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# Absolute Configurational Assignments of Secondary Amines by CD-Sensitive Dimeric Zinc Porphyrin Host

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***Abstract:*** A general chiroptical protocol for determination of absolute configuration of secondary amines including acyclic and cyclic aliphatic amines, aromatic amines, amino acids, and amino alcohols is described. The chiral substrate is linked to the achiral carrier moiety (3-*N*-Boc-amino-propyl-*N*-Boc-amino)acetic acid **1** ($BocHNCH_2CH_2CH_2BocNCH_2COOH$), which after deprotection, yields a bidentate conjugate, capable of forming a 1:1 host/guest complex with dimeric zinc porphyrin host **2**. As in the cases of primary amines and secondary alcohols reported earlier, the complexation of secondary amine conjugates to porphyrin tweezer host **2** represents a stereodifferentiating process, where the large (L) group at the stereogenic center (assigned on the basis of conformational energies *A* value) protrudes from the porphyrin binding pocket. This leads to formation of host/guest complexes with a preferred porphyrin helicity that exhibit intense exciton split CD spectra. It was found that the chiral sense of porphyrin twist is clearly controlled by the stereogenic center despite the *Z*/*E* conformational complexity around the tertiary amide bond of secondary amine conjugates that has greatly hampered previous configurational assignments. Thus, in cases where there is no ambiguity regarding the relative steric size of substituents, the observed CD couplet can be applied for straightforward assignment of absolute configurations. In addition, to extend the application to more difficult cases a molecular mechanics calculation approach using the Merck Molecular Force Field (MMFFs) was developed; this provides conformational information of host/guest complexes and leads to prediction of preferred porphyrin helicity independent of conformational A values. This chiroptical protocol in combination with molecular modeling represents a general method for configurational assignments of secondary amines.

## Introduction

Secondary amines are of great interest due to their roles as plant growth regulators, plant growth promoters, antibiotics, and antitumor agents.[1] They have also been extensively utilized as chiral auxiliaries for asymmetric catalysis, as resolving agents, and as intermediates for the synthesis of biologically active substances.[2] The studies of their absolute configurations by chiroptical methods have been hampered due to the lack of an intense absorption band for direct assignment as well as the conformational complexity of their most common chromophoric derivatives, tertiary amides. In the following, we describe a general method for absolute configurational assignments of secondary amines, in which a chiral secondary amine is derivatized with carrier **1** to generate a bidentate conjugate (Figure 1); the conjugate, upon treatment with a dimeric zinc porphyrin tweezer host **2**, yields the macrocyclic complex **3** through zinc amine coordination. Due to stereodifferentiation leading to preferred porphyrin helicity, the complex exhibits intense exciton-coupled CD that reflects the absolute configuration of the secondary amine. Furthermore, molecular modeling of the complex **3** provides a tool for correlating the observed exciton CD couplet with the absolute configuration.

The absolute configurational assignment of secondary amines has been studied using circular dichroic (CD) methods. Earlier approaches were based on derivatization of secondary amines with fluorescamine or 2-pyridine/pyridine *N*-oxide.[3] The absolute configurations of the amines were empirically correlated with the sign of the CD bands of the chromophoric derivatives at longer wavelength (around 320 nm). Recently, Okamoto and co-workers have synthesized an achiral polyacetylene polymer bearing carboxylic acid groups.[4] Upon formation of acid/base pairs with chiral amines, the polymer gave an induced CD with

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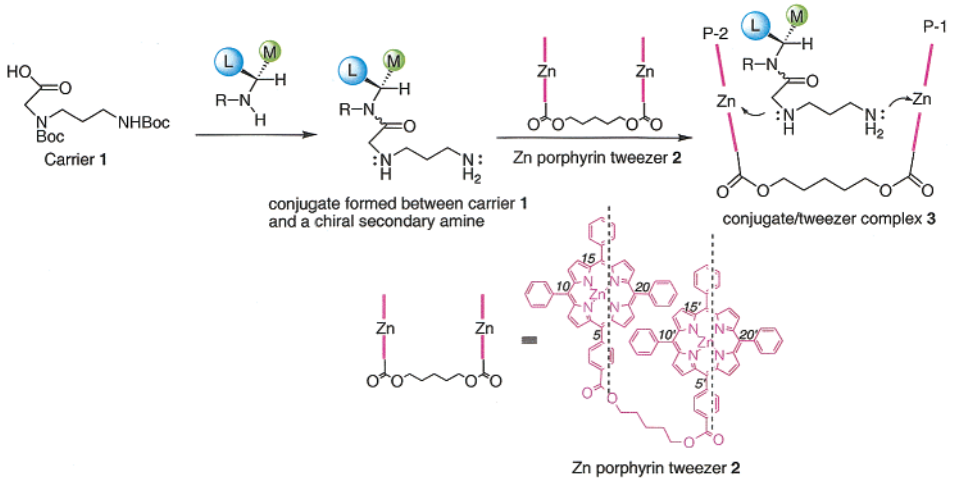


***Figure 1.*** Bidentate conjugate prepared from secondary amine and carrier **1** is capable of forming 1:1 host/guest complex **3** with Zn-porphyrin tweezer **2**. The helicity between the two porphyrins in complex **3**, defined as the projection angle between 5 and 15 and 5′-15′ directions, is governed by the absolute configuration of the secondary amine.

the sign indicating the absolute stereochemistry of the amine. For secondary amines bearing aromatic chromophores, Smith and co-workers derived a semiempirical sector rule for absolute configurational assignments.[5] In all of the aforementioned studies, the CD amplitudes observed are generally very weak ($\Delta\epsilon < 10$). Moreover, the scope of these methods was often restricted since the sign of CD in these cases may be affected by other factors such as the type and bulkiness of substituents.

For secondary amines bearing other functional groups, multiple chromophores can be introduced, and the exciton chirality method can be applied.[6,7] However, this approach has been encumbered by the conformational heterogeneity of the tertiary amides generated after chromophoric derivatization. Tertiary amide bonds are known to exist in two rotamer forms, that is, *Z* and *E*, usually of close energy, with the ratio depending upon the steric sizes of substituents. This often leads to weak CD signals and unreliable or even erroneous assignments. For example, Van Vranken and co-workers have demonstrated that bromobenzoylation of an amino glycoside containing a secondary amine moiety yielded a tertiary amide, which existed as a mixture of *Z* and *E* conformers in approximately 1:1 ratio. The original assignment, which did not take this factor into account, turned out to be incorrect since the observed CD was the sum of CD contributions from these conformers with opposite predicted signs.[6]

The modified Mosher NMR method has been extensively applied to absolute configurational assignments of secondary alcohols,[8] primary amines,[9] and carboxylic acids.[10] Recently, this method has undergone remarkable progress with the development of new reagents and novel procedures. Hoye and workers have extended the Mosher method to cyclic secondary amines by derivatization with the (*R*)- and (*S*)-enantiomers of α-methoxy-α-(trifluoromethyl)phenylacetic acid (MTPA).[11] However, the resulting tertiary amides contain significant populations of two conformers around the tertiary amide bonds. Therefore, the absolute configurations of secondary amines should be derived only from chemical shift differences between the two diastereomers possessing the *same amide conformation*,[11] which in turn requires a detailed conformational analysis of the two MTPA derivatives and NMR assignments of all conformers. Furthermore, this method has not been demonstrated to be widely applicable to acyclic secondary amines. It is thus highly desirable that a more general approach for absolute configurational assignment of secondary amines be developed.

In recent years, porphyrins and zinc porphyrins have become useful as versatile CD reporter groups for structural studies.[12] We have developed a protocol for absolute configurational assignment of diamines utilizing a bis(zinc porphyrin) linked by a pentanediol linker.[13] The resulting achiral zinc porphyrin tweezer **2** is capable of binding various chiral acyclic α,ω-diamines through zinc−amine coordination leading to formation of 1:1 macrocyclic host/guest complexes. Due to stereodifferentiation of substituents at the stereogenic center, the two porphyrins in the complex adopt a preferred chiral twist, as evident from the observed bisignate exciton couplet in the porphyrin Soret band region, the sign of which is governed by the absolute configuration of the bound diamine.

In the case of chiral compounds such as monoamines and monoalcohols with only a single site for attachment of the chromophoric reporter groups, trifunctional carrier moieties exemplified by **1** and **4** were developed (Chart 1). They form

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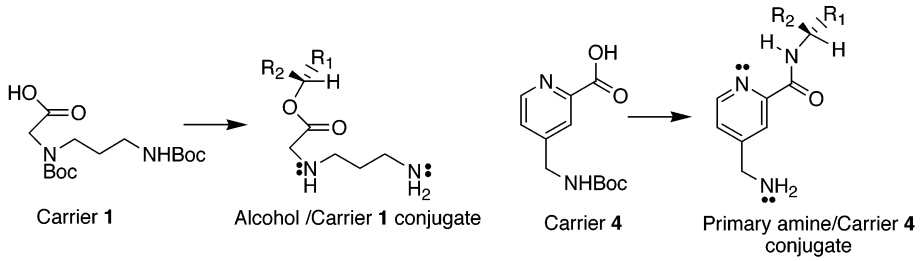
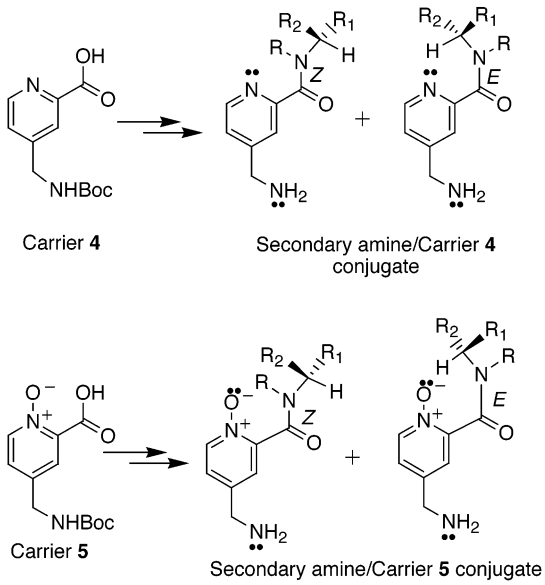
***Chart 1***



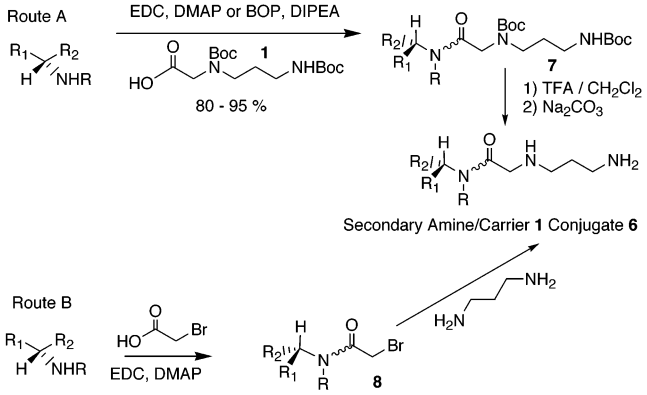

bidentate conjugates with chiral substrates, which are capable of forming 1:1 complexes with the porphyrin tweezer host **2**.[14−16] Steric differentiation between the two substituents at the stereogenic center, assigned large (L) and medium (M), according to their conformational energies expressed in A values as first defined by Winstein and Holness,[17] and later by Eliel,[18] Bushweller,[19] and Lightner,[20] leads to a preferred chiral twist between the porphyrin transition dipole moments;[21] this gives rise to exciton coupled CD with the sign reflecting the absolute configuration of the substrate.

In the present study on secondary amines, we first tested carrier **4** utilized in earlier studies of primary amines.[14] Although certain conjugates formed between carrier **4** and model secondary amines exhibited exciton CD couplet with large amplitudes, the signs of these couplets did not necessarily agree with those expected for the known absolute configurations. We suspected the conformational flexibility of these conjugates as being the most likely reason for this inconsistency. To reduce the conformational freedom, carrier **5** with an *N*-pyridine oxide moiety was prepared (Chart 2). It was hoped that the secondary amine/carrier **5** conjugate might be more rigid through increased electronic repulsion between the negatively charged oxygen of the pyridine oxide and the carbonyl oxygen. While all tested cyclic secondary amines conjugates with carrier **5** yielded exciton CD couplets with signs in agreement with prediction, disappointingly the CD of acyclic secondary amine conjugates were not only much weaker but also exhibited signs inconsistent with the absolute configuration,[22] presumably due to different *Z*/*E* ratios of the conjugates. The unsuccessful attempts with pyridine derivatives **4** and **5** as carriers prompted us to search for a carrier that allows more flexibility between the stereogenic center and the bidentate binding sites, thus leading to a better chiral recognition. Therefore, carrier **1**, developed for successful absolute configurational assignment of secondary alcohols and primary amines,[15,16] became a prospective carrier moiety, which would possibly allow the conjugate to bind tweezer **2** and provide exciton CD couplets reflecting the absolute configuration *regardless* of the conformation of the tertiary amide bond.

***Chart 2***




***Scheme 1.*** Preparation of the Secondary Amine/Carrier **1** Conjugates




## Results and Discussion:

**Preparation of the Conjugates.** The di-Boc-protected carrier **1** was prepared as described previously.[15] Accordingly, the formation of bidentate 2° amine/carrier **1** conjugate **6** was performed by two different procedures (Scheme 1). Secondary amines were coupled with carrier **1** to generate the di-Boc-protected conjugate **7** in greater than 80% yield. Treatment of **7** with trifluoroacetic acid (TFA) and neutralization with $Na_2CO_3$ afforded the free base form of the conjugate **6** (route A), which readily formed a 1:1 host/guest complex with zinc porphyrin tweezer **2**. In the case of acid-sensitive compounds

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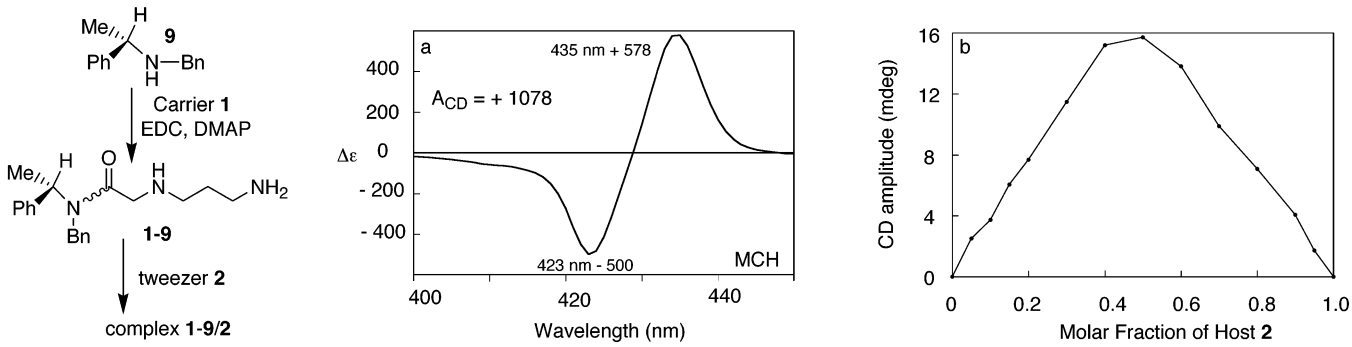


***Figure 2.*** (a) CD spectrum of complex **1-9**/**2** formed between conjugate **1-9** (20 equiv) and Zn-porphyrin tweezer **2** (1 $\mu$M) in methylcyclohexane (MCH). $A_{CD}$ denotes the amplitude of the CD exciton couplet, which represents the difference in $\Delta\epsilon$ between the peak and the trough. (b) Job plot of the complex **1-9**/**2** with the maximum CD amplitude observed when the molar fraction of host **2** is 0.5 indicates that the stoichiometry of binding is 1:1 (total host and guest concentration is 1$\mu$M).

such as 1-*O*-methyl-*N*-methyl-L-acosamine, an alternative route was designed by derivatizing the secondary amine with bromoacetic acid (route B). Treatment of the bromoacetamide **8** with 1,3-diaminopropane gave the free amine conjugate **6** (Scheme 1). Although typical reactions were carried out with milligram quantities of the secondary amine, it was feasible to form the conjugate with as little as 10 $\mu$g of chiral substrate followed by the use of a Pasteur pipet column for purification prior to UV−vis and CD measurements (see Supporting Information).

**Formation and Stoichiometry of Host/Guest Complexes.** Mixing of porphyrin tweezer host **2** with the bidentate guest conjugate **1-9 (1-9** denotes the conjugate formed between carrier **1** and secondary amine **9**), formed between (*S*)-*N*-benzyl-α-phenyethylamine **9** and carrier **1**, gave rise to an intense positive exciton CD couplet with an amplitude $A_{CD} = +1078$ in methylcyclohexane (MCH) (Figure 2a). A Job plot was obtained from CD spectra measured with different molar fractions of the tweezer host **2** with a fixed total amount of host and guest. It is advantageous to monitor CD rather than UV−vis changes for the construction of the Job plot, since in this manner only the host/guest complex is selectively detected while the uncomplexed host remains CD silent. From the Job plot, it is evident that the stoichiometry for the binding of porphyrin tweezer **2** with conjugate **1-9** is 1:1 (Figure 2b) with the maximum CD amplitude observed when the molar fraction of the host **2** was 0.5.

Formation of the 1:1 macrocyclic complex between porphyrin tweezer **2** and the bidentate conjugate guest is also supported by changes in UV−vis spectra observed with different amounts of guest (Figure 3). With increasing concentration of the guest such as conjugate **1-9**, the UV−vis maxima are shifted from 416 nm (Figure 3a, 0 equiv curve) to 422 nm with a shoulder at 435 nm (Figure 3a, 2−40 equiv curve). The absorption maximum of the complex formed between a monoamine, such as isopropylamine, and porphyrin tweezer **2** is at 428 nm.[23] The blue-shifted band of **1-9/2** complex with an absorption maximum at 422 nm and the red-shifted shoulder at 435 nm arise from high-energy (in-phase) and low-energy (out-of-phase) transitions, respectively, due to the strong exciton coupling between the two nearby porphyrins held in a close-to-parallel orientation.[23] This difference clearly indicates the transition from the free tweezer **2** to the 1:1 host/guest macrocyclic complex (Figure 3a,d). It is only after the guest concentration exceeds 200 equiv that UV−vis maxima of the complex are shifted to longer wavelengths beyond 422 nm (Figure 3b, 200−1000 equiv curves). The red-shift beyond 422 nm points to an increase in the population of the flexible 1:2 host/guest complexes. The binding constant ($K_a$) between host **2** and guest **1-9** is determined to be $5 \times 10^6$ $M^{-1}$ through nonlinear curve-fitting of the absorbance changes at 422 nm with increasing amount of guest **1-9** based on a 1:1 stoichiometry. The value of $K_a$ is similar to that measured for the primary amine conjugate of carrier **1**.[15]

The formation of host/guest complexes accompanying the addition of conjugate **1-9** to porphyrin tweezer **2** is also corroborated by changes in the corresponding CD spectra. The CD amplitudes of the host/guest complex increase as the guest concentration increases from 0.2 to 2 equiv and reaches a maximum at 2 equiv, indicating formation of the 1:1 complex (Figure 3c). The CD amplitudes, while varying little between 2 and 500 equiv of guest concentration, decrease above 500 equiv (Figure 3c), suggesting an increase in the population of the 1:2 complex devoid of exciton coupling. Twenty equivalents of the guest were used as the optimal amount for the following CD investigations. In all cases, the CD spectra were measured in different solvents, such as MCH, hexane, benzene, toluene, methylene chloride, and acetonitrile with the data for complex **1-9**/**2** shown in Table 1. The complexes consistently exhibited similar amplitudes in apolar solvents, that is, MCH, hexane, benzene, toluene, with subsequent CD data given only for MCH and hexane. It is noteworthy that, without exception, the sign of CD exciton couplets for a complex remained the same in all solvents tested.

**CD of the Host/Guest Complexes.** The reason that the conjugate/porphyrin tweezer **2** complex yields an exciton-coupled CD spectrum with a specific sign is due to steric differentiation of the three groups at the stereogenic center of the conjugate by porphyrin tweezer **2**. The two substitutents at the stereogenic center are assigned large (L) and medium (M) according to their steric sizes based on their respective conformational energy *A* values,[17−20] while the third group is hydrogen. Upon addition of conjugate **1-9** to porphyrin tweezer **2**, a 1:1 host/guest complex is formed via coordination of the amino nitrogens of **1-9** to the zinc atoms of the porphyrins. In this complex, porphyrin P-1 binds with the primary amino group of the conjugate, while porphyrin P-2 preferably approaches

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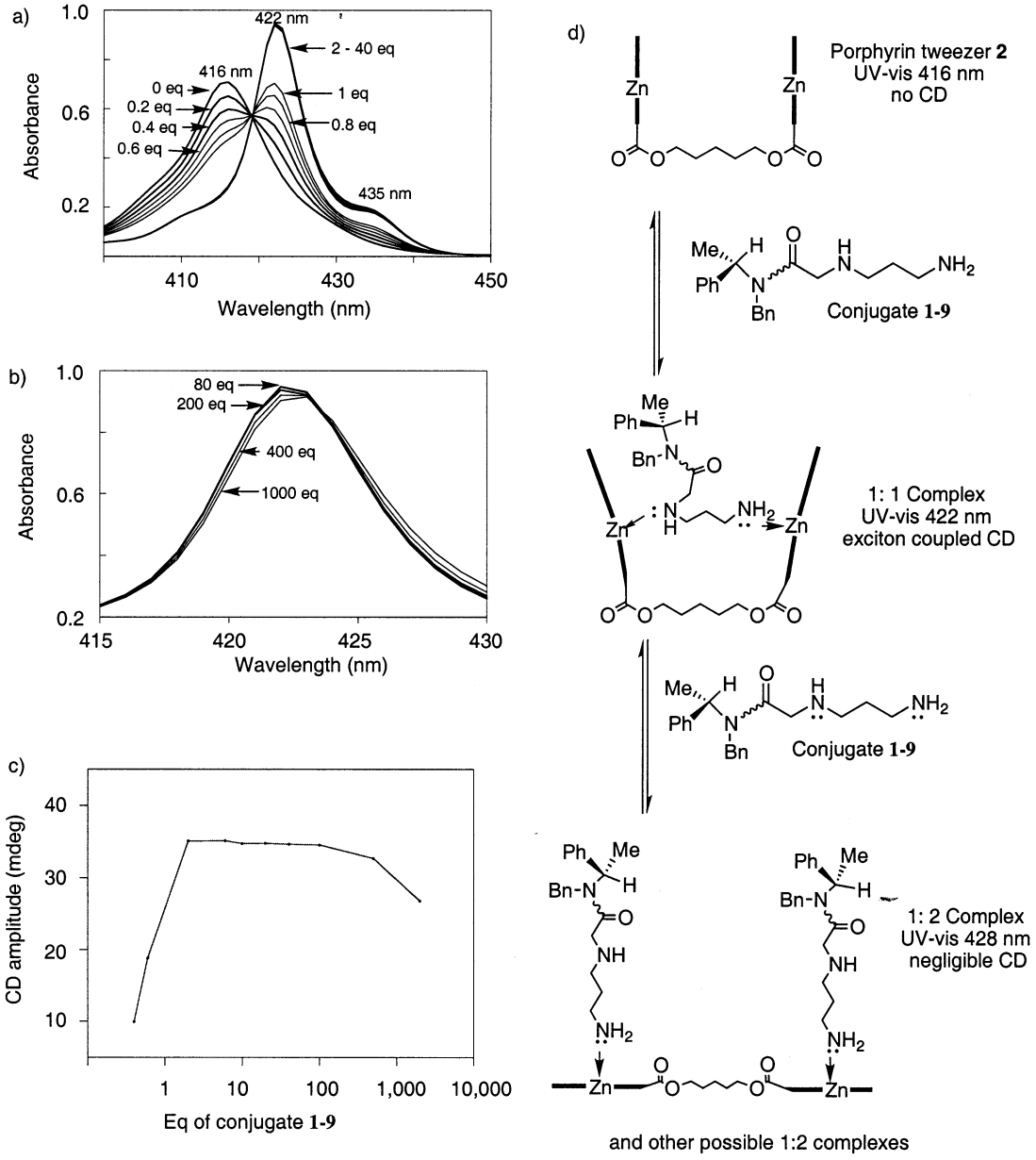
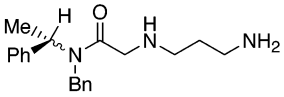



***Figure 3.*** UV−vis and CD of **1-9**/**2** complex with different equivalents of **1-9** in MCH. (The concentration of tweezer **2** is kept constant at 1 $\mu$M in all cases). (a) UV−vis of **2** with 0−40 equiv of **1-9**. **(**b) UV−vis of **2** with 80−1000 equiv of **1-9**. (c) Change in CD amplitudes with different equivalents of **1-9**. (d) Schematic presentation of the equilibria between porphyrin tweezer **2**, 1:1 macrocyclic host/guest complex, and flexible 1:2 host/guest complexes.

the secondary amine from the face of M and H groups, to avoid unfavorable steric interaction with the bulky L group (Figure 4). This stereoselection would render the L group pointing away from the binding pocket formed by the two porphyrins, leading in the case of conjugate **1-9** to a clockwise twist between the transition dipole moments of the two porphyrins, which are along the 5−15 direction of the zinc tetraphenylporphyrins.[21] (For detailed discussion about conformation of the host/guest complex, see the Conformational Analysis section). The overriding clockwise twist between the two porphyrins leads to a positive CD couplet, which is in full agreement with the observed positive CD couplet with an amplitude of +1078 in MCH. Thus, the absolute configuration of the amine can be correlated with the sign of the exciton CD couplet by following the simple rule: if a positive exciton-coupled CD spectrum is observed, the L, M, and H groups are arranged in a clockwise fashion with the amino group in the rear in the Newman projection, and vice versa. This is fully consistent with the primary amine and secondary alcohol conjugates as established previously (conjugates **1-9** vs **1-10**, **1-15** vs **1-16** in Table 2).[15]

The method described here is applicable to chiral secondary amines containing various substituents on the nitrogen (Table 2). While varying the substituents on nitrogen from hydrogen (conjugate **1-10**) to methyl (conjugate **1-11**) or ethyl groups (conjugate **1-12**) does not significantly affect the CD amplitudes, introduction of a benzyl moiety (conjugate **1-9**) leads to a great enhancement in the CD amplitude. It appears that the presence of two aromatic groups, one at the stereogenic center and the other on the nitrogen, is necessary for this enhancement, since the enhancement is much smaller where only one aromatic group is involved (conjugate **1-16** vs **1-11**, **1-16** vs **1-17**). The possible reasons for the observed enhancement of CD amplitude will be discussed in the Molecular Modeling section. Tertiary amides can be formed selectively in the presence of free hydroxyl

***Table 1.*** CD Amplitudes of Complex **1-9/2** in Various Solvents (Concentrations of Tweezer **2** and Conjugate **1-9** Are 1 and 20 $\mu$M, Respectively)

| Guest | Solvent | λ / Δε | $A_{CD}$ amplitude observed |
|---|---|---|---|
| **1-9**  | MCH | 435 nm + 578<br>423 nm - 500 | + 1078 |
| | Hexane | 434 nm + 441<br>422 nm - 381 | + 822 |
| | Methylene chloride | 436 nm + 345<br>424 nm - 301 | + 646 |
| | Benzene | 437 nm + 441<br>426 nm - 387 | + 828 |
| | Toluene | 437 nm + 431<br>426 nm - 384 | + 815 |
| | Acetonitrile | 434 nm + 301<br>422 nm - 255 | + 556 |

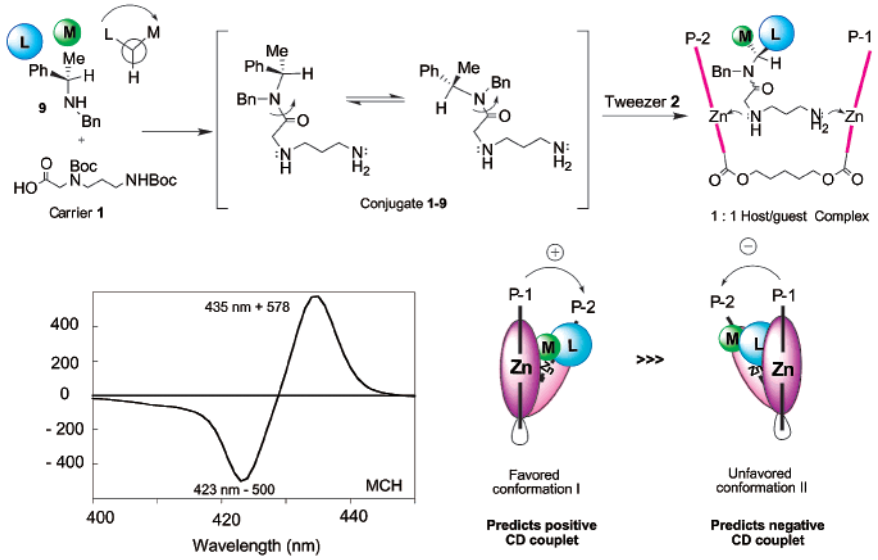



***Figure 4.*** Complex formation between conjugate **1-9** and tweezer **2** leads to two conceivable conformations with opposite sense of twists. The predominant conformation I, the one in which the L (large) group is protruding from the porphyrin binding pocket, gives rise to an CD exciton couplet representing the sense of twist between the two porphyrins and hence the absolute configuration at the stereogenic center.

groups in the chiral substrates (conjugates **1-18**−**1-21**). The existence of multiple chiral centers in these conjugates does not interfere with the results since the amino containing stereogenic center is the sole determinant of the sign of exciton CD couplet.

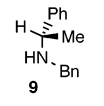
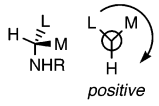
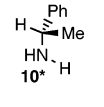
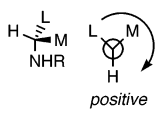
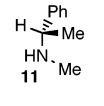
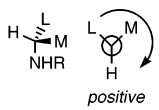
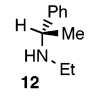
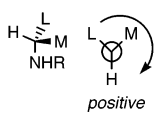
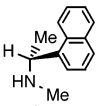
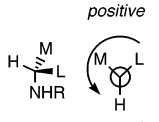
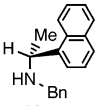
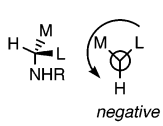
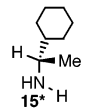
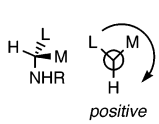
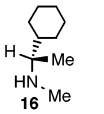
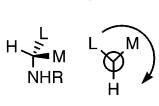
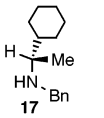
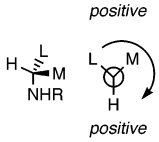
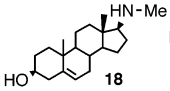
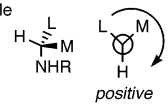
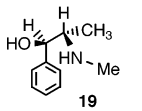
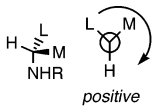
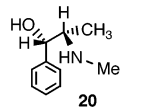
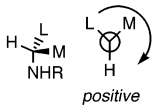
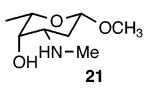
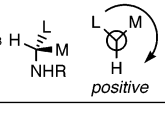
*N*-Me amino acids have also been included in this study since they are important constituents of many biologically active peptides and are often critical for their activities.[24] The carboxyl moiety is assigned M since its conformational energy (∼5 kJ/mol) is smaller than that of the alkyl or aryl substituents (>7.3 kJ/mol).[18]

However, in case of *N*-Me amino esters, unexpected chemical transformation of the conjugates took place, which greatly affected the CD. For example, with (*S*)-*N*-Me-phenylalanine conjugate **1-22a**, the predicted negative CD couplet (0 h curve) completely inverted after 4 h with a much larger amplitude (Figure 5).[25] Moreover, the rate of CD sign inversion was highly dependent upon the size of the substituent groups, for example, in (*S*)-*N*-Me-valine/carrier **1** conjugate the sign inversion took 3 days, while with (*S*)-*N*-Me-alanine/carrier **1** conjugate a positive CD was observed immediately after preparation. It was found that upon deprotonation of the TFA salt of the amino ester conjugate with carrier **1**, the ester conjugate underwent cyclization to yield diketopiperazine **23** in quantitative yield (Figure 5). After isolation, the diketopiperazines functioned as bidentate guests forming 1:1 host/guest complexes with tweezer **2**, which exhibited exciton coupled CD spectra with signs consistent with their absolute stereochemistry (Table 3). This unexpected transformation has prompted our ongoing studies of utilizing tweezer **2** for absolute configurational assignment

(24) Dreyfus, M.; Harri, E.; Hofmann, H.; Kobel, H.; Pache, W.; Tscherter, H. *Eur. J. Appl. Microb.* **1976**, *3*, 125; Takesako, K.; Ikai, K.; Haruna, F.; Endo, M.; Shikamanka, K.; Sono, E.; Nakamura, T.; Kata, I.; Tamaguchi, H. *J. Antibiot.* **1991**, *44*, 919; Luo, Y.; Evindar, G.; Fishlock, D.; Lajoie, G. A. *Tetrahedron Lett.* **2001**, *42*, 3807−3809.

(25) With other conjugates, CD amplitudes of the complexes formed with tweezer **2** did not change significantly with time.

***Table 2.*** Structures and Schematic Representations of Secondary Amines and CD Data of the Conjugate/Tweezer **2** Complexes (Concentrations of Tweezer **2** and the Conjugates Are 1 and 20 $\mu$M, Respectively)

| Chiral substrate | CD Couplet *predicted* | Solvent | λ / Δε | $A_{CD}$ amplitude observed |
|---|---|---|---|---|
|  **9** |  *positive* | MCH | 435 nm + 578<br>423 nm - 500 | + 1078 |
| | | Hex | 435 nm + 441<br>424 nm - 381 | + 822 |
|  **10*** |  *positive* | MCH | 434 nm +130<br>424 nm - 110 | + 240 |
|  **11** |  *positive* | MCH | 435 nm + 120<br>424 nm - 111 | + 231 |
| | | Hex | 434 nm + 148<br>422 nm - 133 | + 281 |
|  **12** |  *positive* | MCH | 434 nm + 162<br>422 nm - 125 | + 287 |
| | | Hex | 433 nm + 144<br>421 nm - 102 | + 246 |
|  **13** |  *negative* | MCH | 435 nm - 223<br>423 nm + 194 | - 427 |
| | | Hex | 435 nm - 181<br>422 nm + 156 | - 337 |
|  **14** |  *negative* | MCH | 435 nm - 503<br>424 nm + 436 | - 939 |
| | | Hex | 432 nm - 326<br>422 nm + 288 | - 593 |
|  **15*** |  *positive* | MCH | 434 nm + 158<br>423 nm - 124 | + 282 |
|  **16** |  *positive* | MCH | 434 nm + 156<br>422 nm - 127 | + 283 |
| | | Hex | 433 nm + 138<br>421 nm - 103 | + 241 |
|  **17** |  *positive* | MCH | 434 nm + 303<br>422 nm - 269 | + 572 |
| | | Hex | 433 nm + 243<br>420 nm - 114 | + 357 |
|  **18** |  *positive* | MCH | 432 nm + 88<br>421 nm - 60 | + 148 |
| | | Hex | 431 nm + 74<br>420 nm - 58 | + 132 |
|  **19** |  *positive* | MCH | 434 nm + 93<br>421 nm - 58 | + 151 |
| | | Hex | 433 nm + 77<br>420 nm - 47 | + 124 |
|  **20** |  *positive* | MCH | 432 nm + 203<br>420 nm - 144 | + 347 |
| | | Hex | 431 nm + 180<br>420 nm - 129 | + 309 |
|  **21** |  *positive* | MCH | 434 nm + 131<br>423 nm - 89 | +220 |
| | | Hex | 434 nm + 42<br>423 nm - 33 | + 75 |

*From ref 15.

of diketopiperazines that have been found to be useful as cell cycle inhibitors,[26] tyrosine kinase inhibitors,[27] and $\beta$-turn mimetics;[28] the results will be reported in due course.

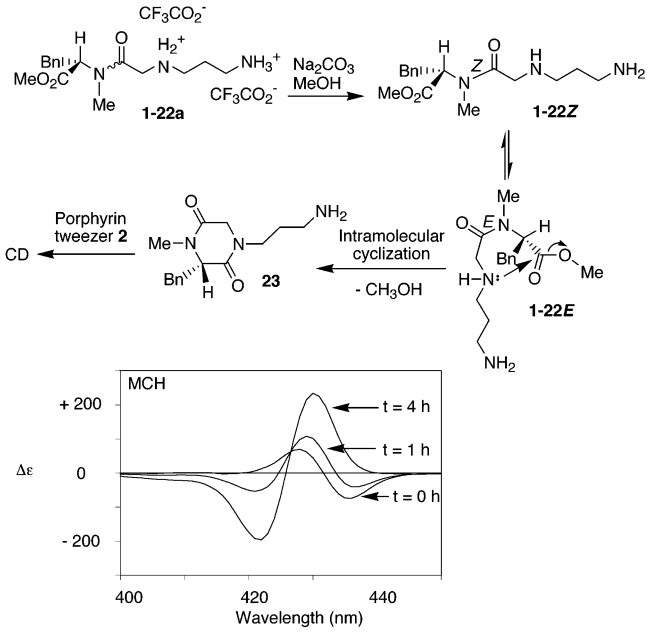
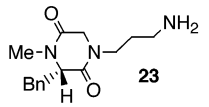
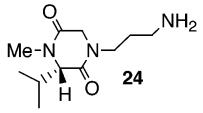
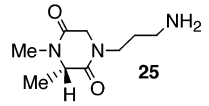
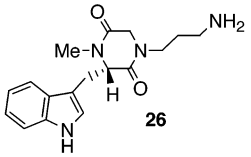



***Figure 5.*** Upon deprotonation of TFA salt of conjugate **1-22a** (CD $t = 0$ h curve), it cyclized to yield diketopiperazine **23** (CD $t = 4$ h curve), which gave opposite sign of exciton-coupled CD upon complexing with Zn-porphyrin tweezer **2**.

***Table 3.*** CD Amplitudes of the Diketopiperazines/Tweezer **2** Complexes in MCH (Concentrations of Tweezer **2** and Diketopiperazines Are 1 and 20 $\mu$M, Respectively)

| Diketopiperazines | λ / Δε | $A_{CD}$ amplitude observed |
|---|---|---|
|  **23** | 428 nm + 240<br>420 nm - 186 | + 426 |
|  **24** | 430 nm + 146<br>421 nm - 117 | + 263 |
|  **25** | 430 nm + 51<br>420 nm - 33 | + 84 |
|  **26** | 430 nm + 276<br>421 nm - 226 | + 512 |

Previously we have reported that the conjugates of primary amino esters (e.g., **1-27**) and hydroxy esters (e.g., **1-28**) with carrier **1** also yielded exciton coupled CD when complexed with porphyrin tweezer **2** (Figure 6).[15,16] Interestingly, it was found that they did not cyclize to yield diketopiperazine or diketomorpholine under the experimental conditions. The lack of cyclization products in these cases is attributed to the propensity of secondary amide in conjugate **1-27** and ester in conjugate **1-28** to adopt the *Z* conformation exclusively.[15,16] The reactive

(26) Wang, H.; Ganesan, A. *Tetrahedron Lett.* **1997**, *38*, 4327−4328; Cui, C.-B.; Kakeya, H.; Osada, H. *Tetrahedron* **1996**, *52*, 12651−12666.
(27) Li, W. R.; Peng, S.-Z. *Tetrahedron Lett.* **1998**, *39*, 7373−7376.
(28) Kim, H.-O.; Nakanishi, H.; Lee, M. S.; Kahn, M. *Org. Lett.* **2000**, *2*, 301−302; Golebiowski, A.; Klopfenstein, S. R.; Shao, X.; Chen, J. J.; Colson, A.-O.; Grieb, A. L.; Russell, A. F. *Org. Lett.* **2000**, 2615−2617.

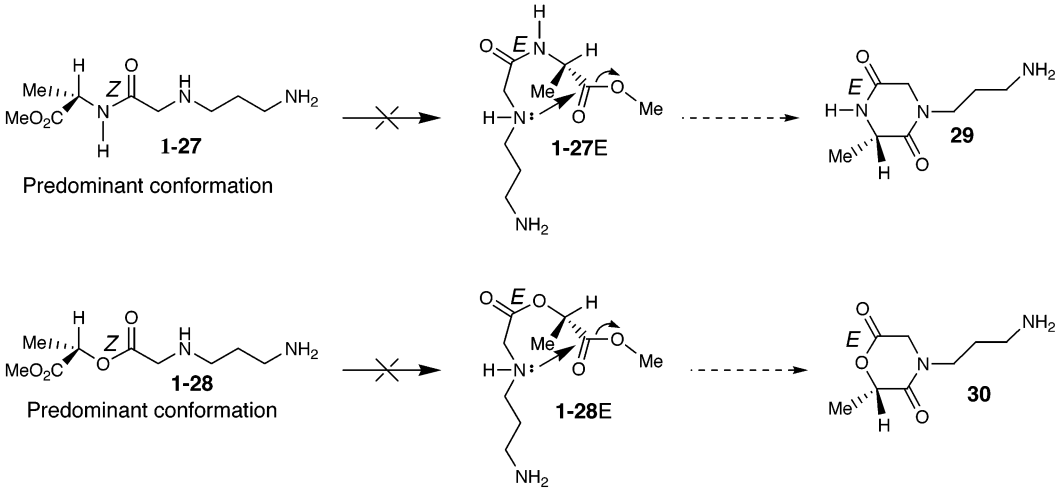



***Figure 6.*** Conjugates **1-27** and **1-28** do not cyclize under experimental conditions due to the predominant *Z* conformations of the amide or ester bonds, respectively.

conformations leading to diketopiperazine **29** or diketomorpholine **30** are presumably *E* (Figure 6).

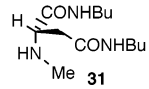
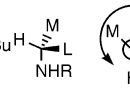
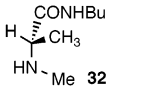
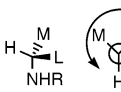
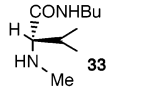
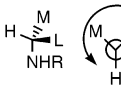
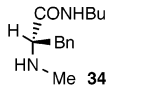
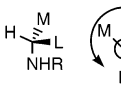
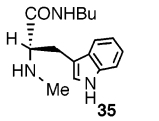
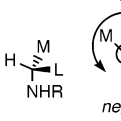
On the basis of these results, and to prevent the formation of diketopiperazines, a modified protocol was developed in which, before conversion to conjugate, the *N*-Me amino acid amides were prepared either through direct amide formation from the free carboxylic acids or aminolysis of the corresponding amino esters.[29] The absolute configuration of *N*-Me amino acid amides can be easily determined by considering the amide moiety as the M group and the alkyl or aromatic moieties as L on the basis of their respective conformational energies (Table 4).

The chiroptical method described here was found to be applicable to cyclic secondary amines as well (Table 5). If a carboxyl substituent is present in the chiral substrate (compounds **36**, **37**, **40**, **41**, **42**), the prior conversion into an amide is necessary to avoid formation of diketopiperazine derivatives. The method has been applied to compounds containing various ring sizes from four- to six-membered rings including pipecolinic acid (**41**)[30] and a tetrahydroisoquinoline derivative (**42**),[31] which are constituents of important biologically active compounds.

This method, however, led to unexpected results with amines containing additional secondary alkyl groups such as cyclohexyl and isopropyl on nitrogen (compounds **43** and **44**). The sign of observed CD of the complexes formed between conjugates **1-43** and **1-44** and porphyrin tweezer **2** turned out to be opposite to that predicted for the corresponding conjugate carrying a primary alkyl group (namely conjugates **1-9** and **1-11**) (Table 6). We find that application of the present chiroptical method to secondary amines containing cyclohexyl and isopropyl on nitrogen is possible in conjunction with molecular modeling due to unusual conformational features of this class of compounds (see the Molecular Modeling section).

In summary, a chiroptical microscale protocol has been developed for absolute configurational assignment of secondary amines. The starting amines are converted into bidentate conjugates with carrier **1** in high yields. Upon mixing of the conjugates with the host tweezer **2**, a 1:1 host/guest complex is formed, which exhibits an exciton CD couplet in the porphyrin Soret band region, with the sign diagnostic of the absolute configuration.

***Table 4.*** Structures and Schematic Representations of N-Me Amino Acid Amides and CD Data of the Conjugate/Tweezer **2** Complexes (Concentrations of Tweezer **2** and the Conjugates Are 1 and 20 $\mu$M, Respectively)

| Chiral substrate | CD Couplet *predicted* | Solvent | λ / Δε | $A_{CD}$ amplitude observed |
|---|---|---|---|---|
|  **31** |  *negative* | MCH | 431 nm - 49<br>420 nm + 39 | - 88 |
| | | Hex | 431 nm - 58<br>419 nm + 47 | - 105 |
|  **32** |  *negative* | MCH | 432 nm - 10<br>422 nm + 16 | - 26 |
| | | Hex | no CD | |
|  **33** |  *negative* | MCH | 432 nm - 114<br>422 nm + 87 | - 201 |
| | | Hex | 431 nm - 93<br>421 nm + 80 | - 173 |
|  **34** |  *negative* | MCH | 431 nm - 101<br>420 nm + 93 | - 204 |
| | | Hex | 431 nm - 102<br>419 nm + 84 | - 186 |
|  **35** |  *negative* | MCH | 432 nm -74<br>421 nm +59 | -133 |
| | | Hex | 432 nm -57<br>420 nm +39 | -96 |

## Structural Studies and Conformational Analysis of the Host/Guest Complexes

To better understand the correlation between the absolute configuration of the secondary amine, the conformational complexity of corresponding amides, and the genesis of porphyrin helicity in host/guest complexes, NMR and molecular modeling studies of these complexes have been performed.

(29) Ghosh, A. K.; Lee, H. Y.; Thompson, W. J.; Culberson, C.; Holloway, M. K.; Mckee, S. P.; Munson, P. M.; Duong, T. T.; Smith, A. M.; Darke, P. L.; Zugay, J. A.; Emini, E. A.; Schleif, W. A.; Huff, J. R.; Anderson, P. S. *J. Med. Chem.* **1994**, *37*, 1177−1188.
(30) Scott, J. D.; Williams, R. M. *Tetrahedron Lett.* **2000**, *41*, 8413−8416; Scott, J. D.; Williams, R. M. *J. Am. Chem. Soc.* **2002**, *41*, 8413−8416.
(31) Sánchez, R.; Luna, H.; Pérez, H. I.; Manjarrez, N.; Solís, A. *Tetrahedron: Asymmetry* **2001**, *12*, 1399−1401.

***Table 5.*** Structures and Schematic Representations of Cyclic Secondary Amines and CD Data of the Conjugate/Tweezer **2** Complexes (Concentrations of Tweezer **2** and the Conjugates Are 1 and 20 $\mu$M, Respectively)

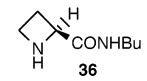
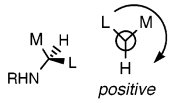
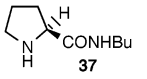
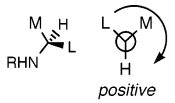
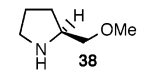
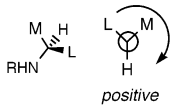
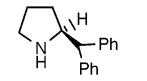
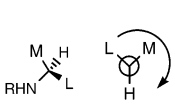
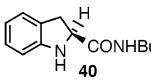
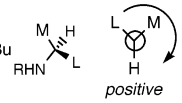
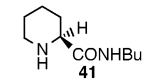
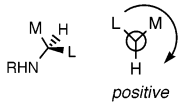
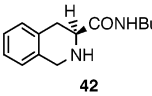
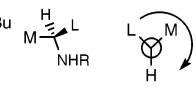
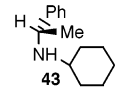
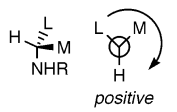
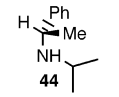
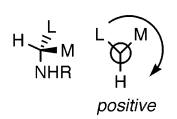
| Chiral substrate | CD Couplet *predicted* | solvent | λ / Δε | $A_{CD}$ amplitude observed |
|---|---|---|---|---|
|  **36** |  *positive* | MCH | 432 nm + 55<br>421 nm - 46 | + 101 |
| | | Hex | 432 nm + 59<br>420 nm - 47 | + 106 |
|  **37** |  *positive* | MCH | 433 nm + 102<br>422 nm - 80 | + 182 |
| | | Hex | 433 nm + 114<br>421 nm - 86 | + 200 |
|  **38** |  *positive* | MCH | 435 nm +154<br>423 nm - 120 | + 274 |
| | | Hex | 434 nm +149<br>422 nm - 118 | + 267 |
|  **39** |  *positive* | MCH | 435 nm + 133<br>424 nm - 125 | + 258 |
| | | Hex | 434 nm + 201<br>422 nm - 147 | + 348 |
|  **40** |  *positive* | MCH | 431 nm + 310<br>420 nm -276 | +586 |
| | | Hex | 420 nm + 285<br>419 nm - 245 | +530 |
|  **41** |  *positive* | MCH | 434 nm + 93<br>422 nm - 70 | + 163 |
| | | Hex | 433 nm + 103<br>421 nm - 70 | + 173 |
|  **42** |  *positive* | MCH | 432 nm + 218<br>421 nm - 177 | + 395 |
| | | Hex | 431 nm + 261<br>420 nm - 214 | + 475 |

***Table 6.*** Structures and Schematic Representations of Secondary Amines Bearing Additional *N*-secondary Alkyl Groups and CD Data of the Conjugate/Tweezer **2** Complexes (Concentrations of Tweezer **2** and the Conjugates Are 1 and 20 $\mu$M, Respectively)

| Chiral substrate | CD Couplet *predicted* | Solvent | λ / Δε | $A_{CD}$ amplitude observed |
|---|---|---|---|---|
|  **43** |  *positive* | MCH | 433 nm - 84<br>422 nm + 75 | - 159 |
| | | Hex | 433 nm - 55<br>421 nm + 58 | - 113 |
|  **44** |  *positive* | MCH | 433 nm - 63<br>421 nm + 37 | - 100 |
| | | Hex | 432 nm - 53<br>420 nm + 33 | - 86 |

## NMR Studies of Host/Guest Complexes

**NMR Assignments of Conjugate 1-9 Upon Complexation with Tweezer 2.** The $^1$H NMR spectrum of the free-base form of conjugate **1-9** in $CDCl_3$ shows the presence of a 2:1 mixture of two conformers arising from rotation around the amide bond. The major conformer (**1-9***Z*), with an H-1 methine quartet at 6.16 ppm, adopts *Z*-amide conformation, while the minor conformer with an H-1 methine quartet at 5.13 ppm, adopts *E*-amide conformation (**1-9***E*) (Figure 7a).

Upon complexation with tweezer **2**, the proton resonances of **1-9** undergo a series of important diagnostic shifts (Table 7).[32] The signals from the propanediamine linker, H-5″, H-6″, and H-7″, are shifted upfield by at least 6 ppm due to the effect of the porphyrin ring current,[33] with the 6″-H showing the largest shift of −7.35 ppm (Figure 7b). The fact that H-6″, located in the middle of the chain, experiences the largest shift demonstrates that both amine nitrogens are coordinated to zinc porphyrins and that the conjugate is sandwiched between the two porphyrins of the zinc porphyrin tweezer. Complexation to a single porphyrin would result in substantially smaller upfield shifts, especially for the protons farther removed from the coordination site.[33]

The host/guest complex **1-9**/**2** (**1-9**/**2** denotes the complex formed by conjugate **1-9** and porphyrin tweezer **2**) showed two sets of signals in a 2:1 ratio for conjugate **1-9** (Figure 7c and Table 7). These are assigned by 2D ROESY measurements as arising from the *Z* and *E* conformers of the bound conjugate. To slow the dynamic equilibrium between the multiple conformations of the complexes, ROESY experiments were carried out at 270 K. Lowering the temperature of the sample resulted in minimal changes in chemical shifts for the guest signals, whose assignments were confirmed by 2D TOCSY and HSQC.[34] In the major conformer of the bound guest, the amide conformation is assigned as *Z*, on the basis of NOE correlations observed between one of the H-1′ benzyl protons at 2.28 ppm with the propanediamine protons at −2.93 ppm (H-3″) and −3.99 ppm (H-5″). The H-1 methine at 4.56 ppm, deshielded by a syn relationship to the amide carbonyl, showed only intraguest NOEs to the 1-Me at 0.55 ppm and to adjacent H-3 aromatic protons at 6.32 ppm (see Figure S in Supporting Information). Strong NOE interactions between the 1-Me group at 0.55 ppm and pyrrole protons of the porphyrin moiety at 8.60 and 8.91 ppm were observed, indicating a close proximity between these two groups in the host/guest complex (Figure 7d). Conversely, in the bound minor *E* conformer the H-1 methine quartet of the guest at 2.75 ppm showed NOE interactions with H-3″ at −2.79 ppm and with H-5″ at −3.88 ppm. The minor conformer failed to exhibit any NOE interactions between host **2** and the guest.

Because the three groups attached to the stereogenic center are differentially accommodated within the intra-tweezer pocket depending on their steric sizes, it is conceivable that they would experience different ring current-induced upfield shifts from zinc porphyrin tweezer **2**.[15] On the basis of our model (Figure 4, see also the Molecular Modeling section), the hydrogen, being the smallest group, will lie between the porphyrins, and be subjected to the strongest ring current effect; the large group L will protrude outside of the pocket in a region of relatively

(32) The NMR measurements were carried out on a sample consisting of 2.8 mM tweezer in the presence of 0.7 equiv of conjugate **1-9** in 0.60 mL of $CDCl_3$, at 300 and 270 K. The NMR signals of the complex of **1-9** with tweezer **2** were assigned by TOCSY, DEPT-HSQC, and ROESY experiments.

(33) Abraham, R. J.; Bedford, G. R.; McNeillie, D.; Wright, B. *Org. Magn. Reson.* **1980**, *14*, 418−425; Abraham, R. J.; Medforth, C. J. *Magn. Reson. Chem.* **1990**, *28*, 343−347; Abraham, R. J.; Marsden, I. *Tetrahedron* **1992**, *48*, 7489−7504; Cross, K. J.; Crossley, M. J. *Aust. J. Chem.* **1992**, *45*, 991−1004.

(34) Wilker, D.; Liebfritz, D.; Kerssebaum, R.; Bermel, W. *Magn. Res. Chem.* **1993**, *31*, 287−292.

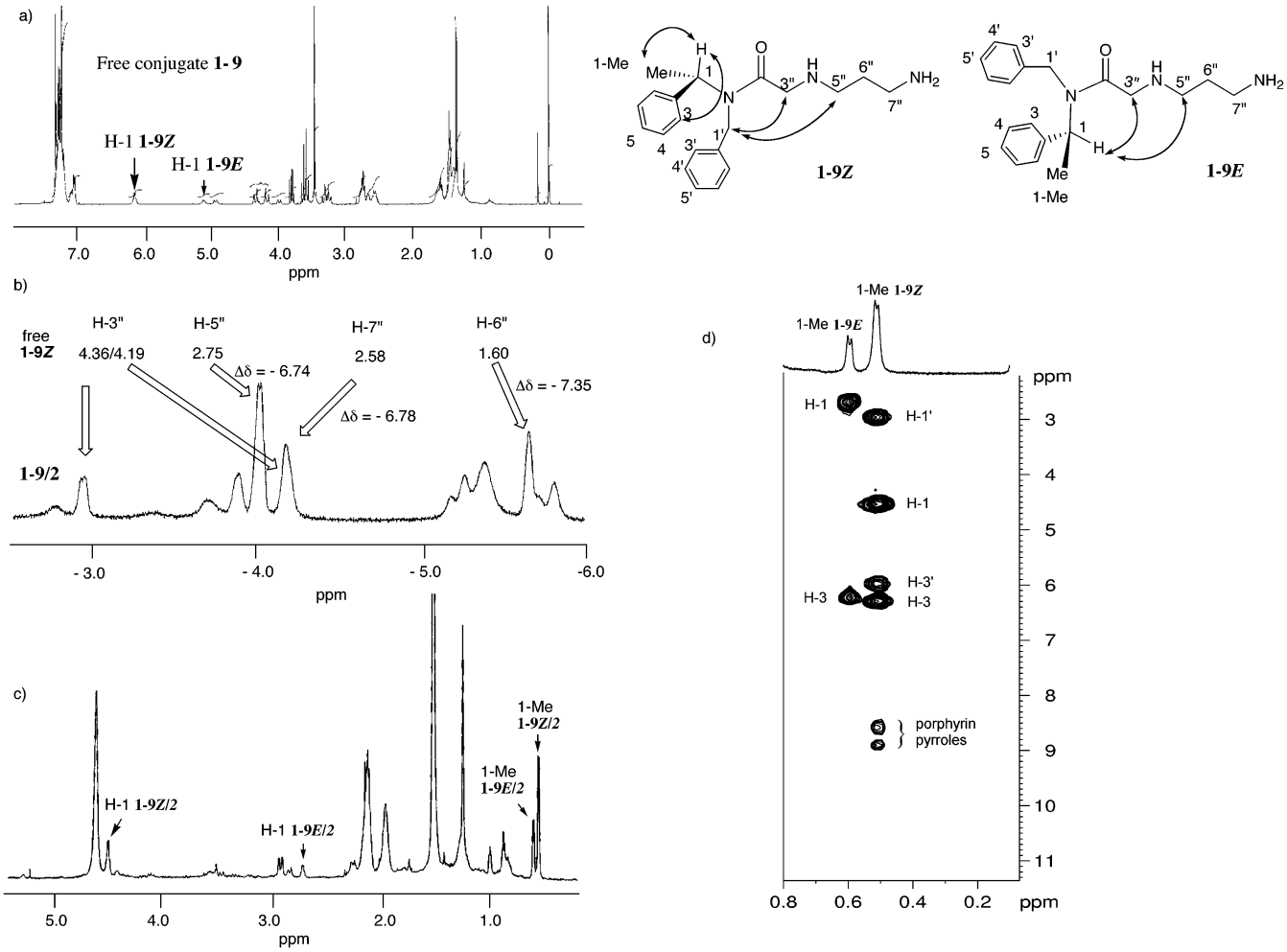



***Figure 7.*** Structures of **1**-**9***Z* and **1**-**9***E* conjugates. Double-headed arrows indicate the intraguest NOE correlations observed in **1**-**9**/**2** complex that are diagnostic of the amide bond conformation. (a) $^1$H NMR spectrum of free conjugate **1**-**9** in $CDCl_3$ (500 MHz) at 300 K. (b) $^1$H NMR spectrum of complex **1**-**9**/**2** from −6 ppm to −2.5 ppm in $CDCl_3$ (500 MHz) at 300K. (c) $^1$H NMR spectrum of complex **1**-**9**/**2** from 0 to 5.5 ppm in $CDCl_3$ (500 MHz) at 300 K. (d) 2D ROESY spectrum of complex **1**-**9**/**2** in $CDCl_3$ (500 MHz) at 270 K, indicating NOE correlation between 1-Me of **1**-**9***Z* and porphyrin pyrrole protons.

***Table 7.*** $^1$H NMR Chemical Shifts $\delta$ (ppm) in $CDCl_3$ (600 MHz) at 300 K for Major and Minor Conformers of Conjugates **1**-**9** and **1**-**9**/**2** Complex, and Ring Current Shift $\Delta\delta = \delta_{1\text{-}9} - \delta_{1\text{-}9/2}$ (ppm) upon Complexation

| | major conformer | | | | | minor conformer | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| proton | free conjugate **1**-**9***Z* | bound conjugate **1**-**9***Z*/**2** | Δδ | | | free conjugate **1**-**9***E* | bound conjugate **1**-**9***E*/**2** | Δδ | | |
| 1 | 6.16 | 4.56 | 1.60 | | S | 5.13 | 2.75 | 2.38 | | S |
| 1-Me | 1.46 | 0.55 | 0.89 | | M | 1.47 | 0.60 | 0.87 | | M |
| 3 | 7.07 | 6.32 | 0.75 | | | 7.12 | 6.25 | 0.87 | | |
| 4 | 7.27 | 7.19 | 0.08 | 0.30 | L | 7.20 | 6.95 | 0.25 | 0.38 | L |
| 5 | 7.25 | 7.19 | 0.06 | | | 7.18 | 7.17 | 0.01 | | |
| 1′a | 3.66 | 2.95 | 0.71 | | | 3.66 | 3.51 | 0.15 | | |
| 1′b | 3.60 | 2.28 | 1.32 | | | 3.60 | 2.82 | 0.78 | | |
| 3′ | ≈7.3 | 5.95 | 1.35 | | | ≈7.3 | 6.02 | 1.28 | | |
| 4′ | ≈7.3 | 6.83 | 0.47 | | | ≈7.3 | 6.95 | 0.35 | | |
| 5′ | ≈7.3 | 7.11 | 0.19 | | | ≈7.3 | 7.07 | 0.23 | | |
| 3″a | 4.36 | −2.93 | 7.29 | | | 4.95 | −2.79 | 7.74 | | |
| 3″b | 4.19 | −3.77 | 7.96 | | | 4.01 | −3.77 | 7.78 | | |
| 5″ | 2.75 | −3.99 | 6.74 | | | 2.79 | −3.88 | 6.67 | | |
| 6″ | 1.60 | −5.75 | 7.35 | | | 1.64 | −5.86 | 7.50 | | |
| 7″ | 2.58 | −4.20 | 6.78 | | | 2.68 | −4.02 | 6.70 | | |

weaker effect, while for the medium group M an intermediate shielding is predicted. This would offer an experimental method to assign their relative steric sizes independent of the conformational energy values. This is corroborated by the NMR shift differences of conjugate **1**-**9** before and after complexation with tweezer **2** (Table 7). For the major conformer, the observed ring current shift is 1.60 ppm for the hydrogen, 0.89 ppm for the M group methyl, and on the average 0.30 for the L group phenyl protons. The same trend is observed for the minor conformer, as the observed ring current shift is 2.38 ppm for the hydrogen, 0.87 ppm for the M group methyl, and on the average 0.38 ppm for the L group phenyl protons. *Thus, in favorable cases*

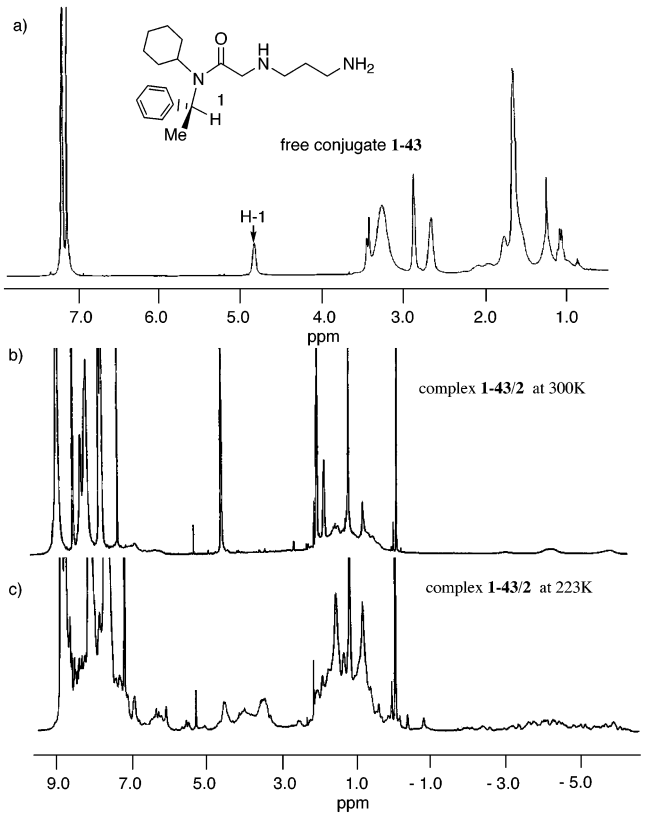



***Figure 8.*** (a) $^1$H NMR spectrum of free conjugate **1-43** in $CDCl_3$ (500 MHz) at 300 K, indicating the presence of only *E* conformer. (b) $^1$H NMR spectrum of complex **1-43**/**2** in CDCl3 (500 MHz) at 300 K. (c) $^1$H NMR spectrum of complex **1-43**/**2** in $CDCl_3$ (500 MHz) at 223K.

*where the proton resonances of L and M groups in the complex can be clearly assigned, the difference in the ring current-induced upfield shifts provides experimental evidence for establishing the relative steric size of the groups attached to the stereogenic center*.

**NMR of Other Conjugates and Host/Guest Complexes.** Free conjugate **1-13** bearing *N*-Me (Table 2) and the cyclic secondary amine conjugate **1-37** (Table 5) in $CDCl_3$ showed the presence of both *Z* and *E* conformers in 3:1 and 1:1 ratio, respectively, at room temperature, while only the *E* conformer was detected for the *N*-cyclohexyl **1-43** conjugate (Figure 8a). The NMR spectra of the complex **1-43**/**2** showed very broad signals for the guests (Figure 8b), as was the case with complexes **1-11**/**2** and **1-37/2**. This was rationalized by the existence of multiple bound guest conformations undergoing rapid exchange inside the host/guest complexes. Lowering the temperature to −50 °C reduced the line broadening but unfortunately revealed extremely complicated NMR spectra (Figure 8c), which rendered further detailed analysis of these particular substrates impossible.

**Molecular Modeling of Host/Guest Complexes.** Molecular modeling studies of host/guest complexes offer an independent tool to predict the expected sign of exciton-coupled CD in addition to conformational energy values (*A* values) and NMR methods presented above. This will be especially helpful in cases where the assignment of L and M groups cannot be accomplished unambiguously because either their *A* values are similar or simply unavailable. Moreover, as discussed above, in many cases the host/guest complexes exhibit complicated NMR spectra, making assignments of L and M groups from chemical shift differences difficult, if not impossible.

**Choice of Computational Method.** The conformations of the host/guest complexes were studied by carrying out molecular mechanics (MM) calculations with the Merck molecular force field (MMFF94)[35] utilizing MacroModel 7.1.[36,37] Various MM methods have been successfully applied to modeling porphyrin-derived structures.[38] MMFF94 differs from the most frequently utilized Allinger-type MM force fields (MM2 and MM3) mainly for its peculiar expressions of van der Waals (VdW) and electrostatic interactions.[35] The choice of MMFF94, whose accuracy was first checked with known structures of porphyrins with axial amino ligands,[39] is based on the possibility of treating zinc ions explicitly (as parametrized in the MacroModel package) within a non-bonded model where the interaction between zinc and nitrogen is only of VdW and electrostatic nature. As a result, the coordination of zinc ions to the amine nitrogens of the conjugate could be reproduced without resorting to dummy atoms with constrained geometry, which will not bias the geometrical freedom around the Zn−N bonds. The "static" version of MMFF94 was employed (referred to as MMFFs) to ensure the planarity of amide and aromatic nitrogen atoms.[35] The conformational space was sampled by means of the Metropolis Monte Carlo (MC) algorithm implemented in MacroModel.[37]

**Modeling of Free Conjugates.** The molecular conformations of the free conjugates **1-9**, **1-10**, **1-11**, and **1-43** were investigated by MC/MMFFs calculations in $CHCl_3$. In the calculated MMFFs structures of the free secondary amine conjugates, the conformation around the amide bond is found to be strongly dependent on the substrate, as summarized in the following:

(1) For the primary amine conjugate **1-10**, only the *Z* conformer is found.

(2) For secondary amine conjugates **1-9** and **1-11** the *Z* conformation is favored with an approximate 2:1 *Z*/*E* ratio calculated from the MMFFs energy differences at room temperature for both compounds. Figure 9 reports the lowest-energy structures for conjugate **1-9** in the two amide conformations.

(3) For the *N*-cyclohexyl conjugate **1-43**, only the *E* conformation is found within 10 kJ/mol.

These calculation results nicely corroborate experimental NMR results of the free conjugates presented above.

**Modeling of Conjugate/Tweezer Complexes: General Approach and Results.** MC/MMFFs calculations were per-

(35) Halgren, T. A. *J. Comput. Chem.* **1996**, *17*, 616−641; Halgren, T. A.; Nachbar, R. B. *J. Comput. Chem.* **1996**, *17*, 587−615; Halgren, T. A. *J. Comput. Chem.* **1996**, *17*, 553−586; Halgren, T. A. *J. Comput. Chem.* **1996**, *17*, 520−552; Halgren, T. A. *J. Comput. Chem.* **1996**, *17*, 490−519.
(36) Mohamadi, F.; Richards, N. G. J.; Guida, W. C.; Liskamp, R.; Lipton, M.; Caufield, C.; Chang, G.; Hendrickson, T.; Still, W. C. *J. Comput. Chem.* **1990**, *11*, 440−467.
(37) Chang, G.; Guida, W. C.; Still, W. C. *J. Am. Chem. Soc.* **1989**, *111*, 4379−4386.
(38) Marques, H. M.; Brown, K. L. *Coord. Chem. Rev.* **2002**, *225*, 123−158.
(39) The MMFFs optimized structure for the complex pyridine-Zn(TPP) has a N−Zn distance of $d$(N−Zn) = 2.291 Å and the Zn atom protrudes by $d$($N_4$−Zn) = 0.300 Å from the average plane defined by the four pyrrole nitrogens. The average values for the 18 entries found in the Cambridge Crystallographic Database (CCD), relative to Zn-tetraphenylporphyrins axially bound to pyridine derivatives, are $d$(N−Zn) = 2.169 ± 0.04 Å and $d$($N_4$−Zn) = 0.327 ± 0.04 Å. More interestingly, in the MC/MMFFs structures for the host/guest complexes, the corresponding average values for the amino groups of the linker are $d$(N−Zn) = 2.245 ± 0.010 Å and $d$($N_4$−Zn) = 0.265 ± 0.015 Å. Only two entries are found in the CCD for Zn-tetraphenylporphyrins axially bound to aliphatic amines; they have $d$(N−Zn) = 2.2415 ± 0.020 Å and $d$($N_4$−Zn) = 0.26 ± 0.12 Å, in very good agreement with our calculations. (TPP = tetraphenylporphyrin).

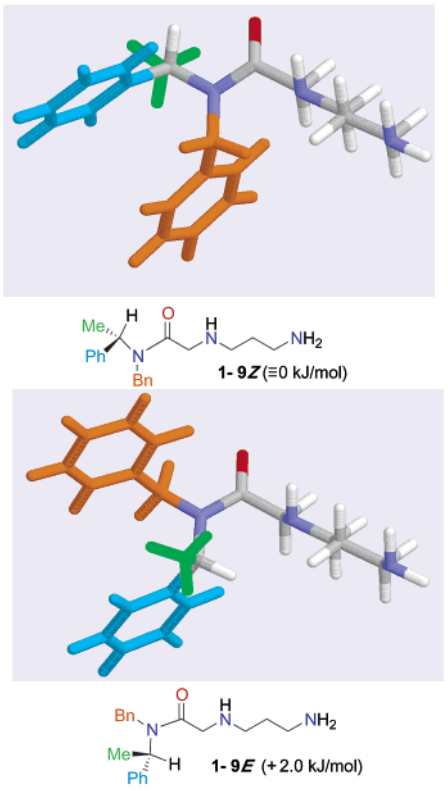
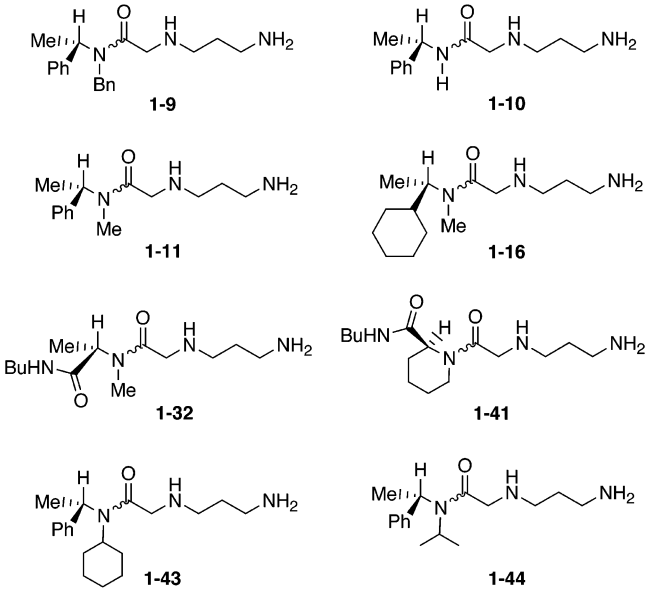



***Figure 9.*** Lowest-energy structures (calculated with MMFFs in $CHCl_3$) and relative energies for conjugate **1-9** in the two amide conformations **1-9***Z* and **1-9***E* with energy for the *Z* isomer set to 0.

***Chart 3***




formed on the complexes between conjugates **1-9**, **1-10**, **1-11**, **1-16**, **1-32**, **1-41**, **1-43**, **1-44** (Chart 3) and tweezer host **2**. Due to the complexity and multiple degrees of freedom of the system, in all cases investigated a number of local minima were found within a limited energy window, usually about 10−15 structures within 10 kJ/mol over a total of 1000 MC steps. Moreover, repetition of the calculation from the same starting structure and with the same parameters and convergence criteria occasionally led to a partially different set of minima. This renders seeking the actual absolute energy minimum an impractical task. This observation agrees well with the conformational heterogeneity demonstrated by NMR spectra of the host/guest complexes. It must be stressed that any discrete species detected by NMR would in principle correspond to an ensemble of fast exchanging structures.

Because of the incomplete parametrization of MMFFs as well as the complexity of the system leading to questionable absolute accuracy of the calculated energies, *the lowest-energy minimum cannot be taken as an entirely faithful representation of the host/guest complex*. It is more appropriate to take into account all the structures within an energy window arbitrarily set to 10 kJ/mol, and to focus on the distribution of structures characterized by the sign and value of the projection angle $\theta$, rather than on the absolute energies. This quantity refers to the projection angle between the transition dipole moments of the two porphyrins running in the 5−15 direction (defined as in Figures 1 and 11),[21,40] which is indicative of the sign of the expected exciton couplet, that is, a positive twist leads to a positive couplet, and vice versa. Following an approach similar to cluster-type analysis,[41] we plotted distribution graphs of $\theta$ values among all minima within 10 kJ/mol, corresponding approximately to 30−40 optimized structures resulting from the MC calculations (3 times 1000 steps). In these graphs (Figure 10), the preferred sign of projection angle $\theta$ for a given complex can be inferred by counting the total number of structures presenting positive and negative $\theta$. Moreover, the value of $\theta$ corresponding to the higher probability cluster detectable in all cases can be taken as the "most probable" $\theta$ value adopted in the host/guest complexes. *For all compounds investigated, the predicted preferred sign of θ based on calculated distribution agreed with the sign of the observed exciton CD couplet.* In most situations, the preferred sign also agrees with the predicted sign based on conformational *A* values.[18] In the two cases (**1-43/2** and **1-44/2** complexes) where prediction based on *A* values seems to fail, MC/MMFFs calculations correctly predicted the observed sign of the porphyrin twist. Thus, the current calculation method in conjunction with the experimental CD host/guest complexes can be used as a reliable tool for assigning the absolute configuration of the secondary amine. We strongly suggest employing this MC/MMFFs calculation approach when an unambiguous prediction of relative steric size of substituents is not possible solely on the basis of conformational energies *A* values and NMR data.

**Modeling of Conjugate/Tweezer Complexes: Analysis of Calculated Structures. (A) Complex 1-9/2 (Figures 10c and 11).** This complex was especially worthy of investigation for the purpose of comparison with experimental conformational information, since its structure is the only one accessible in detail through NMR studies. A large preference for supramolecular conformations with positive $\theta$ values (86%) is found (Figure

(40) The porphyrin Soret transition is doubly degenerate and is more correctly described as a circular oscillator polarized in the whole chromophoric plane (Hsu, M.-C.; Woody, R. W. *J. Am. Chem. Soc.* **1971**, *93*, 3515−3525). However, in many situations the exciton interaction between two porphyrins may be interpretated in terms of an effective transition dipole moment, which in the current case is directed along the 5−15 direction.[21] The theoretical basis of this approach and its application to different bisporphyrin derivatives is currently under investigation: Pescitelli, G., et al., in preparation.

(41) Watts, C. R.; Mezei, M.; Murphy, R. F.; Lovas, S. *J. Biomol. Struct. Dyn.* **2001**, *18*, 733−748; Ripoll, D. R.; Liwo, A.; Scheraga, H. A. *Biopolymers* **1998**, *46*, 117−126; Levy, S.; Schuyler, S. C.; Maglothin, R. K.; Staehelin, L. A. *Biopolymers* **1996**, *38*, 251−272; Wang, C. G.; Langer, T.; Kamath, P. G.; Gu, Z.-Q.; Skolnik, P.; Fryer, R. I. *J. Med. Chem.* **1995**, *38*, 950−957; Bryant, S. D.; Balboni, G.; Guerini, R.; Salvadori, S.; Tomatis, R.; Lazarus, L. H. *Biol. Chem.* **1997**, *378*, 107−114.

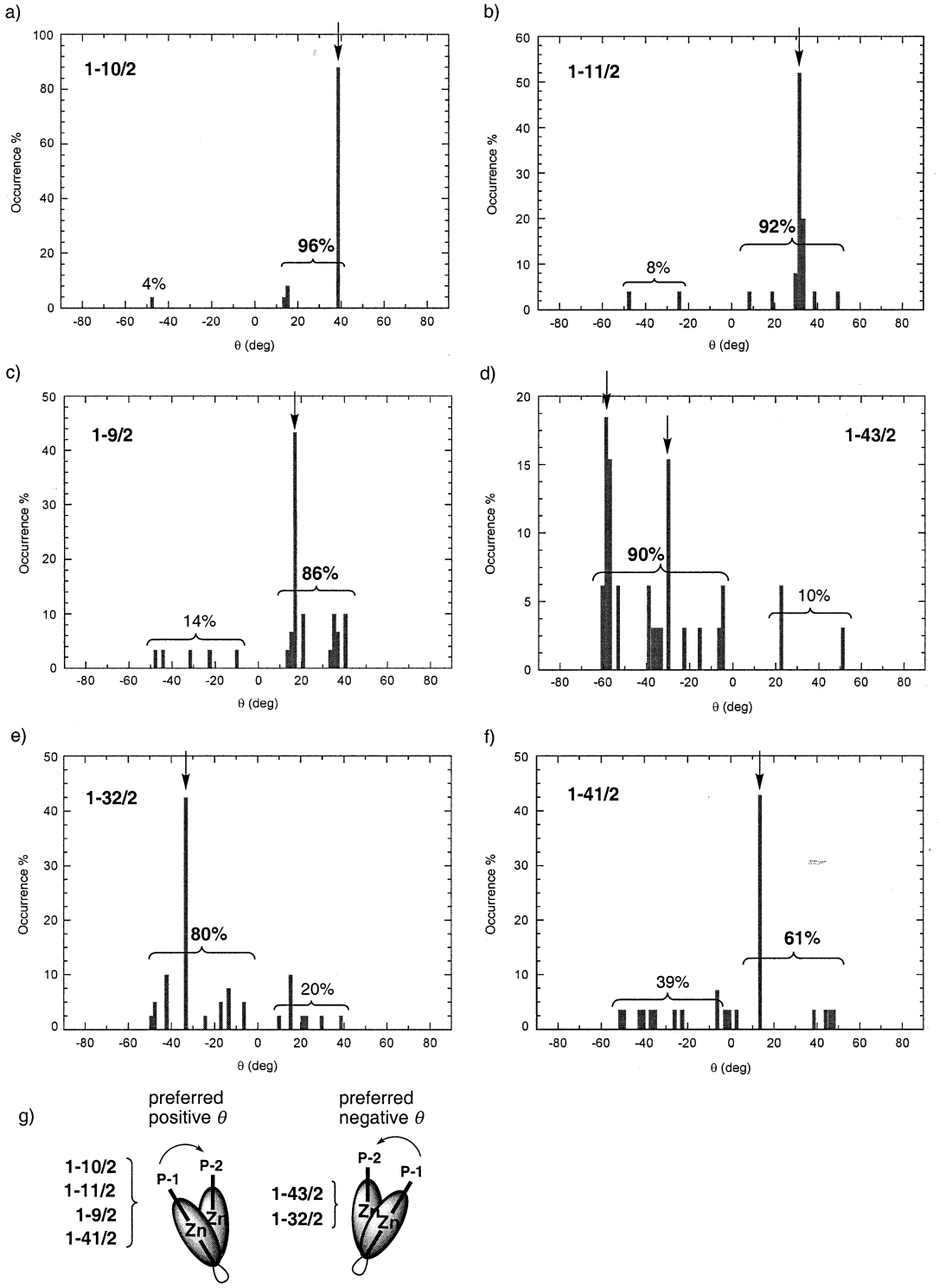



***Figure 10.*** Distribution of $\theta$ values of the MC/MMFFs calculated host/guest complexes within 10 kJ/mol of the lowest-energy conformation for various conjugates (see Chart 3 for structures). The probability of structures with negative and positive values of $\theta$ is reported as percentage. Numbers in bold are for the prevailing sign, also summarized in (g). Arrows indicate the higher probability cluster, to which the "most probable" $\theta$ value is referred.

10c). The calculated structure shown in Figure 11c,d also characterized by a positive twist between the two interacting porphyrins (side view, Figure 11d) is the lowest-energy conformation within the most probable cluster ($\theta \approx +17°$). The amide conformation of the guest in the complex is *Z*, which nicely corroborates the NMR results. In less than 10% of all **1-9/2** complexes, the guests adopt *E*-amide conformation, but interestingly even in this conformation the two porphyrins mostly retain the positive twist. In the most probable structures with the guest in the *Z* conformation, the H-1 is always syn to the carbonyl (Figures 11c,d); the position of the *N*-benzyl group is also in agreement with the observed NOEs between protons 3′/1-Me, 1′/1-Me, and 1′/3. Except for the complexes of **1-43/2** and **1-44/2** (vide infra), the structure depicted in Figure 11c,d is a good representation of a general host/guest arrangement valid for all complexes investigated, which is described in detail

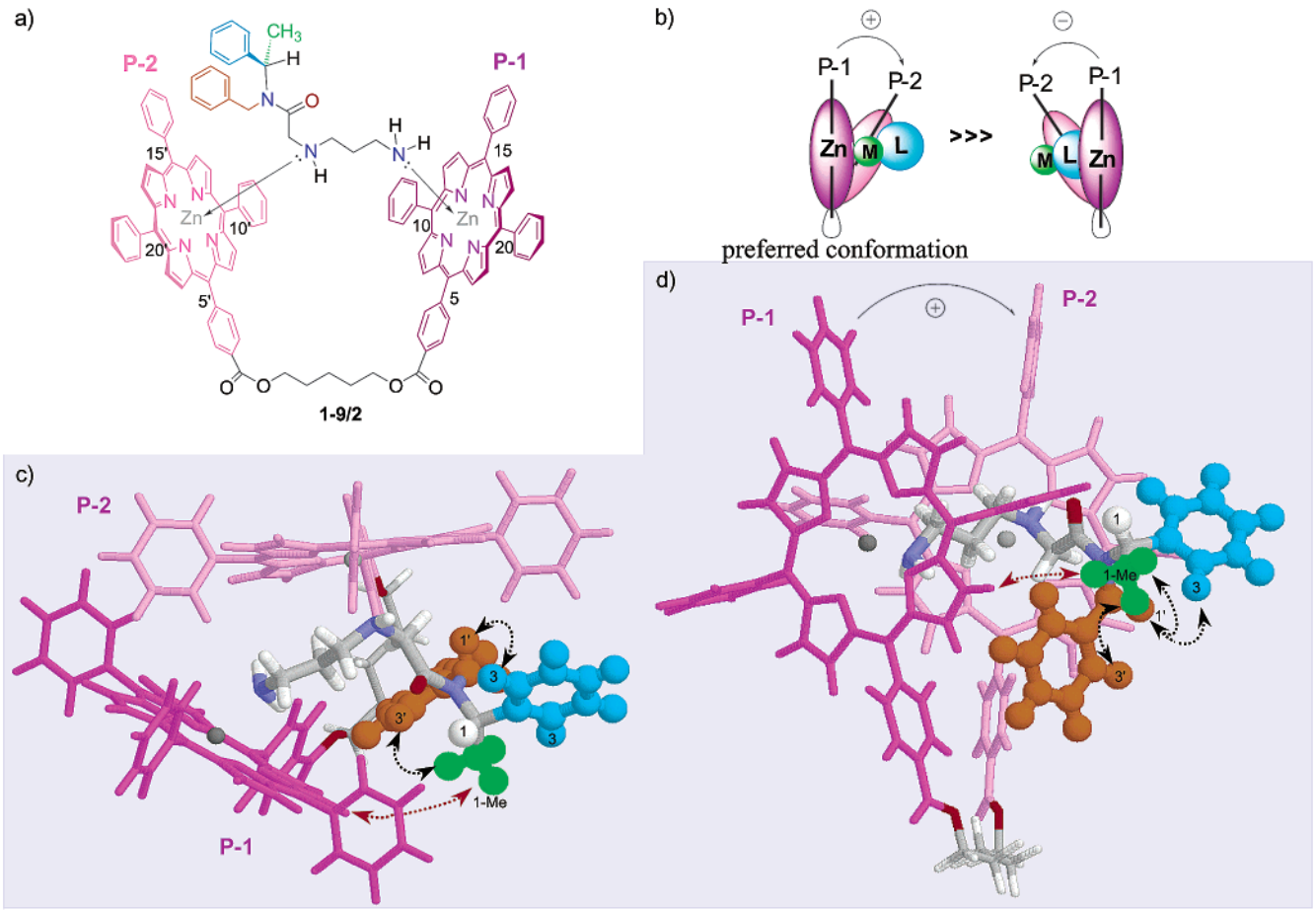



***Figure 11.*** (a) Complex **1-9/2**. (b) Schematic representation of the preferred conformation with a positive $\theta$ value. (c) Top view and (d) front view for the most probable calculated MC/MMFFs structure belonging to the higher-probability cluster in Figure 10c. (Blue) L group; (green) M group; (orange) *N*-benzyl group. Proton numberings are the same as in Figure 7 and Table 7. Dotted double-headed arrows depict intraguest (black) and host/guest (red) NOE's.

as follows: (a) the methylene of the *N*-benzyl group (orange) is directed toward P-2; (b) the chiral center lies approximately in the middle between the 10,20/10′,20′ phenyls (Figure 11a) of the two porphyrins; (c) the M methyl group (green) points toward P-1, reaching in most cases a distance of less than 4 Å with respect to the closest pyrrole protons, which agrees well with the observed host/guest NOE; (d) the L phenyl group (blue) lies in the middle between the two porphyrins, pointing outward (i.e., far from the porphyrin core) and upward in Figure 11d (i.e., toward 15′ phenyl). Thus, the calculation method lends itself as a means of differentiating the M and L groups on the basis of their positions and orientations in the host/guest complex.

The reasons for the surprisingly strong CD ($A = +1078$) observed for tweezer complex of the *N*-benzyl conjugate **1-9** remain unclear, especially because the sign preference and the dispersion of $\theta$ values are quite similar to those of *N*-Me conjugate **1-11** (Figure 10c vs 10b). Moreover, $\pi-\pi$ interactions between the *N*-Bn and phenyl at the stereogenic center do not seem to play an obvious role since these two groups are quite distant in the minimized complex structures (Figure 11c,d), and no NOE between their protons is observable. Only minor geometrical differences are apparent between the molecular models of **1-9/2** and **1-11/2**. In **1-9/2**, the sterically more demanding benzyl group enlarges the whole tweezer chiral pocket more than in the *N*-Me analogue **1-11/2**,[42] pushing the stereogenic center and its substituents more outward and under a stronger influence of 10,20/10′,20′ phenyls, which are perpendicular to the porphyrin planes. It is likely that the ultimate effect of this augmented structural strain leads to greater steric recognition.

**(B) Complex 1-10/2 (Figure 10a).** The calculated structure of primary amine conjugate **1-10** complexed with tweezer **2**, in terms of both $\theta$ dispersion (Figure 10a) and conformation of the host/guest complex (not shown), is consistent with the structures described previously for primary amines.[15,16] An almost complete prevalence of structures with positive $\theta$ values (96%) and a strongly preferred cluster with $\theta = +38°$ are found. A comparison of the molecular model for **1-10/2** with the ones for secondary amines **1-9/2** and **1-11/2**, suggests that the most evident structural difference is that in **1-10/2** the stereogenic center and its substituents lie closer to P-2, with the N−H proton pointing directly toward one of the pyrroles. The N−H bond is almost perpendicular to a pyrrole plane, with an H/plane distance of about 2.70 Å; this suggests a H/$\pi$ interaction,[43] which could be partially responsible for a more rigid and conformationally homogeneous complex, as observed by NMR.[16] The H/$\pi$ interaction is also supported by the strong ring current shift of

(42) The acute angle between the average porphyrin planes is about 42° in **1-9/2** and 30° in **1-13/2**.
(43) Del Bene, J. E.; Cohen, I. *J. Am. Chem. Soc.* **1978**, *100*, 5285−5290; Tarakeshwar, P.; Choi, H. S.; Kim, K. S. *J. Am. Chem. Soc.* **2001**, *123*, 3323−3331.

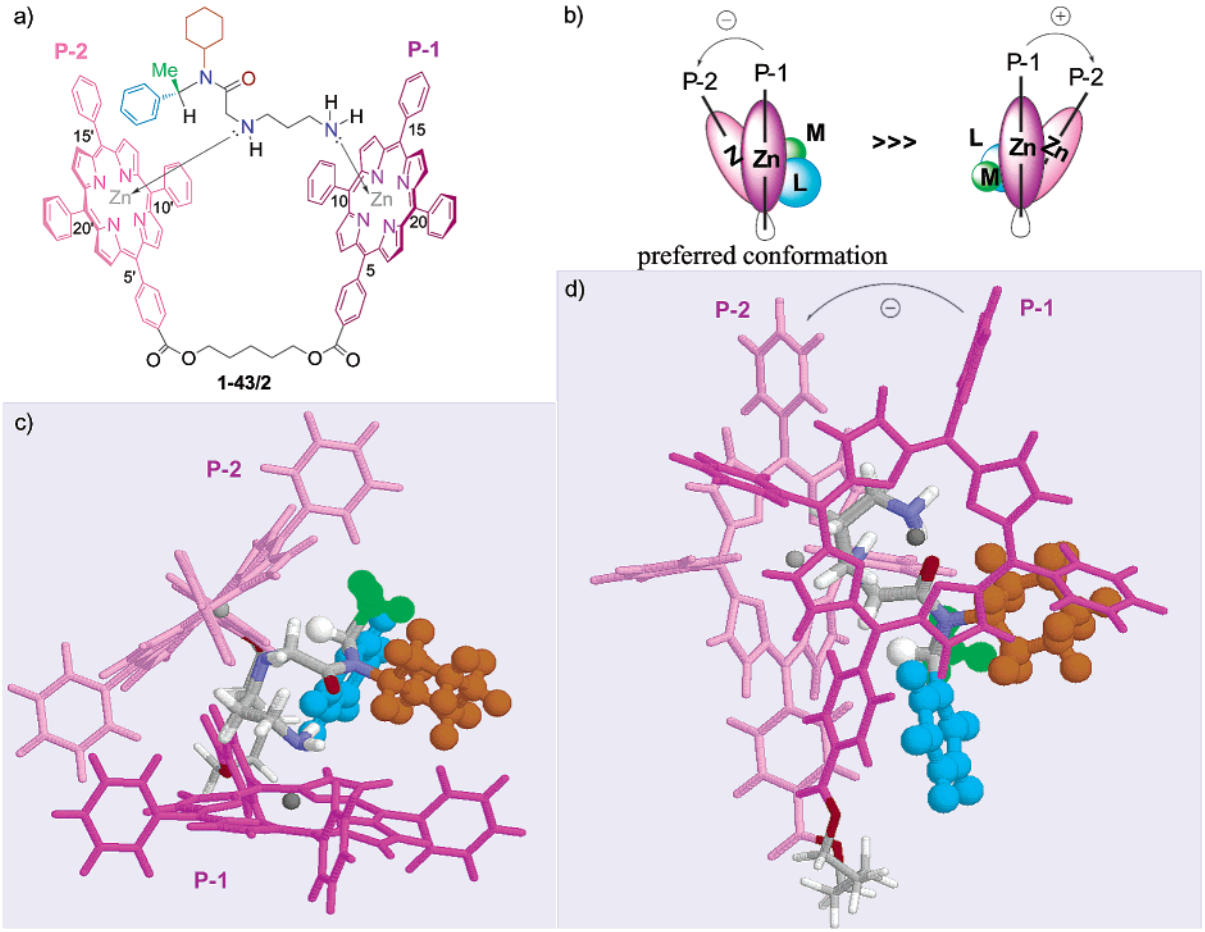



***Figure 12.*** (a) Complex **1-43/2**. (b) Schematic representation of the preferred conformation with a negative $\theta$. (c) Top view and (d) front view for the most probable calculated MC/MMFFs structure belonging to the higher-probability cluster in Figure 10d. (Blue) L group; (green) M group; (orange): *N*-cyclohexyl group.

the N−H (up to 3.70 ppm), the largest reported for protons of the chiral substrate.

**(C) Complexes 1-32/2 and 1-41/2 (Figure 10e,f).** Conformational energy values are not available for complexes **1-32/2** and **1-41/2**. For conjugate **1-32**, the difference between conformational *A* values[18] of methyl group (L) ($A = 7.28$ kJ/mol), and butyl amide group M (*A* value of approximate 4.6−5.4 kJ/mol for esters), is quite small. This leads to a reduced stereodifferentiation, which is reflected in the weak observed CD couplet (amplitude = −26 in MCH, undetectable in hexane). Calculated $\theta$ values are very dispersed (Figure 8e), but still a large preference (80%) for structures with negative $\theta$ values emerges. For conjugate **1-41**, as well as for all cyclic compounds **1-36**−**1-42**, the *A* values for the endocyclic $CH_2$ group are not available; the homogeneity of the CD results for all of these compounds justifies assigning M to the $CH_2$ and L to the exocyclic substituent (Table 5). This hypothesis is substantiated by MC/MMFFs calculations for the complex **1-41/2**, which results in a sharp cluster with $\theta = +14°$ (Figure 10f) within a distribution where positive values are clearly favored (61%).

**(D) Complex 1-43/2 (Figures 10d and 12).** The molecular modeling study of **1-43/2** (Figure 12) was carried out to shed some light on the anomalous behavior of the class of derivatives with secondary *N*-alkyl groups. In fact, the two examples considered in the current study (**1-43** and **1-44**) exhibited porphyrin CD exciton couplets with the sign opposite to the one predicted on the basis of conformational energy *A* values for phenyl and methyl groups (Table 6). A very heterogeneous conformational situation is found for the complex **1-43/2**, as depicted in Figure 10d; however, structures with negative values of $\theta$ are strongly dominating (90%). The favored amide conformation in the complex (>90% of minima within 10 kJ/mol) is found to be *E*, as in the case of the isolated guest (see above). The calculated structure of the *N*-isopropyl bearing conjugate **1-44/2** complex (Table 6) shows similar tendency (results not shown). These findings emphasize the usefulness of our calculations for predicting the preferred porphyrin helicity: *no discrepancies between the absolute configuration predicted by concurrent use of CD spectroscopy and MC/MMFFs conformational analysis on conjugate/tweezer complexes have been found in this study*.

Inspection of a representative structure of the **1-43/2** complex belonging to the cluster around $\theta \approx -37°$ of molecular models (Figure 12) shows clear differences as compared with the common structure represented by the **1-9/2** complex (Figure 11). In **1-43/2**, the *N*-cyclohexyl group (orange) is directed toward P-1, enlarging the whole tweezer pocket, the methyl group M (green) is directed toward P-2, and the phenyl group L (blue) is pointing downward (i.e., toward the benzoate rings). It should be emphasized that these effects only partially result from the favored *E* conformation. It is clear that a completely different discrimination mechanism is operating in this case, which is in

keeping with the exceptional behavior of compounds with secondary *N*-alkyl groups in terms of the relationship between the observed CD couplet and relative sizes of substituents at the stereogenic center.

## Conclusions

A general microscale protocol utilizing zinc porphyrin tweezer **2** to determine the absolute stereochemistry of secondary amines is described. This approach is applicable to cyclic and acyclic aliphatic amines, aromatic amines, amino acids, and amino alcohols. The chiral substrate is derivatized with an achiral carrier moiety **1** to generate a bidentate conjugate, capable of forming 1:1 host/guest complex with zinc porphyrin tweezer host **2**. The absolute configuration of the secondary amine can be readily determined from the sign of the exciton-coupled CD spectrum of the host/guest complex: a positive CD couplet corresponds to a clockwise arrangement of the L, M, and H groups at the stereogenic center in the Newman projection with amino group in the rear, and vice versa. The assignment of large (L) and medium (M) moieties at the stereogenic center is based on their conformational energies. This follows the general trend established previously with primary amines and secondary alcohols,[15,16] even though the complex formed by secondary amine conjugate with tweezer **2** has shown much greater conformational heterogeneity, which has hampered previous attempts for absolute configurational assignment of secondary amines. NMR analysis of some complexes demonstrated that observed differences in the upfield ring current-induced chemical shifts can provide useful information for assignment of the relative steric size of substituents linked to the stereogenic center. Furthermore, conformational studies of host/guest complexes by molecular mechanics calculation using MC/MMFFs revealed that this approach is a reliable tool for predicting the preferred porphyrin helicity upon complexation. In all cases, the sign of calculated preferred porphyrin twist was found to be in agreement with the observed CD exciton chirality. The molecular modeling of the host/guest complex can therefore be utilized in conjunction with the chiroptical procedure as a useful method for absolute configurational assignments, particularly when the relative steric sizes of substituents L and M are ambiguous.

## Experimental Section

Zinc porphyrin tweezer **2** is commercially available from TCI (Japan).

**General Procedure for Preparation of Host/Guest Complex 3 for CD Measurement.** In a typical experiment, tweezer **2** solution (1 $\mu$M) was prepared by the addition of a 10 $\mu$L aliquot of tweezer **2** (0.1 mM in anhydrous $CH_2Cl_2$) solution to the solvent (1 mL) where CD is going to be measured. The free amine solution of conjugate **1-9** (1.8 mg, 3.54 $\mu$mol) was prepared from its TFA salt after the addition of 0.5 mL of MeOH followed by solid $Na_2CO_3$ (10 mg). The solvent (MeOH) was then evaporated under a stream of argon followed by placement under high vacuum (0.2 Torr) for 20 min. Anhydrous $CH_2Cl_2$ (1 mL) was then added to yield the free amine solution of conjugate **1-9** (3.54 mM). An aliquot of 10 $\mu$L of the latter solution (20 equiv) was added to the prepared porphyrin tweezer **2** solution to afford tweezer **2**/conjugate **1-9** host/guest complex. The UV−vis and CD spectra were recorded at 25 °C and corrected for background. The CD spectrum was measured in millidegrees and normalized into $\Delta\epsilon$ ($L \cdot mol^{-1} \cdot cm^{-1}$).

**Computational Section.** Molecular modeling calculations were executed with MacroModel 7.1 package (Schrödinger, Inc., Portland, OR) including Maestro 3.0 as GUI, on a Dell Precision 330 workstation. All molecular mechanics calculations were run using the native MMFFs (MMFF94s) in vacuo with default parameters and convergence criteria, except for the maximum number of minimization steps, set to 50 000. Monte Carlo conformational searches were run with default parameters and convergence criteria, sampling all the structures within 10 kJ/mol over 1000 fully optimized steps. All possible torsional angles were varied during each step, except for the porphyrin ring dihedral angles and the porphyrin-10,15,20 phenyl torsions. $Zn^{2+}$ ions were placed in the middle of porphyrin rings, with −1 charge assigned to one pair of opposite nitrogen atoms; no bonds or other restraints were used. Guest molecules were placed with amine nitrogens close to $Zn^{2+}$ ions; no bonds or other restraints were used, except for a N−Zn distance check set to 2.2 ± 0.5 Å to prevent host/guest dissociation. A typical 1000-step calculation requires approximately 8−10 h.

All the structures resulting from the above calculations with energies within 10 kJ/mol were considered, usually 10−15 structures over 1000 steps; duplicate structures were tallied with their frequency of occurrence. The minima were collected in occurrence graphs as shown in Figure 10 in the main text, and the sign preference of projection angle $\theta$ determined by considering the total probability for $\theta$ of assuming positive and negative values. Figures 11 and 12 in the main text refer to the lowest-energy structures within the most probable clusters, which in many cases include 60−80% of all structures with a given sign of $\theta$.

*E*/*Z* ratios (amide conformations) of free guests were calculated through the Boltzmann formula and absolute MMFFs energies in $CHCl_3$ (GB/SA solvation model) for the two lowest-energy structures corresponding to amide conformers with the linker moiety in the same conformation.

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**Supporting Information Available:** General procedures for the conjugate preparation at both the milligram and microgram scales; procedures for CD measurements of the host/guest complex at the microgram scale; procedures for obtaining the Job plot and binding constant; general procedure for transamidation; procedures for synthesis of **12**, **16**, **17**, and diketopiperazines **23**−**26**, $^1$H NMR and MS data of conjugates **1-9** to **1-11**, **1-14**, **1-16** to **1-21**, **1-31** to **1-44**; 2D ROESY spectrum of complex **1-9/2** (Figure S) (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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