How to cite: *Angew. Chem. Int. Ed.* **2020**, *59*, 21382–21386
International Edition: doi.org/10.1002/anie.202005324
German Edition: doi.org/10.1002/ange.202005324

Chiroptical Sensing

Quantitative Chirality and Concentration Sensing of Alcohols, Diols, Hydroxy Acids, Amines and Amino Alcohols using Chlorophosphite Sensors in a Relay Assay

*F. Yushra Thanzeel, Kaluvu Balaraman, and Christian Wolf**

Abstract: *Analytical methods that allow simultaneous determination of the concentration and enantiomeric composition of small sample amounts and are also compatible with high-throughput multi-well plate technology have received increasing attention in recent years. We now introduce a new class of broadly useful small-molecule probes and a relay sensing strategy that together accomplish these tasks with five classes of compounds including the challenging group of mono-alcohols—a scope that stands out among previously reported UV, fluorescence, and CD assays. Several chlorophosphite probes and aniline indicators have been evaluated and used for on-the-fly CD/UV sensing following a continuous workflow. The wide application range of the readily available sensors is highlighted with almost 30 alcohols, diols, hydroxy acids, amines and amino alcohols, and the accuracy of the stereochemical analysis is showcased with samples covering a wide range of concentrations and enantiomeric ratios.*

The introduction of high-throughput experimentation methodologies has greatly accelerated scientific discoveries and streamlined efforts in numerous academic and industrial laboratories aimed at solving complex chemical and biological tasks under strict time constraints.[1] Despite the undisputable need for quantitative screening methods that can take full advantage of generally available multi-well plate technology with parallel data acquisition and processing capabilities it is still routine to analyze one sample at a time with serial techniques.[2,3] The shortage of experimental advance with these endeavors can at least be partially attributed to the difficulty with simultaneous determination of the concentration and enantiomeric ratio (*er*) of chiral samples, and these tasks are commonly accomplished separately by gravimetric analysis and chiral chromatography, respectively. Recent progress with chiroptical sensing technologies has shown that high-throughput screening of an increasing variety of chiral compounds is now possible.[4] The most impressive examples have been achieved with chiral amines, amino alcohols, amino acids, hydroxy acids and diols.[5] But with regard to other compound classes, for example mono-alcohols, chiroptical on-the-fly concentration and *er* determination needs to be demonstrated. In fact, stereochemical analysis of chiral mono-alcohols by NMR spectroscopy,[6] mass spectrometry[7] or optical methods[8] has remained challenging.[9]

We now wish to introduce a chiral alcohol relay sensing strategy that can accomplish three tasks, that is, determination of the absolute configuration, sample concentration and enantiomeric composition, altogether. We have studied a series of chromophoric phosphite and amidophosphite probes that expedite comprehensive stereochemical analysis of chiral mono-alcohols via simultaneous UV/CD analysis from a single sample. In addition, we prove the usefulness of this new class of sensors by demonstrating a wide application spectrum that includes alcohols but also extends to diols, hydroxy acids, amines and amino alcohols. The unique molecular recognition and chiroptical sensing features are based on irreversible formation of alkyl (amido)phosphite products exhibiting characteristic UV and circular dichroism (CD) signals that allow combined concentration and *er* analysis. While most chirality sensors introduced to date have a narrow substrate scope we show that our phosphite probes are very broadly useful.

To date, a wide range of optical chirality assays operating on the principles of dynamic covalent chemistry, in particular systems involving reversible Schiff base formation, or metal coordination, multicomponent assemblies, host-guest complexation, hydrogen bonding interactions and irreversible substrate binding have surfaced.[4c] To the best of our knowledge, stereochemical analysis with a phosphite or amidophosphite probe has not been demonstrated. We envisioned that fast binding of alcohol substrates with aromatic chlorophosphite probes **1**–**5** should be possible and enable the complicated stereochemical sensing tasks outlined above, Figure 1. Although initial focus was placed on the challenging group of alcohols **6**–**18**, the possibility of optical sensing of diols and hydroxy acids **19**–**22**, amino alcohols **23**–**28** and amines **29**–**33** was also investigated.

The chlorobenzodioxaphosphite **1** was commercially available and the analogues **2**–**4** were prepared in a single step from the corresponding diols and phosphorous trichloride (see SI). The *N*,*N*′-dibenzyl chlorobenzodiazaphosphite **5** was synthesized in three high-yielding steps as shown in Scheme 1. The ability of the sensors **1**–**5** to differentiate between the enantiomers of 1-phenylethanol, **6**, and 1-phenylbutanol, **7**, was then tested under various conditions.

We were pleased to find that all probes generate CD signals that are easily obtained by mixing stoichiometric amounts of the chlorophosphite and the analyte in the presence of diisopropylethylamine, see Scheme 1 and SI. The phosphite formation was verified with several substrates

[*] F. Y. Thanzeel, Prof. Dr. K. Balaraman, Prof. Dr. C. Wolf
Department of Chemistry, Georgetown University
37th and O Streets, Washington, DC 20057 (USA)
E-mail: cw27@georgetown.edu

Supporting information and the ORCID identification number(s) for the author(s) of this article can be found under: https://doi.org/10.1002/anie.202005324.

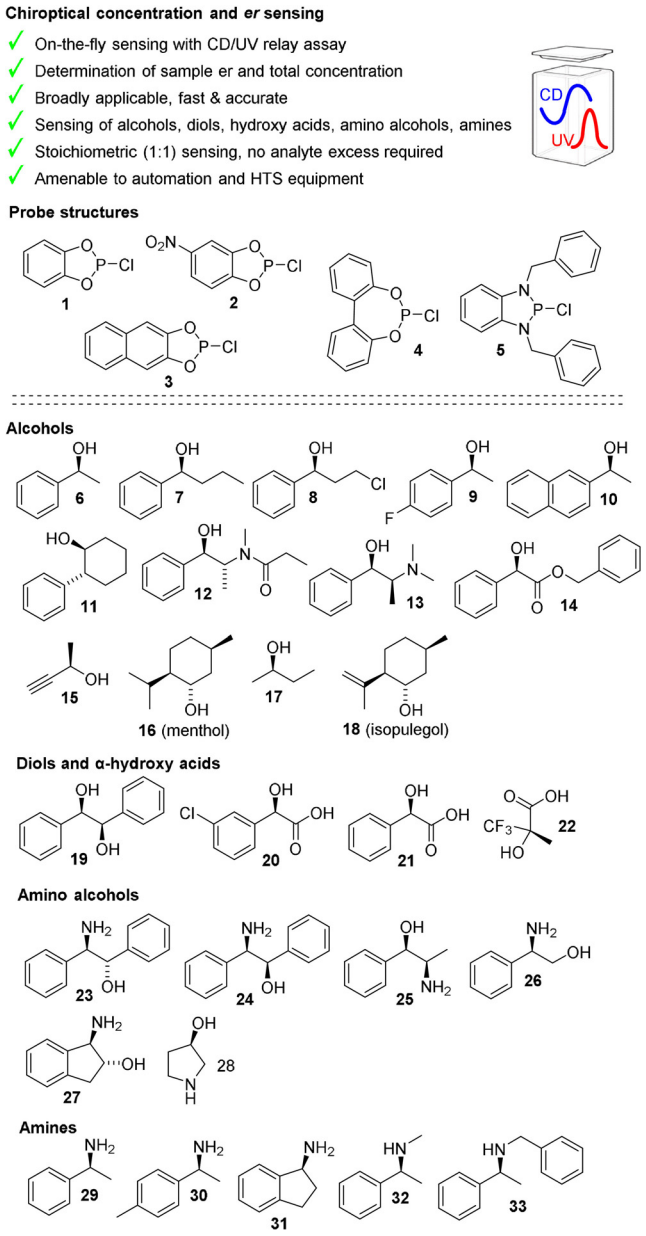
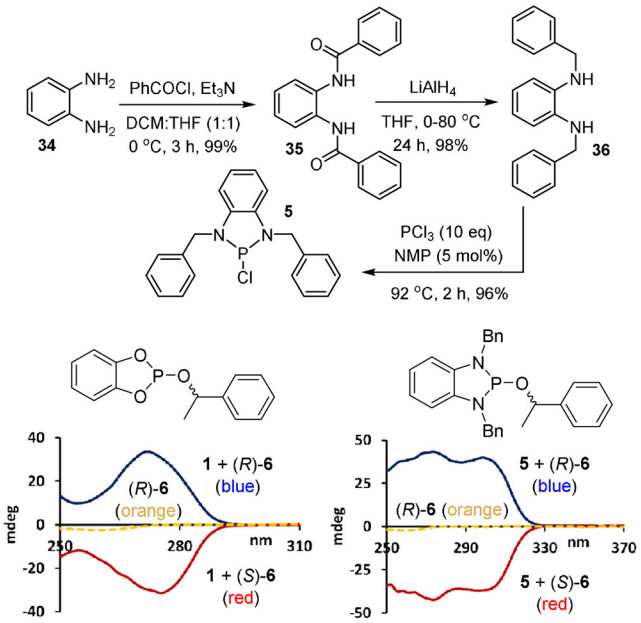
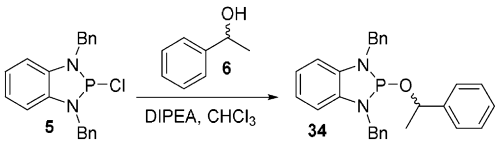


Figure 1. Structures of chlorophosphite probes **1**–**5** and chiral alcohol, diol, hydroxy acid, amino alcohol and amine target compounds **6**–**33**. Only one enantiomer is shown.



Scheme 1. Synthesis of sensor **5** (top) and CD signatures of the phosphites derived from probes **1** and **5**, respectively, and the enantiomers of 1-phenylethanol, **6** (bottom). For comparison, the CD spectrum of the free alcohol (*R*)-**6** under the same conditions is shown in orange. The CD measurements were conducted in chloroform at 0.75 mM (left) and 0.22 mM (right). See SI for details.

Table 1: Chiroptical determination of the enantiomeric ratio and absolute configuration of samples of 1-phenylethanol, **6**, using probe **5**.



Entry	Sample composition			Sensing results	
	Conc. [mM]	Abs. config.	*er*	Abs. config.[a]	*er*[b]
1	5	*R*	95.0:5.0	*R*	94.6:5.4
2	10	*S*	15.0:85.0	*S*	18.8:81.2
3	15	*R*	75.0:25.0	*R*	78.3:21.7
4	18	*S*	35.0:65.0	*S*	34.9:65.1
5	19	*R*	95.0:5.0	*R*	95.5:4.5

[a] Based on the sign of the induced Cotton effects. [b] The *er* was calculated based on the CD signal at 300 nm. See SI for details.

by ESI-MS and NMR analysis. Importantly, the sensing reaction is very fast, it is complete within 3 minutes and readily occurs in common organic solvents, including $CHCl_3$, CH_2Cl_2, THF and ACN, at room temperature which underscores the potential of this sensing assay in high-throughput screening applications.

Encouraged by these initial findings, we decided to test the suitability of the chloroamidophosphite **5** for enantioselective recognition and analysis of the enantiomeric ratio of samples with various amounts of **6**, Table 1 and SI. In all cases, the formation of the amidophosphite **34** allowed correct identification of the absolute configuration of the major enantiomer by comparison with a reference sample and determination of the enantiomeric composition of the non-racemic samples with high accuracy. For example, the sensing of the samples containing the (*R*)- and (*S*)-**6** in a 95.0:5.0 and 35.0: 65.0 ratio gave 94.6:5.4 and 34.9:65.1, respectively, entries 1 and 4.

We then continued with the evaluation of the substrate scope by applying the standard sensing protocol with probes **1** and **5** to a large variety of alcohols **6**–**18** including aliphatic substrates and natural products such as menthol and isopulegol. In all cases, we found that the alcohol binding induces a characteristic CD signal that can be utilized for enantioselective analysis. Representative sensing results with **11**, **12**, **14** and **18** are shown in Figure 2. Based on the general usefulness with mono-alcohols, we expected that the application spec-

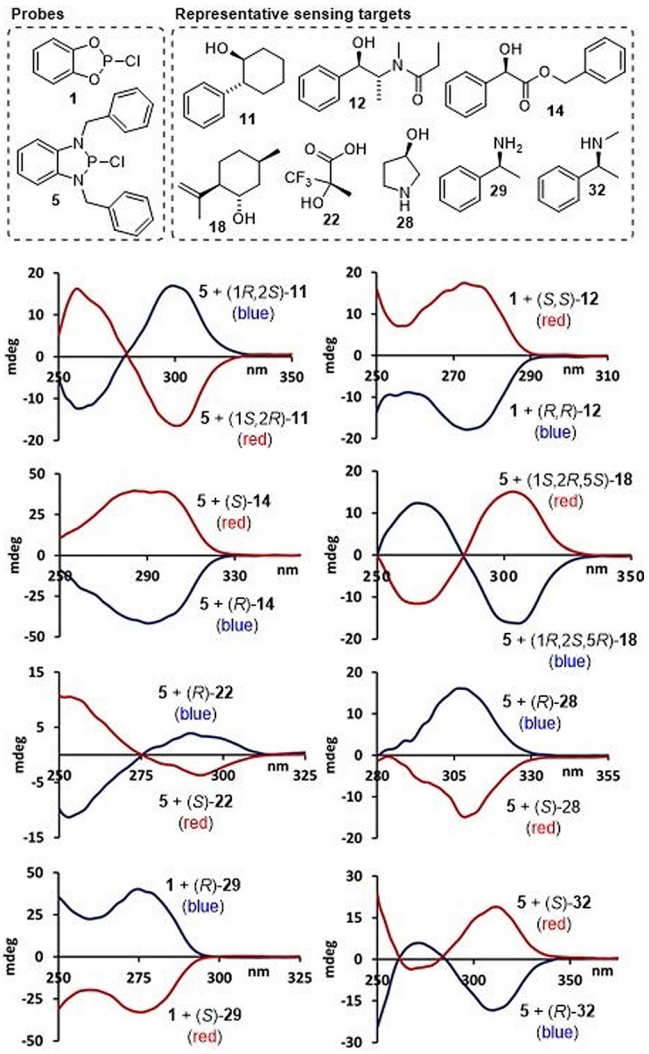
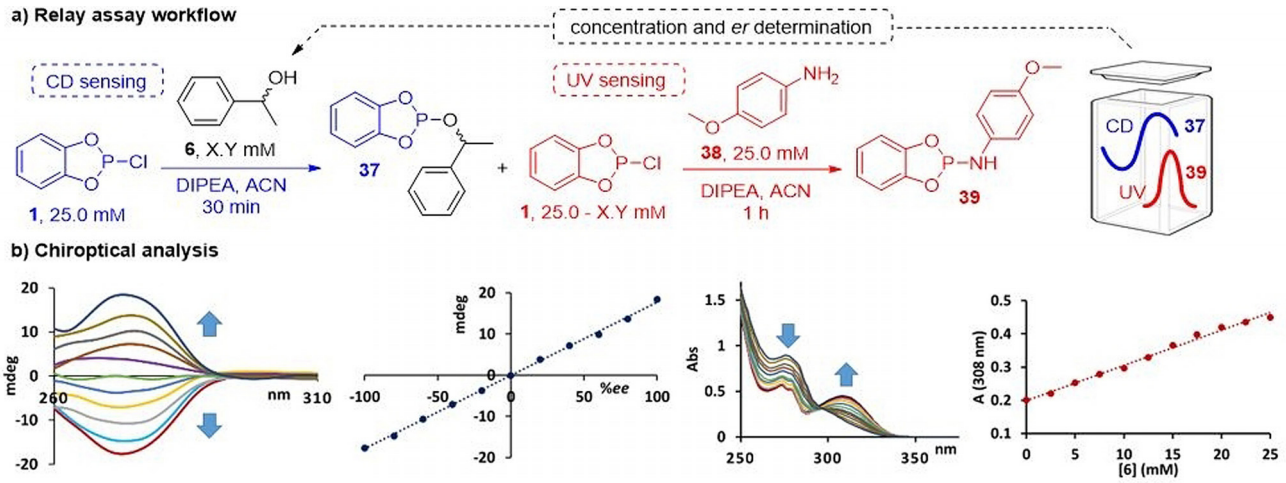


Figure 2. Representative CD responses of probes **1** and **5** to alcohols, hydroxy acids, amino alcohols and amines. All measurements were performed at 0.13–0.40 mM in chloroform. For more details, see SI.

trum of our sensors would extend to other important target compounds and we therefore applied our chiroptical assay to the diols, hydroxy acids, amino alcohols and amines **19**–**33**. Again, we observed smooth phosphite formation under mild conditions in the presence of base and simultaneous induction of strong chiroptical signals at sub-millimolar concentrations, Figure 2. The successful testing with a total of 28 structurally diverse analytes demonstrates that chirality sensing with aromatic chlorophosphites is broadly useful and a practical tool for the determination of the absolute configuration and enantiomeric composition of several compound classes.

To develop a robust optical assay that can accomplish on-the-fly sensing of the enantiomeric ratio and total concentration of chiral compounds we explored the tandem use of sensor **1** and aniline derived UV indicators. The general concept of this relay assay is shown in Scheme 2. First, the phosphite formation of an unknown amount of the alcohol substrate that may be present in up to 25.0 mM with a full equivalent of **1** matching the maximal possible analyte concentration is used for the determination of the absolute configuration and enantiomeric ratio via CD analysis as described above. The remaining excess of unreacted **1** is then captured with an aniline indicator to generate a quantifiable UV signal that is correlated to the original analyte concentration. Several candidates were screened for this purpose and the shortest reaction time together with a distinct UV change that can be used for accurate determination of the original alcohol amount were obtained with *para*-anisidine **38** (SI).

This chiroptical sensing workflow was first tested with samples containing the alcohol **6** in varying amounts and enantiomeric ratios to quantitatively correlate the variation in the corresponding chiroptical readouts to the change of the analyte concentration and *er*. Having established the relay flow, we then attempted the comprehensive sensing analysis of several alcohol mixtures. The samples were subsequently treated with the chlorophosphite and the aniline probe and the resulting mixtures containing **37** and **39** in varying quantities were then subjected to CD/UV analysis, Table 2.



Scheme 2. a) Workflow of the comprehensive UV/CD sensing of 1-phenylethanol using probe **1** and *p*-anisidine as indicator. b) Chiroptical probe responses. Left: CD response of probe **1** at 273 nm to scalemic samples of 1-phenylethanol. Right: UV response at 308 nm to different concentrations of 1-phenylethanol in the presence of **1** and the indicator. See SI for details.

Table 2: Simultaneous chiroptical sensing of the concentration, absolute configuration and enantiomeric ratio of nonracemic samples of **6**.

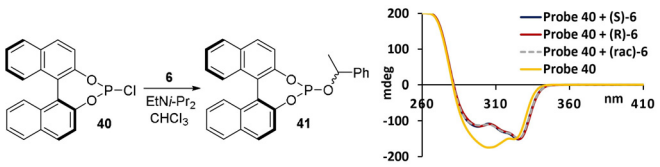
Entry	Sample composition			Sensing results		
	Conc. [mM]	Abs. config.	*er*	Conc.[a] [mM]	Abs. config.[b]	*er*[c]
1	6.0	*S*	83.4:16.6	6.2	*S*	84.1:15.9
2	10.0	*S*	85.0:15.0	9.9	*S*	82.9:17.1
3	13.0	*R*	25.0:75.0	13.8	*R*	22.3:77.7
4	16.0	*S*	71.9:28.1	15.6	*S*	67.5:32.5
5	18.0	*R*	33.3:66.7	18.6	*R*	34.1:65.9
6	20.0	*S*	62.5:37.5	20.5	*S*	61.8:38.2
7	24.0	*S*	97.9:2.1	22.5	*S*	96.9:3.1

[a] Based on the UV change at 308 nm. [b] Based on the sign of the induced Cotton effects. [c] The *er* was calculated based on the CD amplitude measured at 273 nm. See SI for details.

The results prove that the optical relay sensing concept is very reliable and generates accurate concentration and *er* data. The analysis of a nonracemic solution of **6** with an *S*:*R* ratio of 83.4:16.6 and a total concentration (both enantiomers combined) of 6.0 mM gave 84.1:15.9 and 6.2 mM, see entry 1.The sensing of other mixtures with vastly different amounts and enantiomeric compositions was also successful and we obtained relatively small error margins that are acceptable for high-throughput screening purposes, entries 2–7. The recording of CD and UV spectra can be accomplished simultaneously by modern CD spectrophotometers which takes full advantage of the inherent speed of the underlying reactions and the continuous relay workflow. The reproducibility and robustness of this approach were validated with samples containing similar amounts of acetophenone, see SI. We like to point out that chirality sensing with chlorophosphites is not restricted to one particular probe scaffold although the general scope and usefulness were evaluated in depth using **1** and **5**. Overall, these proved equally valuable. However, **1** is commercially available which is certainly advantageous.

Finally, we examined if one could use an atropisomeric BINOL derived chlorophosphite probe for sensing, too. We expected that (*P*)-**40** would have a strong CD signal originating from the BINOL moiety that may be selectively altered upon binding of either (*R*)- or (*S*)-1-phenylethanol, **6**, because diastereomeric products **41** are formed. This would be fundamentally different from the use of the CD-silent chlorophosphites **1**–**5** which are achiral or tropos-type agents that give enantiomeric products with the chiral target compounds **6**–**33** thus generating induced CD effects. Interestingly, we found that sensing of enantiopure **6** and its racemate with (*P*)-**40** yields essentially the same CD spectrum and therefore does not allow chiroptical enantio-differentiation and *ee* determination which altogether underscores the value and practicality of the sensing with **1**–**5** described herein, Scheme 3.

In summary, we have introduced a new class of chiroptical probes and a relay assay sensing strategy that enable accurate on-the-fly stereochemical analysis of mono-alcohols, diols, hydroxy acids, amines and amino alcohols—a variety that stands out among previously reported UV, fluorescence and CD assays. The chlorophosphite CD probes **1**–**5** and several



Scheme 3. Sensing of the enantiomers and a racemic mixture of alcohol **6** with (*P*)-**40**. The reactions were conducted at 20.0 mM and the CD spectra were collected at 0.20 mM in chloroform.

aniline derived UV indicators were evaluated and the commercially available chlorobenzodioxaphosphite and para-anisidine were combined into a continuous sensing workflow. The practicality of this approach and the accuracy of the chiroptical concentration and enantiomeric ratio analysis were demonstrated with seven 1-phenylethanol samples. It is envisioned that this assay can be adapted to high-throughput equipment and multi-well plate technology which would allow fully automated operation and simultaneous screening of hundreds of samples in parallel.

Acknowledgements

We gratefully acknowledge financial support from the U.S. National Science Foundation (CHE-1764135).

Conflict of interest

The authors declare no conflict of interest.

Keywords: chirality · chlorophosphites · circular dichroism · optical sensing · UV spectroscopy

[1] a) A. McNally, C. K. Prier, D. W. C. MacMillan, *Science* **2011**, *334*, 1114–1117; b) A. Buitrago Santanilla, E. L. Regalado, T. Pereira, M. Shevlin, K. Bateman, L.-C. Campeau, J. Schneeweis, S. Berritt, Z.-C. Shi, P. Nantermet, Y. Liu, R. Helmy, C. J. Welch, P. Vachal, I. W. Davies, T. Cernak, S. D. Dreher, *Science* **2015**, *347*, 49–53; c) K. D. Collins, T. Gensch, F. Glorius, *Nat. Chem.* **2014**, *6*, 859–871.

[2] For examples of fast chiral HPLC enantioseparations: a) D. Kotoni, A. Ciogli, C. Molinaro, I. D'Acquarica, J. Kocergin, T. Szczerba, H. Ritchie, C. Villani, F. Gasparrini, *Anal. Chem.* **2012**, *84*, 6805–6813; b) C. L. Barhate, L. A. Joyce, A. A. Makarov, K. Zawatzky, F. Bernardoni, W. A. Schafer, D. W. Armstrong, C. J. Welch, E. L. Regalado, *Chem. Commun.* **2017**, *53*, 509–512.

[3] For recent advances with NMR chiral solvating agents, see a) L. Yang, T. Wenzel, R. T. Williamson, M. Christensen, W. Schafer, C. J. Welch, *ACS Cent. Sci.* **2016**, *2*, 332–340; b) G. Storch, M. Haas, O. Trapp, *Chem. Eur. J.* **2017**, *23*, 5414–5418; c) Q. H. Luu, K. G. Lewis, A. Banerjee, N. Bhuvanesh, J. A. Gladysz, *Chem. Sci.* **2018**, *9*, 5087–5099, and references therein.

[4] a) D. Leung, S. O. Kang, E. V. Anslyn, *Chem. Soc. Rev.* **2012**, *41*, 448–479; b) C. Wolf, K. W. Bentley, *Chem. Soc. Rev.* **2013**, *42*, 5408–5424. For a recent Perspective on this topic: c) B. T. Herrera, S. L. Pilicer, E. V. Anslyn, L. A. Joyce, C. Wolf, *J. Am. Chem. Soc.* **2018**, *140*, 10385–10401.

[5] a) S. H. Shabbir, J. R. Clinton, E. V. Anslyn, *Proc. Natl. Acad. Sci. USA* **2009**, *106*, 10487 – 10492; b) S. Nieto, J. M. Dragna, E. V. Anslyn, *Chem. Eur. J.* **2010**, *16*, 227 – 232; c) K. W. Bentley, D. Proano, C. Wolf, *Nat. Commun.* **2016**, *7*, 12539; d) E. G. Shcherbakova, E. G. V. Brega, V. M. Lynch, T. D. James, P. Anzenbacher, *Chem. Eur. J.* **2017**, *23*, 10222 – 10229; e) K. W. Bentley, P. Zhang, C. Wolf, *Sci. Adv.* **2016**, *2*, e1501162; f) F. Biedermann, W. M. Nau, *Angew. Chem. Int. Ed.* **2014**, *53*, 5694 – 5699; *Angew. Chem.* **2014**, *126*, 5802 – 5807; g) T. A. Feagin, D. P. Olsen, Z. C. Headman, J. M. Heemstra, *J. Am. Chem. Soc.* **2015**, *137*, 4198 – 4206; h) Z. A. De los Santos, C. Wolf, *J. Am. Chem. Soc.* **2016**, *138*, 13517 – 13520; i) L. A. Joyce, E. C. Sherer, C. J. Welch, *Chem. Sci.* **2014**, *5*, 2855 – 2861; j) F. Y. Thanzeel, K. Balaraman, C. Wolf, *Nat. Commun.* **2018**, *9*, 5323; k) F. Y. Thanzeel, A. Sripada, C. Wolf, *J. Am. Chem. Soc.* **2019**, *141*, 16382 – 16387; l) M. E. Shirbhate, S. Kwon, A. Song, S. Kim, D. Kim, H. Huang, Y. Kim, H. Lee, S.-J. Kim, M.-H. Baik, J. Yoon, K. M. Kim, *J. Am. Chem. Soc.* **2020**, *142*, 4975 – 4979.

[6] a) C. Wolf, A. M. Cook, J. E. Dannatt, *Tetrahedron: Asymmetry* **2014**, *25*, 163 – 169; b) L. Yang, T. Wenzel, R. T. Williamson, M. Christensen, W. Schafer, C. J. Welch, *ACS Cent. Sci.* **2016**, *2*, 332 – 340; c) G. Bian, S. Yang, H. Huang, H. Zong, L. Song, H. Fan, X. Sun, *Chem. Sci.* **2016**, *7*, 932 – 938; d) M.-S. Seo, S. Jang, H. Kim, *Chem. Commun.* **2018**, *54*, 6804 – 6808.

[7] a) J. Guo, J. Wu, G. Siuzdak, M. G. Finn, *Angew. Chem. Int. Ed.* **1999**, *38*, 1755 – 1758; *Angew. Chem.* **1999**, *111*, 1868 – 1871; b) M. T. Reetz, M. H. Becker, H.-W. Klein, D. Stöckigt, *Angew. Chem. Int. Ed.* **1999**, *38*, 1758 – 1761; *Angew. Chem.* **1999**, *111*, 1872 – 1875. Asymmetric reaction MS analysis: c) C. Markert, A. Pfaltz, *Angew. Chem. Int. Ed.* **2004**, *43*, 2498 – 2500; *Angew. Chem.* **2004**, *116*, 2552 – 2554; d) C. Markert, A. Pfaltz, *J. Am. Chem. Soc.* **2008**, *130*, 3234 – 3235. A review on this topic: e) S. Piovesana, R. Samperi, A. Lagana, M. Bella, *Chem. Eur. J.* **2013**, *19*, 11478 – 11494.

[8] Examples of optical methods that allow determination of the absolute configuration of mono-alcohols: a) K. Kobayashi, Y. Asakawa, Y. Kikuchi, H. Toi, Y. Ayoma, *J. Am. Chem. Soc.* **1993**, *115*, 2648 – 2654; b) J. M. Lintuluoto, V. V. Borovkov, Y. Inoue, *J. Am. Chem. Soc.* **2002**, *124*, 13676 – 13677; c) S. Hayashi, M. Yotsukura, M. Noji, T. Takanami, *Chem. Commun.* **2015**, *51*, 11068 – 11071; d) S. Shimo, K. Takahashi, N. Iwasawa, *Chem. Eur. J.* **2019**, *25*, 3790 – 3794; e) T. Mądry, A. Czapik, M. Kwit, *ACS Omega* **2019**, *4*, 3244 – 3256. Methods that allow *er* analysis of mono-alcohols: f) L. You, J. S. Berman, E. V. Anslyn, *Nat. Chem.* **2011**, *3*, 943 – 948; g) L. You, J. S. Berman, A. Lucksanawichien, E. V. Anslyn, *J. Am. Chem. Soc.* **2012**, *134*, 7117 – 7125; h) L. You, G. Pescitelli, E. V. Anslyn, L. Di Bari, *J. Am. Chem. Soc.* **2012**, *134*, 7126 – 7134; i) C. Ni, D. Zha, H. Ye, Y. Hai, Y. Zhou, E. V. Anslyn, L. You, *Angew. Chem. Int. Ed.* **2018**, *57*, 1300 – 1305; *Angew. Chem.* **2018**, *130*, 1314 – 1319; j) H. H. Jo, X. Gao, L. You, E. V. Anslyn, M. J. Krische, *Chem. Sci.* **2015**, *6*, 6747 – 6753.

[9] For a general overview of analytical methods: C. Wolf in *Dynamic Stereochemistry of Chiral Compounds—Principles and Applications*, RSC Publishing, Cambridge, **2008**, pp. 136 – 179.

Manuscript received: April 12, 2020
Revised manuscript received: August 3, 2020
Accepted manuscript online: August 6, 2020
Version of record online: September 15, 2020

