboxylate polar groups.



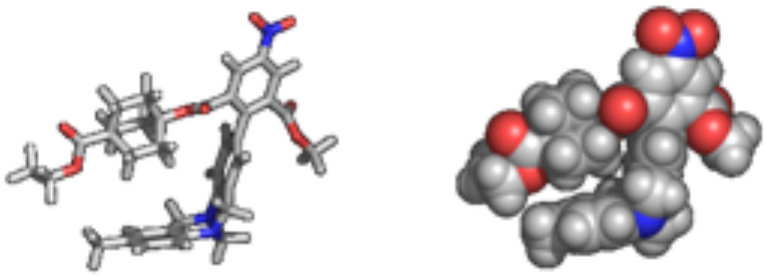


Figure 2.2 (a) Illustration of folding for torsion balance 5l (top);

(b) Two representations of 7h (bottom).

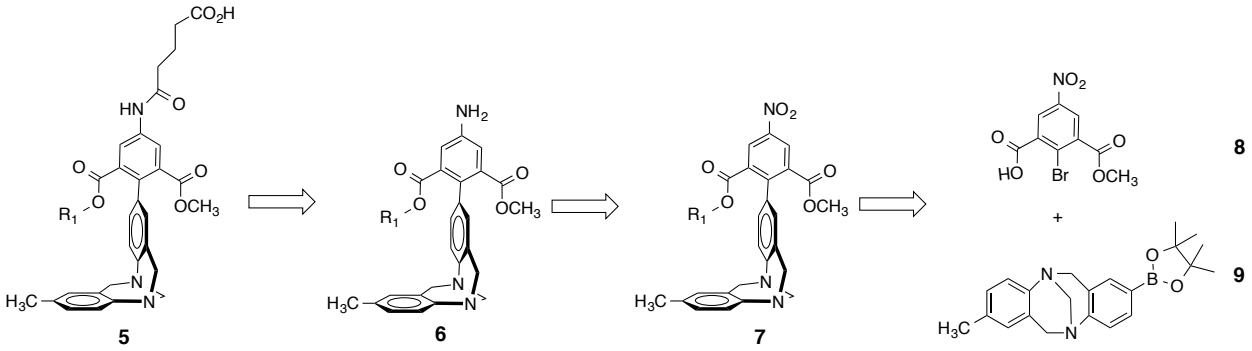
All the torsion balances synthesized were examined with 2D and 1D ^{1}H NMR.[22] For the water-soluble torsion balances, variable temperature ^{1}H NMR experiments were run in CD_3OD and D_2O. It was expected that the folding ratio of the torsion balances might be changed according to temperature because the water structure might be affected as the temperature varied. In this project, we examined the balance behavior at 278 K, 288 K, 298 K and 308 K, four temperatures in each of our torsion balances. Therefore the change in folding free energies among theses torsion balances could be evaluated in a quantitative way at different temperatures. In summary, this project will provide the first quantitative study of the effects of neighboring groups on hydrophobic interactions.

3.0 SYNTHESIS AND ANALYSIS OF TORSION BALANCES FOR EVALUATION OF NEIGHBORING GROUP EFFECTS

3.1 RETROSYNTHETIC ANALYSIS

The retrosynthetic schemes for these torsion balances followed closely the pathways that had been already established in our earlier studies (**Scheme 3.1**).[23] Our target water-soluble torsion balance **5** with hydrophilic moiety on the top of the phenyl ring would be synthesized from amine-torsion balance **6** by a ring opening acylation reaction with glutaric anhydride. Amine torsion balance **6** could be prepared from hydrogenation of nitro torsion balance **7**. Suzuki cross-coupling reaction of pinacolatoborane ester of Tröger's base **9** and diester **8** would furnish torsion balance **7**. Asymmetrical diesters **8** could be prepared by DCC coupling of a monoacid with different alcohols.

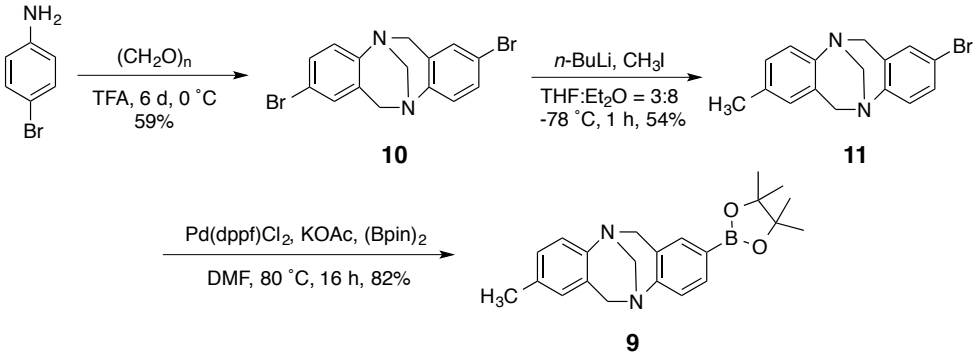
Scheme 3.1 Retrosynthesis of molecular torsion balances 5.



3.2 SYNTHESIS OF THE TORSION BALANCES

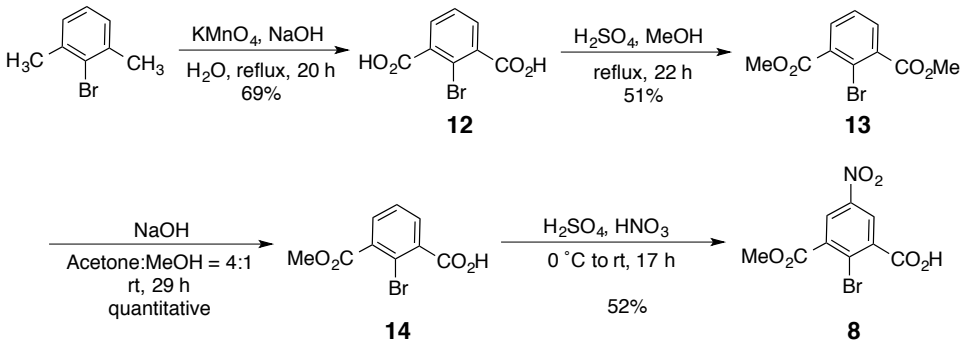
The approach was attractive because pinacolatoborane ester of Tröger's base **9** and diester **8** could be synthesized in parallel before the Suzuki cross coupling reaction. The synthesis of the Tröger's base followed the method of Wärnmark.[24] It started from 4-bromoaniline and paraformaldehyde. The dibromo analog **10** was treated with n-butyllithium and methyl iodine to give the 2-bromo-8-methyl analog **11**.[25] This resulting product was transformed to boron-methyl analog **9** with $Pd(dppf)Cl_2$ (dichlorobis[methylene bis(diphenylphosphine)]-dipalladium-dichloromethane adduct) as catalyst (**Scheme 3.2**).[26]

Scheme 3.2 Synthesis of boron-methyl analog 9.



The isophthalic acid was prepared in a sequence that started with the permanganate oxidation of m-bromo xylene.[27] Esterification of the resulting diacid **12** gave diester **13** as the product.[28] Then diester **13** was treated with 1.1 eq. of sodium hydroxide to yield hemiester **14**, which was then transformed to nitro-hemiester **8** by nitration with nitric acid and sulfuric acid (**Scheme 3.3**).

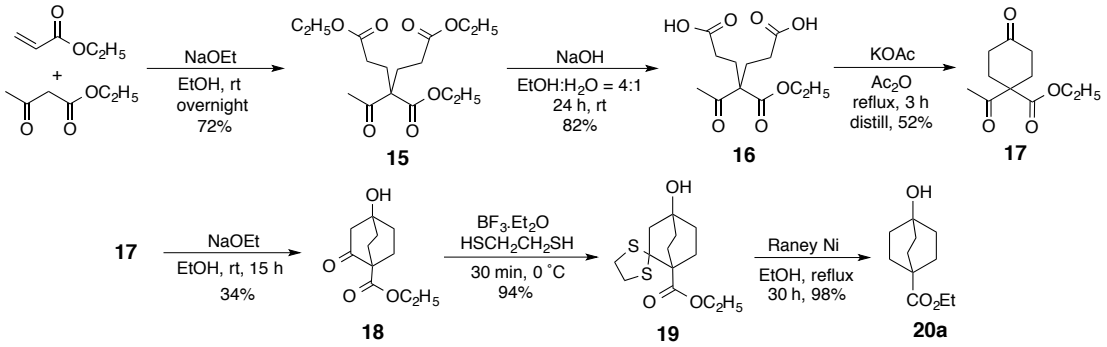
Scheme 3.3 Synthesis of nitro-hemiester 8.



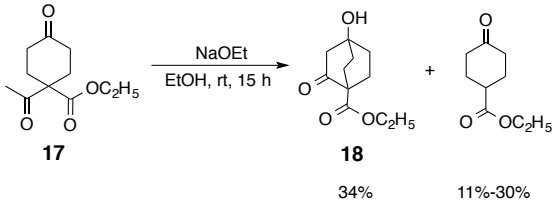
The synthesis of the bicyclo[2.2.2]octane series of alcohols followed Grobb's method (**Scheme 3.4**).[29] It started from Michael addition of ethyl acrylate and ethyl acetoacetate and gave triester **15** in 71% yield. The resulting triester **15** was hydrolyzed with 2.2 equivalent of NaOH in a mixture of ethanol and water (4:1), affording diacid **16**. Since the ester group on the dialkyl acetoacetate backbone of triester **15** is more hindered, the ethyl acrylate esters would tend

to be hydrolyzed more easily. Ketoester **17** was synthesized by Dieckmann condensation and decarboxylation of diacid **16**. Ketoester **17** was treated with sodium ethoxide to generate the bicyclo[2.2.2] structure. The yield in this reaction is relatively low because a significant side product (ethyl 4-oxocyclohexanecarboxylate) was produced (**Scheme 3.5**). This would arise from a nucleophilic attack of ethoxide on the methyl ketone, resulting in a retro-Claisen reaction. The carbonyl group on bicyclo[2.2.2]octane ketoester **18** was removed by treatment with dithiol under acidic conditions, and reduction of the resulting thioketal **19** with Raney Ni. The overall yield of six steps to make ethyl bicyclo[2.2.2]octane carboxylate **20a** is 10%.

Scheme 3.4 Synthesis of bicyclo[2.2.2]octane alcohol analog 20a.

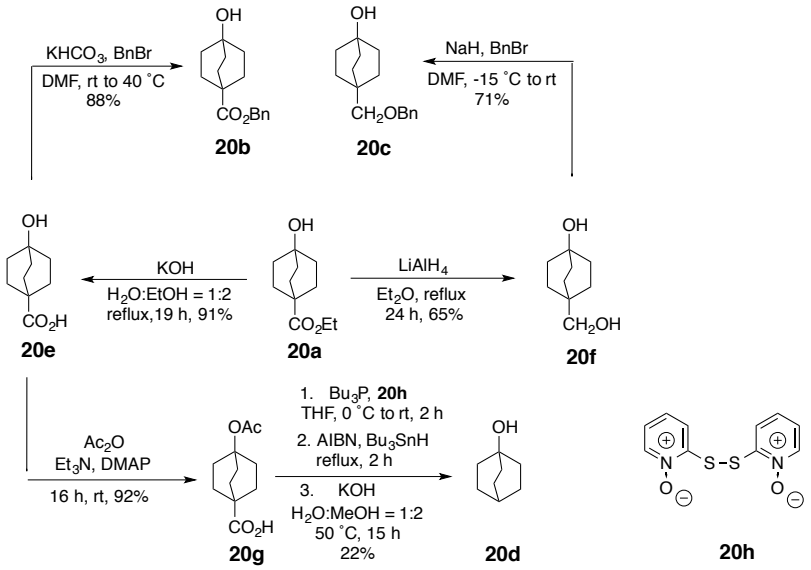


Scheme 3.5 Ring closing reaction of ketoester 17.



The transformations of ethyl ester **20a** to the other needed bicyclo[2.2.2]octane alcohols are described in **Scheme 3.6**. The conversion of ethyl ester **20a** to benzyl ester **20b** was accomplished by hydrolysis then benzylation under basic conditions. Benzyl ether **20c** was formed via reduction of **20a** and benzylation. The synthesis of bicyclo[2.2.2]octane alcohol **20d** is a little tricky. We tried direct Barton decarboxylation on acid **20e**, but product **20d** was not confirmed by TLC and ^{1}H NMR. There were two possibilities: the first is the product **20d** was generated, but it was not detectable with TLC and proton NMR because of serious overlap with Bu_3P and Bu_3SnH; the second is product **20d** was not generated because of the interference of the hydroxyl group. Therefore, we protected the hydroxyl group by acylation.

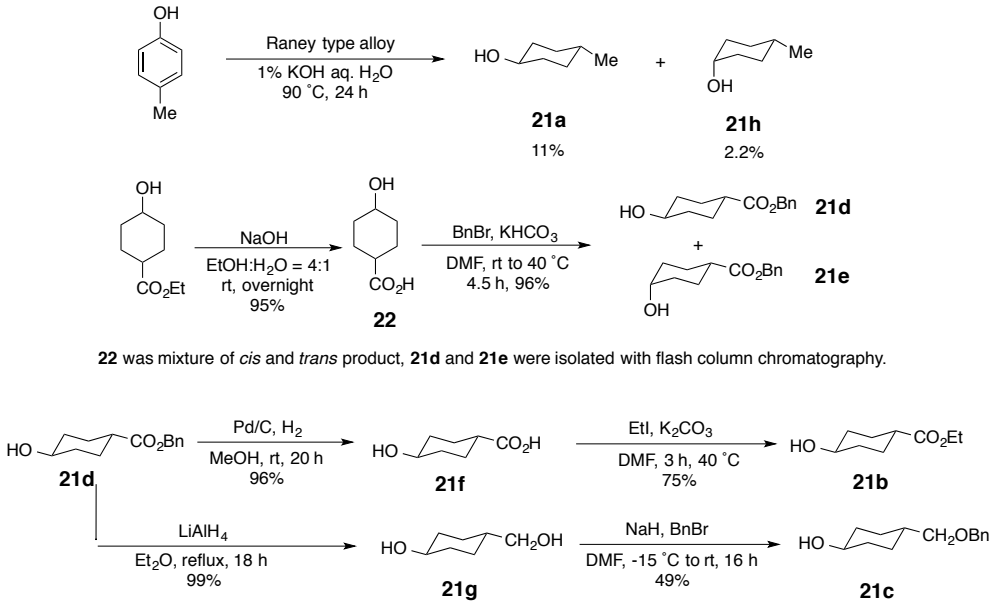
Scheme 3.6 Thansformation of alcohols 20b-20d from 20a.



The decarboxylation of protected derivative acetate **20g** was successful. The resulting product bicyclo[2.2.2]octyl acetate was detected on TLC. Its polarity was very low, and its R_f value was higher than the other reactants. However, because bicyclo[2.2.2]octyl acetate can

sublime easily, it would evaporate if the solvent was removed under reduced pressure. Therefore, distillation was used to remove the solvent after column chromatography. During the following hydrolysis step, because the distilled acetate contained some organic solvents (ether and pentane), a mixed solvent of methanol and water (2:1) was necessary to make the solution homogeneous. Hydrolysis was first tried at room temperature, but without success. At 50 ˚C the reaction was complete after 15 hours of heating. Bicyclo[2.2.2]octyl acetate is a tertiary ester, and is expected to be less accessible to attack by hydroxide, so the reaction at room temperature was too slow. The overall yield of alcohol **20d** was 18% in five steps.

Scheme 3.7 Synthesis of *trans*-cyclohexyl alcohol series.

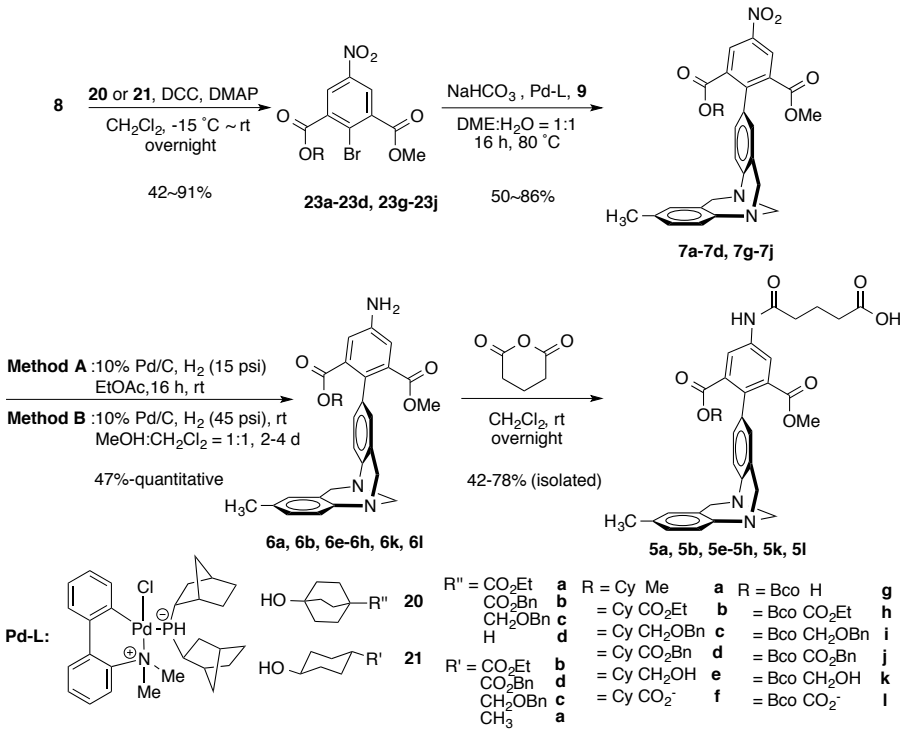


22 was mixture of *cis* and *trans* product, **21d** and **21e** were isolated with flash column chromatography.

The synthesis of cyclohexyl series of alcohols is easier compared to that of the bicyclo[2.2.2]octane series (**Scheme 3.7**). It begins from the *trans* and *cis* mixture of commercially available ethyl 4-hydroxycyclohexane-1-carboxylate. Hydrolysis and benzylation

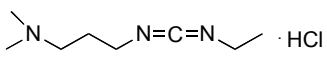
gave benzyl esters **21d** and **21e**. With the more bulky benzyl group instead of the ethyl group, benzyl esters **21d** and **21e** can be isolated by flash chromatography. *trans*-Benzyl ester **21d** was transformed to the other alcohols via a method similar to that used in the bicyclo[2.2.2]octane series. For the *trans* 4-methyl cyclohexanol **21a**, *p*-cresol was used as the starting material. The yield of the hydrogenation product, isolated *trans* product **21a,** was 11%. The low yield can be attributed to the uncompleted reaction after 24 hours of heating.

Scheme 3.8 The synthetic route of torsion balances.

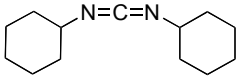


With all the alcohols and monoester **8** in hand, DCC coupling was used to furnish isophthalate esters **23a-d**, **g-j**. We first tried EDC·HCl in the coupling reaction, because it is soluble in water and can be removed more easily. However, it provided poor yields when tertiary

bicyclo[2.2.2] octane alcohols were used. So we changed the coupling reagent to DCC and achieved a good yield of around 70% (**Scheme 3.8**).



EDC·HCl



DCC

In the Suzuki coupling reaction, we followed Brijesh Bhayana's method using Blaser's catalyst (**Scheme 3.8**, Pd-L) and achieved on average about 70% yields.[13c] In order to put a hydrophilic side chain onto the torsion balances, the nitro group was reduced with hydrogen and palladium on carbon. For the torsion balances without benzyl protecting groups, the nitro group was reduced to an amino group under balloon pressure (approximately one standard atmosphere) of hydrogen with Pd/C at room temperature. For the torsion balances with benzyl protecting group, we used a Parr hydrogenator at 45 PSI of hydrogen This ensured that the benzyl group was deprotected when the nitro group is reduced.

The resulting amines **6a, b, e, f, g, h, k, l** were finally treated with glutaric anhydride to furnish water-soluble torsion balances **5a, b, e, f, g, h, k, l**.[13c] During these studies, we found that an amino torsion balance **6h** that had been exposed to air for six months was not able to react with glutaric anhydride. Compared the ^{1}H NMR spectra (**Figure 3.1**) of both freshly made and air exposed torsion balance **6h**, we can see that the proton signals of the top phenyl ring of the air oxidized sample shifted towards low field. Besides, from the IR spectrum (**Figure 3.2**) of the air exposed sample, we can notice that the bending signal (1609 cm^{-1}) of the primary amino group disappeared, which showed up in the freshly made sample. And the two-band signal (3500 cm^{-1} and 3374 cm^{-1}) of the stretching of N-H in the amino group was replaced with the broad peak (3432 cm^{-1}) in the spectrum of the air oxidized sample. The IR data indicated that the amino group -NH_2 of torsion balance **6h** was oxidized to –NHOH after exposure to air for six months.

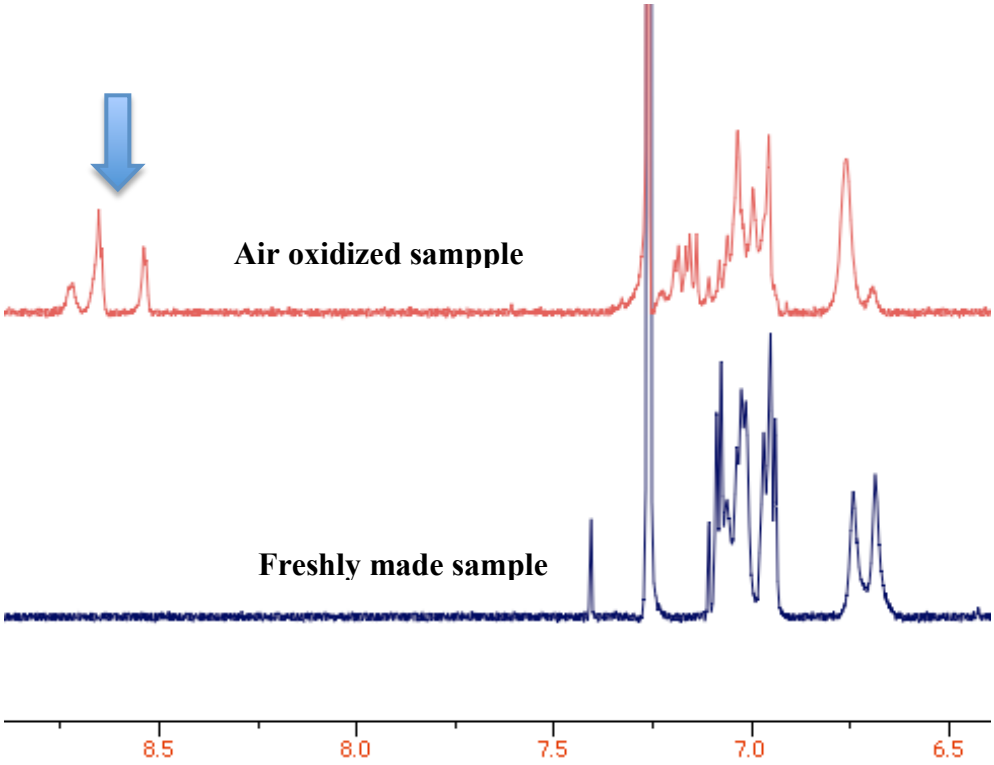


Figure 3.1 ^{1}H NMR spectra of (a), freshly made sample of torsion balance 6h (1 mM in $CDCl_3$) and (b), the same sample after six months

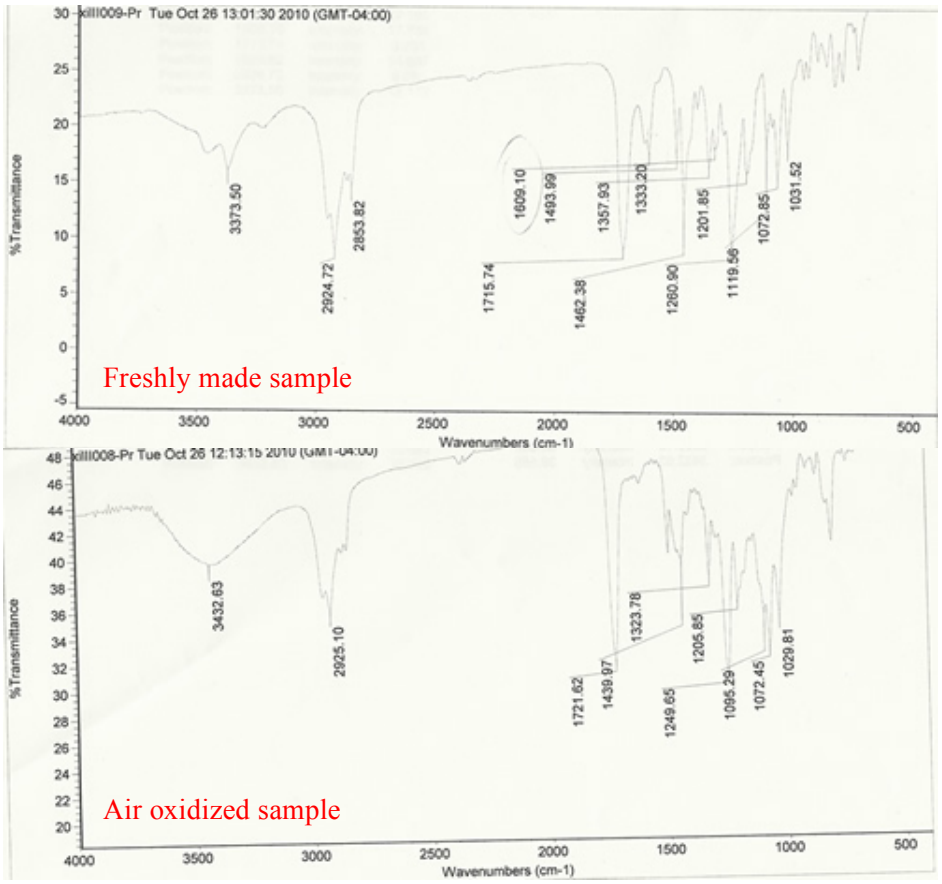


Figure 3.2 IR spectra of air oxidized and freshly made sample of 6h in KBr.

3.3 NMR ANALYSIS AND FOLDING ENERGY CALCULATION

By the methods outlined above, three kinds of torsion balances were made: 1) **7a-d, g-j** are torsion balances with a nitro group ($-NO_2$); 2) **6a, b, e, f, g, h, k, l** are torsion balances with an amino group ($-NH_2$); 3) **5a, b, e, f, g, h, k, l** are torsion balances with glutaric acid side chain ($-CO_2H$). Each of these three sets consists of two series of esters, bicyclo[2.2.2]octyl (Bco) and cyclohexyl (Cy). For those torsion balances with the nitro and amino groups, the 1-D NMR studies were taken in $CDCl_3$. For the torsion balances with the glutaric acid side chain, MeOD and D_2O were used as solvents.

The conformations of these torsion balances were examined by analysis of the methyl ester signals in ^1H NMR spectra. The rotation around the single aryl-aryl bond is slow on the ^1H NMR time scale. When the methyl ester is on the concave side of the Tröger's base, the methyl protons resonate at around 2.8-3.0 ppm. In contrast, when the methyl ester is on the convex side the methyl ester protons resonate at around 3.5-3.7 ppm, as is typical for common methyl esters. This difference unambiguously identifies the folded and unfolded states. **Figure 3.3** shows the cross peaks of the methyl group in **6h**. The methyl on the concave side of the Tröger's base would be at upper field because of the effect of the bottom phenyl ring. All of the calculations of folding percentage in this paper were based on these two signals.

The folding free energies were calculated using the equation below:

$$\Delta G^o = -RT\ln\left(\frac{folded}{unfolded}\right) \qquad \text{Eq. (3.1)}$$

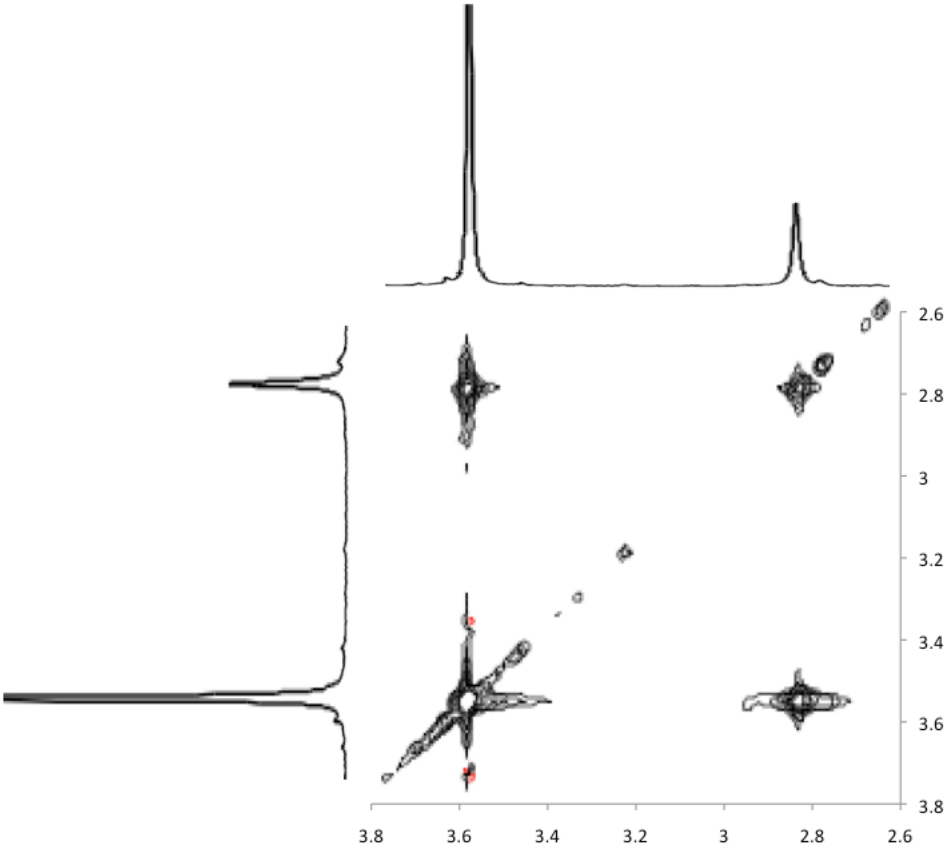


Figure 3.3 Methyl signal of torsion balance 6h in NOESY in $CDCl_3$ at 5 ˚C.

3.3.1 CONTACT SURFACE EFFECTS

The nitro or amino torsion balances were examined in $CDCl_3$ at 1 mM concentration at 5 ˚C (**Table 3.1**, **Table 3.2).** The results are illustrated in **Figure 3.5**, where the front row represents torsion balances with an amino group, while the back row represents torsion balances with a nitro group in the topmost position. Comparing the bicyclo[2.2.2]octyl series and the *trans*-cyclohexyl series (for example comparing entry 3 with entry 4 in **Table 3.1**), the bicyclo[2.2.2]octyl series is found to have higher folding ratios (greater folded stability) than the

cyclohexyl series. This is expected because the bicyclo[2.2.2]octyl esters incorporate a boat-like cyclohexyl ring adjacent to the aromatic plane of the Tröger's base, while cyclohexyl esters have chair-like *trans*-cyclohexyl ring. The more rigid and symmetrical bicyclo[2.2.2]octyl framework gives better contact with the aromatic ring and yields a CH-π interaction stronger than is achievable with the *trans*-cyclohexyl series. The three-fold symmetry of the bicyclo[2.2.2]octyl core will also less disfavor folding.

In **Figure 3.5**, it was observed that torsion balances with ester groups (ethyl or benzyl ester) or carboxylic group have higher folding ratio than those with ether (or hydroxyl) or alkyl group in both nitro and amino series (Most of the folding percentage remained the same after hydrogenation, but there are two exceptions, torsion balances **6f** and **6l**. This will be discussed in **Section 3.3.4**). This may be due to the branching in these groups. The ester group should be in a staggered position relative to bicyclo[2.2.2]octyl core. As the ester group rotates, both oxygens in the ester group have the chance to face to the phenyl plane, though the conformation in **Figure 3.4** may be preferred because the ethyl group is closer to the methyl group on the phenyl ring. In short, branching may make the interaction between ester and the methyl group of the phenyl plane stronger, thus giving a higher folding percentage. We test the effect of branching in **Chapter 4**.

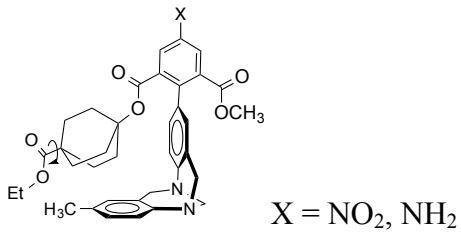


Figure 3.4 Molecular configuration of torsion balances 7h and 6h.

Table 3.1 Folding percentage and energy for torsion balances 7a-d, g-j in $CDCl_3$ at 5 °C.[c, d]

Entry	Name	TB	Folding%[a]	ΔG_{fold}[b] kJ/mol
1	NO_2-Bco-H	**7g**	69	-1.9
2	NO_2-Cy-CH_3	**7a**	63	-1.2
3	NO_2-Bco-CO_2Et	**7h**	72	-2.2
4	NO_2-Cy-CO_2Et	**7b**	64	-1.4
5	NO_2-Bco-CO_2Bn	**7j**	74	-2.4
6	NO_2-Cy-CO_2Bn	**7d**	65	-1.4
7	NO_2-Bco-CH_2OBn	**7i**	60	-0.9
8	NO_2-Cy-CH_2OBn	**7c**	59	-0.7

[a] Indicates ±1.8% of folding error. [b] Indicates ±0.2 kJ/mol absolute error for folding free energy. [c] All the NMR data in this table were determined from line shape analysis of the ^{1}H NMR spectra acquired on a 700 MHz NMR. [d] 1 mM concentration.

Table 3.2 Folding percentage and energy of torsion balances 6a-b, e-h, k-l in $CDCl_3$ at 5 °C. [c, d]

Entry	Name	TB	Folding% [a]	ΔG_{fold}[b] kJ/mol
1	NH_2-Bco-H	**6g**	64	-1.3
2	NH_2-Cy-CH_3	**6a**	62	-1.1
3	NH_2-Bco-CO_2Et	**6h**	72	-2.2
4	NH_2-Cy-CO_2Et	**6b**	66	-1.5
5	NH_2-Bco-CO_2^-	**6l**	67	-1.6
6	NH_2-Cy-CO_2^-	**6f**	54	-0.3
7	NH_2-Bco-CH_2OH	**6k**	67	-1.6
8	NH_2-Cy-CH_2OH	**6e**	57	-0.7

[a] Indicates ±1.8% of folding error. [b] Indicates ±0.2 kJ/mol absolute error for folding free energy. [c] All the NMR data in this table were determined from line shape analysis of the ^{1}H NMR spectra acquired on a 700 MHz NMR. [d] 1 mM concentration.

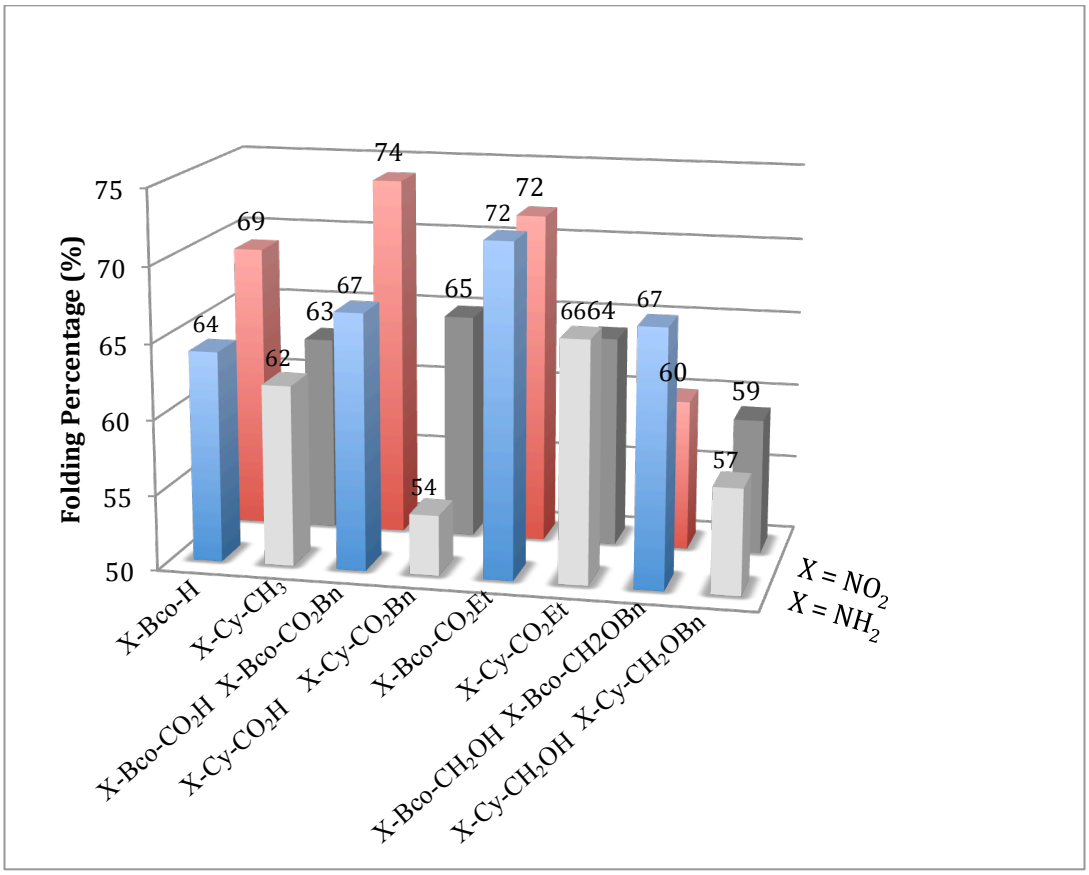


Figure 3.5 Column figure of folding percentage of torsion balances 7a-d, g-j and 6a-b, e-h, k-l in $CDCl_3$ at 5˚C.

3.3.2 TEMPERATURE EFFECTS

In order to test whether temperature would have an effect on the folding percentages of torsion balances, folding percentages of all torsion balances were examined from 5 ˚C to 35 ˚C. This is a limited range due to the freezing point of the aqueous solution and the broadening of signals as temperature rises. As the temperature increases from 5 ˚C to 35 ˚C, the methyl signals broaden due to the faster rotation of the biaryl bond at higher temperature. In this range of temperatures

folded and unfolded methyl ester resonance frequencies changed very little and the differences of folding percentages were within our bounds of error (**Table 3.3**, **3.4**, **3.5**, **Figure 3.6**).

Table 3.3 Folding percentage (Folding%) and folding energy (ΔG^o) of torsion balances 7 in $CDCl_3$ at 5 ˚C, 15 ˚C, 25 ˚C and 35 ˚C. [a, b]

Name	TB	5 ˚C		15 ˚C		25 ˚C		35 ˚C	
		Folding% [c]	ΔG^o [d]	Folding% [c]	ΔG^o [d]	Folding% [c]	ΔG^o [d]	Folding% [c]	ΔG^o [d]
NO_2-Bco-H	**7g**	69	-1.9	69	-1.9	68	-1.9	67	-1.8
NO_2-Cy-Me	**7a**	63	-1.2	62	-1.2	63	-1.3	62	-1.3
NO_2-Bco-CO_2Et	**7h**	74	-2.5	74	-2.6	74	-2.6	73	-2.6
NO_2-Cy-CO_2Et	**7b**	64	-1.4	62	-1.2	65	-1.6	65	-1.6
NO_2-Bco-CO_2Bn	**7j**	74	-2.4	75	-2.6	75	-2.6	74	-2.7
NO_2-Cy-CO_2Bn	**7d**	65	-1.4	64	-1.4	64	-1.5	63	-1.4
NO_2-Bco-CH_2OBn	**7i**	60	-0.9	59	-0.9	57	-0.7	58	-0.8
NO_2-Cy-CH_2OBn	**7c**	59	-0.8	60	-1.0	62	-1.2	67	-1.8

[a] All the NMR data in this table were determined from line shape analysis of the ^{1}H NMR spectra acquired on a 700 MHz NMR. [b] All the torsion balances were examined at 1 mM concentration. [c] Indicates ±1.8% in absolute error in folding percentage. [d] kJ/mol, and indicates ±0.2 kJ/mol absolute error in folding free energy.

Table 3.4 Folding percentage (Folding%) and folding energy (ΔG^o) of torsion balances 6 in $CDCl_3$ at 5 ˚C, 15 ˚C, 25 ˚C and 35 ˚C.

Name	TB	5 ˚C		15 ˚C		25 ˚C		35 ˚C	
		Folding% [c]	ΔG^o [d]	Folding% [c]	ΔG^o [d]	Folding% [c]	ΔG^o [d]	Folding% [c]	ΔG^o [d]
NH_2-Bco-H	**6g**	64	-1.3	65	-1.5	66	-1.6	65	-1.6
NH_2-Cy-Me	**6a**	62	-1.1	61	-1.1	61	-1.1	60	-1.0
NH_2-Bco-CO_2Et	**6h**	72	-2.2	74	-2.5	73	-2.4	73	-2.5
NH_2-Cy-CO_2Et	**6b**	66	-1.5	64	-1.4	63	-1.3	63	-1.4
NH_2-Bco-CO_2H	**6l**	67	-1.6	66	-1.6	69	-2.0	69	-2.0
NH_2-Cy-CO_2H	**6f**	54	-0.3	56	-0.6	59	-0.9	59	-0.9
NH_2-Bco-CH_2OH	**6k**	67	-1.6	67	-1.7	68	-1.8	69	-2.0
NH_2-Cy-CH_2OH	**6e**	57	-0.7	56	-0.6	60	-1.0	60	-1.0

[a] All the NMR data in this table were determined from line shape analysis of the ^{1}H NMR spectra acquired on a 700 MHz NMR. [b] All the torsion balances were examined at 1 mM concentration. [c] Indicates ±1.8% in absolute error in folding percentage. [d] kJ/mol, and indicates ±0.2 kJ/mol absolute error in folding free energy.

Table 3.5 Folding percentage (Folding%) and folding energy (ΔG^o) of torsion balances 5 in MeOD at 5 ˚C, 15 ˚C, 25 ˚C and 35 ˚C.

Name	TB	5 ˚C		15 ˚C		25 ˚C		35 ˚C	
		Folding% [c]	ΔG^o [d]	Folding% [c]	ΔG^o [d]	Folding% [c]	ΔG^o [d]	Folding% [c]	ΔG^o [d]
CO_2H-Bco-H	**5g**	73	-2.3	75	-2.6	74	-2.6	75	-2.8
CO_2H -Cy-Me	**5a**	68	-1.7	68	-1.9	69	-2.0	69	-2.0
CO_2H -Bco-CO_2Et	**5h**	79	-3.0	80	-3.4	82	-3.8	82	-3.9
CO_2H -Cy-CO_2Et	**5b**	69	-1.9	71	-2.1	73	-2.4	74	-2.7
CO_2H -Bco-CO_2H	**5l**	80	-3.3	82	-3.6	82	-3.8	90	-5.7
CO_2H -Cy-CO_2H	**5f**	73	-2.3	74	-2.5	75	-2.8	79	-3.3
CO_2H -Bco-CH_2OH	**5k**	73	-2.3	73	-2.4	73	-2.5	70	-2.2
CO_2H Cy-CH_2OH	**5e**	64	-1.4	66	-1.6	66	-1.6	74	-2.5

[a] All the NMR data in this table were determined from line shape analysis of the ^1H NMR spectra acquired on a 700 MHz NMR. [b] All the torsion balances were examined at 1 mM concentration. [c] Indicates ±1.8% in absolute error in folding percentage. [d] kJ/mol, and indicates ±0.2~0.5 kJ/mol absolute error in folding free energy.

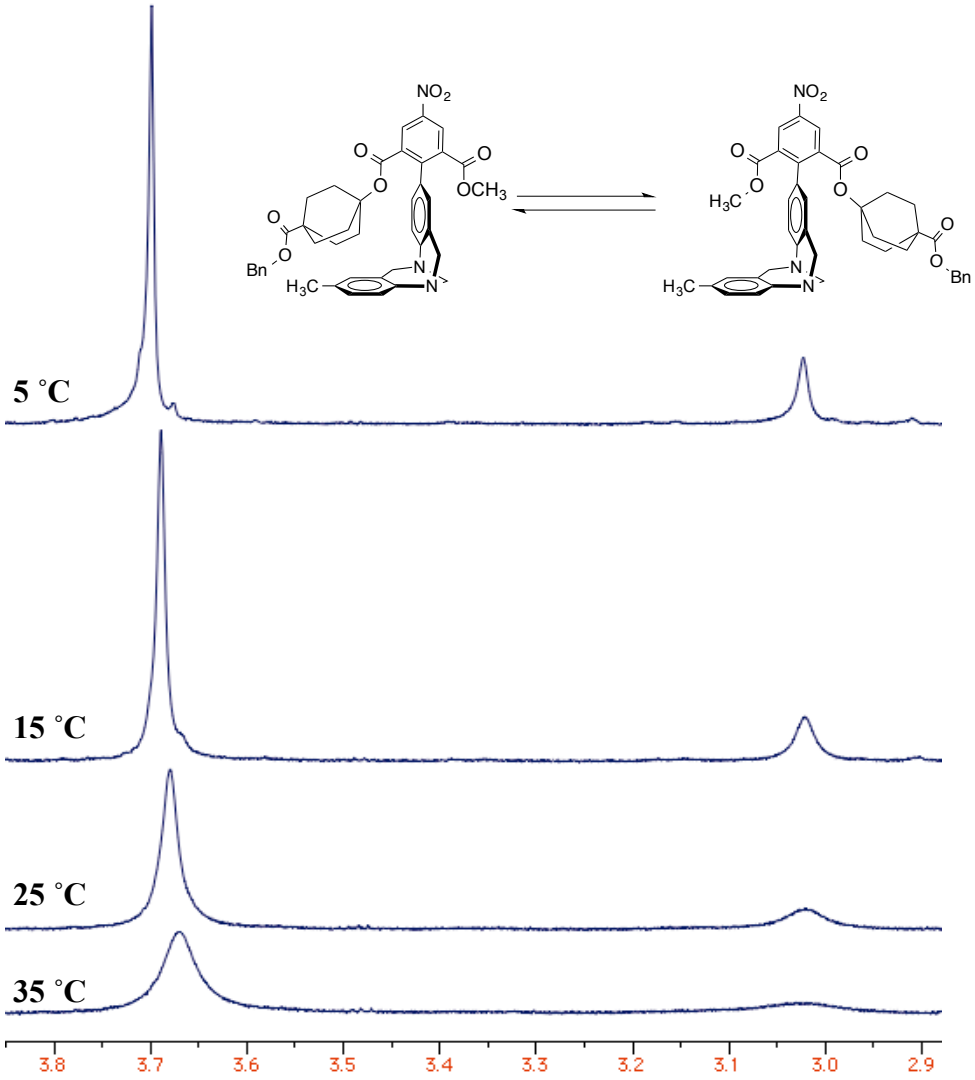


Figure 3.6 Methyl ester signals of torsion balance 7j at 1 mM in $CDCl_3$ from 5 ˚C to 35 ˚C.

3.3.3 SOLVENT EFFECTS

The hydrophilic torsion balances **5a**, **5b**, **5e-5h, 5k,** and **5l** were examined in two solvents (D_2O and MeOD) at 5 ˚C by 700 MHz NMR spectroscopy (**Table 3.6, Figure 3.7**). The folding energy in D_2O is significantly higher than it is in MeOD. This confirms again that a molecular torsion balance is sensitive to nanoscale hydrophobic effects. The bicyclo[2.2.2]octyl series exhibited

higher folding energies than the cyclohexyl series in both D_2O and MeOD. As explained earlier, this is because the bicyclo[2.2.2]octyl core has a larger contact area than the *trans*-cyclohexyl core with the relevant aromatic ring, and the bicyclo[2.2.2]octyl core has a lower rotational entropy loss upon folding. In MeOD, the torsion balances bearing the ester or carboxylic group (**Table 3.6**, entry 2, 4, 5, 6) have higher folding percentages than the balances with simple hydroxymethyl or alkyl groups (**Table 3.6**, entry 1, 3, 7, 8). This might be due to the branching nature of the carbonyl group allowing enhanced London dispersion interactions. In water, however, torsion balance **5g** (**Table 3.6**, entry 1) exhibited folding percentages similar to **5h** and **5l** (**Table 3.6**, entry 2 and 5). The ester and carboxylic groups have hydrogen-bonded interactions with water.

We note that if the exposed surface area of ester and carboxylic group in water is smaller in the folded state than it is in unfolded state, the effect would be a positive contribution to the folding free energy, reducing the magnitude of that energy, and moving the free energies of **5h** and **5l** closer to **5g**. Torsion balances with a hydroxyl group **5e** and **5k** (**Table 3.6**, entry 7 and 8) are expected to form strong hydrogen bonds with water. Inhibition of hydroxyl group solvation in the folded state would decrease the percentage of conformers having the hydroxyl on the concave side (folded). Indeed the data reveal diminished folding of these alcohols **5e** and **5k** (**Table 3.6**, entry 7 and 8) in water compared to their alkyl analogs **5a** and **5g** (**Table 3.6**, entry 1 and 3).

Table 3.6 Folding energy of torsion balances 5a, b, e-h, k, l in MeOD and D_2O at 5 °C.[d, e]

Entry	R	TB	ΔG_{fold} (MeOD) kJ/mol [b]	ΔG_{fold} (D_2O) kJ/mol [c]
1	-Bco-H	**5g**	-2.3	-4.2 [a]
2	-Bco-CO_2^-	**5l**	-3.3	-4.3 [a]
3	-Cy-CH_3	**5a**	-1.7	-3.4 [a], -3.1
4	-Cy-CO_2^-	**5f**	-2.3	-3.8 [a], -3.8
5	-Bco-CO_2Et	**5h**	-3.0	-4.4
6	-Cy-CO_2Et	**5b**	-1.9	-4.1
7	-Bco-CH_2OH	**5k**	-2.3	-3.6
8	-Cy-CH_2OH	**5e**	-1.4	-2.9

[a] Solution was basified with NH_4OH. [b] Indicates ±0.2~0.3 kJ/mol absolute error in folding free energy. [c] Indicates ±0.3 kJ/mol absolute error in folding free energy. [d] All the NMR data in this table were determined from line shape analysis of the ^1H NMR spectra acquired on a 700 MHz NMR. [e] All the torsion balances were examined at 1 mM concentration.

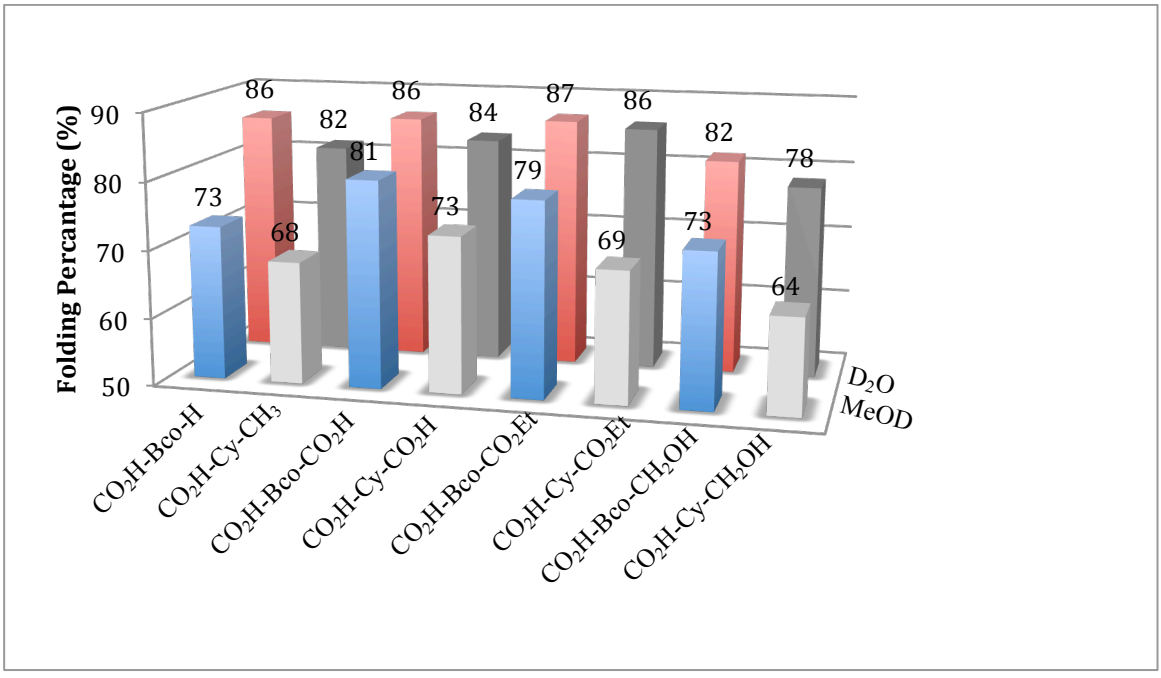


Figure 3.7 Column Figure of folding percentage of torsion balances 5a-b, e-h, k-l in MeOD and D_2O at 5 °C.

3.3.4 CONCENTRATION EFFECTS

As we discussed in **Section 3.3.1**, there are two exceptions. Although the folding percentage of most torsion balances remained similar after reduction on the nitro group, the folding percentage of torsion balances **6f** and **6l** is smaller than their nitro torsion balance analogs **7d** and **7j**. The folding percentage of torsion balance **6f** was only 54%, which is the smallest among the torsion balances with amino group. We think this may be due to intramolecular (**Figure 3.8**) or intermolecular (**Figure 3.9**) interactions between the amino group and the carboxylic acid.

The pKa values of $PhNH_3^+$ and $MeCO_2H$ are 3.8 and 12.6 respectively in DMSO.[42] In our molecule **6f**, the analogous aniline substructure has two electron withdrawing groups, and the pKa of our anilinium ion will be lower than 3.8. We do not know the protonation state of these functional groups. It is unlikely that an intramolecular contact ion pair or intramolecular hydrogen bonded pair (**Figure 3.8**) could form because such a contact would require a twist boat-form conformation for the cyclohexyl group and an s-*cis* conformation of the ester. The total energy cost would be around 7 kcal/mol at room temperature. The high pKa of carboxylic acid in organic solvent also favors the hydrogen bond pair model instead of the ion pair model.

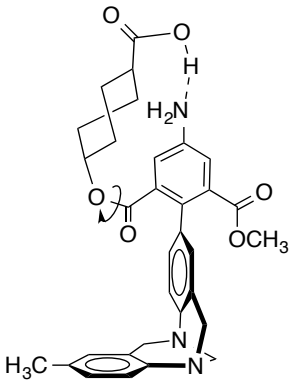


Figure 3.8 Strained conformation required for ionic interactions in torsion balance 6f.

A dimer is therefore more likely (**Figure 3.9)**. The dimer can be formed with a chair form cyclohexane and an s-*trans* ester. The unfolded state is more favorable for dimer formation and, therefore, dimer formation would reduce the folding percentage. Of course dilution should make the dimer less likely, and increase folding.

In order to test the hypothesis that dimer formation decreases folding, torsion balances **6l** and **6f** were examined at different concentrations of $CDCl_3$ (**Table 3.7**). As the concentration decreased, the folding free energy of both torsion balances increased and percentage of the folded state increased as the solution was diluted. This is consistent with our hypothesis that dimer formation is the cause of the exceptional folding ratio in **6l** and **6f**. Concentration would have no effect on folding if folded and unfolded states were unimolecular.

For the torsion balance **6l**, its folding percentage drops from 74% to 67% after hydrogenation. We think this is also due to the intermolecular hydrogen bond formation, since the bulky size of bicyclo[2.2.2]octyl disables the intramolecular approach of the carboxylate to the amine group. Because of the size of bicyclo[2.2.2]octyl, dimer formation is more difficult. This may also explain why the drop off on folding percentage of **6l** caused by dilution is less than the drop seen for **6f**.

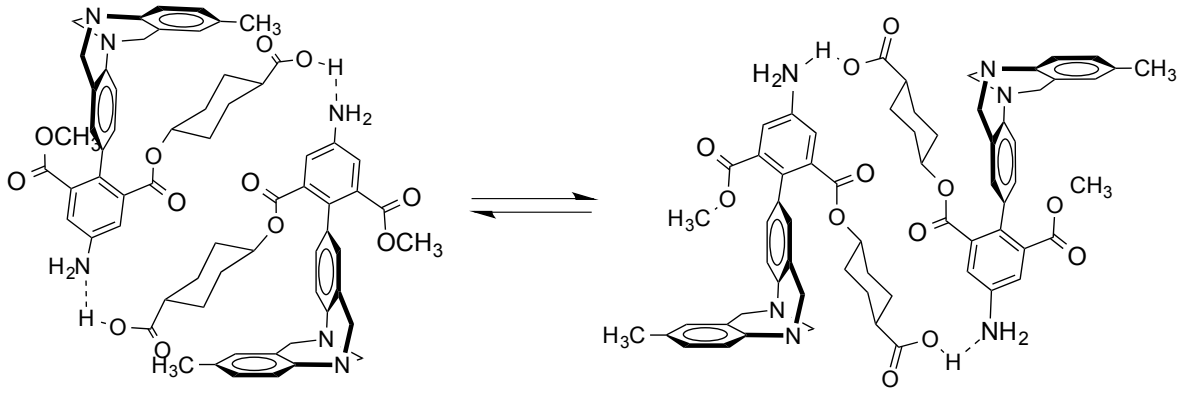


Figure 3.9 Intermolecular protonation of torsion balance 6f.

Table 3.7 Folding percentage of 6l and 6f in different concentrations in $CDCl_3$ at 5 °C. [b]

Entry	R	NH_2-Cy-CO_2H (**6f**) ΔG_{fold} ($CDCl_3$) [a] kJ/mol	NH_2-Bco-CO_2H (**6l**) ΔG_{fold} ($CDCl_3$) [a] kJ/mol
1	1 mM	-0.0	-1.3
2	0.5 mM	-0.2	-1.8
3	0.3 mM	-0.6	-1.9
4	0.1 mM	-1.3	-2.7

[a] Indicates ±0.2 kJ/mol absolute error in folding free energy. [b] All the NMR data in this table were determined from line shape analysis of the ^{1}H NMR spectra acquired on a 700 MHz NMR.

Two torsion balances without the issue of intermolecular protonation were examined in different concentration. In **Table 3.8**, the differences of folding energy for torsion balances **5f** and **5l** are within analytical error in 1mM and 0.1 mM. This indicates that for these more simple balances (that do not contain complementary ions) the concentration has a negligible effect on folding.

Table 3.8 Folding energy for torsion balances 5f and 5l in different pD and concentration at 5 °C. [c]

Entry	pD	Con.	CO_2H-Cy-CO_2H (**5f**) ΔG_{fold} (D_2O) kJ/mol [a]	CO_2H -Bco-CO_2H (**5l**) ΔG_{fold} (D_2O) kJ/mol [b]
1	10	1 mM	-4.1	-4.6
2	10	0.1 mM	-3.9	-4.6
3	6	1 mM	-4.0	-5.0
4	6	0.1 mM	-3.9	-4.6

[a] Indicates ±0.3 kJ/mol absolute error in folding free energy. [b] Indicates ±0.4 kJ/mol absolute error in folding free energy. [c] All the NMR data in this table were determined from line shape analysis of the ^{1}H NMR spectra acquired on a 700 MHz NMR.

3.3.5 pD EFFECTS

In **Section 3.3.3**, we examined the folding percentages of **5a** and **5f** in D_2O with and without NH_4OH. We found out the addition of NH_4OH has negligible effect on the folding percentage. In order to have a more detailed study of folding in basic and acidic environment, we examined the folding percentage of **5f** and **5l** at different pD. The effect of pD on the folding energies of **5f** and **5l** was examined at 5 ˚C (**Table 3.9**). No significant change in folding energies was observed between pD 5.4 and 10.0. As expected, the solubilities of **5f** and **5l** decreased as the pD decreased. This is consistent with our initial observation that NH_4OH had negligible influence on the folding energy these acidic molecules.

Table 3.9 The effect of pD on folding energy of torsion balance 5f and 5l in D_2O at 5 ˚C. [a, b]

Entry	pD	CO_2H-Cy-CO_2H ΔG_{fold} (1 mM) [c] **(5f)** kJ/mol	CO_2H-Bco-CO_2H ΔG_{fold} (1 mM) [d] **(5l)** kJ/mol
1	10	-4.1	-4.6
2	6.9	-3.8	-4.9
3	6.0	-4.0	-5.0
4	5.8	-3.9	-5.1
5	5.4	-4.0	-

[a] The concentrations of **5f** and **5l** were nominally 1 mM in all the experiments, but below pD=6 concentrations were limited by solubility. [b] All the NMR data in this table were determined from line shape analysis of the ^1H NMR spectra acquired at 700 MHz. [c] Indicates ±0.3 kJ/mol absolute error in folding free energy. [d] Indicates ±0.4~0.5 kJ/mol absolute error in folding free energy.

3.3.6 CONCLUSION

The results demonstrate a significant increase in folding for these molecules on changing the solvent from MeOD to D_2O. This is a measure of the greater ability of water (compared to methanol) to enforce solvophobic association. The folding of torsion balance **5l** was not significantly affected by changes in pD in the range of 10 to 5.4. Furthermore, the measured folding energies of **5g** (lacking a polar group) and **5l** (containing a nearby polar carboxylate group) in D_2O were within experimental error. Nearby hydroxyl groups also had insignificant effects on folding. We conclude that in this context a polar group nearby a hydrophobic surface has little or no effect on the magnitude of hydrophobic association.

4.0 EVALUATION OF BRANCHING EFFECT ON FOLDING

In **Section 3.3.1**, it was revealed that the torsion balances bearing a carboxylic ester or carboxylic acid group in the distal position relevant to the ester attachment site have a higher folding energy than torsion balances with simpler groups in that position. We considered that this could be due to the branching nature of the ester/acid functional groups. The ester and acid, due to branching, would allow increased interaction with the methylated aromatic ring spatially close to the ester alkyl group.

In order to test this idea, a torsion balance bearing an isopropenyl group was designed and synthesized. The isopropenyl group is isosteric with a carboxylic acid, and similar to an ester in this context, but is less polar compared to an ester or a carboxylic acid. With this in mind, we reasoned that the effect of branching on folding energy might be evaluated without the confounding effect due to the unknown influence of polarity effects. (**Figure 4.1**)

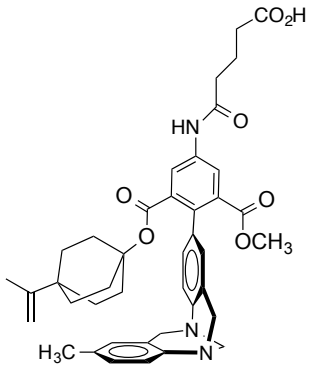


Figure 4.1 Torsion balance with isopropenyl group.

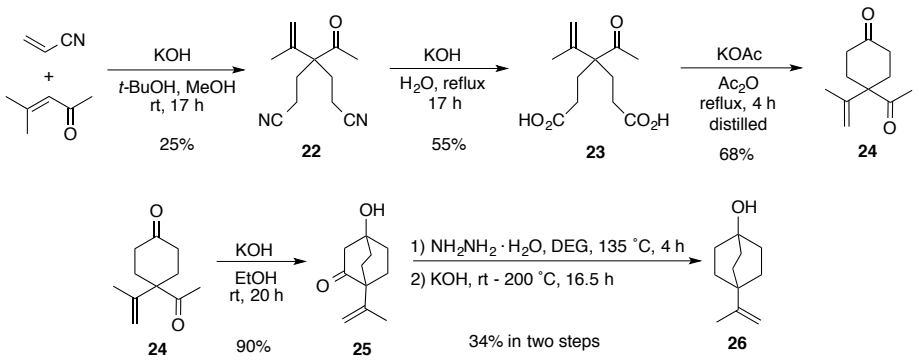
4.1 SYNTHESIS OF THE TORSION BALANCE BEARING A BRANCHED NONPOLAR NEIGHBORING GROUP

Our initial attempt to synthesize 1,4-isopropenylbicyclo[2.2.2]octanol **26** through the use of excess Tebbe reagent on a methyl ketone analog of **26** failed. Our second approach to the synthesis of **26** began from two commercially available compounds (**Scheme 4.1**). The reaction of acrylonitrile and mesityl oxide under basic conditions generated dinitrile **22** through double Michael addition. The hydrolysis of both nitriles furnished dicarboxylic acid **23**. Dieckmann condensation of diacid **23** afforded diketone **24**. The bicyclo[2.2.2]octyl structure was finally formed by the intramolecular aldol reaction of diketone **24** to afford keto-alcohol **25** in 90% yield.

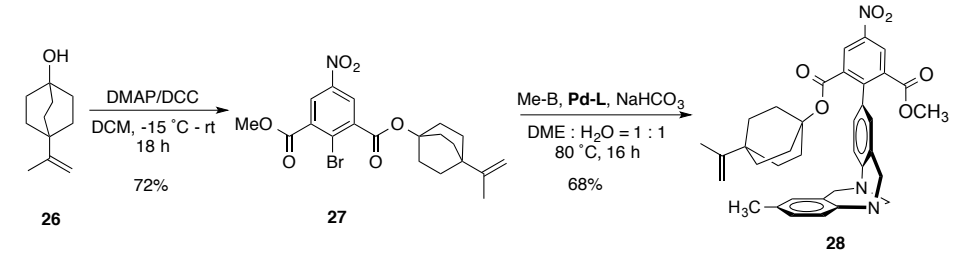
Preliminary attempts to form the thioketal were unsuccessful, so we explored an alternative method for removal of the carbonyl group. Wolff-Kishner reduction with hydrazine afforded the 1,4-isopropenylbicyclo[2.2.2]octyl alcohol **26** in 34% yield over two steps.

The synthesis of torsion balance **28** followed the standard synthetic route of torsion balance preparation already described in this dissertation (**Scheme 4.2**). DCC esterification and Suzuki coupling proceeded with moderate to good yields. Torsion balance **28** was converted to hydrophilic torsion balance **30** in two steps (**Scheme 4.3**). Previously, reduction on the nitro group had been accomplished with hydrogen and palladium on carbon. A different reagent was required in this case, because torsion balance **28** bears a terminal vinyl group, which would be reduced during hydrogenation. Tin chloride reduced the nitro group to amine **29** in good yield.[43] Torsion balance **29** was treated with glutaric anhydride in anhydrous dichloromethane to furnish hydrophilic torsion balance **30**.

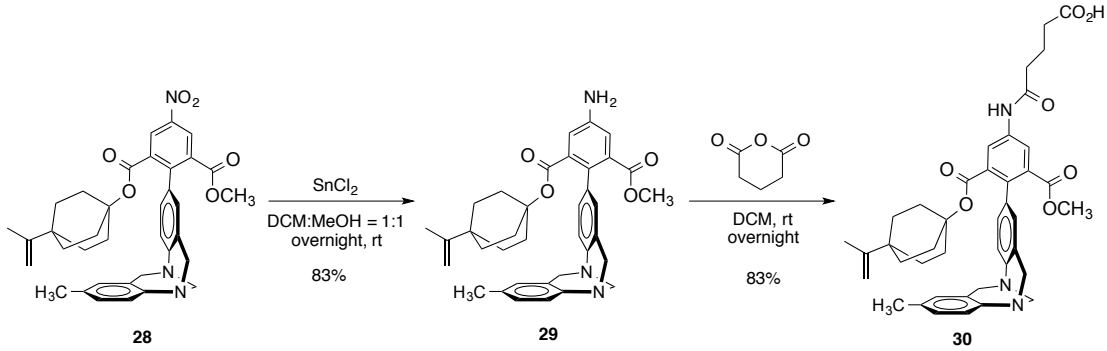
Scheme 4.1 The synthesis of 1,4-isopropenylbicyclo[2.2.2]octanol.



Scheme 4.2 The synthesis of torion balance 28.



Scheme 4.3 The synthesis of hydrophilic torsion balance 30.



4.2 NMR ANALYSIS AND FOLDING ENERGY CALCULATION

Table 4.1 Folding free energy and folding percentage of torsion balances with branch functional groups. [c, d]

Entry	Name	TB	ΔG_{fold} ($CDCl_3$) [f] kJ/mol	Folding% [e]
1	NO_2-Bco-C(Me)=CH_2	**28**	-2.1	71
2	NH_2-Bco-C(Me)=CH_2	**29**	-1.8	68
3 [b]	CO_2H-Bco-C(Me)=CH_2	**30**	-3.0 [a]	79 [a]
4	NO_2-Bco-CO_2Et	**7h**	-2.2	72
5	NH_2-Bco-CO_2Et	**6h**	-2.2	72
6	CO_2H-Bco-CO_2Et	**5h**	-3.0 [a]	79 [a]
7	NO_2-Bco-CO_2Bn	**7j**	-2.4	74
8	NH_2-Bco-CO_2H	**6l**	-1.6	67
9	CO_2H-Bco-CO_2H	**5l**	-3.3 [a]	81 [a]

[a] Examined in MeOD. [b] Insoluble in water, treatment with NH_4OH caused decomposition, which increased analysis error in folding ratio and energy. [c] All the NMR data in this table were determined from line shape analysis of the ^1H NMR spectra acquired on a 700 MHz NMR. [d] All the torsion balances were examined at 1 mM concentration. [e] Indicates ±1.8% in absolute error in folding percentage. [f] Indicates ±0.2 kJ/mol absolute error in folding free energy..

Torsion balances **28** to **30** were examined using 700 MHz ^1H-NMR in 1mM concentration at 5 ˚C. Three series of torsion balances (Entry 1-3, 4-6, 7-9) with branched functional groups were compared. In the listing show in **Table 4.1**, the polarity of these branched neighboring groups increases from top to bottom. However, the change in polarity has little effect on the differences of folding free energy and folding percentage. Both parameters are within our analysis error provided the solvent is the same (entry 1, 4 and 7; entry 2, 5 and 8; entry 3, 6 and 9). The solvent effect is expected. Because CD_3OD is more adhesive than $CDCl_3$

due to its hydrogen bonding character, the torsion balance will be more compact in CD_3OD. Attempts to examine torsion balance **30** in D_2O were foiled by low solubility (much less than 1 mM) and only very weak signals could be detected even after hours of examination time. In an attempt to increase the solubility, 1 μL of NH_4OH was added to the sample, but no useful NMR could be obtained.

4.3 CONCLUSION

The folding free energy of torsion balance **30** (entry 3) is similar to the ones of torsion balances **5h** and **5l** in CD_3OD (entry 6 and 9). Based on this observation, we conclude that the folding percentage of the torsion balance with esters in the distal position is mainly affected by the branching nature of the neighboring group. The closer proximity of the branched group results in greater London dispersion attraction in the folded state. In this instance, the polarity of the functional group has negligible effect on folding.

5.0 EVALUATION OF THE DISTANCE ON NEIGHBORING GROUP EFFECT

LCW (Lum-Chandler-Weeks) theory attempts to analyze the solvation of small and large hydrophobic specimens in water and the resulting cohesive effects between these specimens.[4b] It was calculated by Huang that according to LCW theory excess chemical potential increases quickly as the exposed hydrophobic surface increases, when the radius of the hard sphere is less than 10 Å (**Figure 1-1**).[4a] According to LCW theory, the excess free energy of the hydrophobic-water interface depends on the volume of alkyl group. However, we note that the plot in **Figure 1.1** (For convenience, this plot is shown again on the next page) is based on the radius of interacting spheres. Therefore these results cannot establish whether the effect is due to volume changes or changes in the radius of curvature of the hydrated surface. If only the volume is important, it can be predicted that extending the size of an alkyl group participating in hydrophobically driven association will enhance folding in our models, even in cases where the newly added surface is not involved in contact and remains exposed to water in both folded and unfolded states.

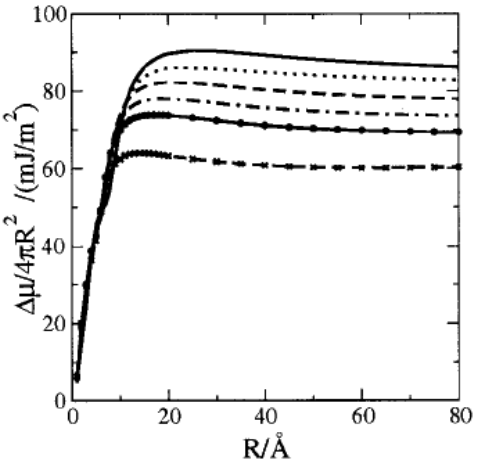


Figure 1.1. The relation between excess chemical potential and the radius of exposed surface for hard spheres, calculated by LCW theory.[4a]

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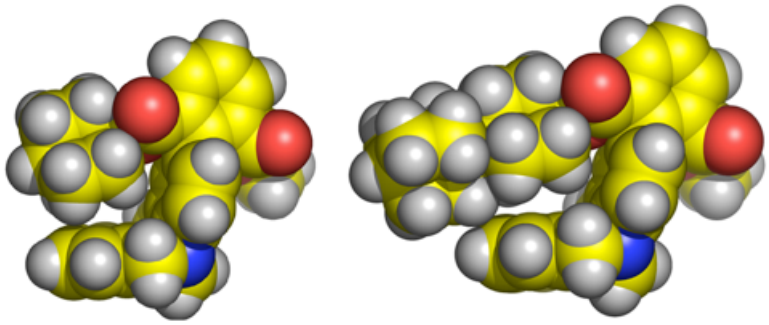
In order to test this prediction, we synthesized torsion balances where folding is favored by hydrophobic contact, and evaluated the effects of alkyl size on folding energy. The molecular torsion balances we designed and synthesized were chosen to have identical contact area. Our designs were guided by the knowledge that branching groups at the distal end of the bicyclooctane esters could still take part in London dispersion interactions and thus could influence folding directly – not through an influence on solvation.

To extend the interacting surface further, in order to be confident that new surface area was not directly attracted to the complementary aromatic surface within our torsion balance, we chose to create bis-bicyclo octane esters (**Figure 5.1).** Our goal was to test the LCW theory inference that volume can affect excess surface free energy, and to do so by comparing the folding energy of these two types of molecular folding balances.



A B

Figure 5.1 Minimized structures of two torsion balances different in size of alkyl group.

Torsion balances that we endeavored to compare are shown in **Figure 5.2**. Within this figure, the torsion balances feature increasing size of alkyl groups from left to right. We sought to measure the difference of folding energies among these torsion balances. It was apparent, from the beginning, that these were very ambitious goals. We were disappointed that we could not achieve all of these goals, but we are happy to report our progress.

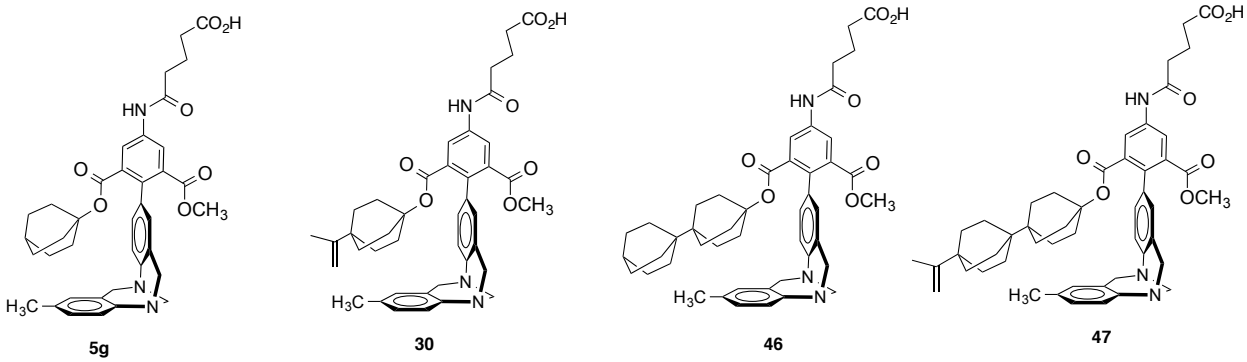
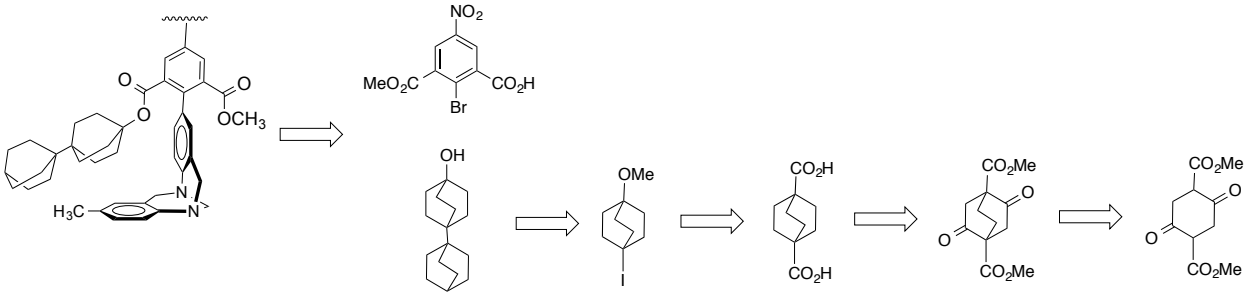


Figure 5.2 Torsion balances with different size of alkyl groups.

5.1 RETROSYNTHETIC ANALYSIS OF BISBICYCLO[2.2.2]OCTYL MOIETY

Because of our successes in many previous torsion balance syntheses, our plan for the synthesis of these new torsion balances focused on the retrosynthesis of the bisbicyclo[2.2.2]octyl moiety needed for ester formation. As described in **Scheme 5.1**, the bisbicyclo[2.2.2]octyl moiety can be created via the coupling reaction of 1-iodo-4-methoxybicyclo[2.2.2]octane. This 1,4-disubstituted bicyclo[2.2.2]octane precursor to the bisbicyclo[2.2.2]octane can be reached from a bicyclo[2.2.2]octane dicarboxylic acid. The symmetrical dicarboxylic acid can be synthesized from the commercially available dimethyl succinylsuccinate.

Scheme 5.1 Retrosynthetic route of torsion balance with bisbicyclo[2.2.2]ocatne moiety.

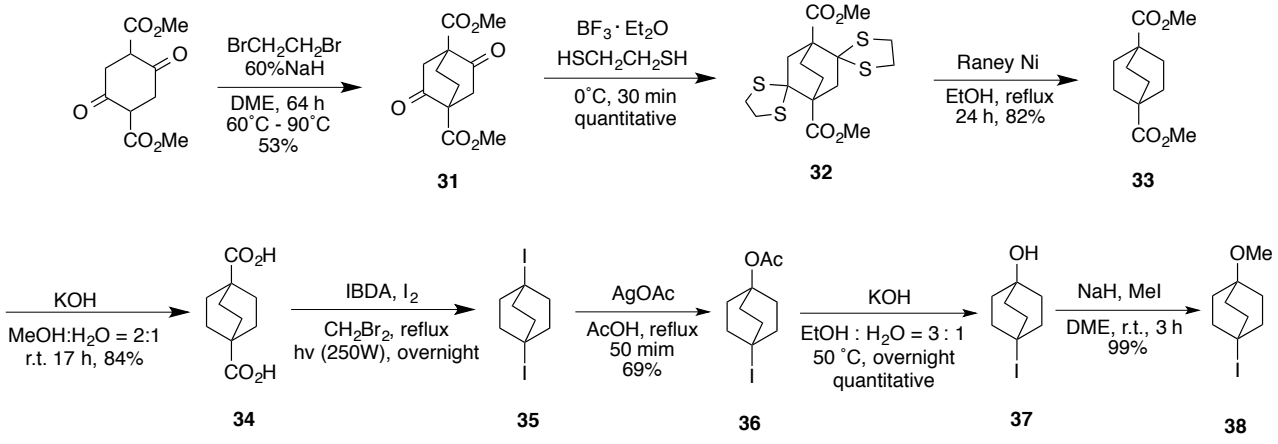


5.2 SYNTHESIS OF THE TORSION BALANCES 46 AND 47

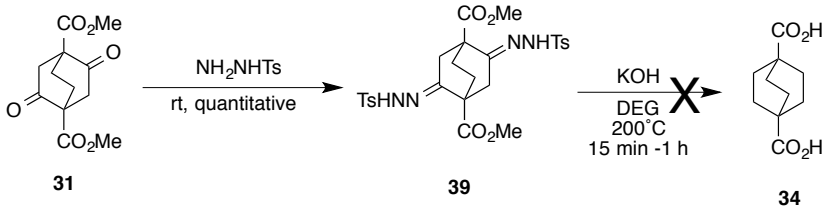
The synthesis of the 1,4-disubstitute bicyclo[2.2.2]octane unit followed **Scheme 5.2**. The bicyclo[2.2.2]octyl moiety of diester **31** was synthesized from commercially available dimethylsuccinylsuccinate through a Michael addition. Removal of the carbonyl groups on

diester **31** was accomplished with dithiol and Raney Ni, affording dimethyl ester **33**. Since the removal of sulfur requires the use of large amounts of pyrophoric Raney Ni, we tried Wolff-Kishner-Huang for deoxygenation at first in order to avoid using dithiol and Raney Ni (**Scheme 5.3**). The generation of tosylhydrazide was quantitative. However, the reduction on the imine group was not successful with base at high temperature. Therefore, Raney nickel method was used in this reaction (82% overall). Diester **33** was hydrolyzed with potassium hydroxide to furnish diacid **34** in good yield. Diiodo bicyclo[2.2.2]octane **35** was converted from diacid **34** through decarboxylation with IBDA and iodine. This reaction proceeded in 60~75% yield. Selective deiodination of diiodo compound **35** with silver acetate afforded mono-iodo ester **36** in good yield. Further hydroxylation of ester **36** at 50 ˚C gave alcohol **37** quantitatively. A first attempt at hydroxylation at room temperature was not successful. This may be due to the space steric hindrance of ester **36**. Alcohol **37** was converted to methyl ether **38** through methylation with methyl iodide. The purification of methyl ether **38** needs to be done very carefully due to its ease of sublimation.

Scheme 5.2 Synthesis of 1,4-disubstituted bicyclo[2.2.2]octane.



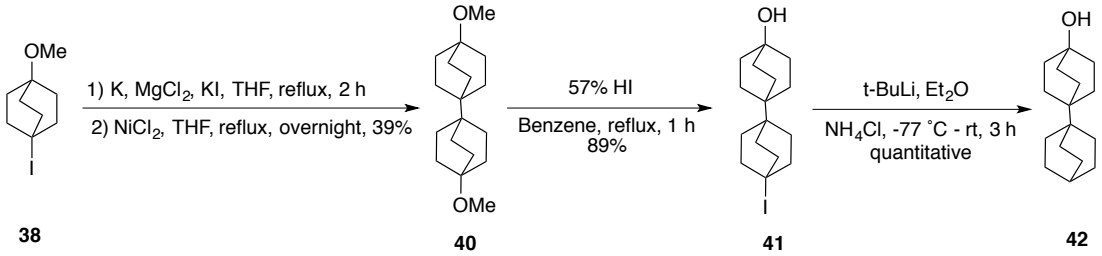
Scheme 5.3 Wolff-Kishner-Huang reaction for deoxygenation of diketone 31.



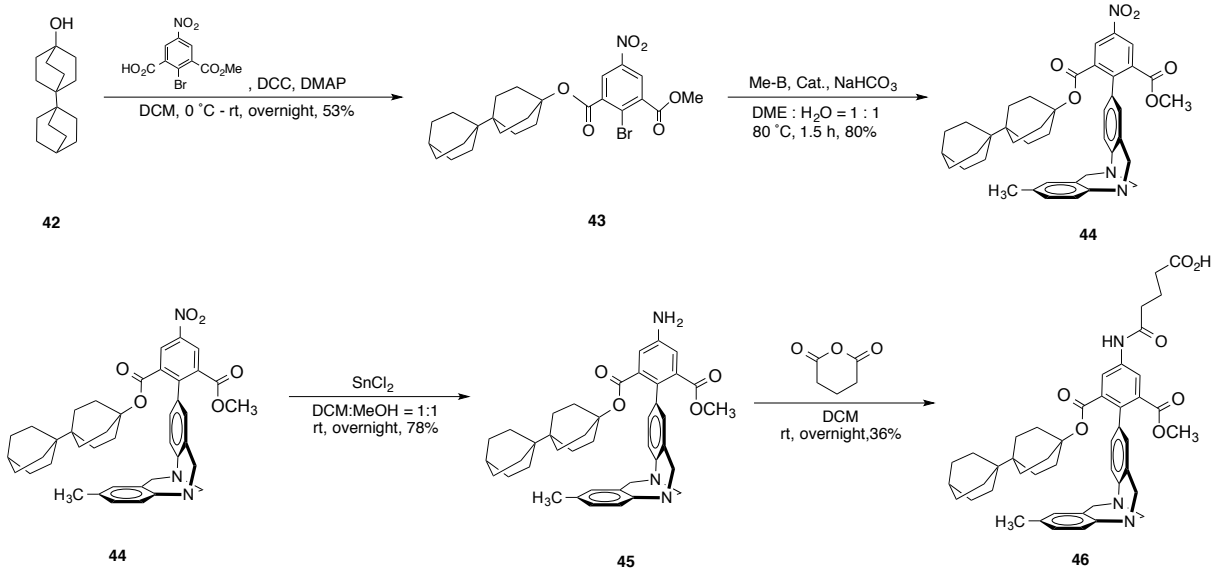
The homocoupling of methyl ether **38** afforded dimethoxy bisbicyclo[2.2.2]octane **40** in moderate yield (**Scheme 5.4**). The generation of the Grignard reagent at the first step was not successful with magnesium and iodine, therefore we turned to the very active Rieke magnesium.[44] The preparation of Rieke magnesium was based on the reaction of potassium and magnesium chloride. Methyl ether **39** was treated with freshly prepared Rieke magnesium to generate the Grignard reagent *in situ*. The following Kumada coupling of Grignard reagents proceeded in 39% yield. The Kumada coupling product dimethoxy bisbicyclo[2.2.2]octane **40** is very symmetrical and has low polarity. The purification with flash column chromatography needs to be done very carefully to avoid loss of the product through sublimation.

The demethylation and monoiodination of **40** to provide the unsymmetrical iodo alcohol **41** proceeded in a one-pot reaction with HI in benzene (89% yield). The large rate difference in the first and second iodination reaction (the diiodide must be forming very slowly) might be due to the biphasic reaction conditions. Bisbicyclo[2.2.2]octyl iodo alcohol **41** was reduced to alcohol **42** in quantitative yield through iodo-lithium exchange followed by protonation. The resulting bisbicyclo[2.2.2]octyl alcohol **42** was incorporated into the torsion balance by the standard DCC esterification and Suzuki coupling reactions, affording torsion balance **46** (**Scheme 5.5**).

Scheme 5.4 The synthesis of bisbicyclo[2.2.2]octyl alcohol 42.

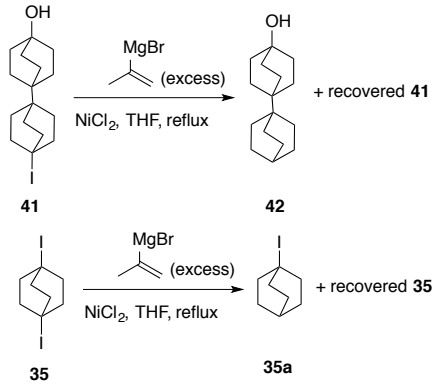


Scheme 5.5 The incorporation of bisbicyclo[2.2.2]octyl alcohol into the torsion balance.



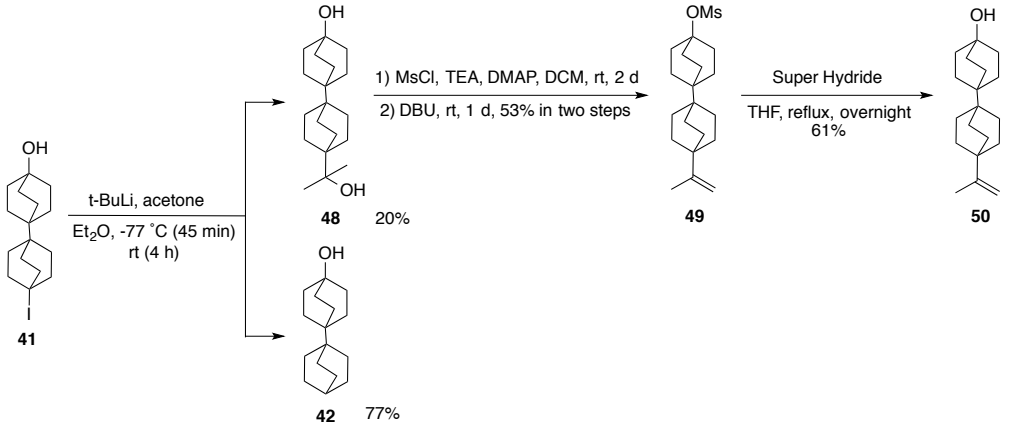
The synthesis of torsion balance **47** is based on the synthesis of the isopropenylbisbicyclo[2.2.2]octane alcohol. Our initial approach was based on the possible Kumada coupling reaction (**Scheme 5.6**) of excess isopropenyl Grignard reagent with the alcohol **41.** However, it generated byproduct alcohol **42** and most of alcohol **41** was recovered. The second attempt was on the reaction of 1,4-diiodo bicyclo[2.2.2]octane **35** and isopropenyl Grignard reagent, which avoided the issue of unprotected hydroxyl group. However, only alcohol **35** and iodide **35a** were detected.

Scheme 5.6 Initial attempt on isopropenylbisbicyclo[2.2.2]octane alcohol synthesis.



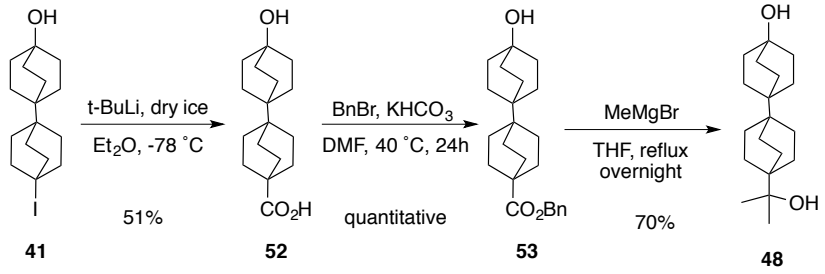
In the third attempt, alcohol **41** was mixed with excess *tert*-butyllithium, and the product of that reaction was then treated with freshly dried acetone (**Scheme 5.7**). We obtained two products. The minor product is the desired diol **48** (20% yield), the major product is the reduction product alcohol **42** (77% yield). The reduction product alcohol **42** may arise from the reaction between the desired organolithium and the enol proton of acetone. It may also arise from rapid iodine-lithium exchange followed by proton abstraction from the alcohol. (Later results show that the lithiation proceeds in at least 50% yield.) To effect dehydration the diol **48** was treated with mesyl chloride to form a dimesylate, which was then treated with DBU, affording elimination product alkene monomesylate **49**. The reductive demesylation of alkene **49** was not successful with Grignard reagent (CH_3MgBr),[45] sodium naphthalenide[46] but Super Hydride $LiBHEt_3$[47] at reflux in THF provided the demesylation product alcohol in 61% yield.

Scheme 5.7 Synthetic route to isopropenylbisbicyclo[2.2.2]octane alcohol 50.



The above route provided only 20% overall yield from alcohol **41** to alcohol **48**, so another route was examined to improve the yield of diol **4**8 (**Scheme 5.8**). The new route featured the formation of the organolithium and carbon dioxide. However, only a moderate yield of desired product **52** was observed. There was still 36% of side product **42** generated. Following this route, after benzyl esterification and Grignard reaction, the overall yield from **alcohol 41** to diol **48** was 35%.

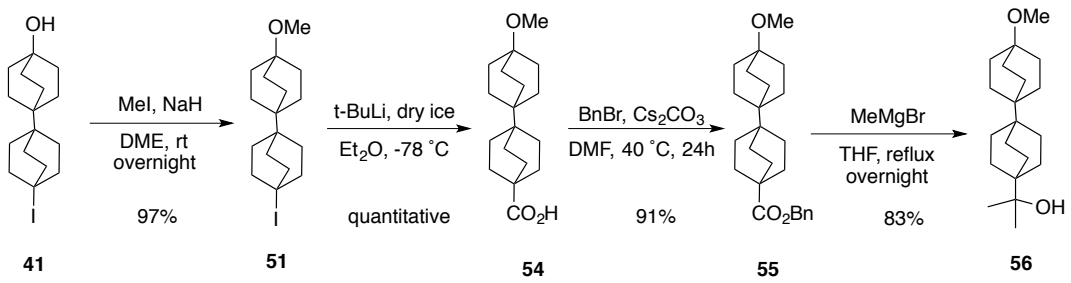
Scheme 5.8 Improved synthetic route of diol 48.



Although the yield was improved, the step of making carboxylic acid **52** was disappointing. Since there was no proton source (other than trace water) brought by carbon dioxide, we considered that the moderate yield was caused by the involvement of the hydroxyl

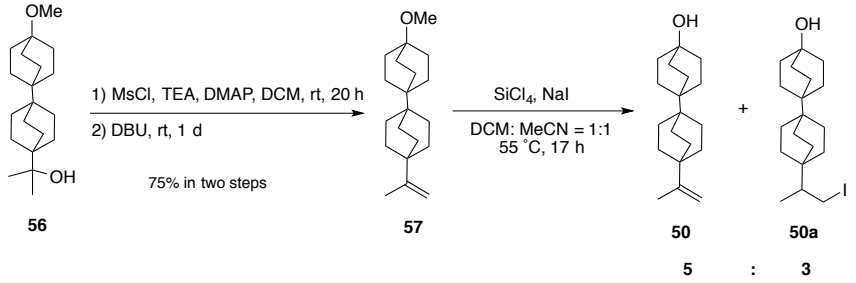
group of alcohol **41**. It is possible that the reaction between hydroxyl group and *tert*-butyllithium is competitive with lithium-iodo exchange. In this case alcohol **42** would be already generated before adding any carbon dioxide. In order to test this assumption, the hydroxyl group of alcohol **41** was protected with a methyl group before subjecting to the product lithium-iodine exchange (**Scheme 5.9**). In the event, methyl protected compound **51** was converted to carboxylic acid **54** in quantitative yield. A conclusion can be draw that lithium-iodine exchange is fast compared with *tert*-butyllithium deprotonation of hydroxyl group. After standard esterification and Grignard reduction, alcohol **41** was converted to alcohol **56** with 72% overall yield.

Scheme 5.9 Synthetic route to alcohol 56 featuring a methyl ether protecting group.



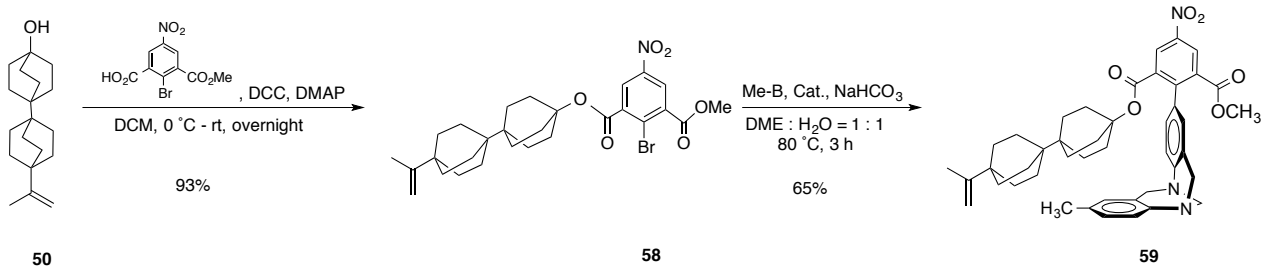
We planned to remove the methyl group after the generation of the vinyl group. The dehydration of **56** to form the vinyl group proceeded as expected to afford methyl ether **57**. The removal of methyl group from ether **57** with silicon tetrachloride and sodium iodide gave completed demethylation, however, it also led to HI addition to the vinyl group according to mass spectrum and ^{1}H NMR results. Therefore, the methyl group would better be removed before the vinyl group formation.

Scheme 5.10 The synthetic route of alcohol 50.



Starting with alcohol **50** synthesized according to **Scheme 5.7**, DCC esterification and Suzuki coupling reaction proceeded to obtain torsion balance **59** (**Scheme 5.11**). Torsion balance **59** was purified by thin layer chromatography. However, it was found to decompose on silica. At this point, we had in hand the NMR results comparing the folding of torsion balance **46** (bisbicyclo ester) with folding of torsion balance **5g** (bicyclo ester) and therefore could answer the question of interest regarding LCW theory. Too many resources were being expended in the attempt to prepare pure torsion balance **59** given the potential benefit to the project. The synthesis of alcohol **50** requires more than ten steps, therefore our campaign to prepare a water soluble version of torsion balance **59** from alcohol **50** was abandoned.

Scheme 5.11 Suzuki coupling for the generation of torsion balance 59.



5.3 NMR ANALYSIS AND FOLDING ENERGY CALCULATION

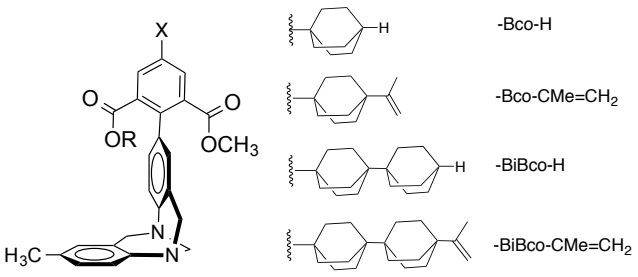


Table 5.1 Folding percentage and free energy of torsion balances with different size of alkyl groups in $CDCl_3$.[a, b]

Entry	R	X	TB	Folding% [d] $CDCl_3$	ΔG_{fold} (kJ/mol) [e] $CDCl_3$
1	-Bco-H	NO_2	**7g**	69	-1.9
2		NH_2	**6g**	64	-1.3
3	-Bco-CMe=CH_2	NO_2	**28**	71	-2.1
4		NH_2	**29**	68	-1.8
5	-Bibco-H	NO_2	**44**	68	-1.7
6		NH_2	**45**	72	-2.2
7	-Bibco-CMe=CH_2	NO_2	**59**	69 [c]	-1.8 [c]
8		NH_2	**-**	-	-

a All the NMR data in this table were determined from line shape analysis of the ^1H NMR spectra acquired on a 700 MHz NMR. [b] All the torsion balances were examined at 1 mM concentration. [c] The NMR of this torsion balance was based on a crude product and examined with a 400 MHz NMR. [d] Indicates ±1.8% in absolute error in folding percentage. [e] Indicates ±0.2 kJ/mol absolute error in folding free energy.

The torsion balances **44**-**46** were examined through 700 MHz NMR spectrometry at 5 ˚C and torsion balance **59** was examined with 400 MHz NMR at room temperature. Torsion balances with a nitro group (**7g**, **28**, **44**, **59**, entry 1, 3, 5 and 7, **Table 5.1**) have very similar folding percentages in $CDCl_3$, though their alkyl groups are increasing in size. After the nitro group of these torsion balances was reduced to an amino group, the folding percentage of torsion balances (**6g**, **29**, **45,** entry 2, 4, 6, **Table 5.1**) was slightly increasing as the size of alkyl group increases.

In **Table 5.2**, the folding energy of torsion balances **30** and **46** (entry 2 and 3) are higher than that of **5g** (entry 1) in MeOD. This can be due to increased σ-π interaction between the alkyl group and the phenyl ring. As indicated in **Figure 5.3**, although most of the second bicyclo[2.2.2]octane moiety is outside the concave pocket, about one third of this alkyl group can contact with the edge of the bottom phenyl ring. This additional interaction in both torsion balances **30** and **46** causes a slightly increase in folding energy.

Table 5.2 Free energy of folding torsion balances with different size of alkyl groups in D_2O.[a,b]

Entry	R	X	TB	MeOD [g] ΔG_{fold} (kJ/mol^{-1})	D_2O [f] ΔG_{fold} (kJ/mol)
1	-Bco-H	$NHCO(CH_2)_3CO_2H$	**5g**	-2.3	-4.2
2	-Bco-CMe=CH_2	$NHCO(CH_2)_3CO_2H$	**30**	-3.0	-3.1[c]
3	-Bibco-H	$NHCO(CH_2)_3CO_2H$	**46**	-2.7	-4.4[d]/-4.1[e]
4	-Bibco-CMe=CH_2	$NHCO(CH_2)_3CO_2H$	**-**	-	-

[a] All the NMR data in this table were determined from line shape analysis of the ^1H NMR spectra acquired on a 700 MHz NMR. [b] All the torsion balances were examined at 1 mM concentration. [c] The solubility of torsion balance **30** was less than 1mM in D_2O. Not enough signal intensity can be analyzed with iNMR, 1 μL of NH_4OH was added to the solution. However, it caused decomposition. [d] The solubility of torsion balance **46** was less than 1mM in D_2O, but enough signal intensity can be analyzed with iNMR. This was measured without NH_4OH. [e] To make comparison, this folding energy was measured with 1 μL of NH_4OH. [f] Indicates ±0.3-0.4 kJ/mol absolute error in folding free energy. [g] Indicates ±0.2-0.3 kJ/mol absolute error in folding free energy.

Results relevant to our key question were revealed when comparison was made between torsion balance **30** and **46**. Torsion balance **30** has a higher folding energy in MeOD than torsion balance **46**. This could be attributed to the π-σ interaction arising from the alkene double bond interacting with the aryl methyl group. This also further confirms that additional size of bisbicyclo[2.2.2]octane compared to isopropenyl group does not contribute to the folding energy.

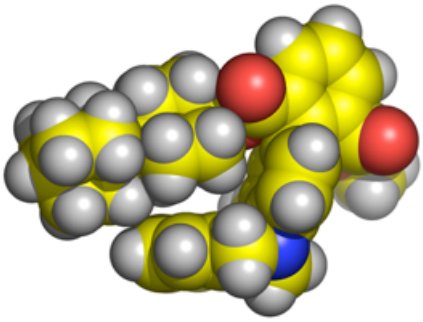


Figure 5.3 Minimized structure of a truncated torsion balance 46.

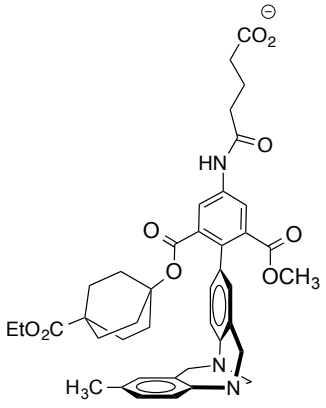
As predicted by LCW theory of hydrophobic effect, the excess free energy should increase rapidly if the radius of the hydrophobic sphere falls on the region of 2-12 Å. As we can see from **Table 5.2**, the folding energy of torsion balances **46** and **5g** is identical within the analytical error. Balance **46** is 0.2 kJ/mol higher than **5g** when it was without ammonium hydroxide, whereas it was 0.1 kJ/mol lower when it was with ammonium hydroxide. Thus the folding energy of torsion balance **5g** is similar to that of **46** in D_2O (entry 1, 3), though they have different length of alkyl groups. Although the difference of folding energy of these two torsion balances can be noticeable in MeOD, it is negligible in D_2O. This could be due to the more adhesive property of water, which caused tighter folding. The significant increase of folding energy in water compared to MeOD, reduced the difference in folding energy arising from the additional interaction. However, if LCW is correct, the folding energy of **46** should be significantly higher than that of **5g**. Based on the above data and analysis, we are unable to confirm that an LCW based prediction is correct.

5.4 CONCLUSION

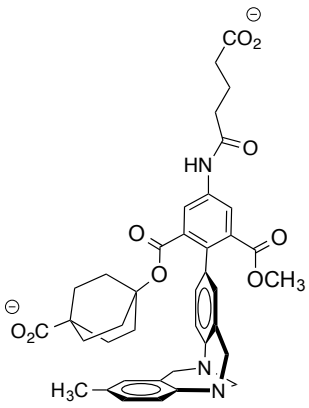
In MeOD, the folding free energy slightly increased as the size of the alkyl group increased. This correlates to the additional interaction between the alkyl group and the bottom phenyl ring. However, the increase in folding energy is not significant when water is used as solvent. This is contrary to the prediction of LCW theory of hydrophobic effect. This research provided a chemical method to test the accuracy of physical theory.

6.0 CHARGE EFFECT ON NEIGHBORING GROUP

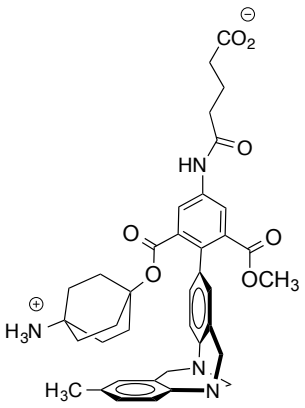
Our final goal in this project was to evaluate the charge effect on folding. The charges in a protein arise from the side chains of some amino acids, and from the N-terminus and C-terminus. It is generally accepted that the internal salt bridge interaction plays a stabilizing role on protein folding. However, most of the charges on proteins are exposed to solvent. Whether these charges on the surface of a protein stabilize protein folding is an open question, because quantification of a single interaction in a complex protein molecule is very difficult. A prior study in our lab indicated that the solvent-exposed salt bridge interaction is indeed a stabilizing factor in folding. However, we also want to know, whether a single charge on the surface will have effect on folding free energy.



5h



5l



69

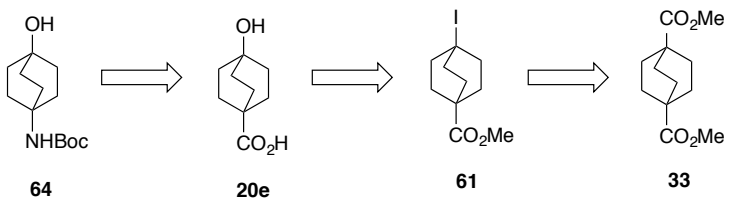
We examined the folding free energy of torsion balance **5l**. This molecule has a negative charge exposed to water due to the neighboring carboxylic acid. As reported in **Chapter 3**, after

evaluation of folding under different pH and temperature conditions, we observed the folding free energy of **51** is similar to that of torsion balance **5h**. Here we describe an effort to evaluate a positive charge effect. The immediate goal was to prepare torsion balance **69** which has a positive charge (generated by a neighboring amine).

6.1 PROGRESS TOWARD TORSION BALANCE 69

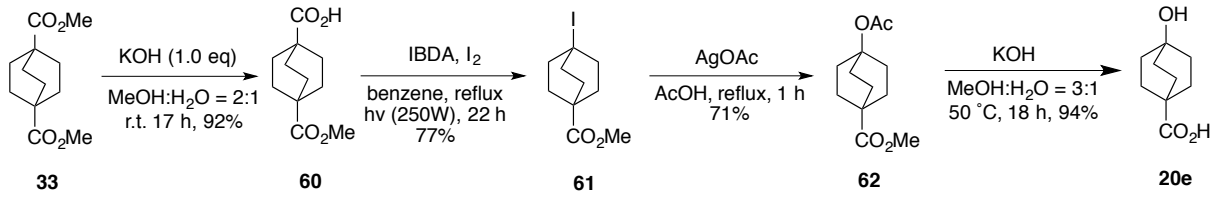
The synthesis of torsion balance **69** requires the synthesis of alcohol **64**. The synthesis of alcohol **64** could be based on the synthetic route illustrated in **Scheme 6.1**. Alcohol **64** can be easily formed by the Curtius rearrangement of the carboxylic acid on **20e**. We planned to synthesize carboxylic acid **20e** from diester **33** in four steps (**Scheme 6.2**). Selective hydrolysis and decarboxylative iodination of **33**, afforded methyl iodoester **61**. The iodide **61** was converted to acetate **62** through the action of silver acetate. The hydrolysis of **62** with potassium hydroxide was first tried at room temperature, but the acetate was not hydrolyzed at an acceptable rate until the temperature was heated to 50 ˚C. This could be attributed to the steric hindrance of the acetate group on **62**.

Scheme 6.1 Retrosynthetic route of alcohol 64.



64 **20e** **61** **33**

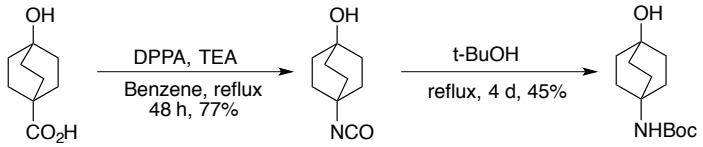
Scheme 6.2 Synthesis of carboxylic acid 20e from diester 33.



33 **60** **61** **62** **20e**

Disubstituded bicyclo[2.2.2]octane **20e** was converted to Boc protected amine **64** by Curtius rearrangement (**Scheme 6.3**). This transformation was first tried as a one-pot reaction, but the yield was less than 10%. The isocyanate **63** was very stable and could be isolated by flash column chromatography. The conversion of isocyanate **63** to Boc protected amine **64** was finished after refluxing the **63** with *t*-BuOH for four days. The unprotected hydroxyl group on **63** should not be a problem, since a good yield of isocyanate was achieved even after refluxing in the basic environment for two days, which indicates self addition (and polymerization) of the isocyanate alcohol is very slow. We sought to shorten the refluxing time in the second step by adding *t*-BuOK, but it made little difference.

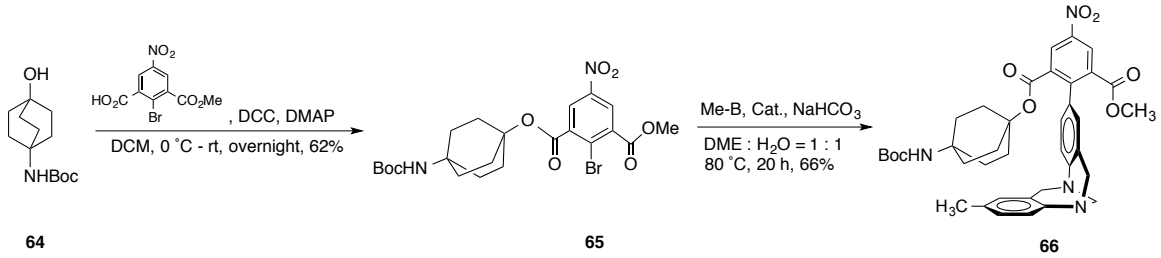
Scheme 6.3 The synthesis of alcohol 64 from carboxylic acid 20e.



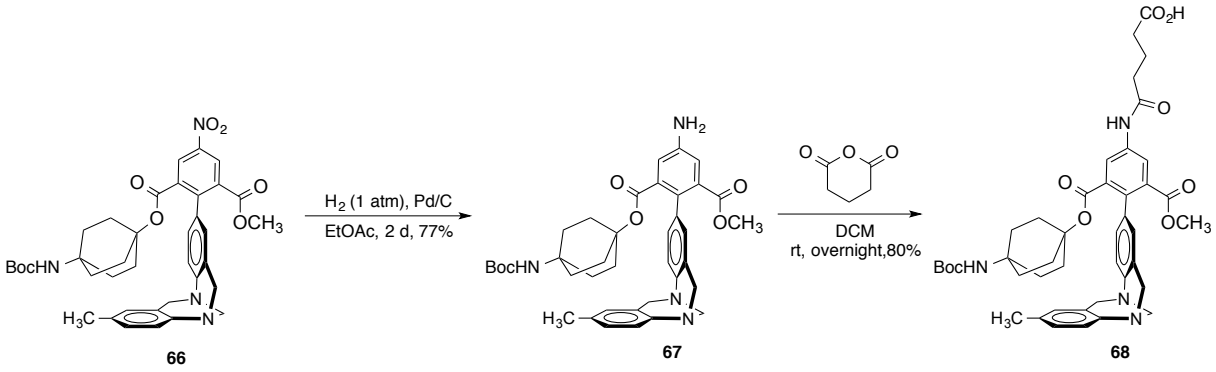
20e **63** **64**

Alcohol **64** was incorporated into torsion balance **66** by the standard DCC esterification with monoacid **8**. The resulting isophthalate undergoes Suzuki coupling to furnish torsion balance **66** (**Scheme 6.4**).

Scheme 6.4 The synthesis of torsion balance 66.



Scheme 6.5 The synthetic route to hydrophilic torsion balance 68.



Torsion balance **66** was converted to the hydrophilic torsion balance **68** by the standard hydrogenation and subsequent reaction with glutaric anhydride (**Scheme 6.5**). The deprotection of Boc is still under investigation. The standard TFA conditions for Boc deprotection caused decomposition of torsion balance **68**. Since only 1-2 mg of torsion balance **68** was left, more **68** will be needed in the future in order to investigate suitable Boc deprotection conditions.

6.2 NMR ANALYSIS

Although deprotected torsion balance **69** has not been synthesized, the NMR results of torsion balances **66**, **67**, **68** can still give us valuable information. Because the Boc protected amine is a large and branched neighboring group, it will be an interesting comparison to the other torsion balances.

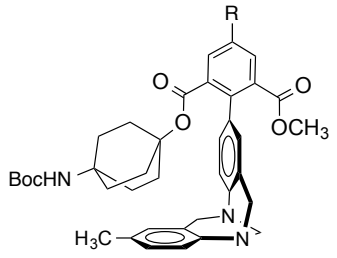


Table 6.1 Folding percentage and folding energy of torsion balance 66, 67, 68.[a, b]

Entry	R	TB	Solvent	Folding% [c]	ΔG_{fold} (kJ/mol) [d]
1	NO_2	**66**	$CDCl_3$	76	-2.7
2	NH_2	**67**	$CDCl_3$	75	-2.5
3	$NHCO(CH_2)_3CO_2H$	**68**	MeOD	81	-3.4
4	$NHCO(CH_2)_3CO_2H$	**68**	D_2O+NH_4OH	92	-5.8

[a] All the NMR data in this table were determined from line shape analysis of the ^1H NMR spectra acquired on a 700 MHz NMR. [b] All the torsion balances were examined at 1 mM concentration. [c] Indicates ±1.8% in absolute error in folding percentage. [d] Indicates ±0.2-0.5 kJ/mol absolute error in folding free energy.

As we can see in **Table 6.1**, torsion balances **66** and **67** have similar folding energy in $CDCl_3$ (entry 1 and 2). In comparison with the nitro and amine analogues of other torsion balances, they have higher folding energy than torsion balances with bisbicyclo[2.2.2]octane or isopropenyl group (torsion balance **28**, **29**, **44**, **45**) and similar folding energy with torsion balances with ethyl ester and benzyl ester groups (torsion balance **7h** and **7j**). We propose this is due to the branching property of the amide group and additional interaction arising from contact

with part of the Boc *t*-butyl group. The glutaric acid analog **68** of this torsion balance was examined in both MeOD and D_2O. The folding energy of **68** is slightly higher than those of torsion balances **30** and **46** in MeOD. However, in water the folding energy of **68** is significantly higher than torsion balances **30** and **46**. Although the length of the neighboring group in torsion balance **68** is similar to that of bisbicyclo[2.2.2]octane in torsion balance **46**, the folding energy of torsion balance **68** is significantly higher. This indicates that the Boc group in torsion balance **68** is the major contribution to this significant increase in folding energy.

6.3 CONCLUSION

The folding energy of torsion balances **66** and **67** is slightly higher than the other torsion balance analogs in organic solvents. This is evidently due to the additional interaction arising from the amide group. In water, however, the folding energy of torsion balance **68** is significantly higher that of torsion balance **46** in water. We propose these facts can be best explained by contact between the Boc group and the aromatic ring.

7.0 CONCLUSIONS AND FUTURE PROSPECTS

Prior investigations to study noncovalent interactions in aqueous media were carried out by Brijesh Bhayana.[13c] It was found that a hydrophilic torsion balance used to study the CH-π interactions had higher (more negative) folding energies in water than in organic solvents. The increase in binding was due to hydrophobic effects, and the CH-π interactions were mainly attributable to the London dispersion force. A molecular torsion balance was also used to investigate the halogen bond effect and salt bridge effect.[16] The folding energies were found to be between -0.2 and -0.3 kcal/mol in $CDCl_3$ and the strength of the halogen bond decreased in magnitude in the following order: Cl ~ Br > I. The folding energies of torsion balances used in the salt bridge study were found to vary between -0.3 to -0.5 kcal/mol. The pD of the buffer solution caused a significant change in folding energies driven by salt bridge interactions.

This project investigated how alkyl group size, functionality, and polarity may affect hydrophobic binding. To complete this investigation, we prepared eleven new torsion balances. The syntheses were convergent, and the synthesis of the most complex torsion balance required 12 steps in the longest continuous path, and 28 steps overall. In this project I developed experimental conditions for 16 new chemical transformations, and characterized 62 new compounds.

Our torsion balances serve as models for two-state protein folding. The models allow a

more accurate and less ambiguous measure of structural effects on side chain interactions. In addition to the synthetic work, this project included detailed physical organic investigations. I used NMR spectroscopy to measure the rates and equilibrium constants associated with the folding and unfolding of these new torsion balances.

To assist future investigators on this project, I have tabulated the characteristic folding information for each of these torsion balances in **Table 7.1**, and the tabulated the rate constants for folding and unfolding in **Table 7.2** to **Table 7.6**.

Table 7.1 A summary of folding free energies in MeOD and D_2O at 5 °C.[a, b, d]

Entry	R	TB	ΔG_{fold} (MeOD) kJ/mol	ΔG_{fold} (D_2O) kJ/mol	$\Delta\Delta G_{solvation}$ [e] kJ/mol
1	-Bco-H	**5g**	-2.3	-4.2 [c]	-1.9
2	-Bco-CO_2^-	**5l**	-3.3	-4.3 [c]	-1.0
3	-Cy-CH_3	**5a**	-1.7	-3.4 [c], -3.1	-1.7
4	-Cy-CO_2^-	**5f**	-2.3	-3.8 [c], -3.8	-1.5
5	-Bco-CO_2Et	**5h**	-3.0	-4.4	-1.4
6	-Cy-CO_2Et	**5b**	-1.9	-4.1	-2.2
7	-Bco-CH_2OH	**5k**	-2.3	-3.6	-1.3
8	-Cy-CH_2OH	**5e**	-1.4	-2.9	-1.5
9	-Bco-CMe=CH_2	**30**	-3.0	-	-
10	-Bibco-H	**46**	-2.7	-4.1 [c], -4.4	-1.4
11	-Bco-NHBoc	**68**	-3.4	-5.8	-2.4

[a] All the NMR data in this table were determined from line shape analysis of the ^1H NMR spectra acquired on a 700 MHz NMR. [b] All the torsion balances were examined at 1 mM concentration. [c] 1 µL of NH_4OH was added. [d] Indicates ±0.2-±0.6 kJ/mol absolute error in folding free energy. [e] Energy of solvation, $\Delta\Delta G_{solvation} = \Delta G_{fold}$ (D_2O) – ΔG_{fold}(MeODD)

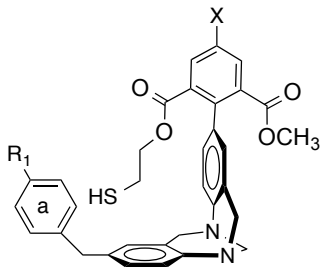
As indicated in **Table 7.1**, the torsion balances bearing a bicyclo[2.2.2]octyl moiety have higher magnitude (more favorable) folding energies than those that have a *trans*-cyclohexyl moiety. Modeling studies indicate that this is due to better contacts of the bicyclo[2.2.2]octyl unit with the adjacent surfaces in the folded state, compared with contacts possible with the *trans*-cyclohexyl unit. We also observed that the polarity of functional groups nearby the contact surfaces has negligible effect on the folding energy. In MeOD, branching functionality caused more favorable folding energies compared to linear functionality. The folding energy in water was significantly higher than in MeOD due to hydrophobic forces. The difference in folding energy between torsion balances with branched and unbranched groups was diminished in D_2O. This diminishment was also observed on contact surface difference (Cy vs Bco). This can be because the London dispersion is larger in water than in MeOD. The London dispersion is larger if the average distance between the surfaces that made contacts in the folded state is shorter in water than in MeOD. The cohesiveness of water is greater than that of methanol. Therefore water will favor more compact folded structures. A more compact structure is expected to have a shorter average distance between the contact surfaces. The added attraction caused by making the structure more compact is greater for poorly bound surfaces (less contact area) than for tightly bound surfaces (more contact area). In other words, a torsion balance with less contact surface has more room for improvement in the London dispersion energy. The result of this line of reasoning is consistent with our observation that the difference in folding energy between any pair of torsion balances that differ in contact surface is smaller in water than in methanol.

The most negative folding energy was found in torsion balance **68**, which bears a Boc protected amine in the distal alkyl position. We think this favorable association might arise due to the relatively free rotation of the Boc group, which provides better contact area between the

surfaces that approach in the folded state. From **Table 7.2** to **Table 7.6**, the rate constants are reported. It is interesting to note that the rates of folding and unfolding are faster in organic solvent compared to water.

In this project, the folding energy of the torsion balance bearing an isopropenyl group was not measured in water due to limited solubility and decomposition in basic solution. Perhaps this should be reinvestigated. Adding one equivalent of base and careful monitoring of pD might prevent decomposition while at the same time giving adequate solubility. A torsion balance with amine group was not synthesized due to decomposition during TFA treatment in the Boc deprotection step. More deprotection conditions should be investigated, including H_3PO_4 or TBAF.[48, 49]

Future goals for this project might include syntheses of torsion balances to study the S-π interactions. S-π interactions are very important in protein folding and stabilization, such as interactions of Cys-Tyr, Cys-Phe and Cys-Trp.[50] There are studies with computer simulation regarding the position between H_2S and benzene (angle θ and distance R), however contrary results were given based on different calculation methods. Using the molecular torsion balance method to investigate S-π interactions will give much-needed experimental data on these interactions. Below are the torsion balances proposed for this future investigation.



R_1 = H or OH
X = NO_2,NH_2 or $NHCO(CH_2)_3CO_2H$

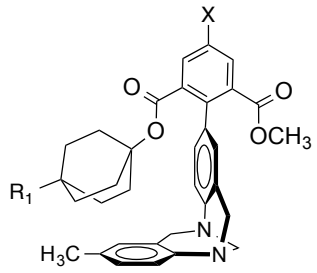


Table 7.2 Rate constants of torsion balances containing a bicyclo[2.2.2]octyl moiety.

Name	Entry	X	R_1	k_u	K_f	Solvent
NO_2-Bco-H	1	NO_2	H	12	27	$CDCl_3$
NO_2- Bco-CO_2Et	2		CO_2Et	8	23.2	
NO_2- Bco-CO_2Bn	3		CO_2Bn	9.5	26.6	
NO_2- Bco-CH_2OBn	4		CH_2OBn	19	28.4	
NH_2-Bco-H	5	NH_2	H	29.5	52.8	$CDCl_3$
NH_2-Bco-CO_2Et	6		CO_2Et	13	33.2	
NH_2-Bco-CO_2H	7		CO_2H	12.5	25.2	
NH_2-Bco-CH_2OH	8		CH_2OH	16	32	
CO_2H-Bco-H	9	NHCO$(CH_2)_3CO_2$H	H	15	39.9	MeOD
CO_2H-Bco-CO_2Et	10		CO_2Et	9	33.2	
CO_2H-Bco-CO_2H	11		CO_2H	6.5	26.8	
CO_2H-Bco-CH_2OH	12		CH_2OH	12.5	33.2	
CO_2H-Bco-H	13	NHCO$(CH_2)_3CO_2$H	H	6	38.1	D_2O
CO_2H-Bco-CO_2Et	14		CO_2Et	2.5	16.5	
CO_2H-Bco-CO_2H	15		CO_2H	2.5	16.1	
CO_2H-Bco-CH_2OH	16		CH_2OH	4	20.4	

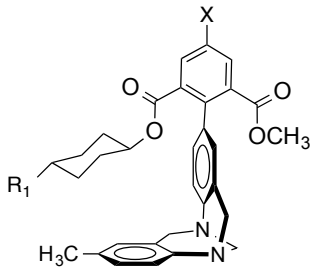


Table 7.3. Rate constants of torsion balances containing a *trans*-cyclohexyl moiety.

Name	Entry	X	R_1	k_u	k_f	Solvent
NO_2-Cy-Me	1	NO_2	H	18	30.4	$CDCl_3$
NO_2-Cy-CO_2Et	2		CO_2Et	13	23.5	
NO_2-Cy-CO_2Bn	3		CO_2Bn	11.5	21.3	
NO_2-Cy-CH_2OBn	4		CH_2OBn	20.5	29.5	
NH_2-Cy-H	5	NH_2	H	23.5	38.1	$CDCl_3$
NH_2-Cy-CO_2Et	6		CO_2Et	16	30.4	
NH_2-Cy-CO_2H	7		CO_2H	21	24.4	
NH_2-Cy-CH_2OH	8		CH_2OH	29	38.7	
CO_2H-Cy-H	9	NHCO$(CH_2)_3CO_2$H	H	18.6	39.5	MeOD
CO_2H-Cy-CO_2Et	10		CO_2Et	17	38.6	
CO_2H-Cy-CO_2H	11		CO_2H	13	35.1	
CO_2H-Cy-CH_2OH	12		CH_2OH	17.5	31.4	
CO_2H-Cy-H	13	NHCO$(CH_2)_3CO_2$H	H	3.5	14.9	D_2O
CO_2H-Cy-CO_2Et	14		CO_2Et	3	17.4	
CO_2H-Cy-CO_2H	15		CO_2H	3	14.8	
CO_2H-Cy-CH_2OH	16		CH_2OH	4	13.3	

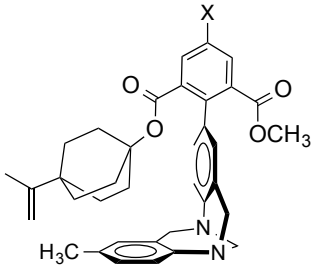


Table 7.4 Rate constants of torion balances containing an isopropenyl group.

Name	Entry	X	k_u	k_f	Solvent
NO_2-Bco-CMe=CH_2	1	NO_2	11.5	27.0	$CDCl_3$
NH_2-Bco-CMe=CH_2	2	NH_2	17.5	34.4	$CDCl_3$
CO_2H-Bco-CMe=CH_2	3	$NHCO(CH_2)_3CO_2H$	7	25.8	MeOD
CO_2H-Bco-CMe=CH_2	4	$NHCO(CH_2)_3CO_2H$	-	-	D_2O

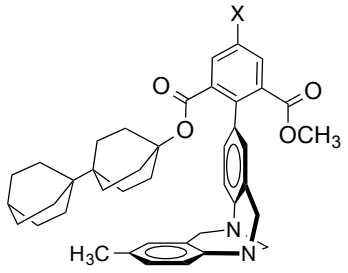


Table 7.5 Rate constants of torsion balances containing a biscyclo[2.2.2]octyl moiety.

Name	Entry	X	k_u	k_f	Solvent
NO_2-Bibco-H	1	NO_2	11	23.9	$CDCl_3$
NH_2- Bibco-H	2	NH_2	12	31.9	$CDCl_3$
CO_2H- Bibco-H	3	$NHCO(CH_2)_3CO_2H$	7.5	23.1	MeOD
CO_2H- Bibco-H	4	$NHCO(CH_2)_3CO_2H$	2	14	D_2O

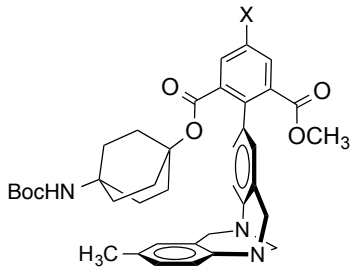


Table 7.6 Rate constants of torsion balances containing a Boc protected amine.

Name	Entry	X	k_u	k_f	Solvent
NO_2-Bco-NHBoc	1	NO_2	11.5	35.1	$CDCl_3$
NH_2- Bco-NHBoc	2	NH_2	12	37.0	$CDCl_3$
CO_2H- Bco-NHBoc	3	$NHCO(CH_2)_3CO_2H$	4.5	24.2	MeOD
CO_2H- Bco-NHBoc	4	$NHCO(CH_2)_3CO_2H$	2.5	32.0	D_2O

8.0 EXPERIMENTAL

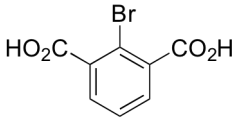
General Information: ^{1}H and ^{13}C NMR spectra were recorded on Bruker Avance 300, 400, 500, 600 and 700 MHz spectrometers. The chemical shifts are given in parts per million (ppm) on the delta scale (δ), and the coupling constant values (*J*) are in Hertz. The solvent peak was used as the reference value. For ^{1}H spectra: $CDCl_3$ = 7.26 ppm; CD_3OD = 3.31 ppm; D_2O = 4.8 ppm. For ^{13}C: $CDCl_3$ = 77.0 ppm, CD_3OD = 49.9 ppm. The abbreviations in proton NMR data are: s = singlet; d = doublet; t = triplet; q = quartet; dd = doublet of doublets; sept = septet; dt = doublet of triplets; dq = doublet of quartets, tt = triplet of triplets, m = multiplet; b = broad. All NMR spectra were obtained at room temperature (18-25 ˚ C) unless otherwise noted.

High resolution mass spectra were recorded on a VG 7070 spectrometer. Infrared (IR) spectra were recorded on an Avatar 380 Nicolet FT-IR spectrometer. Samples of IR were prepared in either solid or liquid method. Liquid method was prepared as a thin layer on a KBr plate by dissolving the sample in CH_2Cl_2 and evaporating the CH_2Cl_2. Solid method was prepared by mixing the sample (0.5-1% w/w) with KBr (IR grade) power with a mortar and pressing the mixture into a clear thin plate. Thin layer chromatography (TLC) was performed on silica gel 60 F_{254} analytical glass plates from EMD Chemicals Inc. Light absorption by compounds was observed using ultraviolet light (254 nm). Melting points were determined using a Mel-Temp apparatus and were uncorrected.

Dry solvents were obtained by distilling the solvents from an appropriate drying agent under nitrogen atmosphere shortly before use. Dichloromethane (CH_2Cl_2) was distilled from CaH_2. THF and Et_2O were distilled from sodium metal and benzophenone was used as indicator. EtOAc and benzene were stored over 4Å molecular sieves. Dry DMSO and DMF were commercial products and used as supplied. Acetylnitrile was distilled from the CaH_2, stored over 4Å molecular sieves and used within one month. References to "removal of volatile components under reduced pressure" refer to rotary evaporation of the sample at 25-65 °C at a pressure of 18-25 mm Hg and then overnight under high vacuum (~ 2 mm Hg) at room temperature (RT, 18-24 ˚C). The degas procedure mentioned below was done by use of three freeze-pump-thaw cycles. All percentage yields are for material of >93% purity as indicated by ^{1}H NMR spectra, unless stated otherwise.

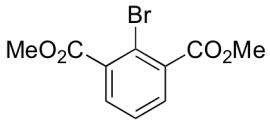
8.1 SYNTHESIS

2-Bromo-isophthalic acid (12). 2-Bromo-*m*-xylene (4.02 g, 21.7 mmol), $KMnO_4$ (16.9 g, 107 mmol), NaOH (0.340 g, 8.50 mmol) and 50 mL of water were placed in a flask and heated to reflux. After 16 h of heating and stirring, the reaction mixture was cooled to RT, and $NaHSO_3$ (8.00 g, 76.9 mmol) was added. The mixture was filtered through Celite, and the filter cake was washed with 60 mL of hot water three times. The filter cake was sonicated with hot water and filtered. All the hot aqueous extracts were combined and reduced to 50 mL volume. When this solution was cooled to RT, the crude product



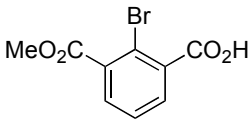
precipitated as a white crystalline solid. This white solid was isolated by filtration, washed with water and dried in vacuo to yield 3.67 g of **12** (15.0 mmol, 69% yield). IR (liquid in CH_2Cl_2, cm^{-1}): υ 3664, 3600-3100 (broad), 2930, 2243, 1643, 1566, 1386, 1282, 1240, 1144, 1027, 950; ^1H NMR (300 MHz, CD_3OD) δ 7.76 (d, 2H, J = 7.8 Hz), 7.52 (t, 1H, J = 7.8 Hz).

2-Bromo-isophthalic methyl ester (13). To **12** (5.00 g, 20.4 mmol) in 90.0 mL of MeOH



solution, 21.5 mL of sulfuric acid was added slowly. The mixture was heated to reflux. After 42 h of stirring and heating, the reaction was cooled to RT, diluted with 100 mL of water and 200 mL of CH_2Cl_2 was added. The isolated aqueous layer was washed with 3x150 mL of CH_2Cl_2. The combined organic solutions were washed with saturated K_2CO_3 solution and water, and then dried over $MgSO_4$. Filtration of this solution and removal of volatile components from the filtrate under reduced pressure afforded 4.50 g (16.5 mmol, 81% yield) of **13** as a colorless oil. IR (liquid in CH_2Cl_2, cm^{-1}): υ 3002, 2953, 2852, 1736, 1587, 1432, 1310, 1284, 1255, 1206, 1149, 1098, 1029, 993, 956, 813, 754; ^1H NMR (300 MHz, $CDCl_3$) δ 7.72 (d, 2H, J = 7.5 Hz), 7.43 (t, 1H, J = 7.8 Hz), 3.96 (s, 6H).

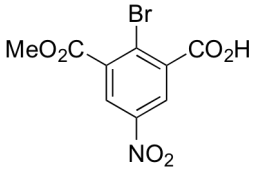
2-Bromo-isophthalic acid monomethyl ester (14). To a solution of **13** (4.01 g, 14.7 mmol) in



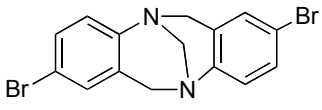
37.5 mL of MeOH/Acetone =1/4 at RT was added NaOH (0.646 g, 16.2 mmol). After stirring overnight at RT, volatile components were removed from this mixture under vacuum. The residue was diluted with 70 mL of EtOAc and 44 mL of 1 M HCl. The separated aqueous layer was extracted with 2x80 mL of EtOAc. The combined

organic portions were washed with brine and then dried over $MgSO_4$. Filtration of this solution and removal of volatile components from the filtrate under reduced pressure afforded a residue that was dissolved in 50 mL of 1 M $NaHCO_3$ solution and extracted with 2x20 mL of EtOAc. The aqueous layer was acidified with 85 mL of 1M HCl (to pH~1) to yield 4.21 g of **14** as a white precipitate (not dry enough to constitute an accurate yield). According to the 1H NMR, the crude product consisted of 93% of monoacid and 7% of diacid. IR (liquid in CH_2Cl_2, cm^{-1}): υ 3583, 1717, 1696, 1400, 1321, 1030, 746; ^1H NMR (300 MHz, CD_3OD) δ 7.78 (dd, 1H, *J* = 7.8 Hz and 1.8 Hz), 7.72 (dd, 1H, *J* = 7.8 Hz and 1.8 Hz), 7.52 (t, 1H, *J* = 7.8 Hz), 3.94 (s, 3H). The product was used in the next step without further purification.

2-Bromo-4-nitro-isophthalic acid monomethyl ester (8). The crude monoacid **14** (3.50 g, 13.5 mmol) was added portionwise to a mixture of cold HNO_3 (4.55 mL) and H_2SO_4 (27.1 mL) acids. After 1 h at 0 ˚C and 16 h at RT, the reaction mixture was added to 450 mL of cold water. The resulting white solid **8** was filtered and dried in vacuo (2.15 g, 7.08 mmol, 52% yield). The product (> 95% pure) was used in the next step without further purification. IR (liquid in CH_2Cl_2, cm^{-1}): υ 3557, 3445, 3091, 1740, 1701, 1604, 1535, 1440, 1354, 1297, 1270, 1201, 1160, 922, 739; ^1H NMR (300 MHz, CD_3OD) δ 8.60 (d, 1H, *J* = 2.7 Hz), 8.56 (d, 1H, *J* = 2.7 Hz), 4.00 (s, 3H).

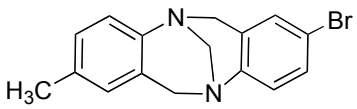


2,8-Dibromo-6*H*,12*H*-5,11-methanodibenzo[*b*,*f*][1,5]diazocine (10). To a stirred mixture of 4-bromoaniline (10.0 g, 58.2 mmol) and paraformaldehyde (3.49 g, 116 mmol) at -10 ˚C was



added dropwise 116 mL of TFA over 1 h via a dropping funnel. Stirring was continued for 150 h at 0 ˚C. TFA was removed in vacuo, 57 mL of water was added followed by addition of 56 mL of conc. NH_4OH. The aqueous layer was extracted with CH_2Cl_2. The combined organic layers were dried with $MgSO_4$, filtered, and volatile components were removed under vacuum. The crude residual product was purified by flash column chromatography (SiO_2, EtOAc/Hexanes = 2/3) to afford 6.50 g (17.1 mmol, 59% yield) of product as a yellow solid (R_f = 0.27, EtOAc/Hexanes = 1/4). IR (liquid in CH_2Cl_2, cm^{-1}): υ 2957, 2900, 2846, 1474, 1435, 1401, 1322, 1298, 1208, 1171, 1147, 1114, 1080, 964, 938, 846, 826, 736; ^1H NMR (300 MHz, $CDCl_3$) δ 7.28 (d, 2H, *J* = 7.5 Hz), 7.03 (m, 4H), 4.65 (d, 2H, *J* = 16.5 Hz), 4.26 (s, 2H), 4.10 (d, 2H, *J* = 16.5 Hz).

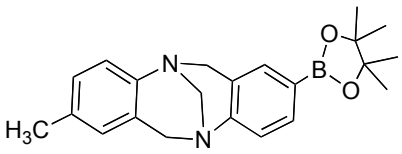
2-Bromo-8-methyl-6*H*,12*H*-5,11-methanodibenzo[*b*,*f*] [1,5]diazocine (11). A flame dried



flask was charged with dibromo analogue **10** (1.00 g, 2.63 mmol). It was dried in vacuo for 10 min and filled with nitrogen before 8 mL of anhydrous THF was added. After the THF solution was cooled to -78 ˚C, n-BuLi (0.185 g, 2.89 mmol, 1.81 mL) was added dropwise to the solution over 4 min. Five min later, the iodomethane (0.560 g, 3.95 mmol, 0.250 mL) was added to the solution slowly over two min. The solution was warmed to RT over 1.5 h, and 32 mL of cold water was then added. The resulting solution was extracted with 3x50 mL of CH_2Cl_2. The combined organic extracts were dried over $MgSO_4$ and filtered. Removal of volatile components under reduced pressure afforded crude product that was purified by flash column chromatography (SiO_2, EtOAc/CH_2Cl_2 = 1/10) to yield 0.450 g of **11** (1.43 mmol, 54.3% yield, R_f = 0.30, EtOAc/CH_2Cl_2 = 1/4). IR (liquid in

CH_2Cl_2, cm^{-1}): υ 2945, 2900, 2849, 1494, 1476, 1437, 1403, 1323, 1297, 1260, 1206, 1180, 1153, 1113, 1096, 1079, 964, 946, 828; ^1H NMR (300 MHz, $CDCl_3$) δ 7.26 (d, 1H, *J* = 7.2 Hz), 7.04 (m, 4H), 6.73 (s, 1H), 4.66 (dd, 2H, *J* = 16.5 and 6.3 Hz), 4.29 (dd, 2H, *J* = 12.0 and 18.5 Hz), 4.11 (dd, 2H, *J* = 16.5 and 9.9 Hz), 2.24 (s, 3H).

(5R,11S)-2-Methyl-8-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-6,12-dihydro-5,11-methano dibenzo[b,f][1,5] diazocine (9). A flask containing **11** (0.100 g, 0.317 mmol), bis(pinacolato)diboron (0.0934 g, 0.349 mmol), KOAc (0.0886 g, 0.952 mmol) and $Pd(dppf)_2Cl_2$ (0.0074 g, 0.0095 mmol) was placed under vacuum for 30 min and filled with N_2. After 2 mL of DMF was added, the reaction mixture was degassed three times. Then the flask was sealed with parafilm and grease. After being stirred at 80 ˚C for 16 h it was cooled to RT, and diluted with 20 mL of CH_2Cl_2. The resulting solution was washed with 6x8 mL of H_2O, dried over $MgSO_4$ and filtered. Removal of volatile components under reduced pressure afforded crude product that was purified by flash column chromatography (SiO_2, Et_2O/Hexanes = 3/2) to afford 0.0094 g of **9** (0.29 mmol, 82% yield, R_f = 0.50, Et_2O/Hexanes = 3/2) as a white solid. IR (liquid in CH_2Cl_2, cm^{-1}): υ 3584, 2976, 1609, 1493, 1359, 1329, 1207, 1145, 1115, 964, 947, 889, 853; ^1H NMR (300 MHz, $CDCl_3$) δ 7.59 (d, 1H, *J* = 7.8 Hz), 7.39 (s, 1H), 7.12 (d, 1H, *J* = 8.1 Hz), 7.01 (d, 1H, *J* = 8.4 Hz), 6.94 (d, 1H, *J* =8.1 Hz), 6.69 (s, 1H), 4.67 (d, 2H, *J* = 16.5 Hz), 4.32 (s, 2H), 4.16 (d, 2H, *J* = 16.5 Hz), 2.20 (s, 3H), 1.27 (s, 12H); ^{13}C NMR (700 MHz, $CDCl_3$) δ 151.20, 145.18, 133.95, 133.62, 133.45, 128.19, 127.36, 127.19, 124.75, 124.28, 83.63, 66.93, 58.75, 58.58, 24.78, 20.78.



tris-**Ethyl 3-acetylpentane-1,3,5-tricarboxylate (15).** To a room temperature solution of sodium (0.290 g, 12.2 mmol) in 93 mL of EtOH prepared in a flame dried flask was added ethyl acetoacetate (8.00 g, 61.5 mmol). Five minutes later, ethyl acrylate (13.5 g, 135 mmol) was added. After 18 h of stirring at RT, 160 mL of H_2O and 3 mL of 1 M HCl was added. The resulting solution was extracted with 4x150 mL of CH_2Cl_2. The combined organic extracts were dried over $MgSO_4$. Filtration of this solution and removal of volatile components from the filtrate under reduced pressure afforded a residue that was purified by flash column chromatography (SiO_2, EtOAc/Hexanes = 2/3) to afford **15** as a colorless oil (14.7 g, 44.4 mmol, 72% yield, R_f = 0.32, EtOAc/Hexanes = 1/4). IR (liquid in CH_2Cl_2, cm^{-1}): υ 2983, 1736, 1449, 1377, 1301, 1186, 1097, 1023; ^1H NMR (300 MHz, $CDCl_3$) δ 4.21 (q, 2H, J = 7.2 Hz), 4.12 (q, 4H, *J* = 7.2 Hz), 2.18 (m, 11H), 1.26 (m, 9H).

4-Acetyl-4-(ethoxycarbonyl)heptanedioic acid (16). A mixture of **15** (1.50 g, 4.54 mmol) in 2 mL of an aqueous solution of NaOH (0.3632 g, 9.08 mmol) and 8 mL of EtOH was stirred at RT for 24 h. EtOH was removed under reduced pressure. The residual mixture was diluted with 20 mL of H_2O, extracted with 40 mL of ether and acidified with 11 mL of 1 M HCl to pH=1. The acidic solution was extracted with 3x60 mL of ether. The combined organic extracts were washed with brine, dried over $MgSO_4$ and filtered. Removal of volatile components under reduced pressure afforded white solid **16** (1.02 g, 3.71 mmol, 82% yield). This crude was not further purified, and used as obtained for the next step. IR (liquid in CH_2Cl_2, cm^{-1}): υ 3400-2800 (broad), 1709, 1415, 1362,

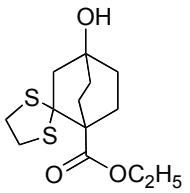
1189, 1096, 1016; ^{1}H NMR (300 MHz, CD_3OD) δ 4.21 (q, 2H, *J* = 7.2 Hz), 2.16 (m, 11H), 1.26 (t, 3H, *J* = 7.2 Hz).

Ethyl 1-acetyl-4-oxocyclohexanecarboxylate (17). A solution of **16** (8.85 g, 32.3 mmol) and KOAc (0.0633 g, 0.640 mmol) in 22.5 mL of acetic anhydride was heated at reflux for 3 h. After the mixture was cooled to RT, the flask was connected to a distillation apparatus. Then it was heated under reduced pressure (11 mmHg). Acetic anhydride was distilled out at 30 ˚C, and the product started to come out as a colorless oil when it was continuously heated to 215 ˚C. The dark brown residue after distillation was purified by flash chromatography (SiO_2, EtOAc/Hexanes = 1/1) to give another portion of product. The combined mass of **17** was 3.57 g (16.8 mmol, 52% yield, R_f = 0.27, EtOAc/Hexanes = 1/3). IR (liquid in CH_2Cl_2, cm^{-1}): υ 3700-3200 (broad), 2977, 1713, 1444, 1364, 1339, 1287, 1219, 1110, 1020, 948, 858, 819, 758; ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.26 (q, 2H, *J* =7.2 Hz), 2.42 (m, 6H), 2.22 (m, 5H), 1.30 (t, 3H, *J* = 7.2 Hz).

Ethyl 4-hydroxy-2-oxobicyclo[2.2.2]octane-1-carboxylate (18). To a flame dried flask with 18.8 mL of freshly made 1M NaOEt in EtOH, was added **17** (2.00 g, 9.40 mmol) in 3.3 mL of absolute EtOH over 8 min under nitrogen. After 15 h of stirring at RT, the reaction was quenched with 7.4 mL of 1M HCl. The alcohol was removed in reduced pressure. The residue was diluted with 40 mL of H_2O, extracted with 4x40 mL of EtOAc. The combined organic solutions were washed with brine, dried over $MgSO_4$,

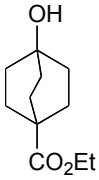
and filtered. Removal of volatile components under reduced pressure afforded crude product which was purified by flash chromatography (SiO_2, EtOAc/Hexanes = 3/2) to give 0.681 g (3.21 mmol, 34% yield, R_f = 0.10, EtOAc/Hexanes = 1/3) of **18** as a colorless oil. IR (liquid in CH_2Cl_2, cm^{-1}): υ 3700-3100 (broad), 2961, 2877, 1736, 1458, 1369, 1334, 1254, 1189. 1105, 1054, 1030; ^1H NMR (300 MHz, $CDCl_3$) δ 4.21 (q, 2H, *J* = 7.2 Hz), 2.51 (s, 2H), 2.28 (m, 2H), 1.90 (m, 6H), 1.30 (t, 3H, *J* = 7.2 Hz).

Ethyl 4-hydroxyspiro[bicyclo[2.2.2]octane-2,2'-[1,3]dithiolane]-1-carboxylate (19): A

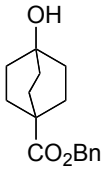


solution of **18** (0.681 g, 3.17 mmol) in 6.34 mL of ethane-1,2-dithiol was cooled to 0 ˚C. It was treated with 3.10 mL of trifluoroborane etherate. The reaction was diluted with 50 mL of CH_2Cl_2 after 30 min. This organic solution was washed with 3x50 mL of 1M NaOH, 3x50 mL of H_2O, 50 mL of brine subsequently. Then it was dried over $MgSO_4$ and filtered. Removal of volatile components under reduced pressure afforded **19** as a white solid (0.865 g, 2.97 mmol, 94% yield, R_f = 0.44, EtOAc/Hexanes = 1/1). The crude was used in the next step without further purification. IR (liquid in CH_2Cl_2, cm^{-1}): υ 3600-3100 (broad), 2948, 2869, 1719, 1456, 1331, 1254, 1235, 1104, 1028; ^1H NMR (300 MHz, $CDCl_3$) δ 4.12 (q, 2H, *J* = 7.2 Hz), 3.33 (m, 2H), 3.20 (m, 2H), 2.54 (s, 2H), 2.43 (m, 2H), 1.95 (m, 2H), 1.73 (m, 4H), 1.27 (t, 3H, *J* = 7.2 Hz).

Ethyl 4-hydroxybicyclo[2.2.2]octane-1-carboxylate (20a): About 15.0 g of wet Raney-Ni was washed with absolute EtOH for three times. The activated Raney-Ni in 29 mL of EtOH was added to **19**, and the mixture was heated at reflux. After 15 h of tirring and refluxing, the reaction was cooled to RT and filtered. The filter cake was washed with CH_2Cl_2 for several times. The combined filtrates were concentrated and afforded **20a** as a white solid (0.566 g, 2.82 mmol, 98% yield, R_f = 038, EtOAc/Hexanes = 1/1). The crude was used in the next step without further purification. IR (liquid in CH_2Cl_2, cm^{-1}): υ 3400-3100 (broad), 2954, 2867, 1718, 1480, 1250, 1108; ^1H NMR (300 MHz, $CDCl_3$) δ 4.08 (q, 2H, *J* = 6.9 Hz), 1.91 (m, 6H), 1.66 (m, 6H), 1.22 (t, 3H, *J* = 7.2 Hz); ^{13}C NMR (300 MHz, $CDCl_3$) δ 177.26, 69.00, 60.19, 38.10, 33.05, 29.36, 14.00.

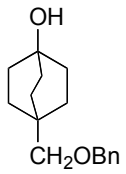


Benzyl 4-hydroxybicyclo[2.2.2]octane-1-carboxylate (20b): ***Step 1) Hydrolysis*****:** A solution of KOH (0.075 g, 1.35 mmol) in 1.5 mL of water and 2 mL of EtOH was added to 1 mL of EtOH solution of **20a** (0.0900 g, 0.454 mmol). The reaction was heated at reflux. The EtOH was removed in reduced pressure after 19 h. The residue was diluted with 10 mL of water and acidified with 2.5 mL of 1M HCl (aq.). This resulting aqueous solution was extracted with EtOAc. The combined organic solutions were washed with brine, dried over $MgSO_4$ and filtered. Removal of volatile components under reduced pressure afforded 0.0704 g of **20e** (0.413 mmol, 91% yield). The crude was used in the next step without further purification. IR (liquid in CH_2Cl_2, cm^{-1}): υ 3500-3300 (broad), 1698, 1104; ^1H NMR (300 MHz, CD_3OD) δ 1.88 (m, 6H), 1.61 (m, 6H); ^{13}C NMR (300 MHz, CD_3OD) δ 181.43, 69.64, 39.39, 33.99, 30.78; ***Step 2) Esterification*****:** To the solution of the above crude **20e** (0.0304 g, 0.179



mmol) in 0.27 mL of DMF was added $KHCO_3$ (0.0215 g, 0.214 mmol). Then it was treated with BnBr (0.0641 g, 0.375 mmol, 0.046 mL) after 15 min. The reaction was stirred at RT for 1 h and 14 h at 40 ˚C. When it was cooled to RT, it was diluted with 5 mL of H_2O and extracted with 5x10 mL of EtOAc. The combined organic solutions were washed with brine for three times, dried over $MgSO_4$ and filtered. Removal of volatile components under reduced pressure afforded crude product, which was purified by flash column chromatography (SiO_2, EtOAc/Hexanes = 3/2) to give 0.0408 g (0.158 mmol, 88% yield, R_f = 0.39, EtOAc/Hexanes = 1/1) of **20b** as a pale yellow oil. IR (liquid in CH_2Cl_2, cm^{-1}): υ 3400-300 (broad), 2943, 2865, 2360, 1716, 1450, 1360, 1229, 1107, 1060, 732; ^1H NMR (300 MHz, $CDCl_3$) δ 7.33 (m, 5H), 5.08 (s, 2H), 1.97 (m, 6H), 1.67 (m, 6H).

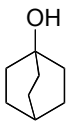
4-(Benzyloxymethyl)bicyclo[2.2.2]octan-1-ol (20c): ***Step 1) Reduction*****:** A suspension of



$LiAlH_4$ (0.0313 g, 0.8243 mmol) in 1.3 mL of anhydrous Et_2O was cooled to 0 ˚C, then 1.2 mL of Et_2O solution of **20a** (0.0817 g, 0.412 mmol) was added to the mixture. The reaction was heated at reflux for 16 h, then allowed to cool to RT. The mixture was cooled to 0 ˚C and treated with 2 mL of 1M NaOH. Then the mixture was filtered through cotton and Celite. The filtrate was dried over $MgSO_4$ and filtered. Removal of volatile components under reduced pressure afforded 45.9 mg (0.585 mmol, 71% yield) of **20f**. The crude was used in the next step without further purification. IR (liquid in CH_2Cl_2, cm^{-1}): υ 3500-3000 (broad), 2936, 2859; ^1H NMR (300 MHz, $CDCl_3$) δ 3.28 (s, 2H), 1.65 (m, 6H), 1.57 (m, 6H); ^{13}C NMR (300 MHz, $CDCl_3$) δ 70.79, 69.68, 33.55, 32.71, 28.97; ***Step 2) Benzylation*****:** A solution of **20f** (0.0300 g, 0.192 mmol) in 0.18 mL of DMF was cooled to -15 ˚C. Then a

suspension of 8.5 mg of NaH (60% in mineral oil) in 0.38 mL of DMF was added to the solution dropwise. It was treated with BnBr (0.0361 g, 0.211 mmol, 0.025 mL) after 15 min and let to warm up to RT. The reaction was stirred at RT for 17 h, then quenched with 6 mL of H_2O. This solution was extracted with 5x10 mL of EtOAc. The combined organic solution was washed with brine, dried over $MgSO_4$ and filtered. Removal of volatile components under reduced pressure afforded crude product which was purified by flash chromatography (SiO_2, EtOAc/Hexanes = 1/1) to give 0.0337 g (0.137 mmol, 71% yield, R_f = 0.52, EtOAc/Hexanes = 1/1) of **20c** as a pale yellow oil. IR (liquid in CH_2Cl_2, cm^{-1}): υ 3650-3300 (broad), 2944, 2863,1718, 1455, 1332, 1275, 1084, 713; ^1H NMR (300 MHz, $CDCl_3$) δ 7.30 (m, 5H), 4.47 (s, 2H), 3.08 (m, 2H), 1.63 (m, 12H); ^{13}C NMR (400 MHz, $CDCl_3$) δ 138.83, 128.25, 127.35, 127.28, 78.17, 73.19, 69.71, 33.69, 32.09, 29.60.

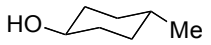
Bicyclo[2.2.2]octan-1-ol (20d): ***Step 1) Acylationn*****:** A mixture of **20e** (0.0638 g, 0.375 mmol) and DMAP (0.0046 g, 0.0375 mmol) was placed in a flask. After vacuated and filled with nitrogen, the mixture was dissolved in acetic anhydride (0.0574 g, 0.562 mmol, 0.053 mL) and Et_3N (0.0948 g, 0.937 mmol, 0.131 mL). The reaction mixture was diluted with 10 mL of H_2O, acidified with 1 mL of 1M HCl after 18 h. The resulting solution was extracted with 5x12 mL of EtOAc. The combined organic extracts were washed with brine, dried over $MgSO_4$ and filtered. Removal of volatile components under reduced pressure afforded crude product, which was purified by flash column chromatography (SiO_2, EtOAc/Hexanes = 2/3) to give 0.073 g (0.345 mmol, 92% yield, R_f = 0.20, EtOAc/Hexanes = 1/3) of **20g** as a white solid. IR (liquid in CH_2Cl_2, cm^{-1}): υ 2965, 2926, 2874, 1735, 1685, 1415, 1287,1253, 954; ***Step 2)***



***Decarboxylation*:** A mixture of **20g** (0.0915 g, 0.431 mmol) and dithio[38] **20h** (0.137 g, 0.539 mmol) was placed in a flask which was wrapped with foil. Then 4.3 mL of THF was added. The reaction was cooled to 0 ˚C and then freshly distilled Bu_3P (0.119 g, 0.586 mmol, 0.146 mL) was added dropwise. After 3 h of stirring at RT, to the reaction were added n-Bu_3SnH (0.628 g, 2.156 mmol, 0.580 mL) and 0.1 mL of THF solution of AIBN (0.0018 g, 0.0108 mmol). After completion, it was heated at refux for 3 h. When it was cooled to RT, the reaction was diluted with 10 mL of Et_2O. This organic extracts were washed with 3x10 mL of saturated $NaHCO_3$, 3x10 mL of 1M HCl, 3x10 mL of H_2O and 3x10 mL of brine in sequence. The resulting solution was dried over $MgSO_4$ and filtered. The volatile components were removed by distillation at 40 ˚C and at atmosphere pressure. The residue was purified by flash column chromatography (SiO_2, Et_2O/Pentane = 1/19). The combined fractions were reduced to 1 mL volume by distillation (R_f = 0.67, Et_2O/Pentane = 7/93). ***Step 3) Hydrolysis*:** The residue in distillation was treated with a solution of KOH (0.0966 g, 1.724 mmol) in 1.1 mL of H_2O and 2.2 mL of MeOH. The mixture was heated at 50 ˚C for 16 h. The MeOH was removed in reduced pressure. The residue was diluted with 2 mL of H_2O and acidified with 2 mL of 1 M HCl. The aqueous solution was extracted with 5x5 mL of Et_2O. The combined organic solutions were washed with brine, dried over $MgSO_4$ and filtered. Removal of volatile components under reduced pressure afforded crude product, which was purified by flash column chromatography (SiO_2, Et_2O/Pentane = 1/1) to give 0.0121 g (0.0959 mmol, 22% yield, R_f = 0.48, Et_2O/Pentane = 1/1) of **20d** as a white solid. IR (liquid in CH_2Cl_2, cm^{-1}): υ 3600-3200 (broad), 2921, 2852, 2359; ^1H NMR (400 MHz, $CDCl_3$) δ 1.69 (m, 6H), 1.62 (m, 6H), 1.56 (m, 1H); ^{13}C NMR (400 MHz, $CDCl_3$) δ 69.13, 33.88, 27.16, 24.04.

Benzyl 4-hydroxycyclohexanecarboxylate (21d): ***Step 1) Hydrolysis*****:** A solution of NaOH (1.36 g, 34.1 mmol) in 16.5 mL of water was added to 66 mL of EtOH solution of the commercial available ethyl 4-oxocyclohexane-1-carboxylate (5.34 g, 31.0 mmol, *trans* and *cis* mixture). After 14 h of stirring at RT, the volatile components were removed in vacuo. The residue was diluted with 20 mL of water and then acidified with 50 mL of 1M HCl (aq.). This resulting aqueous solution was extracted with 4x80 mL of EtOAc. The combined organic solutions were washed with brine, dried with $MgSO_4$ and filtered. Removal of volatile components under reduced pressure afforded 3.56 g of **22** as a white solid (80% yield). According the ^{1}H NMR, the crude was consisted of 67% of *cis* and 33% of *trans* product. ^{1}H NMR (300 MHz, CD_3OD) δ 3.75 (m, 0.67H), 3.45 (tt, 0.33H, J = 3.6 and 10.5 Hz), 2.32 (m, 0.67H), 2.17 (tt, 0.33H, J = 3.6 and 11.7 Hz), 1.93 (m, 2.66H), 1.62 (m, 4.02H), 1.40 (m, 0.66H), 1.22 (m, 0.66H). The product was used in the next step without further purification. ***Step 2) Esterification*****:** To a solution of 3.82 g (26.5 mmol) of **22** in 40 mL of DMF, was added $KHCO_3$ (3.18 g, 31.8 mmol). BnBr (6.80 g, 39.7 mmol, 4.73 mL) was added to the mixture 30 min later. The reaction was stirred at RT for 1.5 h, then heated at 40 ˚C for 3 h. The reaction was quenched with 200 mL of water. The resulting solution was extracted with 5x180 mL of EtOAc. The combined organic extracts were washed with saturated $NaHCO_3$ and brine for several times. The solution was dried with $MgSO_4$ and filtered. Removal of volatile components under reduced pressure afforded 5.96 g (25.4 mmol, 96% yield) of **21d** and **21e** as a waxy oil. The *cis* and *trans* products **21a** (R_f = 0.27, EtOAc/Hexanes = 2/3) and **21d** (R_f = 0.34, EtOAc/Hexanes = 2/3) were isolated by flash column chromatography (SiO_2, EtOAc/Hexanes = 2/3). IR (liquid in CH_2Cl_2, cm^{-1}): υ 3700-3100 (broad), 2937, 2861, 1731, 1497, 1454, 1365,

1270, 1234, 1170, 1067, 1030, 1004, 973, 956, 903, 748; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.34 (m, 5H), 5.11 (s, 2H), 3.61 (m, 1H), 2.31 (tt, 1H, *J* = 3 and 11.7 Hz), 2.02 (m, 4H), 1.55 (m, 2H), 1.30 (m, 2H).

***trans*-4-Methylcyclohexanol (21a):** To 49 mL of Raney type alloy water suspension was added  0.510 g of *p*-creasol (4.7159 mmol). When it was heated to 90 ˚C, 49 mL of 1% KOH aqueous solution was added dropwise to the suspension over 40 min. After 24 h of heating at 90 ˚C, the reaction mixture was filtered. The filtrate was neutralized with 1M HCl, then extracted with 3x100 mL of EtOAc. The filter cake was washed with EtOAc for several times. The combined organic solution was dried over $MgSO_4$ and filtered. Removal of volatile components under reduced pressure afforded crude product, which was purified by flash column chromatography (SiO_2, EtOAc/Hexanes = 1/1). The isolated pure *trans* product **21a** is 0.0567 g (0.497 mmol, 11% yield, R_f = 0.20, EtOAc/Hexanes = 1/4). IR (liquid in CH_2Cl_2, cm^{-1}): υ 3600-3100 (broad), 2921, 2851, 1716, 1460; ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.53 (tt, 1H, *J* = 4.5 and 10.8 Hz), 1.94 (m, 2H), 1.70 (m, 2H), 1.33 (m, 3H), 0.95 (m, 2H), 0.88 (d, 3H); ^{13}C NMR (300 MHz, $CDCl_3$) δ 70.75, 35.45, 33.24, 32.05, 21.86.

***trans*-Ethyl 4-hydroxycyclohexanecarboxylate (21b): *Step 1) Debenzylation*:** The mixture of  **21d** (0.0712 g, 0.304 mmol) and 10% Pd/C (0.0327 g, 0.0304 mmol) was placed in a flask. The flask was evacuated and filled with hydrogen, then 3.0 mL of MeOH was injected inside. The reaction mixture was stirred at RT under balloon pressure (about 1 atm) for

20 h. Then it was filtered through Celite and cotton. Removal of volatile components under reduced pressure afforded 0.0421 g of **21f** (0.292 mmol, 96% yield) as a white solid. The crude was used in the next step without further purification. IR (liquid in CH_2Cl_2, cm^{-1}): υ 3600-3200 (broad), 2928, 2856, 1707, 1453, 1257, 1060; ^1H NMR (300 MHz, CD_3OD) δ 3.53 (tt, 1H, *J* = 10.5 and 3.9 Hz), 2.22 (tt, 1H, *J* = 11.7 and 3.3 Hz), 2.01 (m, 4H), 1.47 (m, 2H), 1.35 (m, 2H); ***Step 2) Esterification*: 21f** (0.0400 g, 0.277 mmol) and $KHCO_3$ (0.0333 g, 0.323 mmol) were placed in a flask. Then 0.42 mL of DMF was added to dissolve the mixture. The solution was warmed up to 40 ˚C. After half an hour later of stirring at 40 ˚C, iodoethane (0.0649 g, 0.416 mmol, 0.0333 mL) was added. After three more hours of heating at 40 ˚C, the reaction was quenched with 5 mL of H_2O. The aqueous solution was extracted with 7x7 mL of EtOAc. The combined organic solutions were washed with 1 M $NaHCO_3$ and water, dried over $MgSO_4$ and filtered. Removal of volatile components under reduced pressure afforded crude product, which was purified by flash column chromatography (SiO_2, EtOAc/Hexanes = 1/1) to yield 0.0358 g (0.208 mmol, 75% yield, R_f = 0.45, EtOAc/Hexanes = 1/3) of **21b**. IR (liquid in CH_2Cl_2, cm^{-1}): υ 3500-3100 (broad), 2933, 2855, 1724, 1453, 1391, 1308, 1280, 1239, 1167, 1132, 1118, 1074, 750; ^1H NMR (300 MHz, $CDCl_3$) δ 4.11 (q, 2H, *J* = 7.2), 3.61 (m, 1H), 2.23 (tt, 1H, *J* = 3.3 and 12 Hz), 2.02 (m, 4H), 1.51 (m, 2H), 1.30 (m, 2H), 1.27 (t, 3H, *J* = 7.2 Hz).

***trans*-4-((Benzyloxy)methyl)cyclohexanol (21c): *Step 1) Reduction*:** A suspension of $LiAlH_4$

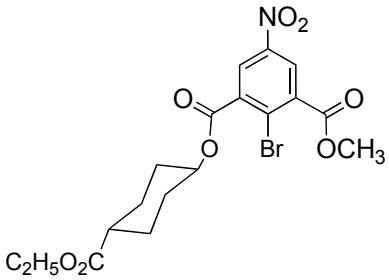
(0.0404 g, 1.07 mmol) in 1.3 mL of anhydrous Et_2O was cooled to 0 ˚C. Then 1.4 mL of Et_2O solution of **21d** (0.124 g, 0.531 mmol) was added to the mixture. After completion, the reaction was heated at reflux for 18 h. When it was cooled to RT, the reaction

was quenched with 0.5 mL of 1M NaOH. Then the mixture was filtered through cotton and Celite. The filtrate was dried over $MgSO_4$ and filtered. Removal of volatile components under reduced pressure to generate 0.104 g of crude. The crude was purified by flash column chromatography (SiO_2, 100% EtOAc) to yield 0.0681g (0.524 mmol, 99% yield, R_f = 0.30, 100% EtOAc) of **21g**. IR (liquid in CH_2Cl_2, cm^{-1}): υ 3650-3100 (broad), 2923, 2854, 1451, 1361, 1096, 1048, 976; ^1H NMR (300 MHz, $CDCl_3$) δ 3.53 (m, 1H), 3.45 (m, 2H), 2.02 (m, 2H), 1.84 (tt, 0.33H), 1.45 (m, 1H), 1.26 (m, 2H), 1.08 (m, 2H); ^{13}C NMR (300 MHz, CD_3OD) δ 71.78, 68.31, 41.03, 35.96, 29.01; ***Step 2) Benzylation*:** To a solution of **21g** (0.0948 g, 0.729 mmol) in 1.46 mL of DMF at -15 ˚C, was added 60% NaH in mineral oil (0.0322 g, 0.802 mmol). After 15 min, BnBr (0.137 g, 0.802 mmol, 0.0945 mL) was added to the suspension. The reaction was stirred at RT for 16 h, then quenched with 10 mL of H_2O. The resulting solution was extracted with 5x10 mL of EtOAc. The combined organic solutions were washed with brine, dried with $MgSO_4$ and filtered. Removal of volatile components under reduced pressure afforded crude product that was purified by flash column chromatography (SiO_2, EtOAc/Hexanes = 1/1) to yield 0.0791 g (0.360 mmol, 49% yield, R_f = 0.39, EtOAc/Hexanes = 1/1) of **21c**. IR (liquid in CH_2Cl_2, cm^{-1}): υ 3700-3100 (broad), 2929, 2856, 1717, 1452, 1363, 1315, 1275, 1113, 1049, 713; ^1H NMR (300 MHz, $CDCl_3$) δ 7.33 (m, 5H), 4.49 (s, 2H), 3.53 (tt, 1H, J = 4.2 and 10.8 Hz), 3.27 (d, 2H), 1.97 (m, 2H), 1.86 (m, 2H), 1.58 (m, 1H), 1.24 (m, 2H), 1.04 (m, 2H); ^{13}C NMR (300 MHz, $CDCl_3$) δ 138.48, 128.24, 127.72, 127.39, 75.32, 72.89, 70.84, 37.08, 34.92, 27.99.

General procedure for esterification.

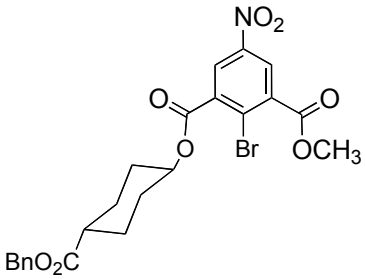
To a stirred solution of monoacid **8 (**0.0873 g, 0.287 mmol), alcohol **21b** (0.0412 g, 0.239 mmol) and DMAP (0.0088 g, 0.0718 mmol) in 1.5 mL of anhydrous dichloromethane at -15 ˚C was added 1,3-dicyclohexylcarbodiimide (0.148 g, 0.718 mmol) in 1.5 mL of dichloromethane over 5 min under nitrogen. The reaction was allowed to stir for overnight during which it was warmed to room temperature. The precipitated urea was filtered off with cotton and Celite. Volatile components were removed under reduced pressure and the crude product was purified using flash chromatography (SiO_2, EtOAc/Hexanes = 2/3) to give 0.0465 g (0.102 mmol, 42% yield, R_f = 0.40, EtOAc/Hexanes = 1/3) of diester **23b** as a waxy solids.

1-(4-(Ethoxycarbonyl)cyclohexyl) 3-methyl 2-bromo-5-nitroisophthalate (**23b**). IR (liquid in

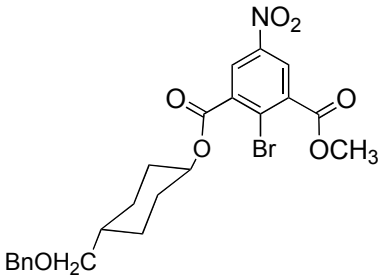


CH_2Cl_2, cm^{-1}): υ 2927, 2868, 1732, 1609, 1536, 1438, 1352, 1306, 1244, 1184, 1060, 734; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.54 (d, 1H, *J* = 2.7 Hz), 8.46 (d, 1H, *J* = 2.4 Hz), 5.05 (m, 1H), 4.14 (q, 2H, *J* = 6.9 Hz), 4.00 (s, 3H), 2.31 (m, 1H), 2.21 (m, 2H), 2.21 (m, 2H), 2.11 (m, 2H), 1.63 (m, 4H), 1.26 (t, 3H, *J* = 7.2 Hz); ^{13}C NMR (400 MHz, $CDCl_3$) δ 174.82, 164.79, 164.03, 146.20, 137.49, 136.39, 126.40, 126.22, 75.19, 60.45, 53.37, 41.71, 30.27, 26.56, 14.18; HRMS (ES) *m/z* calcd for $C_{18}H_{20}BrNO_8$ $[M-e]^+$ 457.0372, found 457.0370.

1-(4-((Benzyloxy)carbonyl)cyclohexyl) 3-methyl 2-bromo-5-nitroisophthalate (23d). By the general procedure, 0.118 g (0.388 mmol) of monoacid **8**, 0.0605 g (0.259 mmol) of **21d**, 0.0095 g (0.0776 mmol) of DMAP, 0.776 g (0.160 mmol) of DCC afforded 0.122 g of **23d** (0.235 mmol, 91% yield, R_f = 0.27, EtOAc/Hexanes = 1/9). IR (liquid in CH_2Cl_2, cm^{-1}): υ 2927, 2868, 1735, 1609, 1535, 1438, 1351, 1306, 1244, 1182, 1135, 1080, 1060, 737; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.54 (d, 1H, *J* = 2.7 Hz), 8.46 (d, 1H, *J* = 2.4 Hz), 7.35 (m, 5H), 5.13 (s, 2H), 5.05 (m, 1H), 4.00 (s, 3H), 2.42 (m, 1H), 2.19 (m, 4H), 1.64 (m, 4H); ^{13}C NMR (300 MHz, $CDCl_3$) δ 174.45, 164.65, 163.89, 146.05, 137.30, 136.21, 135.78, 128.43, 128.09, 127.89, 126.30, 126.11, 74.96, 66.12, 53.26, 41.54, 30.10, 26.42; HRMS (ES) *m/z* calcd for $C_{23}H_{22}BrNO_8$ $[M-e]^+$ 519.0426, found 519.0394.

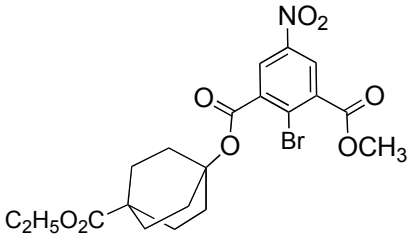


1-(4-((Benzyloxy)methyl)cyclohexyl) 3-methyl 2-bromo-5-nitroisophthalate (23c). By the general procedure, 0.0456 g (0.150 mmol) of monoacid **8**, 0.0220 g (0.100 mmol) of **21c**, 0.0037 g (0.0300 mmol) of DMAP, 0.300 g (0.0619 mmol) of DCC afforded 0.0315 g of **23c** (0.0623 mmol, 62% yield, R_f = 0.40, EtOAc/Hexanes = 1/3). IR (liquid in CH_2Cl_2, cm^{-1}): υ 2944, 2859, 2380, 1736, 1610, 1535, 1450, 1351, 1293, 1293, 1240, 1183, 1020; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.54 (d, 1H, *J* = 2.7 Hz), 8.47 (d, 1H, *J*=2.7 Hz), 7.32 (m, 5H), 5.01 (m, 1H), 4.51 (s, 2H), 4.01 (s, 3H), 2.20 (m, 2H), 2.11 (m, 2H), 1.69 (m, 1H), 1.50 (m, 2H), 1.20 (m, 2H); ^{13}C NMR (300 MHz, $CDCl_3$) δ 164.83, 164.14, 146.17, 138.44,137.67,



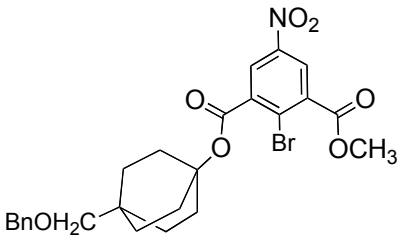
136.33, 128.35, 127.53, 127.51, 126.33, 126.21, 76.56, 74.98, 73.07, 53.37, 37.00, 30.92, 27.73; HRMS (ES) *m/z* calcd for $C_{23}H_{24}BrNO_7$ $[M-e]^+$ 505.0736, found 505.0734.

1-Methyl 3-(4-methylcyclohexyl) 2-bromo-5-nitroisophthalate (23a). By the general procedure, 0.204 g (0.670 mmol) of monoacid **8**, 0.0510 g (0.447 mmol) of **21a**, 0.0167 g (0.134 mmol) of DMAP, 0.277 g (1.34 mmol) of DCC afforded 0.117 g of **23a** (0.293 mmol, 66% yield, R_f = 0.79, EtOAc/Hexanes = 1/3). IR (liquid in CH_2Cl_2, cm^{-1}): υ 2950, 2865, 2360, 1737, 1607, 1579, 1536, 1415, 1439, 1351, 1322, 1293, 1240, 1183, 1153, 1085, 1036, 1017, 989, 918, 740, 713; ^1H NMR (300 MHz, $CDCl_3$) δ 8.54 (d, 1H, *J* = 2.7 Hz), 8.47 (d, 1H, *J* = 2.7 Hz), 5.00 (m, 1H), 4.51 (s, 2H), 4.01 (s, 3H), 2.15 (m, 2H), 1.82 (m, 2H), 1.47 (m, 2H), 1.43 (m, 1H), 1.14 (m, 2H), 0.93 (d, 3H, *J* = 6.6 Hz); ^{13}C NMR (300 MHz, $CDCl_3$) δ 164.80, 164.14, 146.14, 137.72, 136.26, 126.33, 126.25, 126.17, 76.66, 53.32, 32.82, 31.51, 31.37, 21.69; HRMS (ES) *m/z* calcd for $C_{16}H_{19}BrNO_6$ $[M+H]^+$ 400.0396, found 400.0355

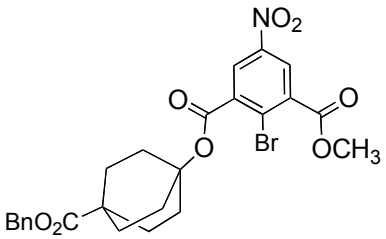


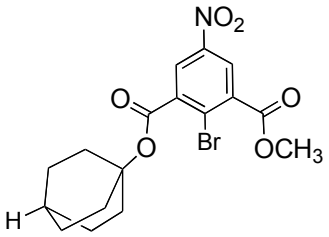
1-(4-(Ethoxycarbonyl)bicyclo[2.2.2]octan-1-yl) 3-methyl 2-bromo-5-nitroisophthalate (23h). By the general procedure, 0.0983 g (0.3235 mmol) of monoacid **8**, 0.0433 g (0.216 mmol) of **20a**, 0.0085 g (0.0647 mmol) of DMAP, 0.134 g (0.647 mmol) of DCC afforded 0.0747 g of **23h** (0.154 mmol, 71% yield, R_f = 0.60,

EtOAc/Hexanes = 1/3). IR (liquid in CH_2Cl_2, cm^{-1}): υ 2955, 2926, 1735, 1609, 1535, 1356, 1303, 1244, 1187, 1153, 1073, 1028, 739; ^1H NMR (300 MHz, $CDCl_3$) δ 8.50 (d, 1H, J = 3.0 Hz), 8.39 (d, 1H, J = 3.0 Hz), 4.11 (q, 2H, J = 7.2 Hz), 4.01 (s, 3H), 2.20 (m, 6H), 2.04 (m, 6H), 1.24 (t, 3H, J = 6.9 Hz); ^{13}C NMR (300 MHz, $CDCl_3$) δ 176.49, 164.80, 163.66, 146.17, 138.47, 136.09, 126.13, 126.03, 125.92, 84.37, 60.50, 53.37, 38.00, 29.21, 29.17, 14.14; HRMS (ES) *m/z* calcd for $C_{20}H_{22}BrNO_8$ $[M\text{-}e]^+$ 483.0529, found 483.0511.

1-(4-((Benzyloxy)methyl)bicyclo[2.2.2]octan-1-yl) 3-methyl 2-bromo-5-nitroisophthalate (23i). 

By the general procedure, 0.0530 g (0.174 mmol) of monoacid **8**, 0.0286 g (0.116 mmol) of **21c**, 0.0043 g (0.0349 mmol) of DMAP, 0.0719 g (0.349 mmol) of DCC afforded 0.0491 g of **23i** (0.0922 mmol, 79% yield, R_f = 0.59, EtOAc/Hexanes = 1/3). IR (liquid in CH_2Cl_2, cm^{-1}): υ 2927, 2868, 1737, 1609, 1535, 1438, 1353, 1310, 1293, 1244, 1183, 1154, 1060, 1025, 739; ^1H NMR (300 MHz, $CDCl_3$) δ 8.52 (d, 1H, J = 2.7 Hz), 8.40 (d, 1H, J = 2.7 Hz), 7.32 (m, 5H), 4.49 (s, 2H), 4.00 (s, 3H), 3.11 (s, 2H), 2.17 (m, 6H), 1.71 (m, 6H); ^{13}C NMR (400 MHz, $CDCl_3$) δ 164.87, 163.69, 146.20, 138.77, 138.69, 136.09, 128.30, 127.43, 127.31, 126.00, 125.91, 85.30, 77.73, 73.24, 53.33, 32.01, 29.61, 29.28; HRMS (ES) *m/z* calcd for $C_{25}H_{26}BrNO_7$ $[M\text{-}e]^+$ 531.0893, found 531.0896.

1-(4-((Benzyloxy)carbonyl)bicyclo[2.2.2]octan-1-yl) 3-methyl 2-bromo-5-nitroisophthalate (23j).  By the general procedure, 0.0916 g (0.301 mmol) of monoacid **8**, 0.0523 g (0.200 mmol) of **20b**, 0.0074 g (0.0603 mmol) of DMAP, 0.124 g (0.603 mmol) of DCC afforded 0.0828 g of **23j** (0.152 mmol, 76% yield, R_f = 0.45, EtOAc/Hexanes = 1/3). IR (liquid in CH_2Cl_2, cm^{-1}): υ 2955, 2926, 2380, 1732, 1610, 1535, 1465, 1357, 1311, 1245, 1285, 1100, 1020; ^1H NMR (300 MHz, $CDCl_3$) δ 8.52 (d, 1H, *J* = 2.7 Hz), 8.39 (d, 1H, *J* = 2.7 Hz), 7.35 (m, 5H), 5.10 (s, 2H), 3.40 (s, 3H), 2.20 (m, 6H), 2.05 (m, 6H); ^{13}C NMR (400 MHz, $CDCl_3$) δ 176.25, 164.82, 163.66, 146.22, 138.47, 136.15, 136.05, 128.56, 128.15,127.78,126.14, 126.03, 125.94, 84.28, 66.23, 53.37, 38.22, 29.22; HRMS (ES) *m/z* calcd for $C_{25}H_{24}BrNO_8$ $[M-e]^+$ 545.0685, found 545.0684.

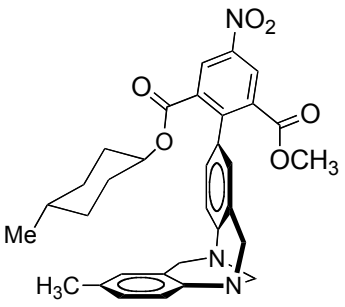
1-(Bicyclo[2.2.2]octan-1-yl) 3-methyl 2-bromo-5-nitroisophthalate (23g).  By the general procedure, 0.0372 g (0.122 mmol) of monoacid **8**, 0.0103 g (0.0816 mmol) of **20d**, 0.0030 g (0.0245 mmol) of DMAP, 0.0505 g (0.245 mmol) of DCC afforded 0.0237 g of **23g** (0.0575 mmol, 71% yield, R_f = 0.62, EtOAc/Hexanes = 1/3). IR (liquid in CH_2Cl_2, cm^{-1}): υ 2927, 2868, 1729, 1609, 1542, 1438, 1355, 1332, 1313, 1291, 1246, 1184, 1025, 737; ^1H NMR (300 MHz, $CDCl_3$) δ 8.51 (d, 1H, *J* = 2.1 Hz), 8.40 (d, 1H, *J* = 2.1 Hz), 4.00 (s, 3H), 2.13 (m, 6H), 1.78 (m, 6H), 1.63 (m, 1H); ^{13}C NMR (400 MHz, $CDCl_3$) δ 164.89, 163.69, 146.21, 138.88, 136.07, 126.00, 125.95, 125.90, 85.38, 53.33, 29.79, 26.90, 23.76; ^{13}C NMR (400 MHz, $CDCl_3$)

δ 164.89, 163.69, 146.19, 138.87, 136.05, 126.00, 125.95, 125.90. 85.38, 53.33, 29.78, 26.89, 23.75; HRMS (ES) *m/z* calcd for $C_{17}H_{18}BrNO_6$ $[M\text{-}e]^+$ 411.0317, found 411.0324.

General procedure for Suzuki coupling.

The flask was charged with **9** (0.0745 g, 0.206 mmol), isophthalate **23a** (0.0750 g, 0.185 mmol) and the Blaser's catalyst **Pd-L** (0.0210 g, 0.0375 mmol). After it was evacuated and filled with N_2, 0.94 mL of DME and 0.94 mL of water were added to the reaction. Then it was treated with $NaHCO_3$ (0.0945 g, 1.13 mmol) and degassed for three times. The reaction was sealed with vacuum grease and parafilm, heated at 80 ˚C for 16h. When it was cooled to RT, the reaction mixture was diluted with H_2O. The resulting suspension was extracted with CH_2Cl_2. The combined organic solutions were washed with brine, dried with $MgSO_4$ and filtered. Removal of volatile components under reduced pressure afforded crude product that was purified by flash column chromatography (SiO_2, Et_2O/Pentane = 9/1) to yield **7a** (0.0746 g, 0.135 mmol, 72% yield, R_f = 0.45, Et_2O/Pentane = 3/1) as a yellow waxy solid.

1-Methyl 3-((1*r*,4*r*)-4-methylcyclohexyl) 2-(8-methyl-6*H*,12*H*-5,11-methanodibenzo[*b*,*f*][1,5] diazocin-2-yl)-5-nitroisophthalate (7a). IR (liquid in CH_2Cl_2, cm^{-1}): υ 2948, 2861, 1723, 1601,



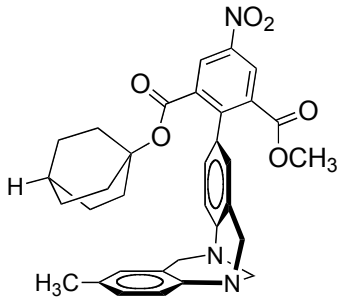
1530, 1494, 1440, 1350, 1323, 1305, 1243, 1207, 1180, 1147, 1097, 992, 965, 946, 911, 833, 737, 717; ^{1}H NMR (700 MHz, $CDCl_3$, 5 ˚C) **Folded (62%):** δ 8.65 (s, 1H), 8.60 (s, 1H), 7.15 (d, 1H, *J* = 8.4 Hz), 7.00 (m, 3H), 6.73 (d, 2H, *J* = 14 Hz), 4.71 (dd, 2H, *J* = 31.5 and 15.4 Hz), 4.32 (dd, 2H, *J* = 20.3 and 13.3 Hz), 4.32 (m, 1H), 4.14 (dd, 2H, *J* = 16.8 and 11.6 Hz), 3.68 (s, 3H), 2.22 (s, 3H), 1.40 (br, 2H), 1.22 (br, 1H), 1.10 (br, 1H), 0.91 (br, 1H), 0.80 (br, 3H), 0.72 (br, 1H), 0.58 (br, 2H), 0.42 (br, 1H); **Unfolded (38%):** δ 8.65 (s, 1H), 8.60 (s, 1H), 7.15 (d, 1H, *J* = 8.4 Hz), 7.00 (m, 3H), 6.73 (d, 2H, *J* = 14 Hz), 4.71 (dd, 2H, *J* = 31.5 and 15.4 Hz), 4.71 (m, 1H), 4.31 (dd, 2H, *J* = 20.3 and 13.3 Hz), 4.14 (dd, 2H, *J* = 16.8 and 11.6 Hz), 3.00 (s, 3H), 2.22 (s, 3H), 1.79 (br, 2H), 1.69 (br, 2H), 1.28 (br, 1H), 1.14 (br, 1H), 1.09 (br, 1H), 0.98 (br, 2H), 0.87 (br, 3H); ^{13}C NMR (700 MHz, $CDCl_3$) δ 166.83, 166.40, 165.65, 148.74, 146.32, 146.11, 145.23, 136.07, 135.78, 135.30, 134.65, 133.52, 132.73, 128.32, 127.66, 127.32, 127.04, 126.76, 126.37, 126.06, 125.79, 125.54, 124.87, 124.11, 75.51, 75.01, 67,33, 66.65, 65.89, 58.61, 58.39, 58.20, 52.81, 51.97, 34.25, 32.57, 31.52, 31.07, 30.54, 30.34, 29.73, 24.89, 22.73, 21.74, 21.22, 20.95, 18.47, 15.31, 14.17; HRMS (ES) *m/z* calcd for $C_{32}H_{33}N_3O_6$ $[M\text{-}e]^+$ 555.2448, found 555.2502.

1-(4-(Ethoxycarbonyl)cyclohexyl) 3-methyl 2-(8-methyl-6*H*,12*H*-5,11-methanodibenzo[*b*,*f*] [1,5] diazocin-2-yl)-5-nitroisophthalate (7b). By the general procedure, 0.0175 g (0.0480 mmol) of **9**, 0.0245 g (0.0530 mmol) of **23b**, 0.0055 g (0.0100 mmol) of Pd catalyst, 0.0245 g (0.291 mmol) of $NaHCO_3$ afforded 0.0215 g of **7b** (0.0350 mmol, 73% yield, R_f = 0.30, EtOAc/Hexanes = 3/1). IR (liquid in CH_2Cl_2, cm^{-1}): υ 3600-3200 (broad), 2948, 1727, 1530, 1494, 1441, 1351, 1325, 1305, 1248, 1206, 1180,1150; ^{1}H NMR (700 MHz, $CDCl_3$, 5 ˚C) **Folded (65%):** δ 8.68 (s, 1H), 8.63 (s, 1H), 7.16 (d, 1H, *J* = 7.7 Hz), 7.00 (m, 3H), 6.74 (d, 2H, *J* = 18.2 Hz), 4.71 (dd, 2H, *J* = 31.5 and 15.4 Hz), 4.41 (m, 1H), 4.31 (dd, 2H, d, 2H, *J* = 20.3 and 13.3 Hz), 4.14 (dd, 2H, *J* = 16.8 and 23.8 Hz), 4.11 (q, 2H, *J* = 7 Hz), 3.69 (s, 3H), 2.20 (s, 3H), 1.80 (m, 1H), 1.62 (br, 1H), 1.47 (br, 1H), 1.45 (br, 1H), 1.27 (br, 1H), 1.26 (br, 3H), 1.14 (br, 1H), 1.11 (br, 1H), 0.66 (br, 1H), 0.44 (br, 1H); **Unfolded (35%):** δ 8.68 (s, 1H), 8.63 (s, 1H), 7.16 (d, 1H, *J* = 7.7 Hz), 7.00 (m, 3H), 6.74 (d, 2H, *J* = 18.2 Hz), 4.71 (dd, 2H, *J* = 31.5 and 15.4 Hz), 4.65 (m, 1H), 4.31 (dd, 2H, d, 2H, *J* = 20.3 and 13.3 Hz), 4.14 (dd, 2H, *J* = 16.8 and 23.8 Hz), 4.11 (q, 2H, *J* = 7 Hz), 2.96 (s, 3H), 2.20 (s, 3H), 2.20 (m, 1H), 1.93 (br, 2H), 1.86 (br, 2H), 1.51 (br, 2H), 1.26 (br, 3H), 1.16 (br, 2H); ^{13}C NMR (700 MHz, $CDCl_3$) δ 174.85, 166.19, 166.06, 148.57, 146.09, 145.95, 144.99, 135.61, 135.52, 134.38, 133.60, 132.57, 128.30, 127.68, 127.51, 127.06. 126.88, 126.52, 126.14, 125.88, 125.47, 124.76, 124.54, 73.80, 66.47, 60.35, 58.52, 58.10, 52.85, 41.17, 34.12, 30.16, 29.63, 29.31, 29.04, 26.10, 20.67, 18.36, 15.23, 14.16; HRMS (ES) *m/z* calcd for $C_{34}H_{35}N_3O_8$ $[M-e]^+$ 613.2502, found 613.2499.

1-(4-((Benzyloxy)methyl)cyclohexyl) 3-methyl 2-(8-methyl-6*H*,12*H*-5,11-methanodibenzo [*b*,*f*][1,5] diazocin-2-yl)-5-nitroisophthalate (7c). By the general procedure, 0.0170 g (0.0470 mmol) of **9**, 0.0216 g (0.0427 mmol) of **23c**, 0.0050 g (0.0085 mmol) of Pd catalyst, 0.0215 g (0.256 mmol) of $NaHCO_3$ afforded 0.0211 g of **7c** (0.0319 mmol, 75% yield, R_f = 0.32, Et_2O/Pentane = 3/2). IR (liquid in CH_2Cl_2, cm^{-1}): υ 3600-3100 (broad), 2943, 2856, 2247, 1722, 1601, 1530, 1494, 1440, 1350, 1323, 1243, 1206, 1178, 1148, 1115, 1096, 995; ^1H NMR (700 MHz, $CDCl_3$, 5 ˚C) **Folded (58%):** δ 8.65 (s, 1H), 8.59 (s, 1H), 7.32 (m, 5H), 7.14 (d, 1H, *J* = 8.4 Hz), 6.99 (m, 3H), 6.72 (d, 2H, *J* = 18.9 Hz), 4.70 (dd, 2H, *J* = 28 and 16.1 Hz), 4.47(s, 2H), 4.38 (m, 1H), 4.32 (dd, 2H, *J* = 25.9 and 16.1 Hz), 4.13 (dd, 2H, *J* = 18.2 and 17.5 Hz), 3.70 (s, 3H), 3.16 (s, 2H), 2.16 (s, 3H), 2-0.3 (br, 9H); **Unfolded (42%):** δ 8.65 (s, 1H), 8.59 (s, 1H), 7.32 (m, 5H), 7.14 (d, 1H, *J* = 8.4 Hz), 6.99 (m, 3H), 6.72 (d, 2H, *J* = 18.9 Hz), 4.70 (dd, 2H, *J* = 28 and 16.1 Hz), 4.65 (m, 1H), 4.47(s, 2H), 4.32 (dd, 2H, *J* = 25.9 and 16.1 Hz), 4.13 (dd, 2H, *J* = 18.2 and 17.5 Hz), 3.25 (s, 2H), 2.97 (s, 3H), 2.21 (s, 3H), 2-0.3 (br, 9H); ^{13}C NMR (700 MHz, $CDCl_3$) δ 146.25, 138.41, 133.46, 132.59, 128.32, 128.24, 127.51, 127.29, 126.97, 125.98, 125.76, 125.48, 124.75, 124.48, 74.94, 73.06, 30.26, 29.64, 27.41, 20.74; HRMS (ES) *m/z* calcd for $C_{39}H_{39}N_3O_7$ $[M-e]^+$ 661.2866, found 661.2853.

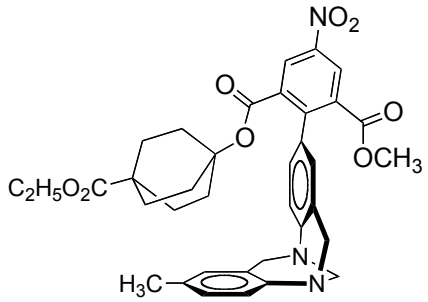
1-(4-((Benzyloxy)carbonyl)cyclohexyl) 3-methyl 2-(8-methyl-6*H*,12*H*-5,11-methanodibenzo [*b*,*f*][1,5] diazocin-2-yl)-5-nitroisophthalate (7d). By the general procedure, 0.0630 g (0.174 mmol) of **9**, 0.0824 g (0.159 mmol) of **23d**, 0.0178 g (0.0317 mmol) of Pd catalyst, 0.0800 g (0.951 mmol) of $NaHCO_3$ afforded 0.0531 g of **7d** (0.0787 mmol, 50% yield, R_f = 0.20, Et_2O/Pentane = 3/1). IR (liquid in CH_2Cl_2, cm^{-1}): υ 2956, 2860, 2360, 1729, 1602, 1530, 1494, 1441, 1350, 1325, 1305, 1252, 1207, 1177, 1148, 1119, 1096, 1042, 1019, 803, 737; ^1H NMR (700 MHz, $CDCl_3$, 5 ˚C) **Folded (64%):** δ 8.66 (s, 1H), 8.61 (s, 1H), 7.36 (m, 5H), 7.15 (d, 1H, *J* = 7.7 Hz), 7.00 (m, 3H), 6.72 (d, 2H, *J* = 27.3 Hz), 5.10 (s, 2H), 4.72 (dd, 2H, *J* = 9.1 and 16.8 Hz), 4.41 (m, 1H), 4.30 (dd, 2H, *J* = 22.4 and 13.3 Hz), 4.13 (dd, 2H, *J* = 16.8 and 22.4 Hz), 3.69(s, 3H), 2.08 (s, 3H), 1.87 (m, 1H), 1.64 (br, 1H), 1.49 (br, 1H), 1.45 (br, 1H), 1.29 (br, 1H), 1.17 (br, 2H), 0.64 (br, 1H), 0.43 (br, 1H); **Unfolded (36%):** δ 8.66 (s, 1H), 8.61 (s, 1H), 7.36 (m, 5H), 7.15 (d, 1H, *J* = 7.7 Hz), 7.00 (m, 3H), 6.72 (d, 2H, *J* = 27.3 Hz), 5.10 (s, 2H), 4.66 (br, 2H), 4.64 (m, 1H), 4.40 (br, 2H), 4.13 (dd, 2H, *J* = 16.8 and 22.4 Hz), 2.97 (s, 3H), 2.27 (m, 1H), 2.21 (s, 3H), 2.00 (br, 2H), 1.87 (br, 2H), 1.54 (br, 2H), 1.17 (br, 2H);^{13}C NMR (600 MHz, $CDCl_3$) δ 174.52, 166.22, 148.72, 146.29, 146.06, 145.10, 135.86, 135.72, 134.64, 133.66, 132.63, 128.56, 128.26, 128.12, 127.63, 127.19, 126.92, 126.30, 126.09, 125.86, 124.77, 124.53, 73.85, 66.64, 66.22, 65.83, 58.66, 58.39, 58.25, 52.79, 41.33, 29.84, 29.67, 29.39, 29.16, 26.18, 20.65, 18.41, 15.25; HRMS (ES) *m/z* calcd for $C_{39}H_{37}N_3O_8$ $[M-e]^+$ 675.2659, found 675.2676.

1-(Bicyclo[2.2.2]octan-1-yl) 3-methyl 2-(8-methyl-6*H*,12*H*-5,11-methanodibenzo[*b*,*f*][1,5] diazocin-2-yl)-5-nitroisophthalate (7g). By the general procedure, 0.0212 g (0.0587 mmol) of



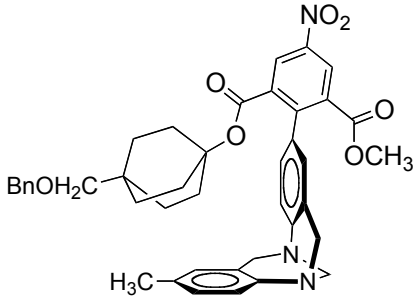
9, 0.0220 g (0.0534 mmol) of **23g**, 0.0060 g (0.0107 mmol) of Pd catalyst, 0.0269 g (0.320 mmol) of $NaHCO_3$ afforded 0.0259 g of **7g** (0.0457 mmol, 86% yield, R_f = 0.65, Et_2O/Pentane = 3/1). IR (liquid in CH_2Cl_2, cm^{-1}): υ 2948, 2868, 2360, 2340, 1718, 1600, 1530, 1494, 1439, 1350, 1326, 1307, 1246, 1206, 1178, 1148, 1116, 1097, 1027, 964, 946, 908, 833, 733; ^{1}H NMR (700 MHz, $CDCl_3$, 5 ˚C) **Folded (66%):** δ 8.60 (s, 1H), 8.51 (s, 1H), 7.16 (d, 1H, *J* = 8.4 Hz), 7.04 (d, 1H, *J* = 8.4 Hz), 6.99 (br, 2H), 6.74 (d, 2H, *J* = 9.1 Hz), 4.72 (m, 2H), 4.33 (dd, 2H, *J* = 23.1 and 12.6 Hz), 4.16 (dd, 2H, *J* = 16.1 and 14.8 Hz), 3.68 (s, 3H), 2.21 (s, 3H), 1.52 (br, 1H), 1.24 (br, 12H); **Unfolded (34%):** δ 8.60 (s, 1H), 8.51 (s, 1H), 7.16 (d, 1H, *J* = 8.4 Hz), 7.04 (d, 1H, *J* = 8.4 Hz), 6.99 (br, 2H), 6.74 (d, 2H, *J* = 9.1 Hz), 4.72 (m, 2H), 4.33 (dd, 2H, *J* = 23.1 and 12.6 Hz), 4.16 (dd, 2H, *J* = 16.1 and 14.8 Hz), 3.00 (s, 3H), 2.21 (s, 3H), 1.73 (br, 6H), 1.62 (br, 7H); ; ^{13}C NMR (600 MHz, $CDCl_3$) δ 166.30, 165.49, 148.61, 145.89, 145.33, 137.12, 134.30, 133.54, 132.91, 128.52, 127.60, 127.19, 126.43, 125.86, 125.48, 124.90, 124.58, 83.40, 66.63, 58.35, 52.77, 28.91, 26.28, 23.56, 20.84; HRMS (ES) *m/z* calcd for $C_{33}H_{34}N_3O_6$ $[M+H]^+$ 568.2442, found 568.2425.

1-(4-(Ethoxycarbonyl)bicyclo[2.2.2]octan-1-yl) 3-methyl 2-(8-methyl-6*H*,12*H*-5,11-methanodibenzo[*b*,*f*][1,5]diazocin-2-yl)-5-nitroisophthalate (7h). By the general procedure,



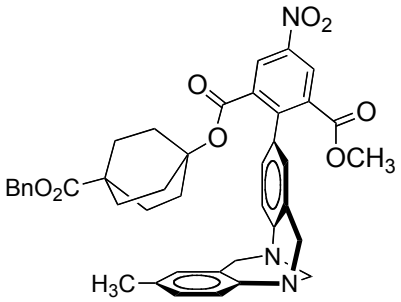
0.0175 g (0.0484 mmol) of **9**, 0.0213 g (0.0440 mmol) of **23h**, 0.0055 g (0.0097 mmol) of Pd catalyst, 0.0244 g (0.290 mmol) of $NaHCO_3$ afforded 0.0191 g of **7h** (0.0299 mmol, 68% yield, R_f = 0.20, Et_2O/Pentane = 3/2). IR (liquid in CH_2Cl_2, cm^{-1}): υ 3600-3100 (broad), 3583, 2924, 2250, 1721, 1601, 1531, 1442, 1312, 1251, 1072, 1028; ^1H NMR (700 MHz, $CDCl_3$, 5 ˚C) **Folded (74%):** δ 8.57 (d, 2H, *J* = 55.3 Hz), 7.17 (d, 1H, *J* = 8.4 Hz), 7.04 (d, 1H, *J* = 7.7 Hz), 6.97 (d, 2H, *J* = 8.4 Hz), 6.74 (d, 2H, *J* = 16.8 Hz), 4.73 (dd, 2H, *J* = 5.6 and 16.8 Hz), 4.33 (dd, 2H, *J* = 25.2 and 12.6 Hz), 4.16 (dd, 2H, *J* = 16.8 and 34.3 Hz), 4.07 (q, 2H, *J* = 7.0 Hz), 3.68 (s, 3H), 2.16 (s, 3H), 1.49 (br, 6H), 1.28 (br, 6H), 1.21 (t, 3H, *J* = 7.0 Hz); **Unfolded (26%):** δ 8.57 (d, 2H, *J* = 55.3 Hz), 7.17 (d, 1H, *J* = 8.4 Hz), 7.04 (d, 1H, *J* = 7.7 Hz), 6.97 (d, 2H, *J* = 8.4 Hz), 6.74 (d, 2H, *J* = 16.8 Hz), 4.65 (br, 2H), 4.37 (br, 2H), 4.16 (dd, 2H, *J* = 16.8 and 34.3 Hz), 4.07 (q, 2H, *J* = 7.0 Hz), 3.00 (s, 3H), 2.19 (s, 3H), 1.87 (br, 6H), 1.81 (br, 6H), 1.21 (t, 3H, *J* = 7.0 Hz); ^{13}C NMR (700 MHz, $CDCl_3$) δ 176.45, 166.20, 165.54, 148.70, 146.31, 145.92, 145.24, 136.73, 134.35, 133.71, 132.86, 128.58, 127.56, 127.09, 126.37, 125.96, 125.66, 124.90, 124.59, 82.43, 66.63, 65.85, 60.39, 58.73, 58.46, 58.33, 52.79, 37.61, 30.30, 29.02, 28.63, 28.32, 20.76, 18.44, 15.27, 14.19; HRMS (ES) *m/z* calcd for $C_{36}H_{37}N_3O_8$ $[M\text{-}e]^+$ 639.2659, found 639.2635.

1-(4-((Benzyloxy)methyl)bicyclo[2.2.2]octan-1-yl) 3-methyl 2-(8-methyl-6*H*,12*H*-5,11-methanodibenzo[*b*,*f*][1,5]diazocin-2-yl)-5-nitroisophthalate (7i). By the general procedure,



0.0194 g (0.0537 mmol) of **9**, 0.0260 g (0.0488 mmol) of **23i**, 0.0060 g (0.0107 mmol) of Pd catalyst, 0.0271 g (0.322 mmol) of $NaHCO_3$ afforded 0.0254 g of **7i** (0.0370 mmol, 76% yield, R_f = 0.26, EtOAc/Hexanes = 1/1). IR (liquid in CH_2Cl_2, cm^{-1}): υ 2922, 2865, 2355, 1718, 1601, 1529, 1493, 1439, 1352, 1313, 1247, 1206, 1179, 1147, 1097, 1027; ^1H NMR (700 MHz, $CDCl_3$, 5 ˚C) **Folded (65%):** δ 8.56 (d, 2H, *J* = 44.1 Hz), 7.36 (s, 5H), 7.17 (d, 1H, *J* = 8.4 Hz), 7.02 (d, 1H, *J* = 8.4 Hz), 6.98 (d, 1H, *J* = 7.7 Hz), 6.93 (s, 1H), 6.72 (d, 2H, *J* = 11.9 Hz), 4.74 (d, 2H, *J* = 14.0 Hz), 4.45 (s, 2H), 4.32 (dd, 2H, *J* = 29.4 and 13.3 Hz), 4.15 (dd, 2H, *J* = 16.8 and 14.4 Hz), 3.68 (s, 3H), 2.91 (s, 2H), 2.09 (s, 3H), 1.29 (br, 6H), 1.12 (br, 6H); **Unfolded (35%):** δ 8.56 (d, 2H, *J* = 44.1 Hz), 7.36 (s, 5H), 7.17 (d, 1H, *J* = 8.4 Hz), 7.02 (d, 1H, *J* = 8.4 Hz), 6.98 (d, 1H, *J* = 7.7 Hz), 6.93 (s, 1H), 6.72 (d, 2H, *J* = 11.9 Hz), 4.68 (br, 2H), 4.45 (s, 2H), 4.34 (br, 2H), 4.15 (dd, 2H, *J* = 16.8 and 14.4 Hz), 3.04 (s, 2H), 3.02 (s, 3H), 2.21 (s, 3H), 1.79 (br, 6H), 1.56 (br, 6H); ^{13}C NMR (600 MHz, $CDCl_3$) δ 166.30, 165.51, 148.66, 146.28, 145.85, 145.28, 138.66, 136.97, 134.35m 133.46, 132.86, 128.41, 128.29, 127.46, 127.36, 127.07, 126.45, 125.89, 125.51, 124.85, 124.56, 83.38, 77.66, 73. 19, 66.60, 58.62, 58.43, 58.25, 52.77, 31.44, 30.30, 29.69, 29.09, 28.72, 24.85, 20.87, 18.43; HRMS (ES) *m/z* calcd for $C_{41}H_{41}N_3O_7$ $[M\text{-}e]^+$ 687.2842, found 687.2877.

1-(4-((Benzyloxy)carbonyl)bicyclo[2.2.2]octan-1-yl) 3-methyl 2-(8-methyl-6*H*,12*H*-5,11-methanodibenzo[*b*,*f*] [1,5]diazocin-2-yl)-5-nitroisophthalate (7j). By the general procedure,

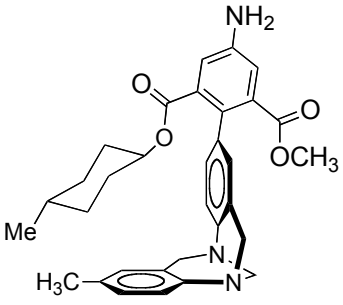


0.0291 g (0.0805 mmol) of **9**, 0.0400 g (0.0732 mmol) of **23j**, 0.0082 g (0.0146 mmol) of Pd catalyst, 0.0369 g (0.244 mmol) of $NaHCO_3$ afforded 0.0323 g of **7j** (0.0479 mmol, 65% yield, R_f = 0.19, Et_2O/Pentane = 3/1). IR (liquid in CH_2Cl_2, cm^{-1}): υ 2951, 2874, 2357, 1723, 1601, 1530, 1493, 1439, 1350, 1313, 1247, 1208, 1178, 1148, 1068, 1029, 833; ^1H NMR (700 MHz, $CDCl_3$, 5 ˚C) **Folded (74%):** δ 8.57 (d, 2H, *J* = 55.3 Hz), 7.33 (m, 5H), 7.18 (d, 1H, *J* = 8.4 Hz), 7.04 (d, 1H, *J* = 8.4 Hz), 6.99 (d, 1H, *J* = 8.4 Hz), 6.92 (d, 1H, *J* = 8.4 Hz), 6.74 (d, 2H, *J* = 7.7 Hz), 5.07 (s, 2H), 4.74 (d, 2H, *J* = 16.8 Hz), 4.32 (dd, 2H, *J* = 28.0 and 12.6 Hz), 4.14 (dd, 2H, *J* = 16.8 and 33.6 Hz), 3.68 (s, 3H), 2.03 (s, 3H), 1.54 (br, 6H), 1.31 (br, 6H); **Unfolded (26%):** δ 8.57 (d, 2H, *J* = 55.3 Hz), 7.33 (m, 5H), 7.18 (d, 1H, *J* = 8.4 Hz), 7.04 (d, 1H, *J* = 8.4 Hz), 6.99 (d, 1H, *J* = 8.4 Hz), 6.92 (d, 1H, *J* = 8.4 Hz), 6.74 (d, 2H, *J* = 7.7 Hz), 5.07 (s, 2H), 4.74 (d, 2H, *J* = 16.8 Hz), 4.32 (dd, 2H, *J* = 28.0 and 12.6 Hz), 4.14 (dd, 2H, *J* = 16.8 and 33.6 Hz), 3.02 (s, 3H), 2.21 (s, 3H), 1.93 (br, 6H), 1.84 (br, 6H); ^{13}C NMR (600 MHz, $CDCl_3$) δ 176.19, 166.22, 165.55, 146.32, 145.93, 145.24, 136.70, 136.05, 134.36, 133.81, 132.86, 128.56, 128.19, 127.87, 127.58, 127.04, 126.36, 125.98, 125.68, 124.89, 124.61, 82.32, 66.62, 66.13, 37.79, 28.63, 28.29, 20.68; HRMS (ES) *m/z* calcd for $C_{41}H_{39}N_3O_8$ $[M-e]^+$ 701.2815, found 701.2781.

General procedures for hydrogenation

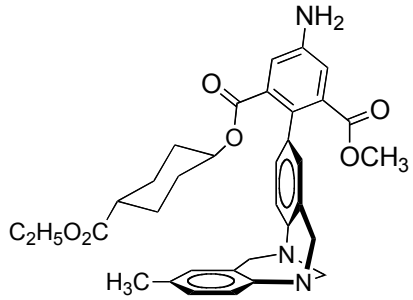
Method A: The torsion balance **7a** (0.0269 g, 0.0485 mmol), 10% Pd/C (0.0052 g, 0.0049 mmol) in 0.97 mL of EtOAc reacted under 1 atm of H_2 pressure for 16 h at RT. The reaction mixture was filtered, concentrated to afford crude product that was purified by flash column chromatography (SiO_2, 100% Et_2O) to yield 0.0125 g (0.0238 mmol, 49% yield, R_f = 0.40, Et_2O/Pentane = 3/1) of **6a**.

1-Methyl 3-((1*r*,4*r*)-4-methylcyclohexyl) 5-amino-2-(8-methyl-6*H*,12*H*-5,11-methanodibenzo [*b*,*f*][1,5]diazocin-2-yl)isophthalate (6a). IR (liquid in CH_2Cl_2, cm^{-1}): υ 2924, 2854, 1741, 1461, 1377, 1260, 1095, 1027, 803; ^1H NMR (700 MHz, $CDCl_3$, 5 ˚C) **Folded (62%):** δ 7.40 (d, 1H, *J* = 7.7 Hz), 7.37 (d, 1H, *J* = 7.7 Hz), 7.10 (s, 1H), 7.03 (d, 1H, *J* = 8.4 Hz), 6.98 (d, 2H, *J* = 18.9 Hz), 6.72 (s, 2H), 4.70 (dd, 2H, *J* = 25.2 and 14.7 Hz), 4.37 (br, 1H), 4.33 (dd, 3H, *J* = 29.4 and 18.9 Hz}, 4.12 (dd, 2H, *J* = 17.5 and 23.8 Hz), 3.88/2.18 (s/s, NH_3^+/NH_2, 3H/2H), 3.59(s, 3H), 2.21 (s, 3H), 1.32 (br, 2H), 1.15 (br, 1H), 1.01 (br, 1H), 0.86 (br, 1H), 0.76 (br, 3H), 0.67 (br, 1H), 0.52 (br, 2H), 0.34 (br, 1H); **Unfolded (28%):** 7.40 (d, 1H, *J* = 7.7 Hz), 7.37 (d, 1H, *J* = 7.7 Hz), 7.10 (s, 1H), 7.03 (d, 1H, *J* = 8.4 Hz), 6.98 (d, 2H, *J* = 18.9 Hz), 6.72 (s, 2H), 4.60 (br, 2H), 4.59 (br, 1H), 4.35 (br, 2H), 4.12 (dd, 2H, *J* = 17.5 and 23.8 Hz), 3.88/2.18 (s/s, NH_3^+/NH_2, 3H/2H), 2.91 (s, 3H), 2.21 (s, 3H), 1.70 (br, 2H), 1.61 (br, 2H), 1.26 (br, 1H), 1.01 (br, 2H), 0.91 (br, 2H), 0.82 (br, 3H); ^{13}C NMR (700 MHz, $CDCl_3$) δ 169.00, 168.64, 149.37, 145.38, 145.17, 135.35, 135.12, 133.50,



128.23, 127.06, 126.81, 125.55, 124.86, 124.11, 117.62, 117.36, 116.64, 116.39, 74.55, 74.42, 66.59, 34.26, 32.62, 31.13, 30.35, 29.73, 21.81, 20.89; HRMS (ES) *m/z* calcd for $C_{32}H_{35}N_3O_4$ $[M\text{-}e]^+$ 525.2549, found 525.2669.

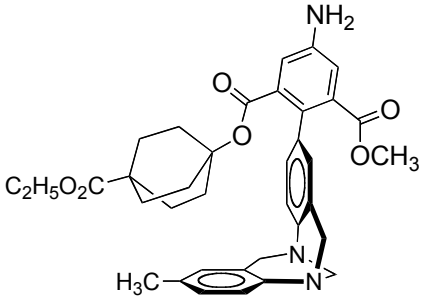
1-(4-(Ethoxycarbonyl)cyclohexyl) 3-methyl 5-amino-2-(8-methyl-6*H*,12*H*-5,11-methano dibenzo[*b*,*f*][1,5] diazocin-2-yl)isophthalate (6b). By the general procedure (Method A),



0.0261 g (0.0426 mmol) of **7b**, 0.0045 g (0.0043 mmol) of 10% Pd/C afforded 0.0146 g of **6b** (0.0250 mmol, 59% yield, R_f = 0.34, EtOAc/Hexanes = 3/1). IR (liquid in CH_2Cl_2, cm^{-1}): υ 2923, 2853, 2239, 2738, 1461, 1377, 1259; ^1H NMR (700 MHz, $CDCl_3$, 5 ˚C) **Folded (66%):** δ 7.40 (s, 1H), 7.35 (s, 1H), 7.08 (br, 1H), 7.01 (d, 1H, *J* = 7.7 Hz), 6.96 (br, 2H), 6.70 (br, 2H), 4.68 (dd, 2H, *J* = 28.7 and 13.3 Hz), 4.34 (br, 1H), 4.30 (br, 2H), 4.11 (m, 4H), 3.85/1.88 (br/br, NH_3^+/NH_2, 3H/2H), 3.56 (s, 3H), 2.19 (s, 3H), 1.77 (br, 1H), 1.58 (br, 1H), 1.40 (br, 2H), 1.18 (br, 1H), 1.07 (br, 2H), 0.57 (br, 1H), 0.34 (br, 1H); **Unfolded (24%):** δ 7.40 (s, 1H), 7.35 (s, 1H), 7.08 (br, 1H), 7.01 (d, 1H, *J* = 7.7 Hz), 6.96 (br, 2H), 6.70 (br, 2H), 4.62 (br, 2H), 4.61 (br, 1H), 4.30 (br, 2H), 4.11 (m, 4H), 3.85/1.88 (br/br, NH_3^+/NH_2, 3H/2H), 2.88 (s, 3H), 2.19 (s, 3H), 2.18 (br, 1H), 1.88 (br, 2H), 1.77 (br, 2H), 1.46 (br, 2H), 1.40 (br, 2H); ^{13}C NMR (500 MHz, $CDCl_3$) δ 175.62, 169.91, 168.80, 149.51, 147.54, 145.86, 135.76, 135.45, 135.12, 134.80, 134.00, 130.85, 128.78, 127.87, 127.56, 126.10, 125.41, 124.56, 118.21, 117.97, 117.47, 117.25, 73.41, 67.40, 60.89, 59.31, 58.96, 52.64, 42.13, 34.81, 30.91, 30.28, 26.93, 21.31, 14.82; HRMS (ES) *m/z* calcd for $C_{34}H_{37}N_3O_6$ $[M\text{-}e]^+$ 583.2761, found 583.2747.

1-(Bicyclo[2.2.2]octan-1-yl) 3-methyl 5-amino-2-(8-methyl-6*H*,12*H*-5,11-methanodibenzo [*b*,*f*][1,5]diazocin-2-yl) isophthalate (6g). By the general procedure (Method A), 0.0169 g (0.0298 mmol) of **7g**, 0.0032 g (0.0030 mmol) of 10% Pd/C afforded 0.0097 g of **6g** (0.0181 mmol, 61% yield, R_f = 0.44, Et_2O/Pentane = 3/1). IR (liquid in CH_2Cl_2, cm^{-1}): υ 3376, 2947, 2923, 2867, 2249, 1711, 1607, 1494, 1461, 1346, 1323, 1255, 1197, 1119, 1032; ^1H NMR (700 MHz, $CDCl_3$, 5 ˚C) **Folded (64%):** δ 7.43 (s, 1H), 7.36 (d, 1H, *J* = 20.3 Hz), 7.04 (m, 4H), 6.73 (d, 2H, *J* = 32.2 Hz), 4.75 (d, 2H, *J* =15.4 Hz), 4.36 (dd, 2H, *J* = 30.8 and 12.6 Hz), 4.16 (dd, 2H, *J* = 16.1 and 44.1 Hz), 3.88/2.03 (br/br, NH_3^+/NH_2, 3H/2H), 3.61 (s, 3H), 2.23 (s, 3H), 1.37 (br, 1H), 1.12 (br, 12H); **Unfolded (36%):** δ 7.43 (s, 1H), 7.36 (d, 1H, *J* = 20.3 Hz), 7.04 (m, 4H), 6.73 (d, 2H, *J* = 32.2 Hz), 4.69 (br, 2H), 4.38 (br, 2H), 4.16 (dd, 2H, *J* = 16.1 and 44.1 Hz), 3.88/2.03 (br/br, NH_3^+/NH_2, 3H/2H), 2.96 (s, 3H), 2.23 (s, 3H), 1.59 (br, 6H), 1.49 (br, 7H); ^{13}C NMR (700 MHz, $CDCl_3$) δ 172.11, 167.86, 167.75, 151.46, 149.23, 146.67, 145.25, 135.79, 135.72, 135.47, 135.23, 133.43, 133.40, 129.83, 128.37, 128.24, 128.20, 127.29, 127.17, 126.73, 125.47, 124.87, 124.06, 124.01, 117.50, 116.99, 116.51, 116.03, 81.94, 66.57, 58.97, 58.54, 58.33, 52.12, 52.00, 34.19, 31.88, 30.27, 29.65, 28.79, 26.19, 23.61, 23.53, 22.65, 21.15, 20.79, 14.10; HRMS (ES) *m/z* calcd for $C_{33}H_{35}N_3O_4$ $[M-e]^+$ 537.2525, found 537.2514.

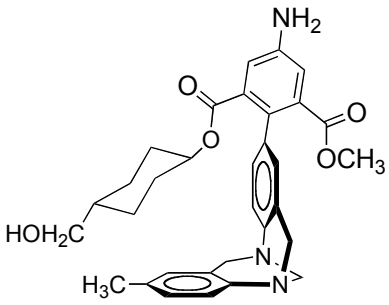
1-(4-(Ethoxycarbonyl)bicyclo[2.2.2]octan-1-yl) 3-methyl 5-amino-2-(8-methyl-6*H*,12*H*-5,11-methano dibenzo[*b*,*f*][1,5]diazocin-2-yl)isophthalate (6h). By the general procedure (Method



A), 0.0127 g (0.0199 mmol) of **7h**, 0.0021 g (0.0020 mmol) of 10% Pd/C afforded 0.0104 g of **6h** (0.0171 mmol, 86% yield, R_f = 0.54, 100% Et_2O). IR (solid in KBr, cm^{-1}): υ 3500, 3373, 2924, 2854, 1716, 1609, 1494, 1462, 1357, 1333, 1261, 1201, 1120, 1073, 1032; ^{1}H NMR (700 MHz, $CDCl_3$, 5 ˚C) **Folded (72%):** δ 7.09 (m, 2H), 7.03 (d, 2H, *J* = 21.7 Hz), 6.97 (dd, 2H, *J* = 8.4 and 18.9 Hz), 6.73 (d, 2H, *J* = 44.8 Hz), 4.73 (d, 2H, *J* = 16.8 Hz), 4.31 (dd, 2H, *J* = 12.6 and 32.9 Hz), 4.13 (dd, 2H, *J* = 16.1 and 39.9 Hz), 4.06 (q, 2H, *J* = 7.0 Hz), 3.84/1.90 (br/br, NH_3^+/NH_2, 3H/2H), 3.56 (s, 3H), 2.18 (s, 3H), 1.26 (br, 12H), 1.24 (t, 3H, *J* = 6.3 Hz); **Folded (28%):** δ 7.09 (m, 2H), 7.03 (d, 2H, *J* = 21.7 Hz), 6.97 (dd, 2H, *J* = 8.4 and 18.9 Hz), 6.73 (d, 2H, *J* = 44.8 Hz), 4.64 (br, 2H), 4.38 (br, 2H), 4.13 (dd, 2H, *J* = 16.1 and 39.9 Hz), 4.06 (q, 2H, *J* = 7.0 Hz), 3.84/1.90 (br/br, NH_3^+/NH_2, 3H/2H), 2.91 (s, 3H), 2.18 (s, 3H), 1.84 (br, 6H), 1.78 (br, 6H), 1.24 (t, 3H, *J* = 6.3 Hz); ^{13}C NMR (700 MHz, $CDCl_3$) δ 168.59, 167.88, 146.93, 145.45, 145.28, 135.73, 135.64, 135.34, 133.70, 133.47, 133.47, 129.90, 128.42, 128.15, 127.40, 127.29, 127.08, 126.68, 125.49, 124.68, 124.00, 117.52, 117.12, 115.68, 106.91, 80.87, 66.70, 60.28, 58.65, 58.39, 52.04, 37.63, 34.20, 30.29, 29.67, 28.60, 28.24, 20.73, 14.18; HRMS (ES) *m/z* calcd for $C_{36}H_{39}N_3O_6$ $[M\text{-}e]^+$ 609.2917, found 609.2906.

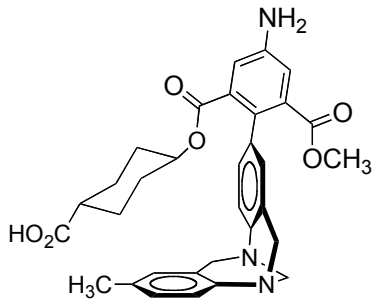
Method B: The vial with torsion **7c** (0.0100 g, 0.0151 mmol), 10% Pd/C (0.0033 g, 0.0030 mmol) in 0.15 mL of CH_2Cl_2:MeOH=1:1 solution was placed in Parr. It was evacuated and filled with H_2 for three times. The reaction was run under 45 PSI of hydrogen for 2-4 days at RT. The reaction mixture was filtered, concentrated. The crude was purified by flash column chromatography (SiO_2, MeOH/CH_2Cl_2 = 1/9) to yield 0.0083 g (0.0153 mmol, quantitative yield, R_f = 0.76, MeOH/CH_2Cl_2 = 15/85) of **6e**.

1-(4-(Hydroxymethyl)cyclohexyl) 3-methyl 5-amino-2-(8-methyl-6*H*,12*H*-5,11-methano dibenzo[*b*,*f*][1,5]diazocin-2-yl)isophthalate (6e). IR (liquid in CH_2Cl_2, cm^{-1}): υ 3700-3200 (broad), 2923, 2854, 1711, 1608, 1494, 1463, 1347, 1261, 1202, 1119, 1036, 1016; ^{1}H NMR (700 MHz, $CDCl_3$, 5 ˚C) **Folded (57%):** δ 7.03 (m, 4H), 6.95 (d, 2H, *J* = 7.7 Hz), 6.70 (br, 2H), 4.63 (br, 2H), 4.34 (br, 1H), 4.31 (br, 2H), 4.11 (dd, 2H, *J* = 16.8 and 22.4 Hz), 3.85/2.05 (br/br, NH_3^+/NH_2, 3H/2H), 3.56 (s, 3H), 3.32 (br, 2H), 2.21 (s, 3H), 1.49 (br, 1H), 1.39 (br, 1H), 1.27 (br, 1H), 1.04 (br, 2H), 0.73 (br, 1H), 0.61 (br, 1H), 0.55 (br, 1H), 0.43 (br, 1H); **Unfolded (43%):** 7.03 (m, 4H), 6.95 (d, 2H, *J* = 7.7 Hz), 6.70 (br, 2H), 4.63 (br, 2H), 4.61 (br, 1H), 4.31 (br, 2H), 4.11 (dd, 2H, *J* = 16.8 and 22.4 Hz), 3.85/2.05 (br/br, NH_3^+/NH_2, 3H/2H), 3.39 (br, 2H), 2.85 (s, 3H), 2.18 (s, 3H), 1.77 (br, 4H), 1.39 (br, 1H), 1.04 (br, 2H), 0.96 (br, 2H); ^{13}C NMR (500 MHz, $CDCl_3$) δ 169.73, 168.72, 146.86, 145.24, 135.72, 135.13, 134.25, 133.27, 130.06, 128.25, 128.10, 127.34, 127.00, 126.79, 125.50, 124.80, 123.95, 117.51, 117.32, 74.10, 67.62, 66.68, 58.48, 51.99, 51.12, 38.82, 34.20,



30.52, 30.28, 29.68, 26.98, 21.16, 20.81, 14.11; HRMS (ES) *m/z* calcd for $C_{32}H_{35}N_3O_6$ $[M\text{-}e]^+$ 541.2474, found 541.2506.

4-((5-Amino-3-(methoxycarbonyl)-2-(8-methyl-6*H*,12*H*-5,11-methanodibenzo[*b*,*f*][1,5] diazocin-2-yl)benzoyl)oxy)cyclohexane-1-carboxylic acid (6f). By the general procedure



(Method B), 0.0430 g (0.0637 mmol) of **7d**, 0.0136 g (0.127 mmol) of 10% Pd/C afforded 0.0244 g of **6f** (0.0440 mmol, 69% yield, R_f = 0.35, 100% EtOAc). IR (liquid in CH_2Cl_2, cm^{-1}): υ 3463, 3371, 3228, 2925, 2856, 1710, 1609, 1495, 1463, 1409, 1349, 1323, 1260, 1200, 1119, 1065, 1036, 1018, 966, 941, 906, 869, 842, 799, 721; ^1H NMR (700 MHz, $CDCl_3$, 5 ˚C) **Folded (54%):** δ 7.08 (m, 4H), 6.97 (br, 2H), 6.71 (dd, 2H, *J* = 25.2 and 25.2 Hz), 4.70 (br, 2H), 4.34 (br, 1H), 4.29 (br, 2H), 4.12 (dd, 2H, *J* = 16.1 and 23.8 Hz), 3.91/2.20 (br/br, NH_3^+/NH_2, 3H/2H), 3.56 (s, 3H), 2.19 (s, 3H), 1.78 (br, 1H), 1.58 (br, 1H), 1.42 (br, 2H), 1.20 (br, 1H), 1.08 (br, 1H), 1.04 (br, 1H), 0.59 (br, 1H), 0.34 (br, 1H); **Unfolded (46%):** δ 7.08 (m, 4H), 6.97 (br, 2H), 6.71 (dd, 2H, *J* = 25.2 and 25.2 Hz), 4.62 (br, 2H), 4.60 (br, 1H), 4.34 (br, 2H), 4.12 (dd, 2H, *J* = 16.1 and 23.8 Hz), 3.91/2.20 (br/br, NH_3^+/NH_2, 3H/2H), 2.96 (s, 3H), 2.19 (s, 3H), 2.18 (br, 1H), 1.93 (br, 1H), 1.89 (br, 1H), 1.73 (br, 1H), 1.67 (br, 1H), 1.42 (br, 2H), 1.04 (br, 1H), 0.86 (br, 1H); ^{13}C NMR (700 MHz, $CDCl_3$) δ 168.85, 168.51, 145.68, 144.76, 144.31, 135.77, 134.75, 134.08, 131.07, 129.96, 128.90, 128.54, 127.54, 127.23, 124.80, 124.06, 123.77, 73.23, 72.90, 66.88, 58.68, 58.52, 58.18, 58.26, 32.06, 30.62, 30.04, 29.83, 29.50, 29.27, 26.20, 22.83, 20.87, 16.39, 14.28; HRMS (ES) *m/z* calcd for $C_{32}H_{33}N_3O_6$ $[M\text{-}e]^+$ 555.2448, found 555.2496.

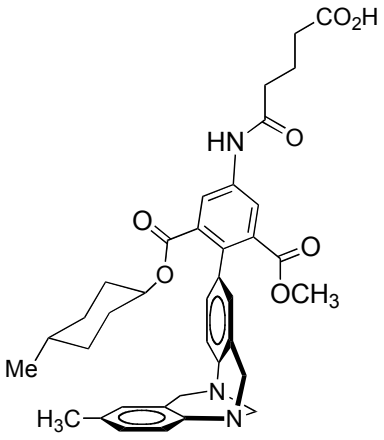
1-(4-(Hydroxymethyl)bicyclo[2.2.2]octan-1-yl) 3-methyl 5-amino-2-(8-methyl-6*H*,12*H*-5,11-methanodibenzo[*b*,*f*][1,5]diazocin-2-yl)isophthalate (6k). By the general procedure (Method B), 0.0148 g (0.0216 mmol) of **7i**, 0.0050 g (0.0043 mmol) of 10% Pd/C afforded 0.0057 g of **6k** (0.0101 mmol, 47% yield, R_f = 0.56, MeOH/EtOAc = 5/95). IR (liquid in CH_2Cl_2, cm^{-1}): υ 3550-3200 (broad), 2923, 1911, 1609, 1494, 1462, 1357, 1262, 1202, 1120, 1029; ^{1}H NMR (700 MHz, $CDCl_3$, 5 ˚C) **Folded (67%):** δ 7.09 (d, 2H, *J* = 11.9 Hz), 7.04 (d, 2H, *J* = 18.2 Hz), 6.96 (d, 2H, *J* = 8.4 Hz), 6.72 (dd, 2H, *J* = 31.2 and 31.2 Hz), 4.70 (d, 2H, *J* = 16.2 Hz), 4.31 (dd, 2H, *J* = 10.5 and 40.6 Hz), 4.14 (dd, 2H, *J* = 16.8 and 33.6 Hz), 3.83/2.01 (br/br, NH_3^+/NH_2, 3H/2H), 3.58 (s, 3H), 3.11 (s, 2H), 2.22 (s, 3H), 1.29 (br, 6H), 1.10 (br, 6H); **Unfolded (33%):** δ 7.09 (d, 2H, *J* = 11.9 Hz), 7.04 (d, 2H, *J* = 18.2 Hz), 6.96 (d, 2H, *J* = 8.4 Hz), 6.72 (dd, 2H, *J* = 31.2 and 31.2 Hz), 4.67 (br, 2H), 4.38 (br, 2H), 4.14 (dd, 2H, *J* = 16.8 and 33.6 Hz), 3.83/2.01 (br/br, NH_3^+/NH_2, 3H/2H), 3.23 (s, 2H), 2.91 (s, 3H), 2.22 (s, 3H), 1.70 (br, 6H), 1.48 (br, 6H); ^{13}C NMR (500 MHz, $CDCl_3$) δ 168.52, 167.81, 147.88, 146.97, 145.23, 135.76, 135.70, 135.42, 133.80, 133.25, 129.98, 128.27, 127.44, 126.74, 125.54, 124.98, 124.03, 117.56, 117.11, 114.86, 114.34, 81.66, 70.88, 66.59, 58.59, 58.34, 52.08, 50.92, 40.37, 34.24, 32.15, 30.59, 30.32, 29.72, 28.59, 28.11, 21.20, 21.06; HRMS (ES) *m/z* calcd for $C_{34}H_{37}N_3O_5$ $[M-e]^+$ 567.2631, found 567.2663.

4-((5-Amino-3-(methoxycarbonyl)-2-(8-methyl-6*H*,12*H*-5,11-methanodibenzo[*b*,*f*][1,5] diazocin-2-yl)benzoyl)oxy)bicyclo[2.2.2]octane-1-carboxylic acid (6l). By the general procedure (Method B), 0.0239 g (0.0341 mmol) of **7j**, 0.0073 g (0.0068 mmol) of 10% Pd/C afforded 0.0140 g of **6l** (0.0241 mmol, 71% yield, R_f = 0.57, MeOH/EtOAc = 1/9). IR (liquid in CH_2Cl_2, cm^{-1}): υ 3583, 3373, 2957, 2926, 1724, 1609, 1495, 1462, 1357, 1263, 1202, 1121, 1073, 1032; ^{1}H NMR (700 MHz, $CDCl_3$, 5 ˚C) **Folded (72%):** δ 7.71 (d, 1H, *J* = 2.8 Hz), 7.53 (d, 1H, *J* = 2.8 Hz), 7.03 (m, 4H), 6.73 (d, 2H, *J* = 56 Hz), 4.71 (d, 2H, *J* = 16.1 Hz), 4.31 (br, 2H), 4.13 (dd, 2H, *J* = 16.1 and 36.0 Hz), 3.84/2.04 (br/br, NH_3^+/NH_2, 3H/2H), 3.56 (s, 3H), 2.19 (s, 3H), 1.49 (br, 6H), 1.31 (br, 6H), 1.26 (br, 3H); **Unfolded (28%):** δ 7.71 (d, 1H, *J* = 2.8 Hz), 7.53 (d, 1H, *J* = 2.8 Hz), 7.03 (m, 4H), 6.73 (d, 2H, *J* = 56 Hz), 4.68 (br, 2H), 4.31 (br, 2H), 4.22 (ddd, 2H, *J* = 5.6 and 11.2 and 27.3 Hz), 3.84/2.04 (br/br, NH_3^+/NH_2, 3H/2H), 2.93 (s, 3H), 2.19 (s, 3H), 1.88 (br, 6H), 1.74 (br, 6H), 1.26 (br, 3H); ^{13}C NMR (700 MHz, $CDCl_3$) δ 168.52, 167.81, 167.74, 146.46, 145.35, 144.85, 135.67, 133.89, 133.62, 132.37, 132.25, 130.89, 130.85, 129.75, 128.75, 128.52, 127.12, 126.47, 124.79, 123.93, 117.56, 117.18, 68.11, 66.34, 60.39, 58.40, 58.10, 52.03, 38.66, 37.53, 29.65, 29.02, 28.87, 28.51, 28.17, 23.69, 22.94, 20.69, 14.02, 10.92; HRMS (ES) *m/z* calcd for $C_{34}H_{35}N_3O_6$ $[M-e]^+$ 581.2424, found 581.2454.

General procedure for synthesis of hydrophilic torsion balances 25.

A flask was charged with the amine torsion balances **6a** (0.0110 g, 0.0209 mmol), and glutaric anhydride (0.0048 g, 0.0419 mmol). It was evacuated and filled with Ar. Then 0.42 mL of anhydrous CH_2Cl_2 was added to dissolve the solid. The reaction was stirred at RT under Ar atmosphere for 16 h. The volatile components were removed under reduced pressure. The crude was purified by flash column chromatography (SiO_2, MeOH/CH_2Cl_2 = 15/85) to yield 0.0095 g (0.0149 mmol, 71% yield, R_f = 0.41, MeOH/CH_2Cl_2 = 15/85) of **5a**.

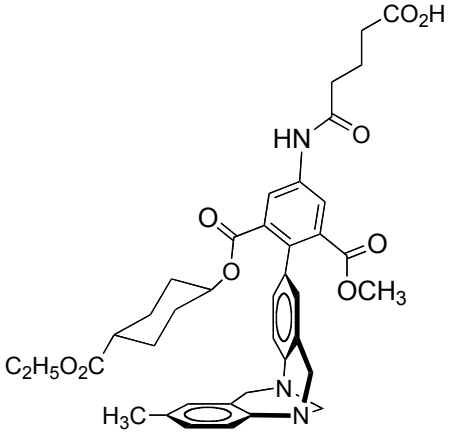
5-((3-(Methoxycarbonyl)-4-(8-methyl-6*H*,12*H*-5,11-metha nodibenzo[*b*,*f*][1,5]diazocin-2-yl)-5-(((4-methylcyclohexy l)oxy)carbonyl)phenyl)amino)-5-oxopentanoic acid (5a). IR (liquid in



CH_2Cl_2, cm^{-1}): υ 3600-3200 (broad), 2947, 2859, 1712, 1595, 1533, 1495, 1459, 1337, 1256, 1202, 1121; ^{1}H NMR (700 MHz, MeOD, 5 ˚C) **Folded (68%):** δ 8.07 (s, 1H), 8.01 (s, 1H), 7.13 (d, 1H, *J* = 7.7 Hz), 7.04 (d, 1H, *J* = 7.7 Hz), 7.00 (d, 1H, *J* = 7.7 Hz), 6.94 (d, 1H, *J* = 7.7 Hz), 6.80 (s, 1H), 6.73 (s, 1H), 4.70 (dd, 2H, *J* = 15.4 and 15.4 Hz), 4.38 (br, 1H), 4.33 (dd, 2H, *J* = 13.3 and 13.3 Hz), 4.17 (dd, 2H, *J* = 16.8 and 25.9 Hz), 3.58 (s, 3H), 2.46 (t, 2H, *J* = 7.0 Hz), 2.36 (t, 2H, *J* = 7.0 Hz), 2.23 (s, 3H), 1.98 (m, 2H, *J* = 7.0 Hz), 1.37 (br, 1H), 1.28 (br, 1H), 1.23 (br, 1H), 1.13 (br, 1H), 0.87 (br, 1H), 0.80 (br, 3H), 0.69 (br, 1H), 0.58 (br, 1H), 0.48 (br, 1H), 0.36 (br, 1H); **Unfolded (32%):** δ 8.07 (s, 1H), 8.01 (s, 1H), 7.13 (d, 1H, *J* = 7.7 Hz), 7.04 (d, 1H, *J* = 7.7 Hz), 7.00 (d, 1H, *J* = 7.7 Hz), 6.94 (d, 1H, *J* = 7.7 Hz), 6.80 (s,

1H), 6.73 (s, 1H), 4.60 (br, 2H), 4.56 (br, 1H), 4.26 (br, 2H), 4.17 (dd, 2H, *J* = 16.8 and 25.9 Hz), 2.95 (s, 3H), 2.46 (t, 2H, *J* = 7.0 Hz), 2.36 (t, 2H, *J* = 7.0 Hz), 2.23 (s, 3H), 1.98 (m, 2H, *J* = 7.0 Hz), 1.71 (br, 2H), 1.67 (br, 2H), 1.28 (br, 1H), 1.05 (br, 2H), 0.96 (br, 2H), 0.87 (br, 3H); ^{13}C NMR (500 MHz, MeOD) δ 174.36, 169.94, 148.37, 126.23, 139.59, 129.39, 136.55, 136.41, 136.01, 135.47, 135.26, 129.49, 129.42, 128.72, 128.49, 128.45, 126.33, 126.08, 125.54, 123.30, 123.10, 76.13, 67.68, 59.56, 59.29, 53.00, 37.27, 35.60, 35.07, 34.44, 33.96, 33.29, 32.54, 31.83, 31.09, 30.96, 22.45, 22.38, 21.43; HRMS (ES) *m/z* calcd for $C_{37}H_{41}N_3O_7$ $[M\text{-}e]^+$ 639.3023, found 639.3015.

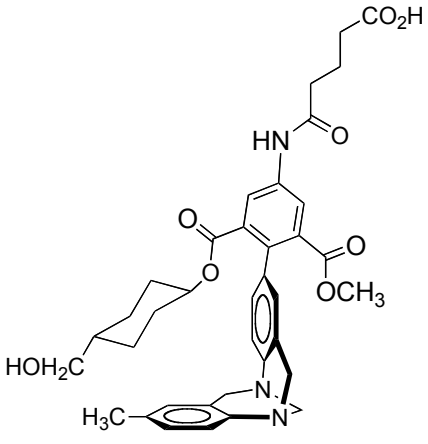
5-((3-(((4-(Ethoxycarbonyl)cyclohexyl)oxy)carbonyl)-5-(methoxycarbonyl)-4-(8-methyl-6*H*, 12*H*-5,11-methanodibenzo[*b*,*f*][1,5]diazocin-2-yl)phenyl)amino)-5-oxopentanoic acid (5b).



By the general procedure, 0.0135 g (0.0232 mmol) of **6b**, 0.0053 g (0.0463 mmol) of glutaric anhydride afforded 0.0123 g of **5b** (0.0176 mmol, 76% yield, R_f = 0.45, MeOH/CH_2Cl_2 = 15/85). IR (liquid in CH_2Cl_2, cm^{-1}): υ 2958, 1724, 1495, 1462, 1339, 1257, 1195, 1041; ^{1}H NMR (700 MHz, MeOD, 5 ˚C) **Folded (69%):** δ 8.09 (s, 1H), 8.04 (s, 1H), 7.09 (d, 1H, *J* = 6.3 Hz), 7.01 (d, 1H, *J* = 8.4 Hz), 6.95 (d, 1H, *J* = 8.4 Hz), 6.91 (d, 1H, *J* = 7.7 Hz), 6.74 (br, 2H), 4.63 (br, 2H), 4.34 (br, 1H), 4.27 (br, 2H), 4.13 (dd, 2H, *J* = 16.8 and 28.0 Hz), 4.08 (q, 2H, *J* = 6.3 Hz), 3.55 (s, 3H), 2.45 (t, 2H, *J* = 7.0 Hz), 2.38 (t, 2H, *J* = 7.0 Hz), 2.16 (s, 3H), 1.96 (m, 2H), 1.79 (br, 1H), 1.51 (br, 1H), 1.41

(br, 1H), 1.31 (br, 1H), 1.14 (t, 3H, *J* = 7.0 Hz), 1.12 (br, 2H), 1.05 (br, 1H), 0.50 (br, 1H), 0.34 (br, 1H); **Unfolded (31%):** δ 8.09 (s, 1H), 8.04 (s, 1H), 7.09 (d, 1H, *J* = 6.3 Hz), 7.01 (d, 1H, *J* = 8.4 Hz), 6.95 (d, 1H, *J* = 8.4 Hz), 6.91 (d, 1H, *J* = 7.7 Hz), 6.74 (br, 2H), 4.58 (br, 3H), 4.39 (br, 1H), 4.30 (br, 2H), 4.13 (dd, 2H, *J* = 16.8 and 16.1 Hz), 4.08 (q, 2H, *J* = 6.3 Hz), 2.89 (s, 3H), 2.45 (t, 2H, *J* = 7.0 Hz), 2.38 (t, 2H, *J* = 7.0 Hz), 2.20 (br, 1H), 2.16 (s, 3H), 1.96 (m, 2H), 1.86 (br, 2H), 1.73 (br, 2H), 1.51 (br, 1H), 1.41 (br, 1H), 1.14 (t, 3H, *J* = 7.0 Hz), 1.12 (br, 1H), 1.05 (br, 1H); ^{13}C NMR (500 MHz, MeOD) δ 177.14, 176.86, 174.24, 169.88, 169.38, 148.44, 146.25, 142.28, 139.60, 139.39, 136.57, 136.44, 135.83, 135.39, 129.55, 129.39, 128.75, 128.51, 128.43, 126.33, 126.13, 125.57, 123.41, 123.18, 74.69, 67.68, 61.70, 60.02, 59.58, 59.33, 52.87, 37.09, 35.60, 34.37, 34.23, 34.04, 33.28, 31.09, 30.96, 30.67, 27.52, 23.95, 22.10, 21.64, 21.54, 21.29, 21.10, 14.75; HRMS (ES) *m/z* calcd for $C_{39}H_{43}N_3O_9$ $[M-e]^+$ 697.3078, found 697.3040.

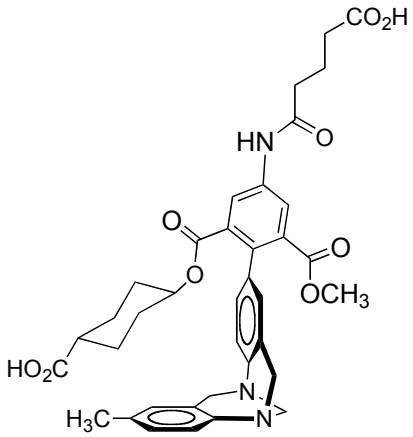
5-((3-(((4-(Hydroxymethyl)cyclohexyl)oxy)carbonyl)-5-(methoxycarbonyl)-4-(8-methyl-6*H*,12*H*-5,11-methanodibenzo[*b*,*f*][1,5]diazocin-2-yl)phenyl)amino)-5-oxopentanoic acid



(5e). By the general procedure, 0.0070 g (0.0129 mmol) of **6e**, 0.0030 g (0.0259 mmol) of glutaric anhydride afforded 0.0053 g of **5e** (0.0081 mmol, 63% yield, R_f = 0.18, MeOH/CH_2Cl_2 = 15/85). IR (liquid in CH_2Cl_2, cm^{-1}): υ 3600-3200 (broad), 2945, 1714, 1596, 1535, 1496, 1461, 1339, 1253, 1203; ^{1}H NMR (700 MHz, MeOD, 5 ˚C) **Folded (64%):** δ 8.07 (s, 1H), 8.01 (br, 1H), 7.13 (d, 1H, *J* = 8.4 Hz), 7.04 (d, 1H, *J* = 6.3 Hz), 7.00 (d, 1H, *J* = 7.7 Hz), 6.94 (d, 1H, *J* = 7.7 Hz), 6.81 (s, 1H), 6.73 (s, 1H), 4.69 (dd, 2H, *J* = 14.7 and 16.8 Hz), 4.39 (br, 1H), 4.33 (dd, 2H, *J* = 12.6 and 12.6

Hz), 4.17 (dd, 2H, *J* = 17.5 and 24.5 Hz), 3.80 (br, 2H), 3.58 (s, 3H), 2.46 (t, 2H, *J* = 7.0 Hz), 2.38 (t, 2H, *J* = 7.0 Hz), 2.20 (s, 3H), 1.97 (m, 2H, *J* = 7.0 Hz), 1.53 (br, 1H), 1.47 (br, 1H), 1.33 (br, 1H), 1.17 (br, 1H), 1.00 (br, 1H), 0.71 (br, 1H), 0.63 (br, 1H), 0.55 (br, 1H), 0.42 (br, 1H); **Unfolded (36%):** δ 8.07 (s, 1H), 8.01 (br, 1H), 7.13 (d, 1H, *J* = 8.4 Hz), 7.04 (d, 1H, *J* = 6.3 Hz), 7.00 (d, 1H, *J* = 7.7 Hz), 6.94 (d, 1H, *J* = 7.7 Hz), 6.81 (s, 1H), 6.73 (s, 1H), 4.60 (br, 3H), 4.28 (br, 2H), 4.17 (dd, 2H, *J* = 17.5 and 24.5 Hz), 3.88 (br, 2H), 2.94 (s, 3H), 2.46 (t, 2H, *J* = 7.0 Hz), 2.38 (t, 2H, *J* = 7.0 Hz), 2.20 (s, 3H), 1.97 (m, 2H, *J* = 7.0 Hz), 1.76 (br, 4H), 1.33 (br, 1H), 1.06 (br, 1H), 1.00 (br, 3H); ^{13}C NMR (500 MHz, MeOD) δ 174.98, 174.34, 139.60, 136.53, 135.38, 129.54, 129.47, 128.48, 126.05, 125.56, 123.31, 123.12, 76.19, 75.72, 69.78, 67.95, 37.21, 34.83, 34.56, 34.36, 30.96, 30.68, 28.38, 23.95, 22.33, 21.77, 21.37, 14.65; HRMS (ES) *m/z* calcd for $C_{37}H_{41}N_3O_8$ $[M-e]^+$ 655.2972, found 655.3007.

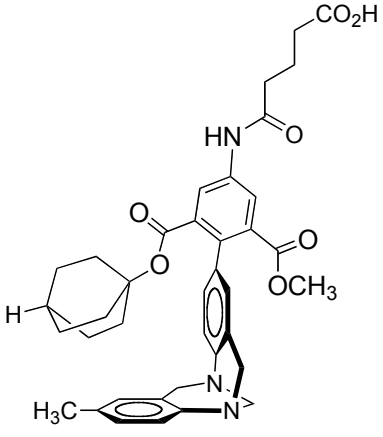
4-((5-(4-Carboxybutanamido)-3-(methoxycarbonyl)-2-(8-methyl-6*H*,12*H*-5,11-methano dibenzo[*b*,*f*][1,5]diazo cin-2-yl)benzoyl)oxy)cyclohexane-1-carboxylic acid (5f). By the



general procedure, 0.0134 g (0.0241 mmol) of 6**f**, 0.0055 g (0.0483 mmol) of glutaric anhydride afforded 0.0079 g of **5f** (0.0118 mmol, 49% yield, R_f = 0.29, MeOH/CH_2Cl_2 = 15/85). IR (liquid in CH_2Cl_2, cm^{-1}): υ 3500-3100 (broad), 2947, 1711, 1594, 1535, 1497, 1460, 1410, 1339, 1253, 1203; ^{1}H NMR (700 MHz, MeOD, 5 ˚C) **Folded (73%):** δ 8.10 (s, 1H), 8.03 (s, 1H), 7.10 (d, 1H, *J* = 7.7 Hz), 7.02 (d, 1H, *J* = 7.7 Hz), 6.97 (d, 1H, *J* = 7.7 Hz), 6.92 (d, 1H, *J* = 7.7 Hz), 6.79 (s, 1H), 6.73 (s, 1H), 4.66 (dd, 2H, *J* = 14.7 and 14.7 Hz), 4.36 (br, 1H), 4.28 (br, 2H), 4.14 (dd, 2H, *J* = 17.5 and 32.9 Hz), 3.56 (s, 3H), 2.46 (t, 2H, *J* =

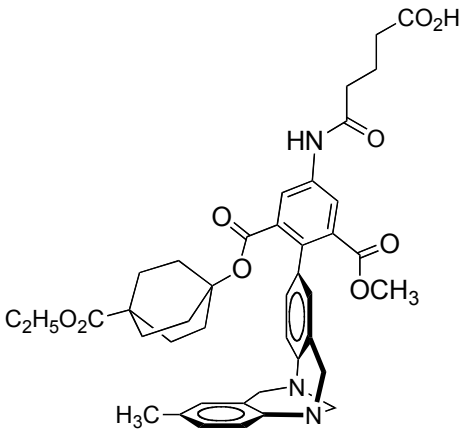
7.7 Hz), 2.38 (t, 2H, *J* = 7.7 Hz), 2.20 (s, 3H), 1.96 (m, 2H, *J* = 7.7 Hz), 1.69 (br, 1H), 1.50 (br, 1H), 1.39 (br, 1H), 1.32 (br, 1H), 1.14 (br, 2H), 1.04 (br, 1H), 0.52 (br, 1H), 0.34 (br, 1H); **Unfolded (27%):** δ 8.10 (s, 1H), 8.03 (s, 1H), 7.10 (d, 1H, *J* = 7.7 Hz), 7.02 (d, 1H, *J* = 7.7 Hz), 6.97 (d, 1H, *J* = 7.7 Hz), 6.92 (d, 1H, *J* = 7.7 Hz), 6.75 (s, 1H), 6.69 (s, 1H), 4.60 (br, 3H), 4.25 (br, 2H), 4.14 (dd, 2H, *J* = 17.5 and 32.9 Hz), 2.90 (s, 3H), 2.46 (t, 2H, *J* = 7.7 Hz), 2.38 (t, 2H, *J* = 7.7 Hz), 2.20 (s, 3H), 2.19 (br, 1H), 1.96 (m, 2H, *J* = 7.7 Hz), 1.86 (br, 2H), 1.75 (br, 2H), 1.44 (br, 2H), 1.14 (br, 1H), 1.04 (br, 1H); ^{13}C NMR (500 MHz, MeOD) δ 177.50, 174.25, 169.83, 169.37, 148.34, 146.09, 139.55, 136.56, 135.59, 135.38, 129.60, 129.40, 128.62, 128.43, 126.05, 125.52, 123.43, 123.21, 74.77, 67.54, 59.51, 59.21, 52.94, 42.67, 37.14, 34.58, 34.43, 30.63, 27.51, 22.15, 21.74, 21.25; HRMS (ES) *m/z* calcd for $C_{37}H_{39}N_3O_9$ $[M-e]^+$ 669.2765, found 669.2741.

5-((3-((Bicyclo[2.2.2]octan-1-yloxy)carbonyl)-5-(methoxycarbonyl)-4-(8-methyl-6*H*,12*H*-5,11-methanodibenzo[*b*,*f*][1,5]diazocin-2-yl)phenyl)amino)-5-oxopentanoic acid (5g). By the general procedure, 0.0084 g (0.0156 mmol) of **6g**, 0.0036 g (0.0313 mmol) of glutaric anhydride afforded 0.0084 g of **5g** (0.0129 mmol, 83% yield, R_f = 0.32, MeOH/CH_2Cl_2 = 5/95). IR (liquid in CH_2Cl_2, cm^{-1}): υ 3600-3100 (broad), 2924, 1712, 1596, 1460, 1342, 1252, 1202; ^{1}H NMR (700 MHz, MeOD, 5 ˚C) **Folded (73%):** δ 8.06 (s, 1H), 7.95 (s, 1H), 7.17 (d, 1H, *J* = 7.7 Hz), 7.08 (d, 1H, *J* = 6.3 Hz), 7.03 (d, 1H, *J* = 7.7 Hz), 6.94 (d, 1H, *J* = 7.7 Hz), 6.86 (s, 1H), 6.74 (s, 1H), 4.72 (br, 2H), 4.35 (br, 2H), 4.21 (dd, 2H, *J* = 16.8



and 21.7 Hz), 3.59 (s, 3H), 2.46 (t, 2H, *J* = 7.7 Hz), 2.38 (t, 2H, *J* = 7.0 Hz), 2.23 (s, 3H), 1.97 (m, 2H), 1.30 (br, 12H), 1.28 (br, 1H); **Unfolded (27%):** δ 8.06 (s, 1H), 7.95 (s, 1H), 7.17 (d, 1H, *J* = 7.7 Hz), 7.08 (d, 1H, *J* = 6.3 Hz), 7.03 (d, 1H, *J* = 7.7 Hz), 6.94 (d, 1H, *J* = 7.7 Hz), 6.86 (s, 1H), 6.74 (s, 1H), 4.72 (br, 2H), 4.35 (br, 2H), 4.21 (dd, 2H, *J* = 16.8 and 21.7 Hz), 2.97 (s, 3H), 2.46 (t, 2H, *J* = 7.7 Hz), 2.38 (t, 2H, *J* = 7.0 Hz), 2.23 (s, 3H), 1.97 (m, 2H), 1.75 (br, 12H), 1.50 (br, 1H); ^{13}C NMR (500 MHz, MeOD) δ 174.31, 139.54, 136.73, 135.37, 131.54, 129.81, 128.81, 128.55, 128.49, 125.64, 123.09, 122.81, 115.72, 83.52, 82.80, 73.87, 67.64, 60.93, 59.68, 59.43, 37.19, 34.75, 34.57, 34.40, 33.28, 30.96, 30.68, 30.17, 27.49, 25.29, 23.95, 22.30, 21.87, 21.25, 14.65; HRMS (ES) *m/z* calcd for $C_{38}H_{41}N_3O_7$ $[M\text{-}e]^+$ 651.3023, found 651.3076.

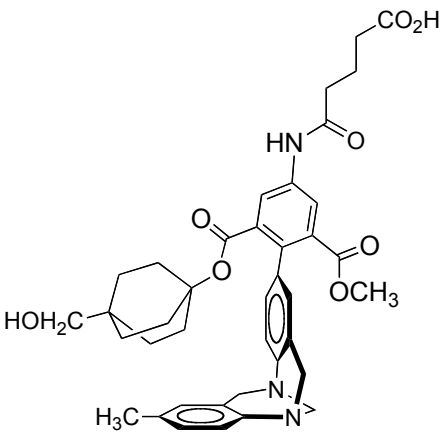
5-((3-(((4-(Ethoxycarbonyl)bicyclo[2.2.2]octan-1-yl)oxy)carbonyl)-5-(methoxycarbonyl)-4-(8-methyl-6*H*,12*H*-5,11-methanodibenzo[*b*,*f*][1,5]diazocin-2-yl)phenyl)amino)-5-oxopentanoic acid (5h). By the general procedure, 0.0094 g (0.0154 mmol) of **6h**, 0.0035 g



(0.0309 mmol) of glutaric anhydride afforded 0.0078 g of **5h** (0.0108 mmol, 70% yield, R_f = 0.42, MeOH/CH_2Cl_2 = 15/85). IR (liquid in CH_2Cl_2, cm^{-1}): υ 3582, 2928, 2360, 2340, 1716, 1465, 1328, 1255, 1206, 1070, 1020; ^{1}H NMR (700 MHz, MeOD, 5 °C) **Folded (79%):** δ 8.07 (s, 1H), 7.98 (s, 1H), 7.16 (d, 1H, *J* = 8.4 Hz), 7.04 (d, 1H, *J* = 8.4 Hz), 6.97 (d, 1H, *J* = 7.7 Hz), 6.89 (d, 1H, *J* = 8.4 Hz), 6.80 (s, 1H), 6.72 (s, 1H), 4.68 (dd, 2H, *J* = 16.1 and 16.1 Hz), 4.29 (dd, 2H, *J* = 22.4 and 12.6 Hz), 4.15 (dd, 2H, *J* = 16.8 and 42.7 Hz), 4.06 (q, 2H, *J* = 7.0 Hz), 3.57 (s, 3H), 2.45 (t, 2H, *J* =

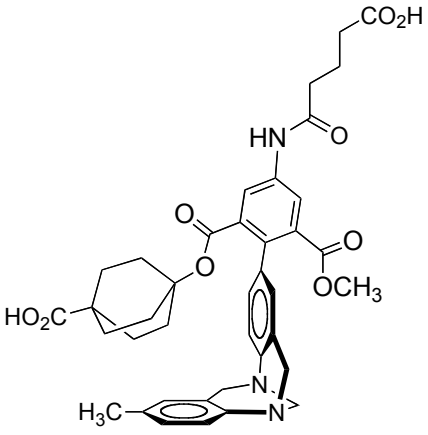
7.0 Hz), 2.38 (t, 2H, *J* = 7.0 Hz), 2.16 (s, 3H), 1.96 (m, 2H, *J* = 7.0 Hz), 1.44 (br, 6H), 1.24 (br, 6H), 1.19 (t, 2H, *J* = 7.0 Hz); **Unfolded (21%):** δ 8.07 (s, 1H), 7.98 (s, 1H), 7.16 (d, 1H, *J* = 8.4 Hz), 7.04 (d, 1H, *J* = 8.4 Hz), 6.97 (d, 1H, *J* = 7.7 Hz), 6.89 (d, 1H, *J* = 8.4 Hz), 6.77 (s, 1H), 6.68 (s, 1H), 4.59 (br, 2H), 4.38 (br, 2H), 4.15 (dd, 2H, *J* = 16.8 and 42.7 Hz), 4.06 (q, 2H, *J* = 7.0 Hz), 2.92 (s, 3H), 2.45 (t, 2H, *J* = 7.0 Hz), 2.38 (t, 2H, *J* = 7.0 Hz), 2.16 (s, 3H), 1.96 (m, 2H, *J* = 7.0 Hz), 1.82 (br, 6H), 1.75 (br, 6H), 1.19 (t, 2H, *J* = 7.0 Hz); ^{13}C NMR (500 MHz, MeOD) δ 178.42, 177.18, 174.23, 169.84, 169.06, 153.08, 148.47, 146.29, 139.56, 139.40, 136.83, 136.72, 136.32, 135.44, 135.22, 129.80, 129.35, 128.80, 128.63, 128.53, 128.47, 126.33, 126.22, 125.61, 123.22, 122.96, 82.53, 67.63, 61.78, 59.64, 59.39, 52.88, 39.17, 37.10, 35.60, 34.40, 31.09, 29.94, 29.58, 22.12, 21.53, 21.38, 14.71; HRMS (ES) *m/z* calcd for $C_{41}H_{45}N_3O_9$ $[M\text{-}e]^+$ 723.3234, found 723.3204.

5-((3-(((4-(Hydroxymethyl)bicyclo[2.2.2]octan-1-yl)oxy)carbonyl)-5-(methoxycarbonyl)-4-(8-methyl-6*H*,12*H*-5,11-methanodibenzo[*b*,*f*][1,5]diazocin-2-yl)phenyl)amino)-5-oxopentanoic acid (5k). By the general procedure, 0.0075 g (0.0132 mmol) of **6k**, 0.0030 g (0.0264 mmol) of glutaric anhydride afforded 0.0070 g of **5k** (0.0103 mmol, 78% yield, R_f = 0.24, MeOH/CH_2Cl_2 = 15/85). IR (liquid in CH_2Cl_2, cm^{-1}): υ 3650-3100 (broad), 2950, 1712, 1495, 1464, 1351, 1254, 1123; ^{1}H NMR (700 MHz, MeOD, 5 ˚C) **Folded (73%):** δ 8.05 (s, 1H), 7.94 (s, 1H), 7.14 (d, 1H, *J* = 8.4 Hz), 7.05 (d, 1H, *J* = 7.0 Hz), 7.01 (d, 1H, *J* = 7.7 Hz), 6.92 (d, 1H, *J* = 8.4 Hz), 6.84



(s, 1H), 6.73 (s, 1H), 4.70 (dd, 2H, *J* = 15.4 and 16.1 Hz), 4.30 (dd, 2H, *J* = 25.2 and 13.3 Hz), 4.20 (dd, 2H, *J* = 16.8 and 35.7 Hz), 3.58 (s, 3H), 3.25 (br, 1H), 3.02 (s, 2H), 2.46 (t, 2H, *J* = 7.0 Hz), 2.39 (t, 2H, *J* = 7.0 Hz), 2.25 (s, 3H), 1.97 (m, 2H, *J* = 7.0 Hz), 1.28 (br, 6H), 1.12 (br, 6H); **Unfolded (27%):** δ 8.05 (s, 1H), 7.94 (s, 1H), 7.14 (d, 1H, *J* = 8.4 Hz), 7.05 (d, 1H, *J* = 7.0 Hz), 7.01 (d, 1H, *J* = 7.7 Hz), 6.92 (d, 1H, *J* = 8.4 Hz), 6.79 (s, 1H), 6.69 (s, 1H), 4.62 (br, 2H), 4.41 (br, 2H), 4.20 (dd, 2H, *J* = 16.8 and 35.7 Hz), 3.41 (br, 1H), 3.12 (br, 2H), 2.95 (s, 3H), 2.46 (t, 2H, *J* = 7.0 Hz), 2.39 (t, 2H, *J* = 7.0 Hz), 2.25 (s, 3H), 1.97 (m, 2H, *J* = 7.0 Hz), 1.74 (br, 6H), 1.48 (br, 6H); ^{13}C NMR (500 MHz, MeOD) δ 174.27, 169.91, 169.10, 148.62, 146.28, 144.40, 139.53, 136.69, 136.26, 135.35, 129.76, 129.39, 128.76, 128.62, 128.46, 126.19, 125.60, 123.14, 122.87, 83.59, 70.80, 67.58, 67.12, 59.58, 59.33, 52.85, 37.13, 34.28, 33.45, 30.03, 29.79, 29.46, 22.21, 21.56, 15.64.

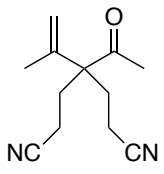
4-((5-(4-Carboxybutanamido)-3-(methoxycarbonyl)-2-(8-methyl-6*H*,12*H*-5,11-methano dibenzo[*b*,*f*][1,5]diazocin-2-yl)benzoyl)oxy)bicyclo[2.2.2]octane-1-carboxylic acid (5l). By



the general procedure, 0.0121 g (0.0208 mmol) of **6l**, 0.0048 g (0.0416 mmol) of glutaric anhydride afforded 0.0061 g of **5l** (0.0088 mmol, 42% yield, R_f = 0.16, MeOH/CH_2Cl_2 = 15/85). IR (liquid in CH_2Cl_2, cm^{-1}): υ 3600-3100 (broad), 2961, 1709, 1596, 1535, 1498, 1461, 1423, 1328, 1258, 1204, 1031; ^{1}H NMR (700 MHz, MeOD, 5 ˚C) **Folded (81%):** δ 8.06 (s, 1H), 7.96 (s, 1H), 7.14 (d, 1H, *J* = 8.4 Hz), 7.06 (d, 1H, *J* = 7.7 Hz), 7.00 (d, 1H, *J* = 7.7 Hz), 6.91 (d, 1H, *J* = 7.7 Hz), 6.83 (s, 1H), 6.73 (s, 1H), 4.71 (dd, 2H, *J* =

15.4 and 16.1 Hz), 4.32 (dd, 2H, *J* = 23.8 and 13.3 Hz), 4.18 (dd, 2H, *J* = 16.8 and 42.0 Hz), 3.58 (s, 3H), 2.46 (t, 2H, *J* = 7.7 Hz), 2.38 (t, 2H, *J* = 7.0 Hz), 2.20 (s, 3H), 1.96 (m, 2H, *J* = 7.0 Hz), 1.47 (br, 6H), 1.27 (br, 6H); **Unfolded (19%):** δ 8.06 (s, 1H), 7.96 (s, 1H), 7.14 (d, 1H, *J* = 8.4 Hz), 7.06 (d, 1H, *J* = 7.7 Hz), 7.00 (d, 1H, *J* = 7.7 Hz), 6.91 (d, 1H, *J* = 7.7 Hz), 6.79 (s, 1H), 6.69 (s, 1H), 4.63 (br, 2H), 4.41 (br, 2H), 4.18 (dd, 2H, *J* = 16.8 and 42.0 Hz), 2.94 (s, 3H), 2.46 (t, 2H, *J* = 7.7 Hz), 2.38 (t, 2H, *J* = 7.0 Hz), 2.20 (s, 3H), 1.96 (m, 2H, *J* = 7.0 Hz), 1.86 (br, 6H), 1.77 (br, 6H); ^{13}C NMR (500 MHz, MeOD) δ 182.04, 177.40, 174.27, 139.56, 136.74, 136.29, 135.70, 135.18, 133.88, 129.31, 128.61, 128.48, 125.64, 124.53, 123.17, 122.93, 82.68, 80.13, 59.64, 52.87, 37.15, 34.60, 34.46, 30.02, 29.66, 22,28, 21.80, 21.48; HRMS (ES) *m/z* calcd for $C_{39}H_{41}N_3O_9$ $[M-e]^+$ 695.2921, found 695.2981.

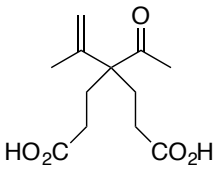
4-Acetyl-4-(prop-1-en-2-yl)heptanedinitrile (22). A solution of 1.503 g (15.315 mmol) of



Mesityl oxide and 0.5 mL of methanolic KOH (KOH: 0.044 g, 0.786 mmol; MeOH: 0.5 mL; 10% KOH in MeOH, w/w) was added to 3.9 mL of *t*-BuOH. The mixture was treated with 1.625 g (30.630 mmol) of acrylonitrile slowly. Reflux apparatus was set up due to exothermic. The reaction was stirred at room temperature for 17 hours. The volatile components were removed in reduced pressure. The residue was diluted with 30 mL of CH_2Cl_2 and 30 mL of water. The isolated aqueous layer was extracted with 5x30 mL of CH_2Cl_2. The combined organic layers were washed with brine, dried over $MgSO_4$ and filtered. The concentration, it afforded 3.437 g of crude. The crude was purified by flash chromatography (SiO_2, EtOAc/Hexanes = 2/3) to give 0.699 g (3.424 mmol, 25% yield) of pure product **22** (R_f =

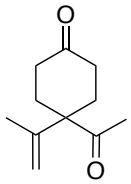
0.26, EtOAc/Hexanes = 3/7). ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.30 (s, 1H), 5.04 (s, 1H), 2.13 (m, 11H), 1.66 (s, 3H).

4-Acetyl-4-(prop-1-en-2-yl)heptanedioic acid (23). A mixture of 0.678 g (3.319 mmol) of **22**



and 0.465 g (8.297 mmol) of KOH in 5.0 mL of deionized water was heated at reflux for 17 hour. To the hot solution, it was added activated charcoal and stirred for one hour. The solution was filtered hot to remove the charcoal. The filtrate was cooled to 0 ˚C, acidified with concentrated HCl to pH = 0. The precipitate was collected by filtration, dried in vacuo, afforded 0.442 g (1.826 mmol, 55% yield) of crude. The crude **23** was used in the next step without further purification. IR (liquid in CH_2Cl_2, cm^{-1}): υ 3650-2900 (broad), 2925, 1708, 1636, 1596, 1416, 1382, 1282, 1195, 906, 734; ^{1}H NMR (400 MHz, MeOD) δ 5.22 (s, 1H), 5.12 (s, 1H), 2.09 (m, 11H), 1.64 (s, 3H); ^{13}C NMR (400 MHz, MeOD) δ 212.18, 177.05, 145.92, 115.84, 115.76, 61.08, 29.73, 26.29, 25.40.

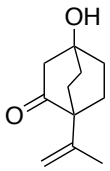
4-Acetyl-4-(prop-1-en-2-yl)cyclohexan-1-one (24). The mixture of **23** (0.400 g, 1.652 mmol)



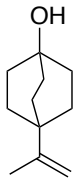
and potassium acetate (3.2 mg, 0.033 mmol) in 1.15 mL of acetic anhydride was heated at reflux for 4 hours, then cooled to room temperature. The solvent was removed by distillation under reduced pressure and the reaction was continuously heated until product came out as colorless oil. The reaction residue was purified by flash chromatography (SiO_2, EtOAc/Hexanes = 2/3) to give another portion of product. The combined product was 0.2023 g (1.129 mmol, 68% yield, R_f = 0.41, EtOAc/Hexanes = 3/7). IR (liquid in

CH_2Cl_2, cm^{-1}): υ 3600-3100 (broad), 2925, 1704, 1591, 1384, 1161, 1018; ^1H NMR (400 MHz, $CDCl_3$) δ 5.19 (s, 1H), 5.13 (s, 1H), 2.36 (m, 6H), 2.17 (s, 3H), 2.05 (m, 2H), 1.71 (s, 3H); ^{13}C NMR (400 MHz, $CDCl_3$) δ 210.21, 209.11, 143.99, 114.32, 56.71, 37.57, 30.69, 24.15, 19.63.

4-Hydroxy-1-(prop-1-en-2-yl)bicyclo[2.2.2]octan-2-one (25). A solution of 0.0275 g (0.492 mmol) of KOH in 0.13 mL of absolute EtOH was added to 0.0206 g (0.114 mmol) of **24**. The resulting yellow solution was stirred at room temperature for 22 hours. The mixture was diluted with 1 mL of deionized water, acidified with 1M HCl to pH = 0. The solution was extracted with 1.5 mL of EtOAc for five times. The combined organic solution was dried over $MgSO_4$, filtered and concentrated. The crude was purified by flash chromatography (SiO_2, EtOAc/Hexanes = 1/1) to give 0.0185 g (0.103 mmol, 90% yield) of pure product **25** (R_f = 0.25, EtOAc/Hexanes = 2/3). IR (liquid in CH_2Cl_2, cm^{-1}): υ 3600-3150 (broad), 2954, 2874, 1719, 1641, 1454, 1375, 1333, 1188, 1100, 894; ^1H NMR (400 MHz, $CDCl_3$) δ 4.97 (s, 1H), 4.73 (s, 1H), 2.45 (s, 2H), 2.04 (m, 2H), 1.92 (m, 2H), 2.80 (m, 4H), 1.76 (s, 3H); ^{13}C NMR (400 MHz, $CDCl_3$) δ 211.71, 144.85, 112.40, 69.86, 51.85, 50.46, 32.97, 26.92, 20.64.

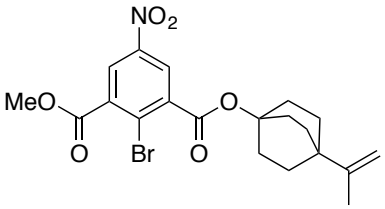


4-(Prop-1-en-2-yl)bicyclo[2.2.2]octan-1-ol (26). A solution of 0.0548 g (0.304 mmol) of **25** in 4.23 mL of diethylene glycol was treated with 1.33 mL (21.905 mmol) of hydrazine monohydrate (80%) at room temperature. The resulting mixture was heated at refluxed for 4 hours. The reaction was cooled to room temperature, added with 0.174 g (3.103 mmol) of KOH. Then it was warmed up to 200 ˚C over a period of 10 hours. After 6.5



hours of heating at 200 ˚C. The reaction was cooled to room temperature, acidified with 18 mL of 1M HCl, diluted with 10 mL of deionized water, extracted with EtOAc (6 X 28 mL). The combined organic layers were washed with brine , dried over $MgSO_4$, filtered and concentrated, affording 0.0623 g of crude. The crude was purified by flash chromatography (SiO_2, EtOAc/Hexanes = 2/3) to give 0.0171 g (0.103 mmol, 34% yield) of product **26** (R_f = 0.54, EtOAc/Hexanes = 1/1). IR (liquid in CH_2Cl_2, cm^{-1}): υ 3500-3200 (broad), 2924, 2854, 2345, 1735, 1460, 1377; ^1H NMR (500 MHz, $CDCl_3$) δ 4.68 (s, 1H), 4.65 (s, 1H), 2.45 (s, 2H), 1.69 (m, 6H), 1.59 (m, 9H); ^{13}C NMR (500 MHz, $CDCl_3$) δ 151.78, 108.74, 69.49, 34.61, 34.02, 31.04, 19.92.

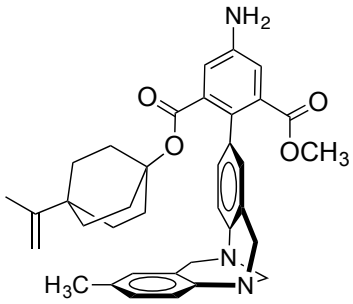
1-Methyl 3-(4-(prop-1-en-2-yl)bicyclo[2.2.2]octan-1-yl) 2-bromo-5-nitroisophthalate (27).



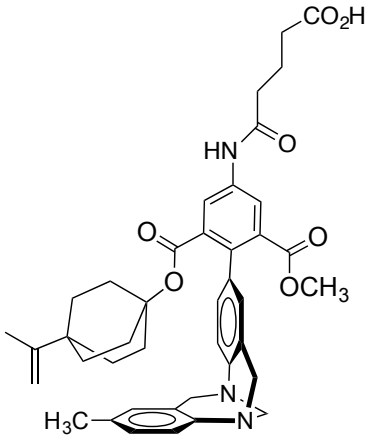
By the general DCC esterification procedure, 0.0439 g (0.145 mmol) of monoacid **9**, 0.0160 g (0.0963 mmol) of **26**, 0.0035 g (0.0289 mmol) of DMAP, 0.0569 g (0.289 mmol) of DCC in 2 mL of CH_2Cl_2 afforded 0.0314 g of **27** (0.0694 mmol, 72% yield, R_f = 0.58, EtOAc/Hexanes = 15/85). IR (liquid in CH_2Cl_2, cm^{-1}): υ 3071, 2924, 1737, 1607, 1532, 1435, 1352, 1294, 1235, 1185, 1153, 1036, 990, 740; ^1H NMR (500 MHz, $CDCl_3$) δ 8.51 (d, 1H, J = 2.4 Hz), 8.40 (d, 1H, J = 2.4 Hz), 4.71 (s, 1H), 4.68 (s, 1H), 4.00 (s, 3H), 2.20 (m, 6H), 1.82 (m, 6H), 1.71 (s, 3H); ^{13}C NMR (500 MHz, $CDCl_3$) δ 164.95, 163.83, 151.05, 146.29, 138.83, 136.17, 126.11, 126.02, 109.22. 85.27, 77.10, 53.44, 35.38, 30.83, 30.01, 19.60. HRMS (ES) *m/z* calcd for $C_{20}H_{23}BrNO_6$ $[M+H]^+$452.0703, found 452.0695.

1-Methyl 3-(4-(prop-1-en-2-yl)bicyclo[2.2.2]octan-1-yl) 2-(8-methyl-6*H*,12*H*-5,11-methano dibenzo[*b*,*f*][1,5]diazocin-2-yl)-5-nitroisophthalate (28). By the general Suzuki coupling procedure, 0.0269 g (0.0743 mmol) of **8**, 0.0280 g (0.0619 mmol) of **27**, 0.0069 g (0.0124 mmol) of Pd catalyst, 0.0312 g (0.372 mmol) of $NaHCO_3$ afforded 0.0257 g of **28** (0.0423 mmol, 68% yield, R_f = 0.19, Et_2O/Pentane = 3/1). IR (liquid in CH_2Cl_2, cm^{-1}): υ 3600-3100 (broad), 2923, 2852, 2360, 1712, 1597, 1458, 1378, 1246, 1155; ^1H NMR (700 MHz, $CDCl_3$, 5 ˚C) **Folded (71%):** δ 8.63 (s, 1H), 8.57 (s, 1H), 7.18 (d, 1H, *J* = 8.4 Hz), 7.04 (d, 1H, *J* = 7.7 Hz), 6.99 (d, 1H, *J* = 7.7 Hz), 6.96 (d, 1H, *J* = 7.7 Hz), 6.74 (d, 2H, *J* = 9.1 Hz), 4.72 (m, 2H), 4.67 (s, 1H), 4.55 (s, 1H), 4.33 (dd, 2H, *J* = 35 and 12.8 Hz), 4.16 (dd, 2H, *J* = 32.2 and 14.8 Hz), 3.69 (s, 3H), 2.16 (s, 3H), 1.61 (s, 3H), 1.30 (br, 12H); **Unfolded (29%):** δ 8.57 (s, 1H), 8.55 (s, 1H), 7.18 (d, 1H, *J* = 8.4 Hz), 7.04 (d, 1H, *J* = 8.4 Hz), 6.99 (d, 1H, *J* = 7.7 Hz), 6.96 (d, 1H, *J* = 7.7 Hz), 6.74 (d, 2H, *J* = 9.1 Hz), 4.72 (m, 2H), 4.67 (s, 1H), 4.61 (s, 1H), 4.33 (dd, 2H, *J* = 35 and 12.8 Hz), 4.16 (dd, 2H, *J* = 32.2 and 14.8 Hz), 3.00 (s, 3H), 2.21 (s, 3H), 1.81 (br, 6H), 1.66 (br, 6H), 1.61 (s, 3H); ^{13}C NMR (700 MHz, $CDCl_3$) δ 160.67, 150.92, 148.74, 146.35, 145.91, 145.28, 133.65, 132.90, 128.47, 127.64, 127.32, 127.15, 127.09, 125.90, 125.55, 124.91, 124.61, 109.03, 83.55, 65.87, 58.69, 43.66, 34.93, 32.02, 30.35, 30.22, 29.10, 24.89, 20.97, 19.44, 17.52, 17.35, 15.30; HRMS (ES) *m/z* calcd for $C_{36}H_{38}N_3O_6$ $(M+H)^+$ 608.2755, found 608.2735.

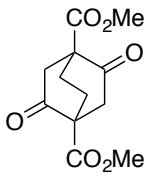
1-Methyl 3-(4-(prop-1-en-2-yl)bicyclo[2.2.2]octan-1-yl) 5-amino-2-(8-methyl-6*H*,12*H*-5,11-methanodibenzo[*b*,*f*][1,5] diazocin-2-yl)isophthalate (29). A solution of 0.0145 g (0.0238



mmol) of **28** in 0.6 mL of CH_2Cl_2 and 0.6 mL of MeOH was treated with 0.0452 g (0.239 mmol) of $SnCl_2$. It was stirred at room temperature for overnight. The volatile components were removed under reduced pressure. The residue was treated with 1 mL of saturated Na_2CO_3 solution, extracted with CH_2Cl_2 (10 X 1 mL). The combine organic layers were dried over $MgSO_4$, filtered, concentrated. The crude generated was 0.0114 g (0.0197 mmol, 83% yield, R_f = 0.20, 100% Et_2O). It was used in the next step without further purification. IR (liquid in CH_2Cl_2, cm^{-1}): υ 3368, 2952, 2924, 2870, 2360, 1735, 1607, 1516, 1462, 1365, 1258, 1204, 1119, 1029, 737; ^{1}H NMR (700 MHz, $CDCl_3$, 5 ˚C) **Folded (68%):** δ 7.08 (d, 2H, *J* = 11.2 Hz), 7.01 (m, 2H), 6.96 (d, 2H, *J* = 7.7 Hz), 6.71 (dd, 2H, *J* = 36.4, 36.0 Hz), 4.65 (m, 4H), 4.32 (m, 2H), 4.14 (dd, 2H, *J* = 16.1 and 30.1 Hz), 3.85/3.12 (br/br, NH_3^+/NH_2, 3H/2H), 3.57 (s, 3H), 2.17 (m, 3H), 1.59 (s, 3H), 1.26 (br, 12H); **Unfolded (32%):** δ 7.08 (d, 2H, *J* = 11.2 Hz), 7.01 (m, 2H), 6.96 (d, 2H, *J* = 7.7 Hz), 6.71 (dd, 2H, *J* = 36.4, 36.0 Hz), 4.65 (m, 4H), 4.32 (m, 2H), 4.14 (dd, 2H, *J* = 16.1 and 30.1 Hz), 3.85/3.12 (br/br, NH_3^+/NH_2, 3H/2H), 2.90 (s, 3H), 2.22 (s, 3H), 1.71 (br, 6H), 1.59 (s, 3H), 1.57 (br, 6H); ^{13}C NMR (400 MHz, $CDCl_3$) δ 168.57, 165.89, 151.23, 145.44, 145.20, 138.02, 135.56, 135,41, 135.35, 133.52, 133.41, 129.94, 128.27, 128.18, 127.31, 127.05, 126.74, 124.84, 124.43, 117.52, 117.05, 108.79, 59.07, 52.04, 34.89, 330.18, 29.68, 29.08, 29.01, 20.93, 19.42; HRMS (ES) *m/z* calcd for $C_{36}H_{40}N_3O_4$ $(M+H)^+$ 578.3019, found 578.3002.

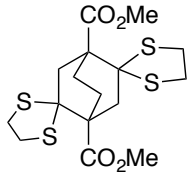
5-((3-(Methoxycarbonyl)-4-(8-methyl-6*H*,12*H*-5,11-methanodibenzo[*b*,*f*][1,5]diazocin-2-yl)-5-(((4-(prop-1-en-2-yl)bicyclo[2.2.2]octan-1-yl)oxy)carbonyl)phenyl)amino)-5-oxopentanoic acid (30).  By the general procedure, 0.0108 g (0.0187 mmol) of **29**, 0.0043 g (0.0374 mmol) of glutaric anhydride in 0.74 mL of CH_2Cl_2 afforded 0.0087 g of **30** (0.0126 mmol, 83% yield, R_f = 0.45, MeOH/CH_2Cl_2 = 15/85). IR (liquid in CH_2Cl_2, cm^{-1}): υ 3600-3100 (broad), 2925, 2855, 1711, 1665, 1596, 1535, 1495, 1461, 1407, 1348, 1327, 1254, 1205, 1122, 1030, 965, 891, 830, 800; ^{1}H NMR (700 MHz, MeOD, 5 ˚C) **Folded (79%):** δ 8.04 (s, 1H), 7.94 (s, 1H), 7.17 (d, 1H, *J* = 7.7 Hz), 7.07 (d, 1H, *J* = 8.4 Hz), 6.98 (d, 1H, *J* = 8.4 Hz), 6.93 (d, 1H, *J* = 8.4 Hz), 6.82 (s, 1H), 6.74 (s, 1H), 4.73 (dd, 2H, *J* = 5.6 and 15.4 Hz), 4.67 (s, 1H), 4.59 (s, 1H), 4.35 (dd, 2H, *J* = 12.6 and 30.1 Hz), 4.21 (dd, 2H, *J* = 16.8 and 33.6 Hz), 3.64 (br, 1H), 3.59 (s, 3H), 2.46 (t, 2H, *J* = 7.7 Hz), 2.38 (t, 2H, *J* = 7.0 Hz), 2.17 (s, 3H), 1.97 (p, 2H, *J* = 7.0 Hz), 1.64 (s, 3H), 1.31 (br, 12H); **Unfolded (21%):** δ 7.99 (s, 2H), 7.17 (d, 1H, *J* = 7.7 Hz), 7.07 (d, 1H, *J* = 8.4 Hz), 6.98 (d, 1H, *J* = 8.4 Hz), 6.93 (d, 1H, *J* = 8.4 Hz), 6.82 (s, 1H), 6.74 (s, 1H), 4.73 (dd, 2H, *J* = 5.6 and 15.4 Hz), 4.64 (br, 2H), 4.35 (dd, 2H, *J* = 12.6 and 30.1 Hz), 4.21 (dd, 2H, *J* = 16.8 and 33.6 Hz), 3.96 (s, 3H), 2.46 (t, 2H, *J* = 7.7 Hz), 2.38 (t, 2H, *J* = 7.0 Hz), 2.21 (s, 3H), 1.97 (p, 2H, *J* = 7.0 Hz), 1.78 (br, 6H), 1.68 (br, 6H), 1.64 (s, 3H); ^{13}C NMR (700 MHz, MeOD) δ 176.18, 173.09, 172.71, 168.31, 157.89, 155.94, 153.41, 151.08, 146.95, 144.28, 143.21, 141.35, 137.96, 133.80, 133.70, 133.61, 132.71, 126.96, 124.81, 124.43, 124.00, 121.55, 121.29, 108.17, 74.25, 65.98, 56.637, 56.64, 51.27, 47.90, 35.63, 34.71, 33.45, 33.28, 29.86, 28.77, 28.74, 26.38, 20.75, 18.32; HRMS (ES) *m/z* calcd for $C_{41}H_{46}N_3O_7$ $(M+H)^+$ 692.3336, found 692.3402.

Dimethyl 2,5-dioxobicyclo[2.2.2]octane-1,4-dicarboxylate (31). Dimethylsuccinylsuccinate



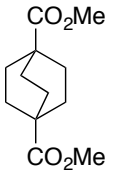
(70.0 g, 0.307 mol) was added by small portions to 34.4 g (0.859 mol, 60% in mineral oil, washed with pentane for 6 times) of NaH suspension in 245 mL of anhydrous DME. After completion, it was heated to 60 ˚C, treated with 122 mL (1.41 mol) of dibromoethane by cannula over 1.5 hours. The reaction was heated at 90 ˚C for 64 hours. The reaction mixture was cooled for a while and filtered through filter paper when it's still warm. The solid was washed twice with CH_2Cl_2. All the volatile components in the combined filtrate were removed under reduced pressure and unreacted dibromoethane was recovered by vacuum distillation. The distillation residue was recrystallized with MeOH, afforded 49.42 g of crude. The crude was purified by flash chromatography (SiO_2, EtOAc/Hexanes = 3/7, then flushed with MeOH/CH_2Cl_2 = 1/9) to give 34.39 g (0.135 mol, 53% yield based on used starting material) of product **31** (R_f = 0.08, EtOAc/Hexanes = 1/4, Melting point: 149 ˚C). 12.11 g (0.053 mol) of dimethyl succinylsuccinate was recovered from the solid of filtration by being suspended in water, acidified with concentrated HCl to pH = 1, extracted with CH_2Cl_2. IR (liquid in CH_2Cl_2, cm^{-1}): υ 3441, 2953, 2853, 1734, 1450, 1399, 1354, 1292, 1260, 1201, 1171, 1144, 1119, 1080, 1027, 950, 904, 833, 808, 785; ^1H NMR (500 MHz, $CDCl_3$) δ 3.80 (s, 6H), 3.07 (dd, 2H, *J* = 19.5 and 3.0 Hz), 2.73 (d, 2H, *J* = 19.5 Hz), 2.51 (dtd, 2H, *J* = 3.5, 11.5 and 3.0 Hz), 2.13 (dt, 2H, *J* = 3.0 and 11.0 Hz).

Dimethyl dispiro[[1,3]dithiolane-2,2'-bicyclo[2.2.2]octane-5',2''-[1,3]dithiolane]-1',4'-dicarboxylate (32). A solution of 22.0 g (85.6 mmol) of **31** in 57.7 mL of dithiolethane was



cooled to 0 ˚C, treated with 84.7 mL (519 mmol) of BF_3 etherate by small portions over one hour. After completion, the reaction was let to warm up to room temperature and stirred for 15 min. It was diluted with 200 mL of CH_2Cl_2, washed with 3 X 200 mL of 1M NaOH, 3 X 200 mL of 1M HCl, 1 X 200 mL of deionized water successively. The organic solution was dried over $MgSO_4$, filtered and concentrated. The crude **32** was 34.9 g (85.9 mmol, quantitative). The crude was used in the next step without further purification. IR (liquid in CH_2Cl_2, cm^{-1}): υ 3431, 2926, 2871, 1728, 1448, 1433, 1336, 1259, 1150, 1082, 1015, 967, 915, 857, 735, 700; ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.68 (s, 6H), 3.33 (m, 6H), 3.00 (m, 2H), 2.87 (d, 2H, *J* = 15.6 Hz), 2.51 (dtd, 2H, *J* = 17.6, 8.8 and 2.4 Hz), 2.13 (dt, 2H, *J* = 18 and 7.6 Hz).

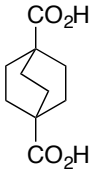
Dimethyl bicyclo[2.2.2]octane-1,4-dicarboxylate (33). A solution of 5.38 g (13.3 mmol) of **32**



in 213 mL of absolute EtOH was treated with 50 mL of Raney nickel (washed with EtOH for six times). The reaction was heated at reflux for one day (TLC checked to make sure the starting material had been depleted. If not, the reaction mixture was filtered, treated with more Raney Nickel in EtOH and run for longer time.). The reaction was cooled to room temperature and filtered through filter paper. The solid was washed with ethanol for several times. The combined organic portions were concentrated under reduced pressure. The greasy oil was diluted in CH_2Cl_2, filtered and concentrated to remove aluminum hydroxide. The afforded crude **33** was 2.446 g (10.82 mmol, 82% yield, R_f = 0.46, EtOAc/Hexanes = 1/4). The

crude was used in the next step without further purification. IR (liquid in CH_2Cl_2, cm^{-1}): υ 3424, 2954, 2924, 2873, 1722, 1456, 1434, 1358, 1257, 1167, 1080, 1001, 983, 945, 909, 853, 787, 704; ^1H NMR (300 MHz, $CDCl_3$) δ 3.65 (s, 6H), 1.81 (s, 12H).

Bicyclo[2.2.2]octane-1,4-dicarboxylic acid (34). A solution of 9.176 g (40.60 mmol) of **33** in



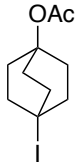
67 mL of MeOH was treated with 13.64 g (0.2430 mol) of KOH in 136 mL of MeOH and deionized water (1:1, v/v). The reaction was stirred at room temperature for overnight. The volume was reduced to about 70 mL under reduced pressure. The resulting aqueous solution was acidified with concentrated HCl to pH = 1. The suspension was filtered through filter paper. The solid was washed with deionized water and dried under reduced pressure. The filtrate was extracted with EtOAc for several times. The combined organic layers were washed with brine, dried over $MgSO_4$, filtered and concentrated. The combined product from filtration and extraction was 6.810 g (34.394 mmol, 85% yield). The crude **34** was used in the next step without further purification. IR (solid in mineral oil, cm^{-1}): υ 2953, 2923, 2854, 1460, 1376, 965, 429; ^1H NMR (400 MHz, MeOD) δ 1.82 (s, 12H).

1,4-Diiodobicyclo[2.2.2]octane (35). The flask was charged with 2.160 g (10.91 mmol) of **34**,

8.780 g (27.27 mmol) of iodobenzene diacetate and 6.921 g (27.268 mmol) of iodine, vacuated and filled with nitrogen. The mixture was treated with 182 mL of benzene. The resulting purple suspension was heated at reflux and irradiated with 250 W tungsten bulb for 4 hours. Then it was cooled to room temperature, 8.780 g (27.268 mmol) of

iodobenzene diacetate and 6.921 g (27.268 mmol) of iodine were added into the reaction under nitrogen. The reaction was again heated at reflux and irradiated with 250 W tungsten bulb for overnight. It was cooled to room temperature, washed with 3 X 180 mL of 10% (w/w) $Na_2S_2O_3$, 3 X 180 mL of brine. The organic layer was dried over $MgSO_4$ and filtered. After concentration, it afforded 15 mL of crude as light yellow oil. The crude was purified by flash chromatography (SiO_2, 100% Hexanes) to give 2.676 g (7.392 mmol, 68% yield) of product **35** (R_f= 0.42, 100% Hexanes). IR (liquid in CH_2Cl_2, cm^{-1}): υ 2954, 2923, 2854, 1462, 1377, 960, 429; ^1H NMR (400 MHz, $CDCl_3$) δ 2.56 (s, 6H); ^{13}C NMR (400 MHz, $CDCl_3$) δ 44.06, 29.71.

4-Iodobicyclo[2.2.2]octan-1-yl acetate (36). A solution of **35** (0.368 g, 1.018 mmol) in 47 mL



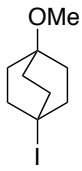
of acetic acid was treated slowly at reflux with increment of a hot suspension of sliver acetate (0.167 g, 0.998 mmol) in 26 mL of acetic acid. More and more yellow precipitate generated as silver acetate was added to the reaction. After the addition of silver acetate was complete, the reaction mixture was stirred at reflux for one hour. The acetic acid was removed by distillation. The residue was cooled to room temperature and treated with deionized water (~ 10 mL). The slurry was filtered through filter paper, and the solid was washed copiously with ether. The aqueous filtrate was further extracted with ether (3 X 10 mL). All the organic solution from filtration and extraction was combined and washed with brine, dried over $MgSO_4$ and filtered. All the volatile components were removed in reduced pressure. It afforded 0.277 g of crude as yellow solid. The crude was purified by flash chromatography (SiO_2, Et_2O/Pentane = 1/9) to give 0.179 g (0.609 mmol, 69% yield) of product **36** (R_f= 0.34, 100%

Hexanes). IR (liquid in CH_2Cl_2, cm^{-1}): υ 2922, 2851, 2352, 1730, 1462, 429; ^1H NMR (300 MHz, $CDCl_3$) δ 2.51 (t, 3H, *J* = 8.1 Hz), 2.07 (t, 6H, *J* = 8.1 Hz), 1.90 (s, 3H); ^{13}C NMR (400 MHz, $CDCl_3$) δ 169.90, 75.65, 41.35, 40.82, 33.31, 22.29.

4-Iodobicyclo[2.2.2]octan-1-ol (37). A mixture of 0.179 g (0.609 mmol) of **36** and 0.136 g

(2.436 mmol) of KOH was dissolved in a mixture of ethanol (3 mL) and water (1 mL). The reaction mixture was heated at 50 ˚C for overnight. The reaction was cooled to room temperature and volatile components were removed at reduced pressure. The residue was diluted with water and acidified with 1 M HCl to pH = 1. The acidic suspension was extracted with Et_2O (5 X 5 mL). The combined organic layers were washed with brine, dried with $MgSO_4$ and filtered. All the volatile components were removed in reduced pressure. It afforded 0.149 g (0.591 mmol, 97% yield) of crude **37** as white solid (R_f= 0.21, Et_2O/Pentane = 1/1). The crude was used in the next step without further purification. IR (liquid in CH_2Cl_2, cm^{-1}): υ 3500-3100 (broad), 2918, 1455, 1330, 1088; ^1H NMR (400 MHz, $CDCl_3$) δ 2.53 (t, 6H, *J* = 8.0 Hz), 1.75 (t, 6H, *J* = 8.0 Hz); ^{13}C NMR (400 MHz, $CDCl_3$) δ 65.20, 42.51, 40.82, 37.38.

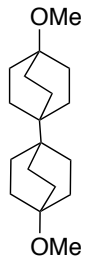
1-Iodo-4-methoxybicyclo[2.2.2]octane (38). A solution of **37** (0.148 g, 0.587 mmol) and methyl



iodide (0.235 g, 1.63 mmol) in 1.2 mL of anhydrous DME was treated with 0.022 g (0.931 mmol) of activated NaH (60% in mineral oil, washed with pentane for five times). The reaction was stirred at room temperature for three hours. The excess NaH in the reaction was quenched with 2 mL of water, acidified with 1M HCl to pH = 1. The isolated

aqueous layer was extracted with CH_2Cl_2 (4 X 5 mL). The combined organic layers were washed with brine, dried over $MgSO_4$ and filtered. All volatile components were removed in reduced pressure. The afforded crude was purified by flash chromatography (SiO_2, Et_2O/pentane = 1/9) to give 0.154 g (0.579 mmol, 99% yield) of product (R_f = 0.42, Et_2O/Pentane = 1/9). IR (liquid in CH_2Cl_2, cm^{-1}): υ 3650-3100 (broad), 2923, 2852, 1593, 1455, 1378, 1278, 1158; ^1H NMR (400 MHz, $CDCl_3$) δ 3.11 (s, 3H), 2.52 (t, 6H, *J* = 8.0 Hz), 1.76 (t, 6H, *J* = 8.0 Hz); ^{13}C NMR (400 MHz, $CDCl_3$) δ 69.11, 49.02, 42.56, 41.48, 33.07.

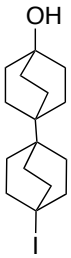
4,4'-Dimethoxy-1,1'-bi(bicyclo[2.2.2]octane) (40). ***1) Preparation of Rieke Magnesium.*** A



flame dried flask was charged with 0.150 g (1.579 mmol) of $MgCl_2$ and 0.266 g (1.601 mmol) of KI. It was vacuated and filled with argon. 3.7 mL of anhydrous THF and 0.111 g (2.842 mmol) of potassium were added to the flask successively. The reaction mixture was heated at reflux for two hours under argon. The grey suspension turned black and it was cooled to room temperature. The crude was used in the next step without further purification. ***2) Coupling reaction.*** The Rieke magnesium was treated with 0.064 g of (0.489 mmol) $NiCl_2$ and 0.106 g (0.398 mmol) of **38** in 2 .0 mL of anhydrous THF under argon. The reaction mixture was heated at reflux for overnight, then cooled to room temperature. THF in the reaction mixture was removed by distillation under nitrogen. The distillation residue was treated with 10 mL of water and filtered through filter paper. The solid was washed with ethanol and ether successively. The filtrate was separated with sep-funnel. The aqueous layer was extracted with Et_2O (10 X 4 mL). The combined organic layers were dried with $MgSO_4$ and filtered. All volatile components were removed in reduced pressure and 0.042 g of crude was

afforded. The crude was purified by flash chromatography (SiO_2, Et_2O/pentane = 1/4) to give 0.022 g (0.077 mmol, 39% yield) of product **40** (R_f = 0.21, Et_2O/Pentane = 1/9). IR (liquid in CH_2Cl_2, cm^{-1}): υ 3600-3200 (broad), 2946, 2865, 2822, 1591, 1458, 1349, 1236, 1198, 1156, 1102, 1031; ^1H NMR (400 MHz, $CDCl_3$) δ 3.11 (s, 6H), 1.56 (m, 12H), 1.45 (m, 12H); ^{13}C NMR (400 MHz, $CDCl_3$) δ 73.28, 48.97, 33.94, 29.26, 26.01; HRMS (ES) *m/z* calcd for $C_{18}H_{30}O_2$ $(M-e)^+$ 278.2246, found 278.2231.

4'-Iodo-[1,1'-bi(bicyclo[2.2.2]octan)]-4-ol (41). A solution of 0.020 g (0.070 mmol) of **40** in 1.6 mL of benzene was treated with 0.39 mL (2.9 mmol) of HI (57% solution). The reaction mixture was heated at reflux for one hour, then treated with 3 mL of ice. The isolated aqueous layer was extracted with ether (5 X 25 mL). The combined organic layers were washed with 3 mL of 10% (w/w) $Na_2S_2O_3$ and 3 mL of brine successively. Then it was dried over $MgSO_4$ and filtered. All the volatile components were removed in reduced pressure. The crude afforded was 0.028 g and purified by flash chromatography (SiO_2, Et_2O/Pentane = 1/4) to give 0.023 g (0.063 mmol, 89% yield) of product **41** (R_f = 0.24, Et_2O/Pentane = 1/1). IR (liquid in CH_2Cl_2, cm^{-1}): υ 3415, 2944, 2859, 1455; ^1H NMR (400 MHz, $CDCl_3$) δ 2.39 (t, 6H, *J* = 7.6 Hz), 1.50 (m, 12H), 1.43 (m, 6H); ^{13}C NMR (400 MHz, $CDCl_3$) δ 69.03, 46.55, 40.81, 34.58, 33.79, 31.52, 29.13, 26.17; HRMS (EI) *m/z* calcd for $C_{16}H_{25}O$ $(M-I)^+$ 233.1905, found 233.1938.

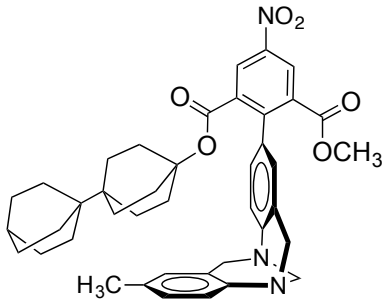


[1,1'-Bi(bicyclo[2.2.2]octan)]-4-ol (42). A flame-dried flask was charged with 0.0184 g (0.0510 mmol) of **41** and 2.0 mL of anhydrous Et_2O. The reaction mixture was cooled to -78 ˚C with acetone/dry ice. It was treated dropwise with 0.60 mL (0.36 mmol) of t-BuLi (0.6 M in pentane) under argon. After completion, it was let to warm up to room temperature, and stirred at room temperature for three hours. The reaction was quenched with 2.0 mL of saturated NH_4Cl solution. The isolated aqueous layer was extracted with Et_2O (5 X 3 mL). The combined organic layers were washed with brine, dried over $MgSO_4$. All the volatile components were removed in reduced pressure. It afforded 0.0124 g (0.0530 mmol, quantitative, Rf = 0.31, Et_2O/Hexanes = 1/1) of crude **42** as white solid. The crude was used in the next step without further purification. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.58 (m, 6H), 1.46 (m, 13H), 1.29 (m, 6H); ^{13}C NMR (400 MHz, $CDCl_3$) δ 69.30, 34.06, 33.73, 32.08, 26.32, 26.01, 24.73, 23.52. HRMS (ES) *m/z* calcd for $C_{16}H_{26}O$ $(M\text{-}e)^+$ 234.1984, found 234.1995.

1-([1,1'-Bi(bicyclo[2.2.2]octan)]-4-yl) 3-methyl 2-bromo-5-nitroisophthalate (43). By the general DCC esterification procedure, 0.0292 g (0.096 mmol) of monoacid **8**, 0.0150 g (0.064 mmol) of **42**, 0.0023 g (0.019 mmol) of DMAP, 0.040 g (0.19 mmol) of DCC in 3.2 mL of anhydrous CH_2Cl_2 afforded 0.020 g of **43** (0.038 mmol, 59% yield, R_f = 0.60, EtOAc/Hexanes = 1/4). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.50 (d, 1H, *J* = 2.4 Hz), 8.38 (d, 1H, *J* = 2.4 Hz), 3.99 (s, 3H), 2.08 (br, 6H), 1.57 (br, 6H), 1.48 (br, 7H), 1.31 (br, 6H); ^{13}C NMR (400 MHz, $CDCl_3$) δ 164.89, 163.76, 146.18, 138.85, 136.05, 126.02, 125.95, 125.91, 85.19, 53.33, 34.40, 33.76,

30.02, 26.18, 25.94, 24.66, 23.49; HRMS (ES) *m/z* calcd for $C_{25}H_{30}NO_6$ $(M\text{-}e)^+$ 519.1256, found 519.1249.

1-([1,1'-Bi(bicyclo[2.2.2]octan)]-4-yl) 3-methyl 2-(8-methyl-6*H*,12*H*-5,11-methanodibenzo[*b*,*f*][1,5]diazocin-2-yl)-5-nitroisophthalate (44). By the general procedure of

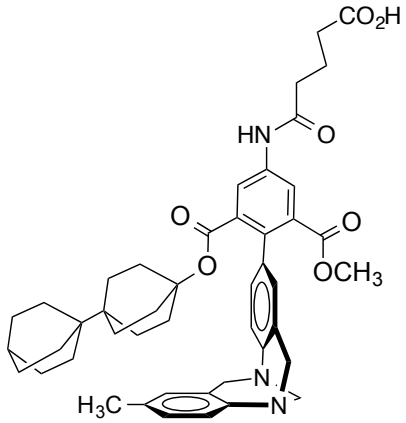


Suzuki coupling, 0.017 g (0.046 mmol) of **9**, 0.020 g (0.039 mmol) of **43**, 0.008 g (0.014 mmol) of Pd catalyst, 0.023 g (0.278 mmol) of $NaHCO_3$ in 0.2 mL of DME and 0.2 mL of water afforded 0.021 g of **44** (0.031 mmol, 80% yield, R_f = 0.25, EtOAc/Hexanes = 1/1). IR (liquid in CH_2Cl_2, cm^{-1}): υ 3388, 2924, 2854, 1598, 1454, 1378, 1275, 1156, 1117, 913, 745; ^1H NMR (700 MHz, $CDCl_3$, 5 ˚C) **Folded (68%):** δ 8.62 (s, 1H), 8.52 (s, 1H), 7.16 (d, 1H, *J* = 8.4 Hz), 7.03 (d, 1H, *J* = 8.4 Hz), 6.99 (br, 2H), 6.77 (s, 1H), 6.73 (s, 1H), 4.72 (m, 2H), 4.21 (m, 2H), 4.16 (dd, 2H, *J* = 16.8 and 27.3 Hz), 3.68 (s, 3H), 2.22 (m, 3H), 1.70-0.88 (br, 25H); **Unfolded (32%):** δ 8.55 (s, 2H), 7.16 (d, 1H, *J* = 8.4 Hz), 7.03 (d, 1H, *J* = 8.4 Hz), 6.99 (br, 2H), 6.77 (s, 1H), 6.73 (s, 1H), 4.72 (m, 2H), 4.21 (m, 2H), 4.16 (dd, 2H, *J* = 16.8 and 27.3 Hz), 3.03 (s, 3H), 2.22 (m, 3H), 1.70-0.88 (br, 25H); ^{13}C NMR (500 MHz, $CDCl_3$) δ 171.44, 166.84, 153.98, 153.12, 148.65, 146.27, 145.83, 133.42, 132.90, 130.36, 128.28, 127.60, 127.10, 127.02, 126.53, 125.78, 125.46, 124.81, 124.49, 117.22, 75.02, 60.39, 43.63, 36.65, 34.09, 33.64, 29.70, 25.93, 24.86, 24.65, 23.48, 21.05, 14.20; HRMS (ES) *m/z* calcd for $C_{41}H_{46}N_3O_6$ $(M+H)^+$ 676.3387, found 676.3372.

1-([1,1'-bi(bicyclo[2.2.2]octan)]-4-yl) 3-methyl 5-amino-2-(8-methyl-6*H*,12*H*-5,11-methanodibenzo[*b*,*f*][1,5]diazocin-2-yl)isophthalate (45). A solution of 0.0077 g (0.0114 mmol) of starting material **44** in 0.6 mL of CH_2Cl_2 and MeOH (1:1) was treated with 0.0216 g (0.114 mmol) of $SnCl_2$. The reaction was stirred at room temperature for overnight under N_2. The reaction was diluted with 1 mL of CH_2Cl_2 and quenched with 1 mL of saturated Na_2CO_3. The isolated aqueous layer was extracted with CH_2Cl_2 (5 X 1 mL). The combined organic solution was washed with brine, dried over $MgSO_4$ and filtered. All the volatile components were removed in reduced pressure. The afforded crude was 5.7 mg (0.00884 mmol, 78% yield, R_f = 0.25, EtOAc/Hexanes = 1/1). The purity of the crude **45** was checked by 1H NMR and was used in the next step without further purification. IR (liquid in CH_2Cl_2, cm^{-1}): υ 3600-3100 (broad), 2923, 2857, 1714, 1603, 1461, 1377, 1261, 1198, 1119, 1035, 799; ^{1}H NMR (700 MHz, $CDCl_3$, 5 ˚C) **Folded (72%):** δ 7.33 (d, 2H, *J* = 23.8 Hz), 7.02 (d, 1H, *J* = 12.6 Hz), 6.97 (m, 3H), 6.71 (br, 2H), 4.68 (d, 2H, *J* = 16.2 Hz), 4.31 (dd, 2H, *J* = 10.5 and 40.6 Hz), 4.14 (dd, 2H, *J* = 16.8 and 33.6 Hz), 3.83/2.01 (br/br, NH_3^+/NH_2, 3H/2H), 3.57 (s, 3H), 2.22 (s, 3H), 1.80-0.80 (br, 25H); **Unfolded (28%):** δ 7.33 (d, 2H, *J* = 23.8 Hz), 7.02 (d, 1H, *J* = 12.6 Hz), 6.97 (m, 3H), 6.71 (br, 2H), 4.68 (d, 2H, *J* = 16.2 Hz), 4.31 (dd, 2H, *J* = 10.5 and 40.6 Hz), 4.14 (dd, 2H, *J* = 16.8 and 33.6 Hz), 3.83/2.01 (br/br, NH_3^+/NH_2, 3H/2H), 2.94 (s, 3H), 2.22 (s, 3H), 1.80-0.80 (br, 25H); ^{13}C NMR (500 MHz, $CDCl_3$) δ 173.37, 167.70, 153.97, 149.02, 145.34, 145.18, 133.31, 133.04, 130.43, 129.04, 128.28, 128.16, 127.23, 127.01, 126.82, 124.82, 123.98, 120.56, 116.67, 116.27, 77.22, 75.05, 43.64,

43.51, 34.09, 33.63, 29.71, 25.96, 24.86, 24.63, 23.51, 22.70, 14.13; HRMS (ES) *m/z* calcd for $C_{41}H_{48}N_3O_4$ $(M+H)^+$ 646.3645, found 646.3668.

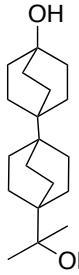
5-((3-(([1,1'-bi(bicyclo[2.2.2]octan)]-4-yloxy)carbonyl)-5-(methoxycarbonyl)-4-(8-methyl-6*H*,12*H*-5,11-methanodibenzo[*b*,*f*][1,5]diazocin-2-yl)phenyl)amino)-5-oxopentanoic acid (46). By the general procedure of ring opening acylation with glutaric anhydricd, 0.0101 g



(0.0156 mmol) of **45**, 0.0036 g (0.0313 mmol) of glutaric anhydride in 0.31 mL of anhydrous CH_2Cl_2 afforded 0.0023 (0.0030 mmol, 36% yield based on used starting material) g of **46** and 0.0047 g (0.0073 mmol, R_f = 0.40, MeOH/CH_2Cl_2 = 15/85) of **45** recovered. IR (liquid in CH_2Cl_2, cm^{-1}): υ 3600-3100 (broad), 2923, 2854, 1730, 1650, 1574, 1495, 1456, 1378, 1248, 1204, 1124, 832; ^{1}H NMR (700 MHz, MeOD, 5 ˚C) **Folded (76%):** δ 8.14 (s, 1H), 8.04 (s, 1H), 7.16 (d, 1H, *J* = 8.4 Hz), 7.07 (d, 1H, *J* = 6.3 Hz), 7.03 (d, 1H, *J* = 12.6 Hz), 6.96 (d, 1H, *J* = 7.0 Hz), 6.86 (s, 1H), 6.74 (s, 1H), 4.72 (m, 2H), 4.32 (m, 2H), 4.14 (dd, 2H, *J* = 16.1 and 23.1 Hz), 3. 62 (br, 1H), 3.59 (s, 3H), 2.36 (t, 2H, *J* = 7.7 Hz), 2.28 (s, 3H), 2.20 (t, 2H, *J* = 7.7 Hz), 1.96 (p, 2H, *J* = 7.7 Hz), 2.0-0.80 (br, 25H); **Unfolded (24%):** δ 8.08 (br, 2H), 7.16 (d, 1H, *J* = 8.4 Hz), 7.07 (d, 1H, *J* = 6.3 Hz), 7.03 (d, 1H, *J* = 12.6 Hz), 6.96 (d, 1H, *J* = 7.0 Hz), 6.81 (s, 1H), 6.71 (s, 1H), 4.72 (m, 2H), 4.32 (m, 2H), 4.14 (dd, 2H, *J* = 16.1 and 23.1 Hz), 3.62 (br, 1H), 2.99 (s, 3H), 2.36 (t, 2H, *J* = 7.7 Hz), 2.25 (s, 3H), 2.20 (t, 2H, *J* = 7.7 Hz), 1.96 (p, 2H, *J* = 7.7 Hz), 2.0-0.80 (br, 25H); ^{13}C NMR (500 MHz, $CDCl_3$) δ 173.37, 167.70, 153.97, 149.02, 145.34, 145.18, 133.31, 133.04, 130.43, 129.04, 128.28, 128.16, 127.23, 127.01, 126.82, 124.82,

123.98, 120.56, 116.67, 116.27, 77.22, 75.05, 43.64, 43.51, 34.09, 33.63, 29.71, 25.96, 24.86, 24.63, 23.51, 22.70, 14.13; HRMS (ES) *m/z* calcd for $C_{46}H_{54}N_3O_7$ $(M+H)^+$ 760.3956, found 760.3951.

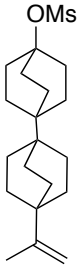
4'-(2-Hydroxypropan-2-yl)-[1,1'-bi(bicyclo[2.2.2]octan)]-4-ol (48). ***Method A) Direct Addition:*** A flame-dried flask was charged with 0.0500 g (0.139 mmol) of **41** and 3.5 mL of



anhydrous Et_2O. The reaction mixture was cooled to -78 ˚C with acetone/dry ice bath. It was treated dropwise with 0.61 mL (0.97 mmol) of t-BuLi (1.6 M in pentane) under argon. After completion, it was stirred at -78 ˚C for 45 min, then treated with 0.10 mL (1.39 mmol) of freshly distilled acetone. The reaction was let to warm up to room temperature, and stirred at room temperature for four hours. The reaction was quenched with 5 mL of saturated NH_4Cl solution. The isolated aqueous layer was extracted with Et_2O (5 X 15 mL). The combined organic layers were washed with brine, dried over $MgSO_4$, filtered. All the volatile components were removed in reduced pressure. It afforded 0.1072 g of crude as white solid. The crude was purified by flash chromatography (SiO_2, EtOAc/Hexanes = 3/2) to give 0.0082 g (0.028 mmol, 20% yield) of product **48** (R_f= 0.31, EtOAc/Hexanes = 1/1) and 0.0249 mg (0.106 mmol, 77% yield) of **42** (R_f= 0.67, EtOAc/Hexanes = 1/1) as side product. ***Method B) Grignard Reaction:*** A flame dried flask was charged with 0.0160 g (0.0435 mmol) of **53** and 4.3 mL of anhydrous THF. It was treated with 0.87 mL of methyl Grignard reagent (MeMgBr, 3.0 M, 2.609 mmol) at room temperature under nitrogen. The reaction was heated at reflux for overnight, then cooled to room temperature. The excess Grignard reagent was quenched with

several drops of deionized water until no more bubbles generated. All volatile organic components were removed in reduced pressure. The residue was diluted with 2.0 mL of deionized water and acidified with 1M HCl to pH=1. It was extracted with 5 X 4 mL of ether. The combined organic layers were dried over $MgSO_4$, filtered. After all volatile components were removed in reduced pressure, it afforded crude as white solid. The crude was purified by flash chromatography (SiO_2, EtOAc/Hexanes = 3/2) to give 0.0089 g (0.031 mmol, 70% yield, R_f = 0.21, EtOAc/Hexanes = 1/1) of product **48**. IR (liquid in CH_2Cl_2, cm^{-1}): υ 3500-3300 (broad), 2949, 2924, 2863,1730, 1457, 1376, 1332, 1261, 1187, 1102, 938; ^1H NMR (300 MHz, $CDCl_3$) δ 1.57 (m, 6H), 1.46 (m, 6H), 1.41 (m, 6H), 1.35 (m, 6H), 1.25 (s, 6H); ^{13}C NMR (400 MHz, $CDCl_3$) δ 67.48, 67.25, 34.33, 34.19, 32.24, 31.86, 26.47, 25.84, 25.34, 25.15. HRMS (ES) *m/z* calcd for $C_{19}H_{31}O$ $(M\text{-}OH)^+$ 275.2375, found 275.2386.

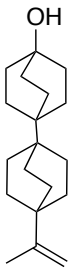
4'-(Prop-1-en-2-yl)-[1,1'-bi(bicyclo[2.2.2]octan)]-4-yl methanesulfonate (49). A mixture of



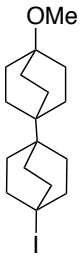
0.0140 g (0.0479 mmol) of **48** and 0.0176 g (0.144 mmol) of 4-dimethylaminopyridine was dissolved in 1.0 mL of anhydrous CH_2Cl_2 and added with 0.080 mL of triethyl amine under nitrogen. The reaction was cooled to 0 ˚C and treated with 0.05 mL (0.62 mmol) of distilled mesyl chloride dropwise. After completion it was let to warmed up to room temperature and stirred at room temperature for two days. To the reaction it was added 0.10 mL of 1,8-Diazabicyclo[5.4.0]undec-7-ene (DBU) and stirred continuously for another 24 h. The reaction was diluted with 2 mL of water and 2 mL of CH_2Cl_2. The isolated aqueous layer was extracted with 5 X 3 ML of CH_2Cl_2. The combined organic layers were dried over $MgSO_4$ and filtered. All the volatile components were removed in reduced pressure, and it afforded 0.106 g of crude. The crude was purified by flash chromatography (SiO_2, EtOAc/Hexanes =

3/17) to give 0.0089 g (0.0253 mmol, 53% yield) of product **49** (R_f = 0.37, EtOAc/Hexanes = 3/17). IR (liquid in CH_2Cl_2, cm^{-1}): υ 2957, 2927, 2873, 1636, 1459, 1353, 1333, 1175, 954, 918, 869, 831, 764; ^1H NMR (400 MHz, $CDCl_3$) δ 4.63 (d, 2H, *J* = 10.4 Hz), 2.96 (s, 3H), 2.06 (m, 6H), 1.66 (s, 3H), 1.56 (m, 6H), 1.49 (m, 6H), 1.34 (m, 6H); ^{13}C NMR (400 MHz, $CDCl_3$) δ 152.98, 108.00, 91.93, 40.81, 35.18, 34.01, 33.84, 31.42, 29.78, 26.60, 25.23, 19.54. HRMS (ES) *m/z* calcd for $C_{20}H_{33}O_3S$ $(M+H)^+$ 353.2150, found 353.2133.

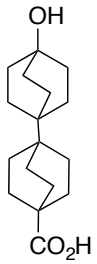
4'-(Prop-1-en-2-yl)-[1,1'-bi(bicyclo[2.2.2]octan)]-4-ol (50). A solution of 0.087 g (0.025 mmol) of **49** in 2.0 mL of anhydrous THF was treated with 0.50 mL of Super Hydride ($LiBHEt_3$, 1.0 M in THF) under nitrogen. The reaction as heated at reflux for overnight, then cooled to room temperature. It was quenched with 1.0 mL of MeOH and heated at reflux for another 5 min. The reaction was again cooled to room temperature and treated with 1.0 mL of water. All the volatile components were removed in reduced pressure. The residue was acidified with 1M HCl to pH = 2, extracted with 5 X 5 mL of Et_2O. The combined organic layers were dried over $MgSO_4$, filtered and concentrated in reduced pressure, afforded 0.0147 g of crude. The crude was purified by flash chromatography (SiO_2, EtOAc/Hexanes = 7/18) to give 0.0034 g (0.012 mmol, 61% yield) of product **50** (R_f = 0.25, EtOAc/Hexanes = 18/82) and 0.0015 g (0.0043 mmol) of recovered starting material **49**. IR (liquid in CH_2Cl_2, cm^{-1}): υ 3600-3100 (broad), 2946, 2922, 2854, 1709, 1592, 1458, 1377, 1278, 1114; ^1H NMR (400 MHz, $CDCl_3$) δ 4.63 (d, 2H, *J* = 10.4 Hz), 1.66 (s, 3H), 1.57 (m, 6H), 1.49 (m, 12H), 1.36 (m, 6H); ^{13}C NMR (400 MHz, $CDCl_3$) δ 153.30, 107.87, 69.29, 35.31, 34.27, 34.12, 34.05, 29.93, 26.38, 25.35, 19.56; HRMS (ES) *m/z* calcd for $C_{19}H_{31}O$ $(M+H)^+$ 275.2375, found 275.2382.



4-iodo-4'-methoxy-1,1'-bi(bicyclo[2.2.2]octane) (51). The mixture of methyl iodide (0.0405 g, 0.2843 mmol) and alcohol **41** (0.0023 g, 0.0064 mmol) was treated with 0.0123 g (0.3067 mmol, washed with hexanes for three times) of NaH in 0.16 mL of anhydrous DME at room temperature under nitrogen atmosphere. The reaction was stirred for 4.5 h. The reaction was treated with 0.5 mL of water and acidified with 1 M HCl to pH = 1. The isolated aqueous layer further extracted with CH_2Cl_2 for five times. The combined organic layers were washed with brine, dried over $MgSO_4$ and filtered. All the volatile components were removed in reduced pressure. It afforded 3.5 mg of crude. The crude was purified by flash chromatography (SiO_2, Et_2O/Pentane = 1/4) to give 0.0025 g (0.0067 mmol, quantitative, R_f = 0.23, Et_2O/Pentane = 1/3) of product **51**. IR (liquid in CH_2Cl_2, cm^{-1}): υ 2943, 2862, 1592, 1454, 1228, 1178, 1102, 1000, 944, 817; ^1H NMR (400 MHz, $CDCl_3$) δ 3.14 (s, 3H), 2.34 (t, 6H, J = 7.6 Hz), 1.55 (m, 12H), 1.42 (t, 6H, J = 4.0 Hz); ^{13}C NMR (400 MHz, $CDCl_3$) δ 73.12, 49.01, 46.69, 40.84, 34.50, 31.56, 29.15, 29.10, 25.86.

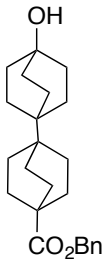


4'-Hydroxy-[1,1'-bi(bicyclo[2.2.2]octane)]-4-carboxylic acid (52). A flame-dried flask was charged with 0.0512 g (0.1422 mmol) of **41** and 10 mL of anhydrous Et_2O. The reaction mixture was cooled to -78 ˚C with acetone/dry ice bath. It was treated dropwise with 1.07 mL (1.71 mmol) of t-BuLi (1.6 M in pentane) under argon. After completion, it was stirred at -78 ˚C for 45 min. Freshly crushed dry ice (~10 mL) was added to the reaction under Argon. It was let to warm up to room temperature and stirred for three hours. The reaction mixture was treated with 1 mL of 1M NaOH. The yellow suspension



was extracted with 1M NaOH (5 X 10 mL). The combined aqueous layers were acidified with concentrated HCl to pH = 1, then extracted with Et_2O (5 X 25 mL). The combined organic layers were dried over $MgSO_4$ and filtered. All the volatile components were removed in reduced pressure. It afforded crude 0.0565 g. The crude was purified by flash chromatography (SiO_2, EtOAc/Hexanes = 3/1) to give 0.0203 g (0.073 mmol, 51% yield, R_f= 0.23, EtOAc/Hexanes = 7/3) of product **53**. The organic layer after basic extraction was dried over MgSO4, filtered and concentrated in reduced pressure. It afforded 0.0113 g (0.0483 mmol, 34% yield, R_f = 0.83, EtOAc/Hexanes = 7/3) of **52**. IR (liquid in CH_2Cl_2, cm^{-1}): υ 3600-3300 (broad), 2945, 2864, 1691, 1455, 1346, 1261, 1095; ^{1}H NMR (300 MHz, MeOD) δ 1.70 (m, 6H), 1.53 (m, 12H), 1.41 (m, 6H); ^{13}C NMR (500 MHz, MeOD) δ 182.57, 69.64, 39.29, 35.77, 35.46, 34.70, 29.76, 27.56, 25.84.

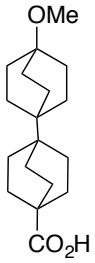
Benzyl 4'-hydroxy-[1,1'-bi(bicyclo[2.2.2]octane)]-4-carboxylate (53). A solution of 0.0180 g



(0.0647 mmol) of **52** and 0.0194 g (0.194 mmol) of $NaHCO_3$ in 0.65 mL of anhydrous DMF was stirred at room temperature for 15 min, then treated with 44 μL of benzyl bromide under Argon. The reaction was heated at 40 ˚C for two days. It was cooled to room temperature and added with 4 mL of water. The aqueous solution was extracted with 5 X 5 mL of ether. The combined organic layers were dried over $MgSO_4$ and filtered. All the volatile components were removed in reduced pressure and afforded crude. The crude was purified by flash chromatography (SiO_2, EtOAc/Hexanes = 3/7) to give 0.0129 g (0.0351 mmol, 99% yield based on used starting material) of product **53** (R_f= 0.28, EtOAc/Hexanes = 1/3) and 0.0082 g (0.0295 mmol) of recovered starting material. IR (liquid in CH_2Cl_2, cm^{-1}): υ 3400-3100

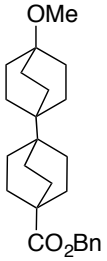
(broad), 2947, 1722, 1456, 1222, 1094; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.32 (m, 5H), 5.07 (s, 2H), 1.73 (m, 6H), 1.57 (m, 6H), 1.48 (m, 6H), 1.37 (m, 6H); ^{13}C NMR (400 MHz, $CDCl_3$) δ 177.97, 136.44, 128.52, 128.45, 128.19, 127.91, 127.63, 69.15, 65.78, 38.25, 34.46, 34.19, 33.92, 28.33, 26.39, 24.56.

4'-Methoxy-[1,1'-bi(bicyclo[2.2.2]octane)]-4-carboxylic (54). A flame-dried flask was charged

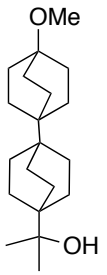


with 0.0047 g (0.013 mmol) of **51** and 1.25 mL of anhydrous Et_2O. The reaction mixture was cooled to -78 ˚C with acetone/dry ice bath. It was treated dropwise with 0.03 mL (0.048 mmol) of t-BuLi (1.6 M in pentane) under argon. After completion, it was stirred at -78 ˚C under argon for 45 min, then under carbon dioxide (generated by dry ice over drying tube) for 3 h. The reaction mixture was let to warn up to room temperature and treated with 1 mL of 1M NaOH. The organic layer was extracted with 1M NaOH (5 X 1 mL). The combined aqueous layers were acidified with concentrated HCl to pH = 1, then extracted with Et_2O (5 X 5 mL). The combined organic layers were dried over $MgSO_4$ and filtered. All the volatile components were removed in reduced pressure. It afforded crude 0.0047 g. The crude was purified by flash chromatography (SiO_2, MeOH/CH_2Cl_2 = 5/95) to give 0.0043 g (0.014 mmol, quantitative, R_f = 0.57, MeOH/CH_2Cl_2 = 7/93) of product **54**. IR (liquid in CH_2Cl_2, cm^{-1}): υ 3600-3000 (broad), 2918, 2865, 1688, 1593, 1455, 1410, 1273, 1101; ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.15 (s, 3H), 1.72 (t, 6H, *J* = 7.8 Hz), 1.58 (t, 6H, *J* = 8.1 Hz), 1.46 (t, 6H, *J* = 7.5 Hz), 1.38 (t, 6H, *J* = 7.5 Hz); ^{13}C NMR (400 MHz, $CDCl_3$) δ 184.38, 73.40, 48.94, 37.99, 34.47, 34.08, 29.24, 28.17, 26.09, 24.44.

Benzyl 4'-methoxy-[1,1'-bi(bicyclo[2.2.2]octane)]-4-carboxylate (55). A mixture of 0.0250 g (0.0856 mmol) of **54** and 0.116 g (0.343 mmol) of Cs_2CO_3 in 0.86 mL of anhydrous DMF was stirred at room temperature for 15 min, then treated with 41 μL (0.0586 g, 0.3425 mmol) of benzyl bromide under nitrogen. The reaction was heated at 40 ˚C for overnight. It was cooled to room temperature and added with 6 mL of water. The aqueous solution was extracted with 5 X 6 mL of EtOAc. The combined organic layers were dried over $MgSO_4$ and filtered. All the volatile components were removed in reduced pressure and afforded 0.0877 g of crude. The crude was purified by flash chromatography (SiO_2, EtOAc/Hexanes = 16/84) to give 0.0298 g (0.078 mmol, 91% yield) of product **55** (R_f = 0.42, EtOAc/Hexanes = 15/85). IR (liquid in CH_2Cl_2, cm^{-1}): υ 3600-3100 (broad), 2947, 2866, 1724, 1589, 1456, 1345, 1224, 1064; ^1H NMR (300 MHz, $CDCl_3$) δ 7.31 (m, 5H), 5.07 (s, 2H), 3.15 (s, 3H), 1.73 (t, 6H, *J* = 7.8 Hz), 1.58 (t, 6H, *J* = 5.4 Hz), 1.46 (t, 6H, *J* = 8.1 Hz), 1.38 (t, 6H, *J* = 8.1 Hz); ^{13}C NMR (400 MHz, $CDCl_3$) δ 177.94, 136.46, 128.44, 127.89, 127.62, 73.27, 65.75, 48.96, 38.27, 34.46, 29.25, 28.34, 26.07, 24.50.

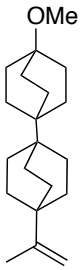


2-(4'-Methoxy-[1,1'-bi(bicyclo[2.2.2]octan)]-4-yl)propan-2-ol (56). A flame-dried flask was charged with 0.0280 g (0.0733 mmol) of **55** and 1.5 mL of anhydrous THF. It was treated with 0.49 mL of methyl Grignard reagent (MeMgBr, 3.0 M in Et_2O, 1.466 mmol) at room temperature under nitrogen. The reaction was heated at reflux for 20 h, then cooled to room temperature. The excess Grignard reagent was quenched with several drops of deionized water until no more bubbles generated. All volatile organic components were removed in reduced pressure. The residue was diluted with 5.0 mL of



deionized water and acidified with 1M HCl to pH = 1. It was extracted with 6 X 5 mL of EtOAc. The combined organic layers were dried over $MgSO_4$, filtered. After all volatile components were removed in reduced pressure, it afforded 0.0251 g of crude as white solid. The crude was purified by flash chromatography (SiO_2, EtOAc/Hexanes = 1/2) to give 0.0185 g (0.0605 mmol, 83% yield, R_f = 0.54, EtOAc/Hexanes = 2/3) of product **56**. IR (liquid in CH_2Cl_2, cm^{-1}): υ 3600-3100 (broad), 2950, 2864, 1595, 1457, 1361, 1237, 1195, 1167, 1138, 1089, 1029, 937; ^1H NMR (300 MHz, $CDCl_3$) δ 3.15 (s, 3H), 1.56 (m, 6H), 1.45 (m, 12H), 1.34 (m, 6H), 1.08 (s, 6H); ^{13}C NMR (400 MHz, $CDCl_3$) δ 74.44, 73.39, 48.92, 37.10, 34.01, 33.99, 29.31, 25.95, 25.65, 25.08, 24.97. HRMS (ES) *m/z* calcd for $C_{20}H_{35}O_2$ $(M+H)^+$ 307.2632, found 307.2630.

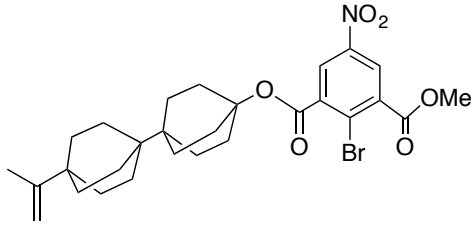
4-Methoxy-4'-(prop-1-en-2-yl)-1,1'-bi(bicyclo[2.2.2]octane) (57). A mixture of 0.0034 g



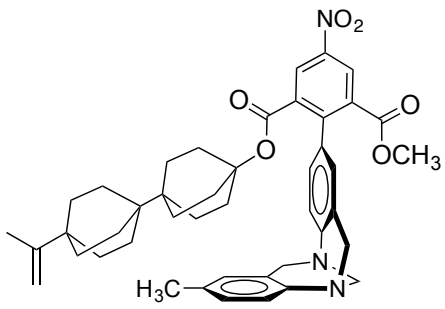
(0.011 mmol) of **56** and 0.0041 g (0.033 mmol) of 4-dimethylaminopyridine was dissolved in 0.22 mL of anhydrous CH_2Cl_2 and added with 9.0 µL of triethylamine under nitrogen. The reaction was cooled to 0 ˚C and treated with 5 µL (0.0067 g, 0.067 mmol) of distilled mesyl chloride dropwise. After completion it was let to warmed up to room temperature and stirred at room temperature for two days. To the reaction it was added 23 µL of 1,8-Diazabicyclo[5.4.0]undec-7-ene (DBU) and stirred continuously for another 24 h. The reaction was diluted with 1 mL of water and 1 mL of CH_2Cl_2. The isolated aqueous layer was extracted with 6 X 1 ML of CH_2Cl_2. The combined organic layers were dried over $MgSO_4$ and filtered. All the volatile components were removed in reduced pressure, and it afforded 0.0155 g of crude. The crude was purified by flash chromatography (SiO_2, Et_2O/Pentane = 24/76) to give 0.0024 g (0.0083 mmol, 75% yield) of product **57** (R_f= 0.63, EtOAc/Hexanes =

15/85). ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.63 (d, 2H, *J* = 4.8 Hz), 3.15 (s, 3H), 1.67 (s, 3H), 1.54 (m, 6H), 1.48 (m, 6H), 1.36 (m, 6H), 1.26 (m, 6H).

1-Methyl 3-(4'-(prop-1-en-2-yl)-[1,1'-bi(bicyclo[2.2.2]octan)]-4-yl) 2-bromo-5-nitroisophthalate (58). By the general procedure of DCC esterification, 0.0113 g (0.0374 mmol) of monoacid **9**, 0.0034 g (0.012 mmol) of **50**, 0.0023 g (0.019 mmol) of DMAP, 0.0154 g (0.0745 mmol) of DCC in 0.75 mL of anhydrous CH_2Cl_2 afforded 0.0064 g of **58** (0.011 mmol, 93% yield, R_f = 0.44, EtOAc/Hexanes = 12/88). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.51 (d, 1H, *J* = 2.4 Hz), 8.38 (d, 1H, *J* = 2.4 Hz), 4.64 (d, 2H, *J* = 8.8 Hz), 4.00 (s, 3H), 2.09 (m, 6H), 1.67 (s, 3H), 1.60 (m, 6H), 1.50 (m, 6H), 1.38 (m, 6H); ^{13}C NMR (400 MHz, $CDCl_3$) δ 164.90, 163.77, 153.09, 146.21, 138.84, 136.09, 126.04, 125.98, 125.93, 107.98, 85.11, 53.34, 35.26, 34.25, 34.13, 30.00, 29.85, 26.22, 25.27, 19.57; HRMS (ES) *m/z* calcd for $C_{28}H_{34}NO_6Br$ $(M\text{-}e)^+$ 559.1569, found 559.1574.



1-Methyl 3-(4'-(prop-1-en-2-yl)-[1,1'-bi(bicyclo[2.2.2]octan)]-4-yl) 2-(8-methyl-6*H*,12*H*-5,11-methanodibenzo[*b*,*f*][1,5]diazocin-2-yl)-5-nitroisophthalate (59). By the general Suzuki coupling procedure, 0.0026 g (0.0047 mmol) of **58**, 0.034 g (0.0093 mmol) of **8**, 0.0016 g (0.0028 mmol) of Pd catalyst, 0.0047 g (0.056 mmol) of $NaHCO_3$ in 0.1 mL of DME and 0.1 mL of water afforded 0.0021 g of **59** (0.0029 mmol, 62% yield, R_f = 0.41, EtOAc/Hexanes = 1/1). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.56 (d, 2H, *J* = 18.4 Hz), 7.16 (d, 1H, *J* = 8.4 Hz), 7.01 (m, 3H), 6.73 (d, 2H, *J*

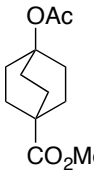


= 8.8 Hz), 4.71 (m, 4H), 4.20 (m, 2H), 4.13 (m, 2H), 3.65 (br, 3H), 2.09 (s, 3H), 2-0.8 (br, 27 H); HRMS (ES) *m/z* calcd for $C_{44}H_{50}N_3O_6$ $(M+H)^+$ 716.3700, found 716.3712.

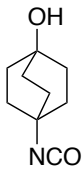
Methyl 4-iodobicyclo[2.2.2]octane-1-carboxylate (61). ***1) Hydrolysis.*** A solution of 1.054 g (4.665 mmol) of **33** in 5.2 mL of MeOH was treated with 0.261 g (4.67 mmol) of KOH solution. The reaction was stirred at room temperature for overnight. The volatile components were removed in reduced pressure. The aqueous residue was diluted with water and acidified with 1 M HCl to pH = 1. The resulting solution was extracted with EtOAc (5 X 15 mL). The combined organic layers were washed with brine, dried over $MgSO_4$ and filtered. It was concentrated in reduced pressure, affording 0.928 g of crude. The crude was purified with acid-base extraction to remove the starting material (0.230 g of diester recovered), affording 0.680 g of mixture of monoacid **60** and diacid **34**. The mixture was used in the next step without further purification. ***2) Decarboxylation.*** The flask was charged with 0.680 g (10.907 mmol) of mixture of **60** and **34**, 1.280 g (3.975 mmol) of iodobenzene diacetate and 1.009 g (3.975 mmol) of iodine, vacuated and filled with nitrogen. The mixture was treated with 53 mL of benzene. The resulting purple suspension was heated at reflux and irradiated with 250 W tungsten bulb for 4 hours. Then it was cooled to room temperature, 1.280 g (3.975 mmol) of iodobenzene diacetate and 1.009 g (3.975 mmol) of iodine were added into the reaction under nitrogen. The reaction was again heated at reflux and irradiated with 250 W tungsten bulb for overnight. It was cooled to room temperature, washed with 3 X 40 mL of 10% (w/w) $Na_2S_2O_3$, 3 X 40 mL of brine. The organic layer was dried over $MgSO_4$, filtered and concentrated in reduced pressure. The crude was purified by flash chromatography (SiO_2, Et_2O/pentane = 1/4) to give

0.716 g of product **61** (2.436 mmol, 67% yield in two steps, R_f= 0.51, Et_2O/pentane = 1/9) and 0.332 g (0.918 mmol) of **35**. IR (liquid in CH_2Cl_2, cm^{-1}): υ 2951, 2920, 2866, 1727, 1454, 1322, 1242, 1071, 1008; ^1H NMR (400 MHz, $CDCl_3$) δ 3.57 (s, 3H), 2.41 (s, 6H), 1.87 (s, 6H); ^{13}C NMR (400 MHz, $CDCl_3$) δ 176.73, 52.17, 39.83, 31.82, 30.78, 28.10.

Methyl 4-acetoxybicyclo[2.2.2]octane-1-carboxylate (62). The mixture of **61** (0.703 g, 2.391 mmol) and silver acetate (0.479 g, 2.869 mmol) was dissolved in 24 mL of acetic acid. It was heated at reflux for one hour. The acetic acid was removed by distillation. The residue was diluted with 10 mL of water and filtered. The solid was washed with Et_2O copiously. The isolated aqueous phase was Et_2O extracted for five times. The combined organic layers were washed with saturated $NaHCO_3$, dried over $MgSO_4$, filtered and concentrated in reduced pressure, affording 0.384 g (1.700 mmol, 71% yield) of crude. The crude **62** was used in the next step without further purification. IR (liquid in CH_2Cl_2, cm^{-1}): υ 2921, 2851, 2354, 1731, 1455, 1203; ^1H NMR (300 MHz, $CDCl_3$) δ 3.55 (s, 3H), 1.92 (br, 6H), 1.87 (br, 9H); ^{13}C NMR (300 MHz, $CDCl_3$) δ 176.72, 169.73, 79.38, 51.28, 37.74, 28.77, 21.91.

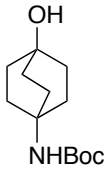


4-Isocyanatobicyclo[2.2.2]octan-1-ol (63). A solution of 0.050 g (0.29 mmol) of **20e** in 3.0 mL of benzene was treated with 0.1 mL (0.074 g, 0.73 mmol) of triethyl amine and 0.121 g (0.440 mmol) of diphenolphosphory azide under nitrogen. The reaction was heated at reflux for 48 hours under nitrogen. The reaction was cooled to room temperature, concentrated in reduced pressure. The residue was diluted with 5.0 mL of EtOAc, washed with



saturated $NaHCO_3$ and brine, dried over $MgSO_4$ and filtered. All the volatile components were removed in reduced pressure. The resulting crude was purified by flash chromatography (SiO_2, EtOAc/Hexanes = 1/1) to give 0.038 g (0.23 mmol, 77% yield) of product **63** (R_f = 0.40, EtOAc/Hexanes = 1/1). IR (liquid in CH_2Cl_2, cm^{-1}): υ 3400-3000 (broad), 2954, 2922, 2868, 2260, 1475, 1349, 1104, 1039, 968; ^1H NMR (400 MHz, $CDCl_3$) δ 1.93 (br, 6H), 1.75 (br, 6H); ^{13}C NMR (400 MHz, $CDCl_3$) δ 122.62, 67.64, 53.91, 34.87, 33.95. HRMS (ES) *m/z* calcd for $C_9H_{13}NO_2$ $(M-e)^+$ 167.0946, found 167.0992.

***Tert*-butyl (4-hydroxybicyclo[2.2.2]octan-1-yl)carbamate (64).** A solution of 0.037 g (0.22 mmol) of **63** in 4.4 mL of anhydrous *tert*-butanol was heated at reflux for five days under nitrogen. Then it was cooled to room temperature and concentrated in reduced pressure. The resulting crude was purified by flash chromatography (SiO_2, EtOAc/Hexanes = 1/1) to give 0.024 g (0.098 mmol, 45% yield) of product **64** (R_f = 0.36, EtOAc/Hexanes = 1/1). IR (liquid in CH_2Cl_2, cm^{-1}): υ 3500-3300 (broad), 2925, 2870, 1694, 1526, 1460, 1390, 1356, 1330, 1303, 1245, 1170, 1095, 1047, 970, 737; ^1H NMR (400 MHz, $CDCl_3$) δ 4.34 (br, 1H), 1.92 (br, 6H), 1.70 (br, 6H), 1.38 (s, 9H); ^{13}C NMR (400 MHz, $CDCl_3$) δ 154.45, 78.98, 68.10, 49.14, 33.86, 31.67, 28.38. HRMS (ES) *m/z* calcd for $C_{13}H_{23}NO_3Na$ $(M+Na)^+$ 264.1600, found 264.1588.

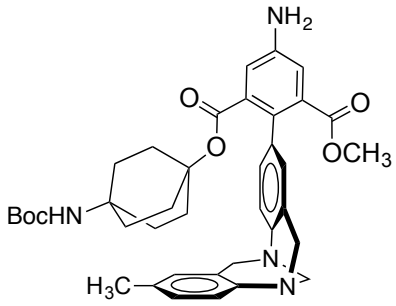


1-(4-((*Tert*-butoxycarbonyl)amino)bicyclo[2.2.2]octan-1-yl) 3-methyl 2-bromo-5-nitroisophthalate (65). By the general DCC esterification procedure, 0.045 g (0.15 mmol) of monoacid **8**, 0.024 g (0.098 mmol) of **64**, 0.0036 g (0.030 mmol) of DMAP, 0.061 g (0.30 mmol) of DCC in 5.0 mL of CH_2Cl_2 afforded 0.032 g of **65** (0.061 mmol, 62% yield, R_f = 0.30, EtOAc/Hexanes = 1/4). IR (liquid in CH_2Cl_2, cm^{-1}): υ 3600-3100 (broad), 2928, 2868, 1715, 1605, 1535, 1347, 1246, 1163, 1029; ^1H NMR (300 MHz, $CDCl_3$) δ 8.51 (d, 1H, *J* = 2.7 Hz), 8.37 (d, 1H, *J* = 2.7 Hz), 4.35 (br, 1H), 3.99 (s, 3H), 2.25 (m, 6H), 2.05 (m, 6H), 1.42 (s, 9H); ^{13}C NMR (400 MHz, $CDCl_3$) δ 164.82, 163.61, 154.20, 146.23, 138.48, 136.18, 126.10, 125.99, 125.92, 83.34, 53.33, 48.77, 31.34, 29.84, 28.39.

1-(4-((*Tert*-butoxycarbonyl)amino)bicyclo[2.2.2]octan-1-yl) 3-methyl 2-(8-methyl-6*H*,12*H*-5,11-methanodibenzo[*b*,*f*][1,5]diazocin-2-yl)-5-nitroisophthalate (66). By the general procedure of Suzuki coupling, 0.0138 g (0.0383 mmol) of **9**, 0.0183 g (0.0348 mmol) of **65**, 0.0043 g (0.0077 mmol) of Pd catalyst, 0.0193 g (0.230 mmol) of $NaHCO_3$ in 0.35 mL of DME and 0.35 mL of water afforded 0.0173 g of **66** (0.0254 mmol, 66% yield, R_f = 0.27, EtOAc/Hexanes = 3/2). IR (liquid in CH_2Cl_2, cm^{-1}): υ 3600-3100 (broad), 2926, 1717, 1598, 1531, 1495, 1441, 1366, 1310, 1246, 1166, 1050, 1029; ^1H NMR (700 MHz, $CDCl_3$, 5 ˚C) **Folded (76%):** δ 8.64 (s, 1H), 8.54 (s, 1H), 7.17 (d, 1H, *J* = 9.1 Hz), 7.05 (d, 1H, *J* = 7.7 Hz), 6.99 (dd, 2H, *J* = 8.4 and 21.0 Hz), 6.79 (s, 1H), 6.73 (s, 1H), 4.72 (m, 2H), 4.32 (m, 2H), 4.16 (dd, 2H, *J* = 16.8 and 39.9 Hz), 3.69 (s, 3H), 2.23 (s, 3H), 1.40 (s, 9H), 1.24 (br, 12H); **Unfolded (24%):** δ 8.55 (br, 2H), 7.17 (d, 1H, *J* = 9.1

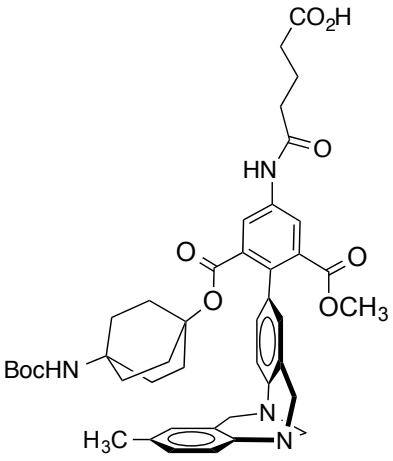
Hz), 7.05 (d, 1H, *J* = 7.7 Hz), 6.99 (dd, 2H, *J* = 8.4 and 21.0 Hz), 6.79 (s, 1H), 6.73 (s, 1H), 4.72 (m, 2H), 4.33 (m, 2H), 4.16 (dd, 2H, *J* = 16.8 and 39.9 Hz), 3.00 (s, 3H), 2.28 (s, 3H), 1.73 (br, 6H), 1.52 (br, 6H), 1.40 (s, 9H); ^{13}C NMR (400 MHz, $CDCl_3$) δ 171.27, 171.15, 155.76, 150.00, 146.40, 146.34, 145.97, 136.67, 136.27, 134.41, 133.67, 132.99, 128.565, 127.57, 127.30, 127.21, 125.93, 125.66, 124.95, 124.59, 119.41, 77.34, 60.40, 48.29, 43.02, 30.85, 29.70, 28.93, 28.41, 21.06, 20.85, 14.20; HRMS (ES) *m/z* calcd for $C_{38}H_{43}N_4O_8$ $(M+H)^+$683.3081, found 683.3134.

1-(4-((*Tert*-butoxycarbonyl)amino)bicyclo[2.2.2]octan-1-yl) 3-methyl 5-amino-2-(8-methyl-6*H*,12*H*-5,11-methanodibenzo[*b*,*f*][1,5]diazocin-2-yl)isophthalate (67). By the general procedure of hydrogenation (Method B), 0.0087 g (0.013 mmol) of **66**, 0.0027 g (0.0026 mmol) of 10% Pd/C afforded 0.0064 g of **67** (0.0098 mmol, 77% yield, R_f = 0.33, EtOAc/Hexanes = 3/1). IR (liquid in CH_2Cl_2, cm^{-1}): υ 3600-3150 (broad), 2924, 2853, 1712, 1608, 1495, 1463, 1364, 1262, 1198, 1168, 1120, 1050, 1032, 799, 737; ^{1}H NMR (700 MHz, $CDCl_3$, 5 ˚C) **Folded (75%):** δ 7.10 (d, 2H, *J* = 11.9 Hz), 7.05 (d, 1H, *J* = 8.4 Hz), 7.02 (d, 1H, *J* = 8.4 Hz), 6.90 (d, 1H, *J* = 9.8 Hz), 6.96 (d, 1H, *J* = 7.0 Hz), 6.80 (s, 1H), 6.70 (s, 1H), 6.6 (br, 1H), 4.74 (dd, 2H, *J* = 8.4 and 16.8 Hz), 4.33 (m, 2H), 4.15 (dd, 2H, *J* = 16.8 and 45.5 Hz), 3.83/2.02 (br/br, NH_3^+/NH_2, 3H/2H), 3.58 (s, 3H), 2.24 (s, 3H), 1.42 (br, 6H), 1.40 (br, 9H), 1.34 (br, 6H); **Unfolded (25%):** δ 7.10 (d, 2H, *J* = 11.9 Hz), 7.05 (d, 1H, *J* = 8.4 Hz), 7.02 (d, 1H, *J* = 8.4 Hz), 6.90 (d, 1H, *J* = 9.8 Hz), 6.96 (d, 1H, *J* = 7.0 Hz), 6.80 (s, 1H), 6.70 (s, 1H), 6.6 (br, 1H), 4.65 (m, 2H), 4.33 (m, 2H), 4.15 (dd, 2H, *J* = 16.8



and 45.5 Hz), 3.83/2.02 (br/br, NH_3^+/NH_2, 3H/2H), 2.89 (s, 3H), 2.22 (s, 3H), 1.57 (br, 6H), 1.49 (br, 6H), 1.40 (s, 9H); ^{13}C NMR (600 MHz, $CDCl_3$) δ 168.51, 166.86, 154.41, 145.22, 135.68, 133.45, 130.02, 128.27, 127.24, 124.93, 124.16, 117.55, 117.17, 66.78, 58.40, 55.99, 48.41, 31.91, 30.74, 29.69, 29.35, 28.82, 28.39, 22.68, 20.84, 14.12; HRMS (ES) *m/z* calcd for $C_{38}H_{45}N_4O_6$ $(M+H)^+$ 653.3339, found 653.3389.

5-((3-(((4-((*tert*-butoxycarbonyl)amino)bicyclo[2.2.2]octan-1-yl)oxy)carbonyl)-5-(methoxycarbonyl)-4-(8-methyl-6*H*,12*H*-5,11-methanodibenzo[*b*,*f*][1,5]diazocin-2-yl)phenyl)amino)-5-oxopentanoic acid (68). By the general procedure of ring opening acylation with glutaric anhydricd, 0.0042 g (0.0064 mmol) of **67**, 0.0015 g (0.0013 mmol) of glutaric anhydride in 0.54 mL of anhydrous CH_2Cl_2 afforded 0.0039 (0.0051 mmol, 80% yield, R_f = 0.32, MeOH/CH_2Cl_2 = 15/85) g of **68**. IR (liquid in CH_2Cl_2, cm^{-1}) υ 3600-3100 (broad), 3055, 2924, 2854, 2540, 1908, 1712, 1595, 1530, 1497, 1461, 1409, 1256, 1199, 1172, 1051, 1032, 966, 887, 844, 801, 737, 703; ^1H NMR (700 MHz, MeOD, 5 ˚C) **Folded (81%):** δ 8.06 (s, 1H), 7.94 (s, 1H), 7.16 (d, 1H, *J* = 7.7 Hz), 7.07 (d, 1H, *J* = 8.4 Hz), 7.03 (d, 1H, *J* = 7.7 Hz), 6.93 (d, 1H, *J* = 8.4 Hz), 6.87 (s, 1H), 6.73 (s, 1H), 4.68 (m, 2H), 4.31 (dd, 2H, *J* = 12.6 and 27.3 Hz), 4.14 (dd, 2H, *J* = 16.8 and 42.7 Hz), 3.58 (s, 3H), 2.42 (t, 2H, *J* = 7.0 Hz), 2.28 (t, 2H, *J* = 7.0 Hz), 2.25 (s, 3H), 1.97 (p, 2H, *J* = 7.0 Hz), 1.51 (br, 6H), 1.40 (s, 9H), 1.32 (br, 6H); **Unfolded (19%):** δ 8.02 (s, 1H), 8.00 (s, 1H), 7.16 (d, 1H, *J* = 7.7 Hz), 7.07 (d, 1H, *J* = 8.4 Hz), 7.03 (d, 1H, *J* = 7.7 Hz), 6.93 (d, 1H, *J* = 8.4 Hz), 6.80 (s, 1H),



6.70 (s, 1H), 4.68 (m, 2H), 4.31 (dd, 2H, J = 12.6 and 27.3 Hz), 4.14 (dd, 2H, J = 16.8 and 42.7 Hz), 2.97 (s, 3H), 2.42 (t, 2H, J = 7.0 Hz), 2.28 (t, 2H, J = 7.0 Hz), 2.25 (s, 3H), 1.97 (p, 2H, J = 7.0 Hz), 1.92 (s, 9H), 1.89 (br, 6H), 1.83 (br, 6H); ^{13}C NMR (700 MHz, $CDCl_3$) δ 178.43, 171.06, 170.53, 166.72, 161.52, 150.33, 149.92, 143.56, 137.52, 136.00, 135.95, 130.04, 128.59, 127.22, 123.47, 122.22, 121.77, 120.77, 118.94, 117.58, 122.36, 114.16, 110.48, 53.44, 49.41, 49.39, 35.51, 35.50, 31.61, 31.55, 29.73, 28.47, 22.72, 22.68, 18.89, 14.15, 12.40; HRMS (ES) *m/z* calcd for $C_{43}H_{51}N_4O_9$ $(M+H)^+$ 767.3651, found 767.3646.

8.2 PREPARATION OF BUFFER SOLUTIONS USED AS NMR SOLVENTS IN FOLDING ENERGY STUDY OF TORSION BALANCES

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.

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.

Preparation of the 0.050 M Potassium Deuterium Phthalate Buffers

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 (0.00050 mol) 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 3.1, which was determined by adding 0.4 to a reading taken from a glass electrode pH meter.

A solution of 0.1026 g (0.00050 mol) of the deuterated potassium salt and 2.2 mL of the 0.10 M NaOD 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 5.5.

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 mixture was redissolved in another portion of D_2O, and the solvent again was 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 7.2.

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 10.0.

8.3 ERROR ANALYSIS

All the torsion balance NMR simulations were conducted by simulation of the shapes and intensities of the folded and unfolded methyl ester signals using the iNMR package.® In **Figure 7.1**, a simulation of the methyl ester in folded and unfolded state was overlaid with the experimental spectrum of torsion balance **25l** in MeOD. As we can see, the simulation spectrum (red) could be well matched to the experimental one (blue) by adjusting the population and dynamic constant of the peaks. The folding percentage with these optimum parameters is 80.5%. In Figure 4.2, the dynamic constant increased by 0.5. So the populations of both two peaks have to be adjusted to fit the spectrum, giving 82.3% of folding percentage. In Figure 4.3, vice versa and the folding percentage is 80.7%. Therefore, the error of folding percentage is within ±1.8% and we concluded that the peak simulation would represent the true populations.

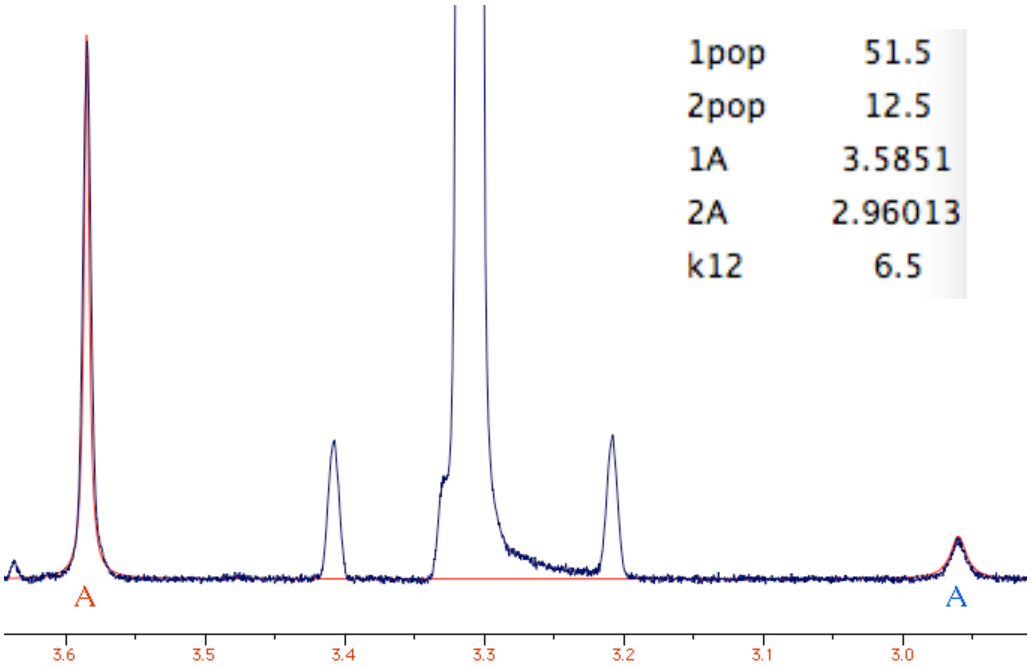


Figure 4.1 A simulation of torsion balance 25l overlaid with the experimental spectrum at 5 ˚C with dynamic constant at 6.5.

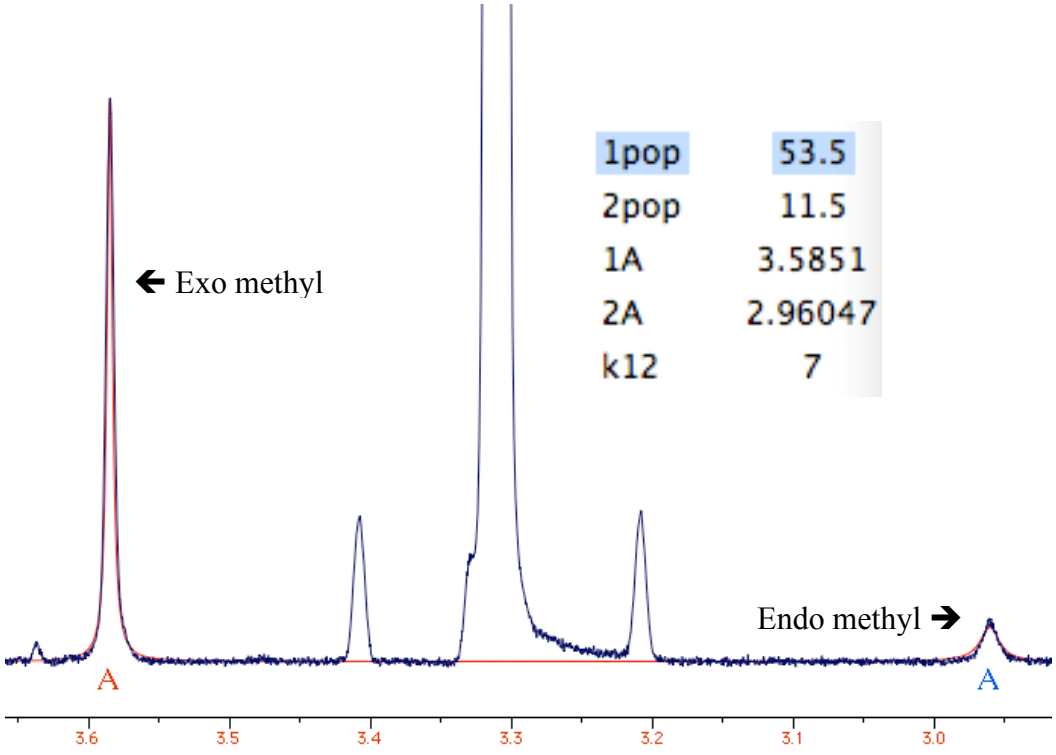


Figure 4.2 Simulation of torsion balance 25l with a 1.8% increase in folding ratio.

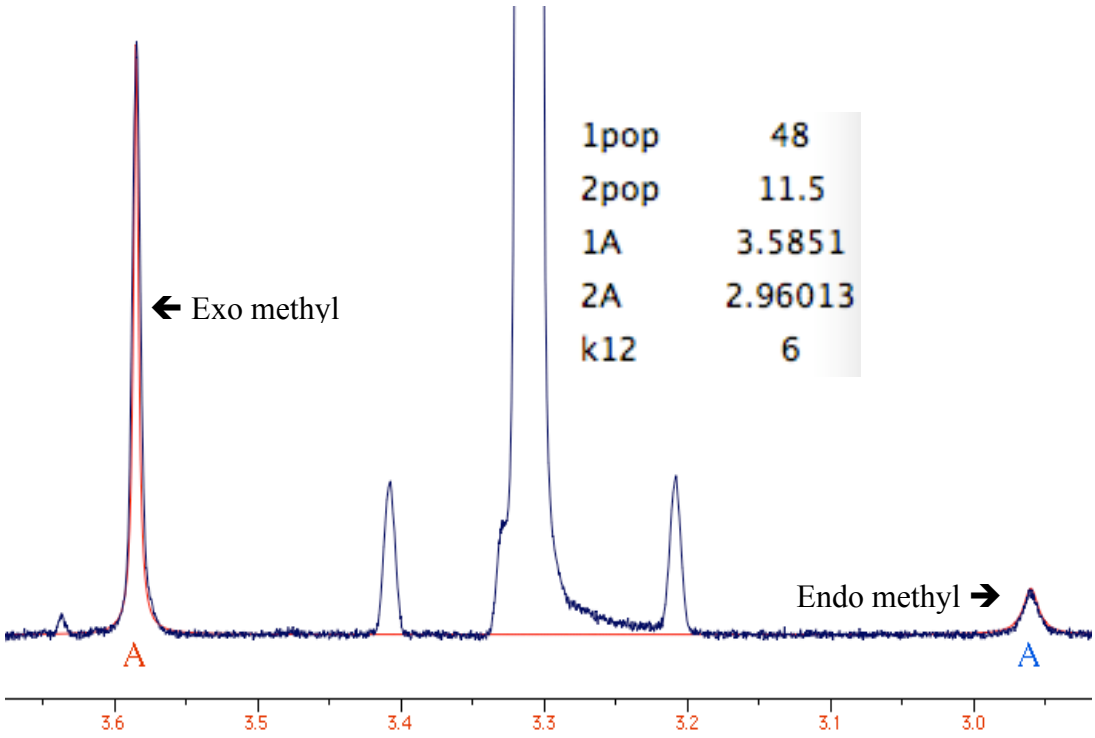


Figure 4.3 Simulation of torsion balance 25l with a 0.2% increase in folding ratio.

The error of the folding free energy was calculated with the equations below. The folding energy was determined with equation 4.1. The equilibrium constant was written in terms of folding percentage x (Eq. 4.2). Then the folding free energy could be expressed in terms of folding percentage (Eq. 4.3). Equation 4.3 was first differentiated with respect to the folding percentage (Eq.4.4), then multiplied by the error of folding percentage to give the absolute folding energy error (Eq. 4.5). The relative folding energy error was achieved by dividing absolute folding energy error by the folding free energy (Eq. 4.6).

$$\Delta G^o = -RT \ln K_{eq} \qquad \text{(Eq. 4.1)}$$

$$K_{eq} = \frac{k^1}{k^{-1}} = \frac{folded}{unfolded} = \frac{x}{100 - x} \qquad \text{(Eq.4.2)}$$

$$\Delta G^o = -RT \ln \frac{x}{100 - x} \qquad \text{(Eq.4.3)}$$

$$\frac{d(\Delta G^o)}{dx} = -RT \frac{100}{x(100 - x)} \qquad \text{(Eq.4.4)}$$

$$\Delta(\Delta G^o) = \left(\frac{d(\Delta G^o)}{dx} \right) \Delta x = -\frac{100RT}{x(100 - x)} \Delta x \qquad \text{(Eq.4.5)}$$

$$\frac{\Delta(\Delta G^o)}{\Delta G^o} = -\frac{\frac{100RT}{x(100 - x)} \Delta x}{-RT \ln\left(\frac{x}{100 - x} \right)} = \frac{-100 \Delta x}{x(100 - x) \ln\left(\frac{x}{100 - x} \right)} \qquad \text{(Eq.4.6)}$$

For a torsion balance with 53.8% of folding percentage at 278K, the folding energy is -0.345 kJ/mol with an absolute error of ±0.164 kJ/mol and a relative error of ±47.6%. When it is at 70% of folding, the folding energy is -1.917 kJ/mol with an absolute error of ±0.194 kJ/mol and a relative error of ±10.1%. When it is at 86.4% of folding, the folding energy is -4.184 kJ/mol with an absolute error of ±0.347 kJ/mol and a relative error of ±8.3%. A graph that describes the relative error in folding energy with relation to the folding percentage is shown in Figure 4.4. Therefore, we estimated the relative error in folding energy is ±47.6% to ±8.3% in the range of 53.8% to 86.4% of folding.

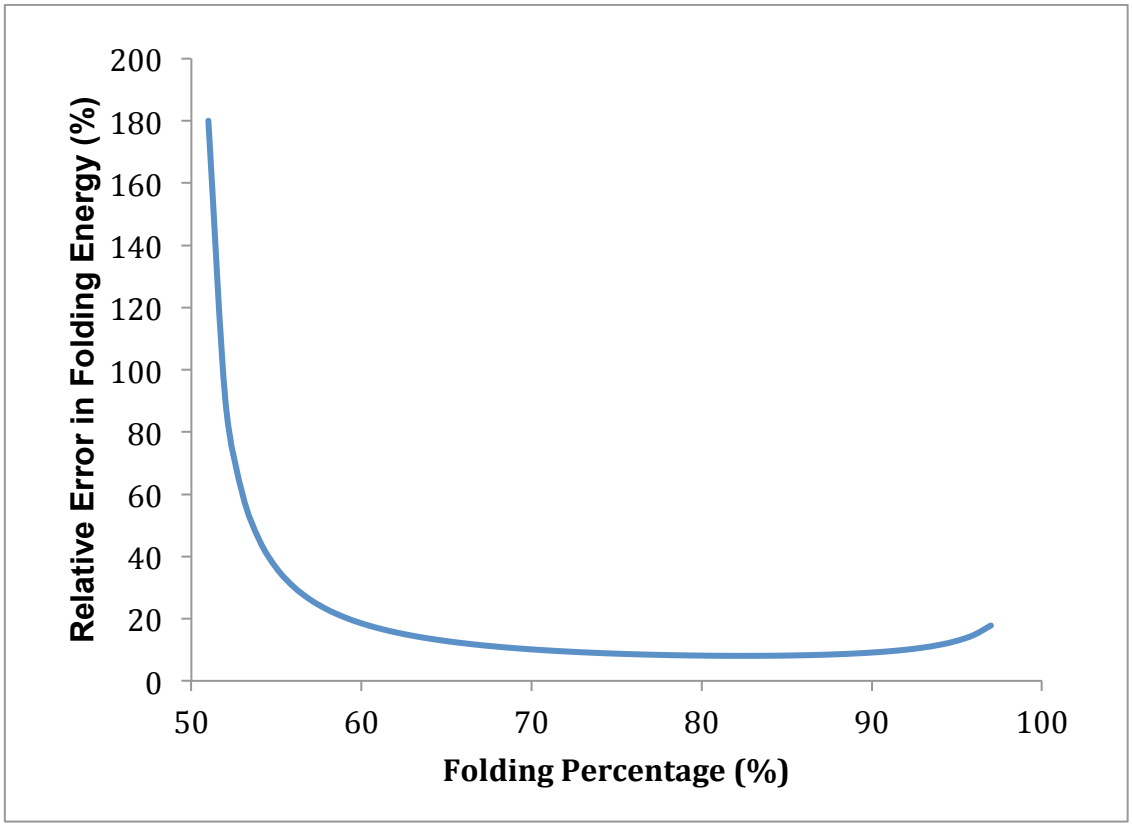


Figure 4.4 Relation of folding percentage and relative error in folding energy for a ±1.5% error in folding percentage

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