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Structure Elucidation

# Absolute Configuration of Small Molecules by Co-Crystallization

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***Abstract:*** *The most reliable method to determine the absolute configuration of chiral molecules is X-ray crystallography, but small molecules can be difficult to crystallize. We report rapid co-crystallization of tetraaryladamantanes with small molecules as different as n-decane to nicotine to produce crystals for X-ray analysis and the assignment of absolute configuration when the molecules are chiral. A screen of 52 diverse compounds gave inclusion in co-crystals for 88 % of all cases and a high-resolution structure in 77 % of cases. Furthermore, starting from three milligrams of analyte, a combination of NMR spectroscopy and X-ray crystallography produced a full structure in less than three days using an adamantane crystallization chaperone that encapsulates the analyte at room temperature.*

Molecules with different absolute configuration behave differently in chiral environments, such as the active sites of enzymes or receptors. This is also true for active pharmaceutical ingredients.[1] Natural sources and synthetic methods provide a plethora of new molecules of unknown stereochemical configuration. Without a reliable method to determine the absolute configuration of new molecules a most undesirable situation arises, such as that for the enantiomers of sugar molecules whose stereochemical configuration was arbitrarily assigned to D/L,[2] long before the true absolute configuration was determined by anomalous dispersion X-ray crystallography.[3]

When a new compound is isolated in pure form, it is usually characterized spectroscopically. Spectroscopic methods, such as NMR of underivatized analytes in an achiral solvent, do not yield the absolute configuration of enantiomers, nor do separations on chiral HPLC or GC columns, making the search for new methods for assigning stereochemistry an active field of research.[4,5] The most important method to determine the three-dimensional structure of molecules is single-crystal X-ray crystallography. However, many small or highly flexible molecules do not crystallize readily, producing oils, glasses, amorphous precipitates, or poorly ordered solids instead.

Several approaches are being pursued to address this limitation. Some approaches take inspiration from structural biology. For proteins, crystallization chaperones have been described that bind covalently or non-covalently to the analyte to help it assemble into a crystalline lattice.[6] Thus far, there are no general chaperones that co-crystallize with small-molecule analytes,[7] and few of the potential host systems, such as porphyrins, calixarenes, or cyclodextrins, have a proven ability to provide the absolute configuration of a broad range of different organic molecules.[8–10] Ternary mixtures of aromatic sulfonates, guanidinium cations and analytes give crystalline hydrogen-bonded frameworks, if a salt suitable for capturing the molecule of interest is identified from a large library of potential hosts. In a recent study, seven different hosts were employed to accommodate ten different medium-size organic compounds.[11] Furthermore, there is an approach using crystalline sponges, i.e., pre-crystallized metal–organic frameworks, into which analyte molecules can be diffused,[12] that has been successful in several cases.[13,14] A related approach that has worked well for alcohols, phenols, and carboxylic acids uses coordinative alignment in chiral frameworks.[15] The success of these methods relies on the ability of the analyte to take up identical positions in the pre-formed crystalline lattice without disorder, and to produce sufficiently strong signals in the X-ray diffraction pattern often dominated by reflexes of the host material.[16]

One option to solve the crystallization problem of small analytes is to generate inclusion complexes with larger organic hosts.[17] We have recently described tetraaryladamantanes (TAAs) that readily form crystalline inclusion complexes with small molecules.[18] Both 1,3,5,7-tetrakis(2,4-dimethoxy-phenyl)adamantane (TDA) and 1,3,5,7-tetrakis(2,4-diethoxy-phenyl)adamantane (TEO) were found to accommodate a range of guest molecules in several crystal systems.[19] The ability to encapsulate structurally diverse guest molecules has been used to create reagents with a crystalline coat,[20] to capture aromatic hydrocarbons from the gas phase,[19] and to stabilize starting materials for organic synthesis,[21] but it has not been utilized for structure elucidation. Herein, we report that co-crystallization of difficult-to-crystallize small molecules and tetraaryladmantanes can be used to rapidly determine the absolute configuration of small molecules.

The three tetraaryladamantanes employed as crystallization chaperones are shown in Figure 1. Octaethers TDA and TEO have been previously shown to co-crystallize with guest molecules.[18–21] The third, new chaperone, is 1,3,5,7-tetrakis(2-bromo-4-phenyl)adamantane (TBro). This chaperone was developed to accommodate lipophilic analytes, assuming that a halogenated TAA will interact well with such compounds, much like halogenated solvents do with lipophilic or

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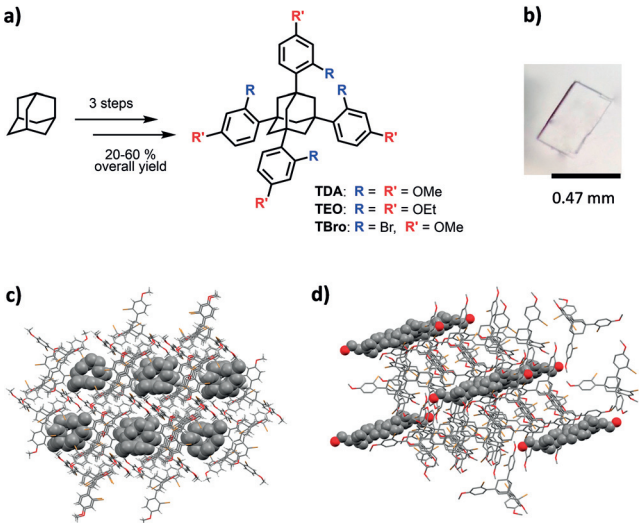


*Figure 1.* Tetraaryladamantane chaperones and their co-crystals with analytes. a) Synthesis and structures of tetraaryladamantanes. b) Photograph of crystal from a nicotine/TEO mixture (3:1 *w*/*w*). Packing arrangement in representative co-crystals: c) α-humulene/TBro, and d) farnesol/TBro.

amphiphilic solutes. The synthesis of TBro is detailed in the Supporting Information. Like the other two TAAs, TBro is accessible from adamantane in three synthetic steps. Besides the ability to interact well with saturated hydrocarbons, tetrabromide TBro also gives strong anomalous dispersion signals in X-ray crystallography.

At the outset of our study, 48 crystalline inclusion complexes of tetraaryladamantanes had been found in our laboratories,[18–21] none of which contained a chiral guest molecule. We chose the structurally diverse analytes of Figure 2 for single-run thermal crystallization experiments with TDA or TBro. A mixture of the analyte and the adamantane was heated for a maximum of 30 seconds on a laboratory hotplate until a clear solution formed. This solution was then allowed to cool to room temperature by switching off the heating of the hotplate. Crystals formed within minutes or hours. Details of the crystallization experiments are listed in Table S1. Crystals for X-ray diffraction were harvested after allowing crystallization to go to completion overnight. Inclusion was confirmed by X-ray crystallography, leading to the structures shown in Figure 2. All structures of the analytes shown were obtained in the first crystallization attempt. Typical examples of crystal packing are displayed in Figure 1 c,d for α-humulene (**5**) and farnesol (**4**), co-crystallized with TBro.

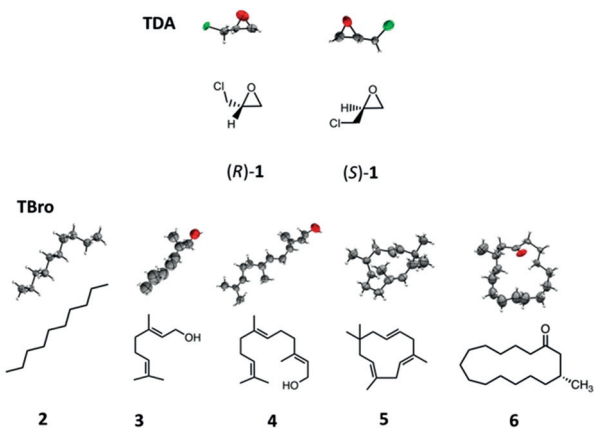


*Figure 2.* X-ray crystal structures of analytes obtained by co-crystallization with TDA and TBro (ORTEP plots of the encapsulated molecules on the 50% probability level).

The same rapid, single-run approach was then tested for TEO, the TAA known to encapsulate up to 30 wt% of guest molecules.[19] The results for this TAA are shown in Figure 3. Overall, the screen was designed to demonstrate breadth and the ability to determine absolute configuration, even though the asymmetric unit of the co-crystals are larger than those of small molecules. To achieve the former, achiral molecules were included where necessary. The chiral molecules studied included five pairs of enantiomers, namely (*R*)- and (*S*)-epichlorohydrin (**1**), a chiral starting material for an inhibitor,[22] limonene (**7**), carvone (**8**), 2-phenylethanol (**15**), and methylbenzylamine (**16**). All ten gave co-crystals, with stoichiometries ranging from 1:1 to 4:3 (TAA/analyte) and a triclinic or monoclinic crystal system. Refined structures of both of the enantiomers were obtained in less than two days. Flack parameters were in the range of −0.1 to 0.2 (Table S1), allowing for unambiguous assignment of absolute configuration. For small-molecule crystals, a threshold of 0.1 is commonly used, but the larger host–guest complexes with their light atoms, including many host atoms with centrosymmetric positions, require a more tailored approach, including inversion tests.[23] When the Flack parameter exceeded 0.1 for a chiral analyte, validation included test calculations with the opposite chirality (inversion of the coordinates), which invariably gave a much higher Flack value, confirming the assignment (see Table S3 in the Supporting Information for difficult cases). Since unambiguous assignments were obtained for either enantiomer, the information which enantiomer is which was indeed obtained twice for each pair of enantiomers.

Our screen also included reactive molecules, such as hexamethylene diisocyanate (**19**), an important industrial reagent, cyclopentylisocyanate (**17**), and phenylisocyanate (**18**), for which no previous high-resolution structures have been deposited in databases. Furthermore, we co-crystallized structurally diverse and hard-to-crystallize compounds, such as *n*-decane (**2**) and [12]-crown-4 (**14**), a flexible saturated heterocycle. Among our analytes was a series of alcohols of increasing chain length (**20**–**23**) and an amino acid ester (**11**). The screen also gave high-resolution structures of the natural products (−)-α-thujone (**9**), nicotine (**10**), (−)-linalool (**12**), α-humulene (**5**), geraniol (**3**), eugenol (**13**), and farnesol (**4**). Needless to say, an achiral host will not differentiate between two enantiomers of a chiral compound when it forms a co-crystal. Accordingly, crystallization of TEO with a racemic mixture of (±)-limonene gave a racemic structure (last entry in the list of crystal data in the Supporting Information).

The largest test compound was muscone (**6**) with a molecular weight of 238 g mol$^{-1}$ with a single small substituent on a large flexible ring. In this case, thermal crystallization with

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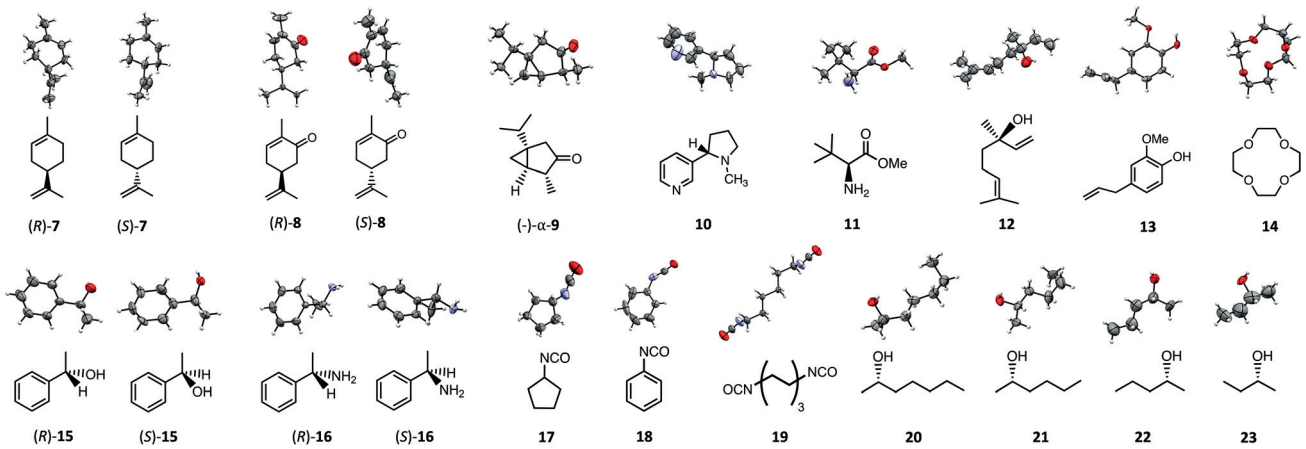


***Figure 3.*** Three-dimensional structures of analytes, as obtained by co-crystallization with TEO and X-ray crystallography (ORTEP plots at 50% probability).

a single chaperone gave a Flack parameter that did not allow assignment of the absolute configuration, indicating an upper limit for the rapid, single-run crystallization approach. A high-resolution structure may require slower crystallization or a different chaperone in this case (only TBro was tested thus far).

The overall success rate of our method can be gleaned from the full list of all 52 small molecules tested in this study (Table S2). In terms of encapsulation of the analyte in a co-crystal, the success rate was 88%, even though not all three TAAs were tested for every analyte. Furthermore, 77% of the analytes yielded at least one high-resolution structure. We are unaware of similar statistics for any of the other methods for structure elucidation by host-facilitated X-ray crystallography.

The molecular weight limit for encapsulation is not yet clear. Stoichiometries of 4:1 (TBro/farnesol) and the diversity of shapes encapsulated suggest that it is not defined by the size of a simple cavity, left unfilled when the tetraaryladamantanes assemble. This naive notion is also in disagreement with the observation that all three chaperones are able to form densely packed crystals without inclusions;[24] for TBro, such a structure is shown in Figure S3. Rather, the ability of the analyte to act as solvent in the crystallization of the TAA appears to be the limiting factor.

The broad range of molecules encapsulated by the TAAs is visualized in Figure S5 as a plot of size and lipophilicity of guest molecules. The lipophilicity was expressed as the logarithm of the partitioning coefficient (logP), calculated with the program Molinspiration.[25,26] The numerical values can be found in Table S4. The plot shows that the ability of the three TAAs to encapsulate analytes overlaps. Analytes that are large and lipophilic appear to be most easily encapsulated by TBro, which dissolves more readily in such "poor solvents", but this TAA was developed by us recently, and therefore the number of data points is more limited than for the other adamantanes. Of the remaining two TAAs, TDA shows the greater diversity in crystal systems and a larger propensity to have partially disordered guest molecules in its unit cells. This makes TEO our default choice for structure elucidation by co-crystallization.

Rapid, single-run crystallizations are attractive for automated analysis. New molecules synthesized or isolated in milligram quantities will almost invariably be characterized by NMR spectroscopy and their absolute configuration may remain unclear. Other combinations of techniques, such as HPLC–NMR analysis, are known to achieve high throughput.[27] As a first foray into a combined NMR–X-ray method, we performed exploratory experiments on what we call "spectroscopy cum crystallization" (SCC). For this, we miniaturized crystallization, and we also checked for crystal formation without heating. Isothermal crystallization avoids the need to heat precious compounds. We had noticed previously that an amorphous form of TDA crystallizes from organic liquids upon standing at 25 °C.[20] Furthermore, hexamethylene diisocyanate (**19**), a reactive compound, also co-crystallized with TEO at room temperature, encouraging us to proceed.

Obtaining basic one- and two-dimensional NMR spectra requires low milligram quantities of small-molecule analytes. In the experiment shown in Figure 4, we dissolved 3 mg of nicotine in 0.2 mL $CD_2Cl_2$, a solvent that does not get encapsulated by TEO. After acquiring the $^1$H NMR spectrum in a standard micro-NMR tube, the bulk of the solvent was removed with a flow of nitrogen, followed by addition of 1 mg of finely powdered TEO. Crystals formed spontaneously from the mixture at room temperature overnight. One such crystal was harvested and gave the X-ray structure shown, confirming that SCC is a valid option for automatable, in-depth structure elucidation.

Seemingly promiscuous co-crystallization with many small molecules that are then well-packed is an unusual phenomenon. We suspect that this phenomenon is due to the structural characteristics of our TAAs because close structural derivatives, such as 1,3,5,7-tetrakis(4-methoxy-2-ethylphenyl)adamantane, do not show this behavior.[18,19] The lack of hydrogen-bond donors leads to an absence of highly directional interactions between host molecules, as found in

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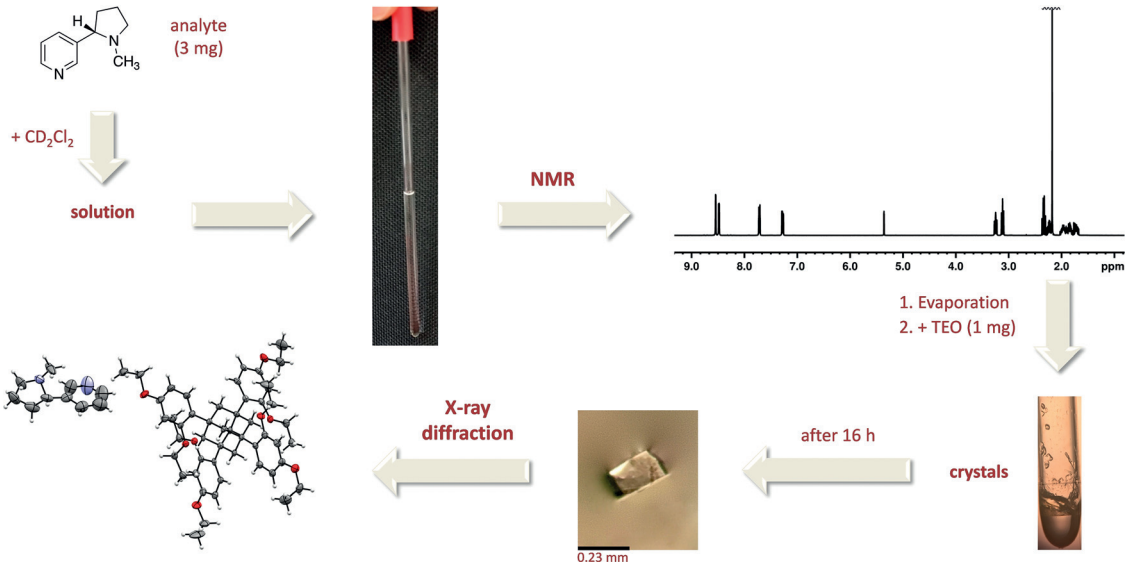


***Figure 4.*** Flow chart for spectroscopy-cum-crystallography (SCC) method. A solution of nicotine in $CD_2Cl_2$ was used for NMR analysis, and evaporation of the solvent under a stream of nitrogen was followed by addition of finely powdered TEO as the crystallization chaperone. After 16 h, the crystal shown was picked for X-ray crystallography, producing the three-dimensional structure shown in the lower left-hand corner (ORTEP plot). The entire protocol took 3 days, including sample preparation, wait and acquisition times on spectrometer and diffractometer and computational time. Figure S4 shows an analogous experiment for (*R*)-carvone.

the crystals of Dianin's compound, which encapsulates many different small molecules, but does not force a strong crystalline order on them.[28] The TAAs also have a shape and symmetry that favors rapid crystallization. The *ortho*-substituents of their phenyl rings probably slow the rotation about the phenyl–adamantane axis, without causing atropisomerism. Furthermore, their alkoxy groups can adopt different tweezer-like conformations that help to accommodate different guest molecules.[18] With a fairly rigid, but still adaptable general structure, the inclusion of analytes during rapid crystallization is probably a kinetic phenomenon.

The ease with which a well-ordered crystalline arrangement with guests is found is apparently greater than for hosts that take up the analyte into a preformed crystal lattice. Whereas a crystalline sponge stays rigid as an analyte tries to find a binding site, the adamantane undergoes nucleation and crystal growth solvated by guest molecules and apparently sheds only those small molecules that do not prevent it from achieving crystallinity. As it settles into one of the accessible conformations and crystal systems, it finds a molecular arrangement that suits both analyte and itself. As our data shows, there are many more such mutually acceptable arrangements than for rigid, preformed lattices.[29]

In conclusion, we report a method for structure elucidation of small molecules by using tetraaryladamantanes as crystallization chaperones. One-step crystallization occurs without the need for covalent bonds or strong directional interactions. The small molecule acts as the solvent and is then solidified with the aid of the chaperone. The method produces high-resolution co-crystal structures with analytes covering more than five orders of magnitude in calculated octanol–water partitioning coefficient and sizes up to at least 222 $g\,mol^{-1}$. The procedure is rapid, does not require a screen for a suitable solvent or crystallization conditions, and can be combined with NMR spectroscopy. It is simple and robust, yielding data for many linear and cyclic compounds, with or without a functional group. This method can be used to determine the absolute configuration where it usually originates, both for targets of multistep syntheses and for classes of related natural products: on the level of chiral small molecules.

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## Conflict of interest

The authors declare no conflict of interest.

**Keywords:** absolute configuration · crystallization · stereochemistry · structure elucidation · X-ray crystallography

[1] T. Eriksson, S. Björkman, B. Roth, A. Fyge, P. Höglund, *Chirality* **1998**, *10*, 223.
[2] E. Fischer, *Ber. Dtsch. Chem. Ges.* **1891**, *24*, 1836.
[3] J. M. Bijvoet, A. F. Peerdeman, A. J. van Bommel, *Nature* **1951**, *168*, 271–272.
[4] J. M. Seco, E. Quinoa, R. Riguera, *Chem. Rev.* **2004**, *104*, 17.
[5] G. Bringmann, T. Bruhn, K. Maksimenka, Y. Hemberger, *Eur. J. Org. Chem.* **2009**, 2717.
[6] J. L. Johnson, K. C. Entzminger, J. Hyun, S. Kalyoncu, D. P. Heaner, Jr., I. A. Morales, A. Sheppard, J. C. Gumbart, J. A.

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Maynard, R. L. Lieberman, *Acta Crystallogr. Sect. D* **2015**, *71*, 896.
[7] M. B. Bhatt, G. R. Desiraju, *CrystEngComm* **2008**, *10*, 1747.
[8] M. P. Byrn, C. J. Curtis, Y. Hsiou, S. I. Khan, P. A. Sawin, S. K. Kendrick, A. Terzis, C. E. Strouse, *J. Am. Chem. Soc.* **1993**, *115*, 9480.
[9] T. Gruber, C. Fischer, W. Seichter, P. Bombicz, E. Weber, *CrystEngComm* **2011**, *13*, 1422.
[10] M. Ceborska, *Chem. Phys. Lett.* **2016**, *651*, 192.
[11] Y. Li, S. Tang, A. Yusov, J. Rose, A. N. Borrfors, C. T. Hu, M. D. Ward, *Nat. Commun.* **2019**, *10*, 4477.
[12] Y. Inokuma, S. Yoshioka, J. Ariyoshi, T. Arai, Y. Hitora, K. Takada, S. Matsunaga, K. Rissanen, M. Fujita, *Nature* **2013**, *495*, 461.
[13] N. Zigon, M. Hoshino, S. Yoshioka, Y. Inokuma, M. Fujita, *Angew. Chem. Int. Ed.* **2015**, *54*, 9033; *Angew. Chem.* **2015**, *127*, 9161.
[14] N. Zigon, T. Kikuchi, J. Ariyoshi, Y. Inokuma, M. Fujita, *Chem. Asian J.* **2017**, *12*, 1057.
[15] S. Lee, E. A. Kapustin, O. M. Yaghi, *Science* **2016**, *353*, 808.
[16] Y. Inokuma, S. Yoshioka, J. Ariyoshi, T. Arai, Y. Hitora, K. Takada, S. Matsunaga, K. Rissanen, M. Fujita, *Nature* **2013**, *501*, 262.
[17] F. Cramer, *Einschluss-Verbindungen*, Springer, Berlin, **1954**, p. 4.
[18] A. Schwenger, W. Frey, C. Richert, *Chem. Eur. J.* **2015**, *21*, 8781.
[19] P. E. Alexandre, A. Schwenger, W. Frey, C. Richert, *Chem. Eur. J.* **2017**, *23*, 9018.
[20] A. Schwenger, W. Frey, C. Richert, *Angew. Chem. Int. Ed.* **2016**, *55*, 13706; *Angew. Chem.* **2016**, *128*, 13910.
[21] F. Krupp, S. He, W. Frey, C. Richert, *Synlett* **2018**, *29*, 1707.
[22] R. Kanada, M. Tanabe, R. Muromoto, Y. Sato, T. Kuwahara, H. Fukuda, M. Arisawa, T. Matsuda, M. Watanabe, S. Shuto, *J. Org. Chem.* **2018**, *83*, 7672.
[23] H. D. Flack, G. Bernardinelli, *Chirality* **2008**, *20*, 681.
[24] C. Richert, F. Krupp, *Synlett* **2017**, *28*, 1763.
[25] Accessible via http://www.molinspiration.com.
[26] For a review, see: R. Mannhold, G. I. Poda, C. Ostermann, I. V. Tetko, *J. Pharm. Sci.* **2009**, *98*, 861.
[27] J. J. van der Hooft, V. Mihaleva, R. C. de Vos, R. J. Bino, J. Vervoort, *Magn. Reson. Chem.* **2011**, *49*, S55.
[28] W. Baker, A. J. Floyd, J. F. W. McOmie, G. Pope, A. S. Weaving, J. H. Wild, *J. Chem. Soc.* **1956**, 2010.
[29] Deposition Numbers 1970857 (for (*R*)-**1**), 1970865 (for (*S*)-**1**), 1970895 (for **2**), 1970897 (for **3**), 1970899 (for **4**), 1970896 (for **5**), 1978250 (for **6**), 1970875 (for (*R*)-**7**), 1970877 (for (*S*)-**7**), 1970880 (for (*R*)-**8**), 1970881 (for (*S*)-**8**), 1970886 (for **9**), 1970888 (for **10**), 1970909 (for **10**), 1994691 (for **11**), 1970887 (for **12**), 1970893 (for **13**), 1970889 (for **14**), 1970919 (for (*R*)-**15**), 1970892 (for (*S*)-**15**), 1970890 (for (*R*)-**16**), 1970891 (for (*S*)-**16**), 1978246 (for **17**), 1978248 (for **18**), 1970894 (for **19**), 1994690 (for **20**), 1994689 (for **21**), 1994687 (for **22**), 1994686 (for **23**) contain the supplementary crystallographic data for this paper. These data are provided free of charge by the joint Cambridge Crystallographic Data Centre and Fachinformationszentrum Karlsruhe Access Structures service www.ccdc.cam.ac.uk/structures.

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