ChemComm

Employing a Chiroptical Sensor for the Absolute Stereochemical Determination of α-Amino and α-Hydroxyphosphonates

Journal:	*ChemComm*
Manuscript ID	CC-COM-04-2023-001757.R2
Article Type:	Communication

COMMUNICATION

Employing a Chiroptical Sensor for the Absolute Stereochemical Determination of α-Amino and α-Hydroxyphosphonates

Received 00th January 20xx,
Accepted 00th January 20xx

DOI: 10.1039/x0xx00000x

Debarshi Chakraborty, Li Zheng, Yijing Dai, Jeff Gwasdacus, James E. McTighe, William D. Wulff,* Babak Borhan*

The absolute stereochemistry of the α-amino and α-hydroxyphosphonates is determined using a chiroptical sensor. The induced helicity of the host-guest complex is correlated to the chirality of the guest molecule via a simple binding model. The relative size of the substituents dictates the predominant helical population, leading to an easy circular dichroic readout.

α-Amino and α-hydroxyphosphonates constitute an important family of biologically active molecules because of their desirable pharmacological properties.[1] In particular, α-aminophosphinic acids are considered as structural analogues of natural α-amino acids, and thus are used as bioisosters in drug discovery platforms. Their structural similarity with the tetrahedral transition state for amide and ester hydrolysis has led to the development of α-amino and α-hydroxyphosphonates as potent enzyme inhibitors.[2] Furthermore, their importance as antiviral and anticancer agents, antibiotics, neuro-modulators, plant growth regulators, herbicides, and many other applications have drawn significant attention from medicinal as well as synthetic chemists for their streamlined asymmetric preparation.[3] Not surprisingly, the efficacy and potency of this class of molecules is often determined by their absolute stereochemistry. Recent advancements in asymmetric synthesis have resulted in several enantioselective strategies to obtain this class of compounds.[4] Nonetheless, methodologies for rapid absolute stereochemical determination are less developed and remain as a critical bottleneck for structural analysis.

Absolute stereochemical assignment of α-amino and α-hydroxyphosphonates relies heavily on Mosher-type amide or ester analysis (Figure 1a).[5] This protocol necessitates the formation of both diastereomers (double derivatization) with the chiral analyte and subsequent analysis of their ^{1}H and ^{31}P NMR. Often, conformational analysis of the diastereomers is required to predict the differences in the NMR data based on optimized conformations. Despite its success, there are issues that complicate Mosher analyses, as well, the overall process is slow, requiring chemical derivatization and chromatographic separation prior to analysis. Chiral solvating agents (CSA) that form diastereomers via non-covalent interactions have also been employed for the absolute stereochemical determination of α-amino and α-hydroxyphosphonates.[6] Nonetheless, they do not provide a general solution, as the changes in NMR are empirical and cannot be readily predicted. A general chiroptical protocol that can determine the absolute stereochemistry of both α-amino and α-hydroxyphosphonates in a rapid manner would be ideal. To this end, we sought to take advantage of supramolecular host-guest chemistry where translation of chirality from a chiral guest molecule to the host-guest complex would result in a predictable circular dichroic output (Figure

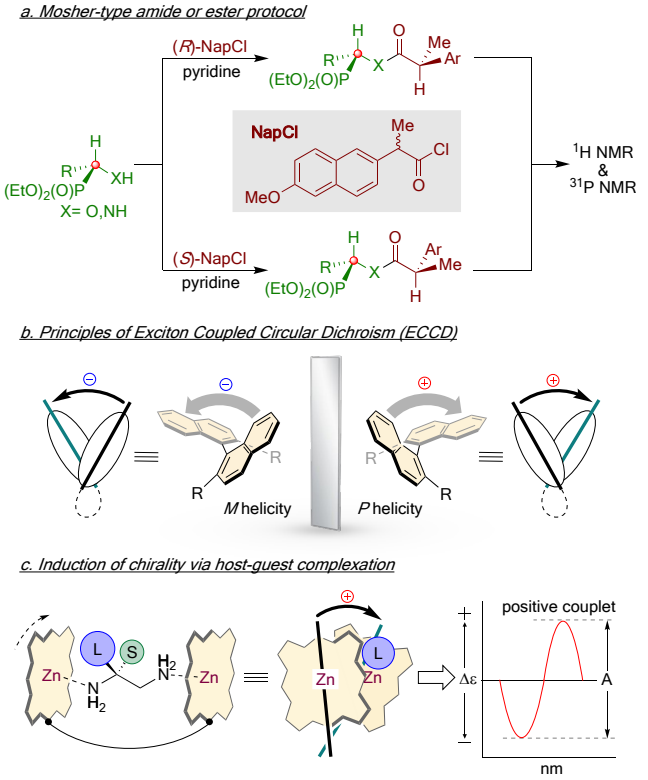


Figure 1. a. Absolute stereochemical determination of α-hydroxy and α-aminophosphonates via double derivatization with both the enantiomers of naproxen chloride. b. Correlation of the sign of the ECCD spectra with the helicity of the coupling chromophores. c. Determination of absolute stereochemistry of α-chiral diamines through host-guest complexation with bis-porphyrin tweezers.

Michigan State University, Department of Chemistry, East Lansing, MI 48824. Email: babak@chemistry.msu.edu

†Electronic Supplementary Information (ESI) available: [details of any supplementary information available should be included here]. See DOI: 10.1039/x0xx00000x

1b).[7] This has led to the development of a rapid, general, micro-scale, derivatization-free chiroptical procedure to assign the absolute stereochemistry of α-amino and α-hydroxyphosphonates in a non-empirical fashion utilizing the principles of Exciton Coupled Circular Dichroism (ECCD). Herein, we report the use of C_3-Zn-TPFP Tz **1** as a "reporter of chirality" for asymmetric α-amino and α-hydroxy phosphonates.

The use of ECCD is critical for the success of the strategy outlined in this manuscript, thus, a brief introduction to the technique is warranted. ECCD arises from the coupling of the electric transition dipole moments of two or more independently conjugated chromophores that are oriented in a helical fashion in space (Figure 1b).[7c, d, f, g, 8] The coupling results in a bisignate CD signal that is defined as either positive or negative. The sign is directly related to the helicity of the interacting chromophores, thus providing a non-empirical assignment of asymmetry. The chromophores that are involved in generating an ECCD signal are either present in the molecule or can be introduced through derivatization or coordination. As is often the case, molecules of interest lack the appropriate chromophores that are required to generate the ECCD signal. Investigations in this field have focused on approaches that introduce the requisite chromophores, frequently via coordination, to enable analysis of absolute stereochemistry. In particular, the porphyrin tweezer methodology pioneered by Nakanishi and Berova,[9] has provided a general solution for the absolute stereochemical determination of chiral molecules that do not possess these required chromophores (Figure 1c). This methodology has been implemented successfully for the stereochemical determination of a variety of molecular families such as alcohols, amines, diols, diamines, amino acids, amino alcohols, epoxy alcohols, carboxylic acids, etc.[7g, 9-10] Although the ECCD method is uniquely suited for non-empirical determination of absolute stereochemistry, it is important to note that the strength of the ECCD couplet (the amplitude, denoted as A), is linearly correlated with the enantioenrichment of the analyte. Thus, with the help of a standard curve, the measured amplitude can report on the *ee* of the sample, as well as its absolute stereochemistry.[11]

Molecules typically used as guests for this protocol can be divided in two groups; those with two sites of attachment that can interact in a bidentate fashion with the porphyrin tweezer, and others with only one coordinating site available requiring alternate strategies for complexation with porphyrin tweezers or must rely on the use of different host molecules altogether.[7g] α-Amino and α-hydroxyphosphonates fall in the first category, where the phosphonate oxygen atom can provide one binding site and the nitrogen or oxygen atom in α-amino and α-hydroxyphosphonates, respectively, provide the second binding element. Our prior investigations with porphyrin tweezers have shown that success of this protocol in producing consistent ECCD signals is dependent on limiting the number of possible conformations upon host-guest complexation.[10c, d]

To achieve the latter, two strategies have emerged; 1. Strong binding affinity ensures a robust host-guest complexation, reducing energetically close-lying conformations that can complicate the ECCD spectrum;[10d] and 2. A smaller and/or rigid linker between the two porphyrin rings reduces conformational flexibility.[10c] To this end, we envisioned the use of the fluorinated C_3-Zn-TPFPtz-**1**,[10c] where the porphyrin rings are connected by a short C_3 linker (Figure 2). The fluorinated aryl rings lead to a more Lewis acidic Zn metal center that enhances binding with α-amino and α-hydroxyphosphonates, while the C_3-linker limits conformational freedom upon binding.

Gratifyingly, a strong positive ECCD signal (A = -198, A corresponds to the amplitude of the bisignate curve, the sum of the absolute values of the positive and negative Cotton effects) was observed when only 5 equivalents of compound *R*-**2a** was complexed with C_3-Zn-TPFPtz **1** (1 μM) in hexane at 0 °C (Table 1). Hexane was chosen as the solvent since it neither competes with the guest molecule in binding with the host, nor does it interact with the guest molecule to diminish the host-guest interaction. The success in generating a strong ECCD signal with *R*-**2a** bound to **1** prompted the examination of several structurally diverse a-aminophosphonates exhibiting branched alkyl or aryl groups (see compounds *S*-**2b**, *R*-**2c**, *S*-**2d**, Table 1). All these molecules produced strong and consistent ECCD signals. Next, the system was challenged with a-aminophosphonates that featured linear alkyl groups of different chain lengths (*S*-**2e** - *R*-**2g**), as well as alkene (*S*-**2h**), alkyne (***S*-2i**), and heteroatoms containing substituents (*S*-**2k** and *S*-**2l**). As with the first set of molecules tested, there were no anomalies, with all complexes generating ECCD spectra centered about the porphyrin Soret band without any complications.

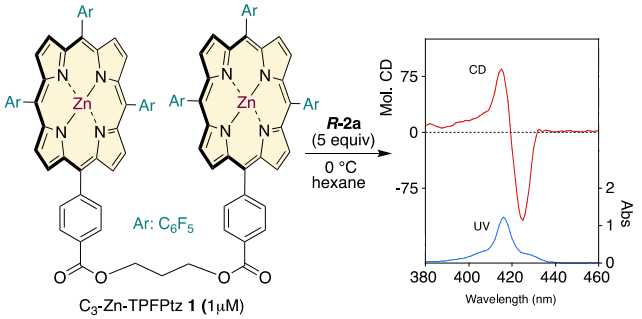


Figure 2. Binding of R-**2a** with C_3-Zn-TPFPtz **1** leads to a negative ECCD signal.

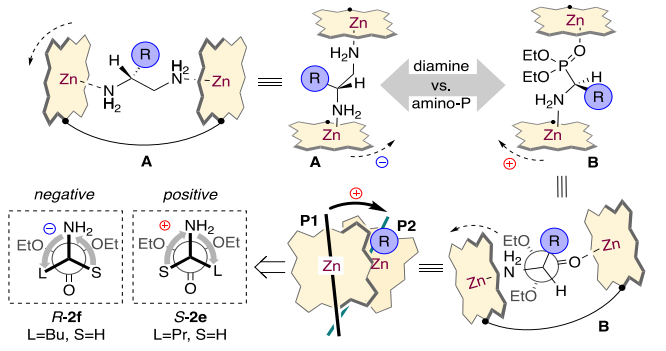
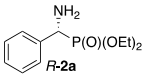
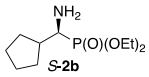
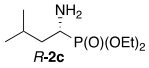
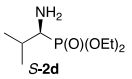
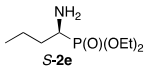
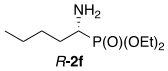
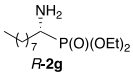
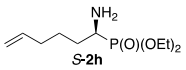
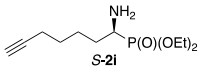
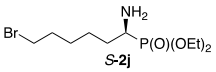
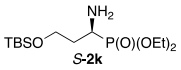
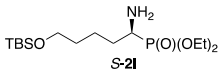
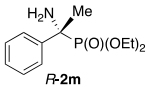
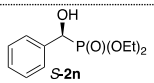
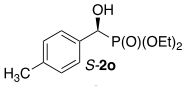
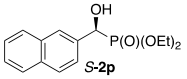


Figure 3. Comparison of the binding model for diamines (**A**) vs. α-aminophosphonates (**B**) complexed with tweezer **1**. Unlike diamines that are not sterically demanding, the two ethoxy subsituents lead to the rotation of the long chain to avoid interaction with the linker connecting the porphyrins. The porphyrin bound to the amino group slides away from the larger R group on the asymmtric center (movement illustrated by the dashed arrows) and favors the P helicty (illustrated by the bold arrow) for the depicted stereochemistry. A simple mnemonic is shown in the dashed box to correlate the observed sign of the ECCD spectrum to the chirality of the guest molecule.

Table 1. Predicted and observed ECCD signal for α-amino/α-hydroxyphosphonates complexed with C_3-Zn-TPFPtz **1**.

phosphonate	predicted sign	λ (nm), (Δε)	A/Acorr[a,b]
 *R*-**2a**	negative	425, -117 414, +81	-198/-220[c]
 *S*-**2b**	positive	424, +129 412, -92	+222/+313[c]
 *R*-**2c**	negative	424, -117 415, +136	-253/-278[c]
 *S*-**2d**	positive	421, +106 414, -77	+183/+229[c]
 *S*-**2e**	positive	426, +32 417, -19	+51/+57[c]
 *R*-**2f**	negative	423, -27 414, +27	-54/-60[c]
 *R*-**2g**	negative	422, -28 415, +26	-54/-67[d]
 *S*-**2h**	positive	426, +52 414, -36	+88/+100[c]
 *S*-**2i**	positive	425, +21 416, -45	+66/+72[d]
 *S*-**2j**	positive	426, +122 416, -93	+215/+247[e]
 *S*-**2k**	positive	425, +90 413, -51	+141/+170[c]
 *S*-**2l**	positive	423, +144 413, -96	+240/+282[e]
 *R*-**2m**	negative	425, -150 414, +185	-335[e]
 *S*-**2n**	positive	424, +81 412, -30	+111/+173[e]
 *S*-**2o**	positive	424, +40 412, -11	+51/+88[e]
 *S*-**2p**	positive	424, +122 412, -72	+194/+198[e]

[a]CD measurements (10 scans) of C_3-Zn-TPFPtz **1** (1 mM) complexed with phosphonates were performed at 0 °C in hexane. [b]A corresponds to the amplitude for the CD spectrum where A_{corr} is the amplitude after correcting for the enantiopurity of the guest molecule. [c]5 equiv of guest. [d]10 equiv of guest. [e]1 equiv of guest.

With a good set of data on hand, we next looked to develop a mnemonic that would relate the absolute stereochemistry of the guest molecules with the observed ECCD. In general, (*R*) α-aminophosphonates produced a negative ECCD signal whereas (*S*) α-aminophosphonates generated a positive signal. Presumably, the stereochemical differentiation that leads to the induced ECCD of the host is driven by steric demands of the guest substituents. Thus, caution is warranted as Cahn-Ingold-Prelog stereochemical assignment may not always follow steric preferences. Nonetheless, in all examples listed in Table 1 (*S*-**2a** - *S*-**2l**), the larger substituent based on A strain values[8b, 12] also has the third priority (phosphonate first and amino group being second) by CIP rule.

At first glance, the binding of α-aminophosphonates with the zinc porphyrin tweezers would resemble the binding of diamines, a system that has been described before in detail (Complex **A**, Figure 3).[9] The conventional binding model developed for a-chiral diamines places the guest molecule parallel to the alkyl chain linker that connects the two porphyrins. Under such a scenario the porphyrin closer to the chiral center slides away from the larger R group and a negative helicity is formed for the molecule depicted in Complex **A**. Employing the exact same binding scenario with α-aminophosphonates results in prediction of ECCD opposite to that observed experimentally. The cause for this is in the dissimilar binding conformation of α-aminophosphonates as compared to diamines. If bound in the same manner as diamines, α-aminophosphonates would direct the two ethoxy substituents on the phosphorus center towards the linker, leading to unfavorable steric interactions. To avoid steric congestion, we postulate the bound α-aminophosphonate is rotated along the linear chain extended between the two porphyrin rings such that the ethoxy groups are positioned outside the cavity. In this manner, neither the ethoxy substituents, nor the R group are directed towards the linker or the bound porphyrins (Complex **B**, Figure 3). Porphyrin **P2** that is coordinated with the phosphonate oxygen atom is not influenced greatly by the asymmetric center, and therefore, does not dictate the helicity of the host-guest complex. On the other hand, **P1** binds the nitrogen atom close to the asymmetric center and must choose to either swing towards the bulky R group (larger substituent based on A strain value) or shy away to avoid steric crowding. The former conformation would lead to the less favorable *M*-helical arrangement, whereas the latter conformation would yield the more stable *P*-helicity. The totality of the latter discussion is distilled into a simple to follow mnemonic (see dashed box, Figure 3).

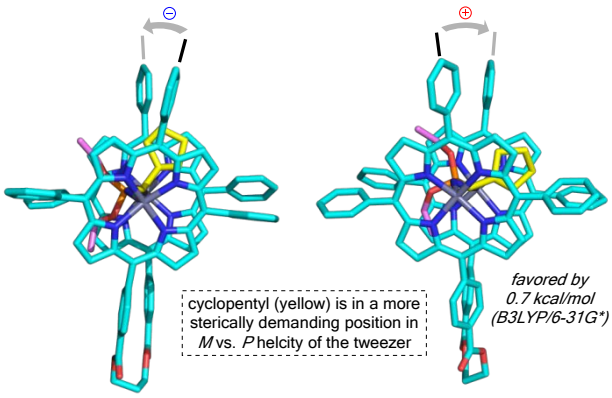


Figure 4. Energy optimized (DFT-B3LYP/6-31G*) structures of P and M helical conformers of C_3-Zn-TPFPtz **1** complexed with S-**2b.** In the P conformer, favored by 0.7 kcal/mol, the porphyrin bound to the amino group moves away from the cyclopentyl group to minimize steric interaction.

Energy minimization (DFT/B3LYP/6-31G*) for the *S*-**2b** complexed with C3-Zn-TPFPtz **1** also agrees with the same intuitive reasoning, illustrating that α-aminophosphonates bind through a "side on" approach (Figure 4). Further analysis of the

calculated structures for the *P* and *M* bound *S*-**2b** reveals that the *P*-helical complex is ~0.7 kcal/mol more stable as compared to the *M*-helical conformer. The predicted signs of all α-aminophosphonates presented in Table 1 are consistent with the proposed binding model. However, given the small energy difference, the large numbers of potential conformations, and the challenges of accurate energy evaluations for such large structures, it must be understood that these models are computationally guided "best guesses" to aid the reader in visualizing and rationalizing the experimental behavior. While NMR studies would have been helpful to investigate the complex formed between the porphyrins and the chiral guest molecules, the substantially higher concentrations needed for NMR analysis (100-fold) led to sever aggregation of the porphyrin tweezers, yielding indecipherable results.

With the success of C_3-Zn-TPFPtz **1** in determining the absolute stereochemistry of secondary α-aminophosphonates, the host system was challenged with a tertiary aminophosphonate, *R*-**2m**. Notably, the Mosher methodology is limited to secondary chiral centers and cannot be extended directly to determine the absolute configuration of a tertiary center.[13] When treated with C_3-Zn-TPFPtz **1** under identical conditions, compound *R*-**2m** produced a negative ECCD signal. The observed signal agrees with that predicted from the mnemonic (Figure 3).

We surmised that the C_3-Zn-TPFPtz **1** host should report the absolute stereochemistry of α-hydroxyphosphonates in the same manner. We also inferred that C_3-Zn-TPFPtz **1** should bind α-hydroxyphosphonates in a similar fashion with the hydroxyl oxygen serving as the secondary binding element. Pleasingly, when the amine of *R*-**2a** was converted to the alcohol *S*-**2n**, the resultant ECCD signal was inverted. Consistent with the latter discussion, complexation of *S*-**2n** with C_3-Zn-TPFPtz **1** leads to a positive ECCD signal, which is opposite to that observed with *R*-**2a**. The observed ECCD spectra with *S*-**2m** and *S*-**2o** are also in full agreement with the predicted signs based on the proposed mnemonic (Table 1 and Figure 3).

In summary, we have demonstrated a simple chiroptical procedure for the determination of absolute stereochemistry of α-amino and α-hydroxyphosphonates. This protocol does not require derivatization or chromatographic separation prior to analysis. Strong host-guest complex formation leads to a discernable ECCD signal at a micromolar level. A simple binding model based on the size of the substituents can easily correlate the substrate chirality with the observed ECCD signal.

We are grateful to the NSF (CHE-2247261) for their support of this research.

Conflicts of interest

There are no conflicts to declare.

Notes and references

[1] a) H. Seto and T. Kuzuyama, *Nat. Prod. Rep.* 1999, **16**, 589; b) K. Moonen, I. Laureyn and C. V. Stevens, *Chem. Rev.* 2004, **104**, 6177; c) F. Palacios, C. Alonso and J. M. de Los Santos, *Chem. Rev.* 2005, **105**, 899; d) O. I. Kolodiazhnyi, *Tetrahedron: Asymmetry* 2005, **16**, 3295; e) H. Groger and B. Hammer, *Chem. Eur. J.* 2000, **6**, 943.

[2] a) P. P. Giannousis and P. A. Bartlett, *J. Med. Chem.* 1987, **30**, 1603; b) S. Ikeda, J. A. Ashley, P. Wirsching and K. D. Janda, *J. Am. Chem. Soc.* 1992, **114**, 7604.

[3] a) C. Giordano and G. Castaldi, *J. Org. Chem.* 1989, **54**, 1470; b) E. J. Glamkowski, G. Gal, R. Purick, A. J. Davidson and M. Sletzinger, *J. Org. Chem.* 1970, **35**, 3510; c) H. P. Guan, Y. L. Qiu, M. B. Ksebati, E. R. Kern and J. Zemlicka, *Tetrahedron* 2002, **58**, 6047.

[4] a) Y. Dai, L. Zheng, D. Chakraborty, B. Borhan and W. D. Wulff, *Chem. Sci.* 2021, **12**, 12333; b) G. D. Joly and E. N. Jacobsen, *J. Am. Chem. Soc.* 2004, **126**, 4102; c) B. Saito, H. Egami and T. Katsuki, *J. Am. Chem. Soc.* 2007, **129**, 1978; d) M. Ordonez, J. L. Viveros-Ceballos, C. Cativiela and F. J. Sayago, *Tetrahedron* 2015, **71**, 1745.

[5] a) K. Blazewska, P. Paneth and T. Gajda, *J. Org. Chem.* 2007, **72**, 878; b) K. M. Blazewska and T. Gajda, *Tetrahedron: Asymmetry* 2009, **20**, 1337.

[6] a) F. Hammerschmidt, W. Lindner, F. Wuggenig and E. Zarbl, *Tetrahedron: Asymmetry* 2000, **11**, 2955; b) M. Skwarczynski, B. Lejczak and P. Kafarski, *Chirality* 1999, **11**, 109; c) C. V. Ward, M. L. Jiang and T. P. Kee, *Tetrahedron Lett.* 2000, **41**, 6181; d) Y. Li, G. H. Yang, C. Q. He, X. Li, K. N. Houk and J. P. Cheng, *Org. Lett.* 2017, **19**, 4191.

[7] a) V. V. Borovkov, G. A. Hembury and Y. Inoue, *Acc. Chem. Res.* 2004, **37**, 449; b) H. Lu and N. Kobayashi, *Chem. Rev.* 2016, **116**, 6184; c) C. Wolf and K. W. Bentley, *Chem. Soc. Rev.* 2013, **42**, 5408; d) L. You, D. Zha and E. V. Anslyn, *Chem. Rev.* 2015, **115**, 7840; e) M. Liu, L. Zhang and T. Wang, *Chem. Rev.* 2015, **115**, 7304; f) D. Pasini and A. Nitti, *Chirality* 2016, **28**, 116; g) H. Gholami, D. Chakraborty, J. Zhang and B. Borhan, *Acc. Chem. Res.* 2021, **54**, 654.

[8] a) N. Harada and K. Nakanishi, *University Science Books: Mill Valley, CA* 1983; b) N. Harada, S. L. Chen and K. Nakanishi, *J. Am. Chem. Soc.* 1975, **97**, 5345; c) N. Berova, P. L. Polavarapu, K. Nakanishi and R. W. Woody, *Comprehensive chiroptical spectroscopy*, Wiley, Hoboken, NJ, **2012**; d) G. Pescitelli, *Chirality* 2022, **34**, 333.

[9] X. F. Huang, B. H. Rickman, B. Borhan, N. Berova and K. Nakanishi, *J. Am. Chem. Soc.* 1998, **120**, 6185.

[10] a) X. Huang, N. Fujioka, G. Pescitelli, F. E. Koehn, R. T. Williamson, K. Nakanishi and N. Berova, *J. Am. Chem. Soc.* 2002, **124**, 10320; b) X. Li and B. Borhan, *J. Am. Chem. Soc.* 2008, **130**, 16126; c) X. Li, C. E. Burrell, R. J. Staples and B. Borhan, *J. Am. Chem. Soc.* 2012, **134**, 9026; d) X. Li, M. Tanasova, C. Vasileiou and B. Borhan, *J. Am. Chem. Soc.* 2008, **130**, 1885; e) M. Tanasova, M. Anyika and B. Borhan, *Angew. Chem. Int. Ed.* 2015, **54**, 4274; f) Q. Yang, C. Olmsted and B. Borhan, *Org. Lett.* 2002, **4**, 3423; g) N. Berova, L. Di Bari and G. Pescitelli, *Chem. Soc. Rev.* 2007, **36**, 914; h) G. Pescitelli, L. Di Bari and N. Berova, *Chem. Soc. Rev.* 2011, **40**, 4603; i) N. Berova, G. Pescitelli, A. G. Petrovic and G. Proni, *Chem. Commun.* 2009, 5958.

[11] a) B. T. Herrera, S. L. Pilicer, E. V. Anslyn, L. A. Joyce and C. Wolf, *J. Am. Chem. Soc.* 2018, **140**, 10385; b) J. R. Howard, A. Bhakare, Z. Akhtar, C. Wolf and E. V. Anslyn, *J. Am. Chem. Soc.* 2022, **144**, 17269; c) S. L. Pilicer, J. M. Dragna, A. Garland, C. J. Welch, E. V. Anslyn and C. Wolf, *J. Org. Chem.* 2020, **85**, 10858; d) A. Sripada, Y. Thanzeel and C. Wolf, *Chem* 2022, **8**, 1734; e) F. Y. Thanzeel, K. Balaraman and C. Wolf, *Angew. Chem. Int. Ed.* 2020, **59**, 21382.

[12] E. L. Eliel, S. H. Wilen and L. N. Mander, *New York: Wiley.* 1994.

[13] E. Kuliszewska, M. Hanbauer and F. Hammerschmidt, *Chem. Eur. J.* 2008, **14**, 8603.