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**PAPER**

# Sensitive detection of enantiomeric excess in different acids through chiral induction in an oligo(*p*-phenylenevinylene) aggregate

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Induction of chirality in achiral aggregates of an oligo(*p*-phenylenevinylene) has been used to detect the enantiomeric excess in acids used in the resolution of chiral compounds. The chiral acids which induce helicity in the aggregates are present at only 10% of the concentration of the chromophore, whose chiroptical activity can be detected using circular dichroism spectroscopy. An ee of 10% in mixtures of (+) and (−) acids has been clearly evidenced in a series of samples using only 1.5 μg and concentration of approximately 10 μM of the chiral compound. The composition of both carboxylic and phosphoric acid derivatives can be detected, thanks to their binding to the dimerised core ureidotriazine unit attached at one end of the oligo(*p*-phenylenevinylene) which induces a preferred twist in the aggregated aromatic rod. This chiral arrangement is reflected in the Cotton effects that the assemblies show. The sign of the induced dichroic signal can be affected by the substituents around a stereogenic centre of otherwise identical configuration, and can lead to ambidextrous assemblies as seen in Cotton effects at different positions for different acids. While this technique can be used to detect enantiomeric excess, screening of the pure enantiomers is wise prior to the use of the method to detect scalemic mixtures. This supramolecular approach to evaluation of chiral content in samples could also be applied to other types of aggregates based on achiral molecules which show sensitivity to molecular chiral inducers.

## Introduction

One of the most important challenges of the pharmaceutical industry remains the preparation of enantiopure chiral compounds through asymmetric synthesis or resolution of racemic mixtures. As a key step in the development of such processes, the use of new routine methods to determine the presence of an enantiomeric excess in a mixture of enantiomers has a tremendous importance. A large choice of techniques is available for the detection of a scalemic mixture,[1] with NMR,[2] polarimetry and circular dichroism (CD) spectroscopy[3] and chiral chromatography[4] the most frequently used, each differing from the others in their speed, reliability and the amount of sample necessary for any measurement. Compared with NMR and polarimetry, circular dichroism can be an extremely sensitive technique depending on the absorbance and the strength of Cotton effects, but naturally requires chromophores with absorbance in the visible and/or ultraviolet regions of the electromagnetic spectrum. Chiral HPLC is by far the most sensitive method with the possibility of coupling the chromatographic apparatus to detectors using UV/visible absorption or the more sensitive mass spectrometry. The latter permits the detection of the ee using a few μL of low-concentration solutions, which correspond to nanogram scale samples. While chiral HPLC is used widely, it is not routine to implement it with every compound. Often, difficult separation and/or detection of enantiomers requires a preliminary derivatization and even purifications which can reduce the enantiomeric excess. Furthermore, the more common absorption detectors are of limited use for compounds with weakly absorbing chromophores. For this reason, new ways to detect the presence of an imbalance in a mixture of enantiomers through the possible amplification of chiral properties of a molecule exploiting its influence on the self-organization of achiral assemblies are being explored.[5] In this respect, the introduction of chiral guests in crystals,[6] liquid crystals,[7] polymers[8] and supramolecular aggregates[9] including lyotropic polypeptides[10] has been performed to show the induction of chirality in the structures of assemblies through the appearance of new properties and for chiral analysis.

The crystallization of chiral carboxylic and phosphoric acids in diastereoisomeric salts with chiral bases is a frequently used method for resolution,[11] and it is useful to be able to determine easily if the acid crystallized is scalemic or racemic. Here we focus our attention on a supramolecular assembly which presents a high selectivity towards these molecules. The dimers of oligo(*p*-phenylenevinylene)s (OPVs) held together by four hydrogen-bonds are able to stack in apolar solvents giving long fibrillar

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aggregates. While chiral derivatives form chiral stacks spontaneously,[12] the helicity of the stacks has also been induced during the self-assembling of achiral derivatives (A-OPV4) through the interaction with chiral carboxylic acids by additional two-point complementary hydrogen bonds.[13] Furthermore, these studies revealed a chiral amplification phenomenon, appearing as a non-linear dependence of the induction of helicity toward the amount of chiral acid introduced, which is particularly promising for chirality detection. Here we used A-OPV3 dimers[14] (Fig. 1) and their self-assembly to detect chirality in the presence of phencyphos derivatives which are used as resolving agents in Pasteurian diastereomeric crystallisation processes. We would like to encounter a routine technique to detect an enantiomeric excess after resolution experiments involving enantiopure amines. Considering the small volume of diastereoisomeric crystals usually obtained in the resolution attempts with these resolving agents, we considered the possibility of working directly with such samples without further purification. Thus, our study focuses on the selectivity of induction towards acids as well as the limits of detection of such assemblies through CD measurements exploiting the strong Cotton effects arising from the helical OPV stack aggregates.

## Results and discussion

We performed the first induction experiments in small vials filled with 3 mL of an A-OPV3 solution ($c = 10^{-4}$ M) in methylcyclohexane (MCH), an apolar solvent which allows aggregation of A-OPV3.[14] After adding 1 equivalent of different guests to each vial, we treated the samples in a sonic bath at 40 °C for 15 min to achieve complete dissolution of the additive. The entire batch was then heated to 60 °C in an oil bath and then stored overnight on the bench at room temperature to permit the formation of the stacked aggregates. It appears that in spite of its low solubility in MCH, (−)-phencyphos induced a CD signal indicative of the presence of a preferential helicity in the assemblies of the OPV (Fig. 2). On the other hand, in the presence of ephedrine or quinine, two resolving agents of phencyphos derivatives (Fig. 1), no CD signals were observed, even in the presence of several equivalents of the amines (Fig. 2). The lack of any induced signal in the case of quinine or ephedrine confirms that these bases do not interact with the hydrogen bond accepting stacked aggregates, a hypothesis confirmed by the lack of changes in the absorption spectra.

Working with low-concentration solutions of phencyphos as an additive to the OPV stacks, we observed that only

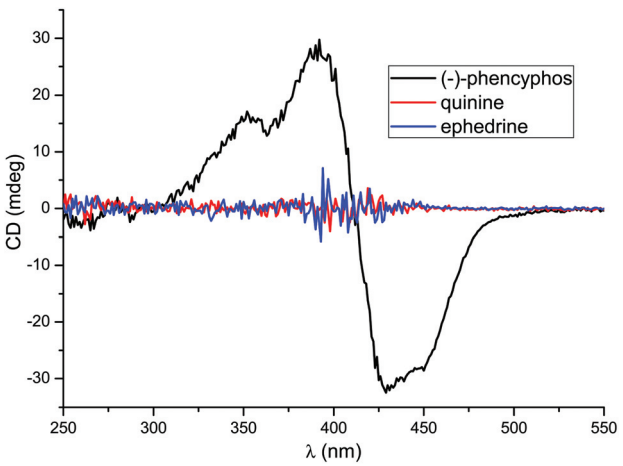


**Fig. 2** CD spectra at 293 K of an A-OPV3 solution in methylcyclohexane ($10^{-4}$ M) after dissolution, heating and overnight cooling with different chiral molecules (nominal ratio 1 : 1, cell: 1 cm path length).

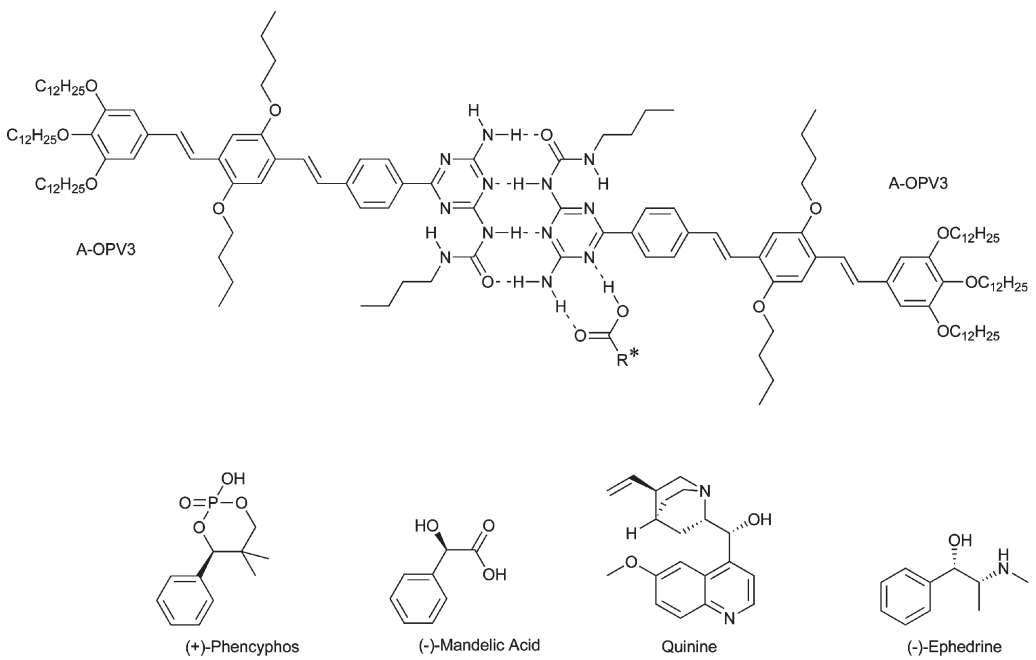


**Fig. 1** Proposed hydrogen-bonded structure of the A-OPV3-carboxylic acid complex and the chiral compounds used as possible guests for induction experiments.

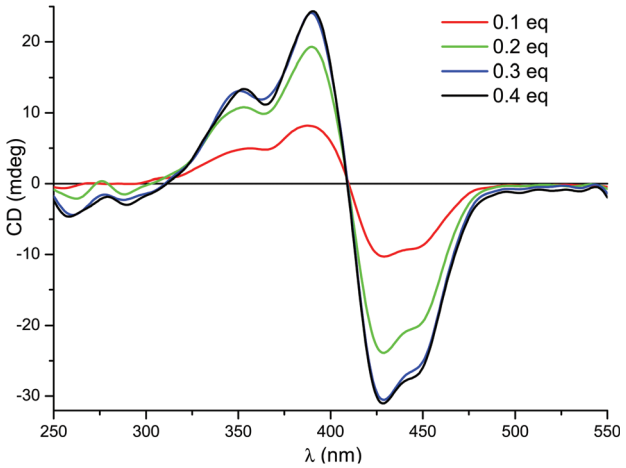


**Fig. 3** An overlay of CD spectra of A-OPV3 solutions ($10^{-4}$ M in methylcyclohexane) on increasing the amount of (−)-phencyphos from 0.1 to 0.4 equivalents (cell: 1 cm path length).

0.1 equivalents of this phosphoric acid per molecule were sufficient to obtain a clear and reproducible CD signal. The non-linear dependence of the induction phenomenon appeared clearly when varying proportions of phencyphos : A-OPV3 were used for the formation of the chiral stacks, and a maximum intensity was obtained with 0.3 equivalents (Fig. 3). The saturation of the CD signal at this proportion and the absence of increasing optical activity with higher concentrations of the acid cannot be explained only by a limiting twist to the fibres, and is probably a result of reaching the solubility limit of the acid in the MCH solution of A-OPV3, a hypothesis which is supported by the observation of precipitation of (−)-phencyphos in the $5 \times 10^{-5}$ M solution upon cooling. Thus, in order to avoid any perturbation of the CD signals arising from precipitation of the acid, we decided to work at a concentration of $2 \times 10^{-5}$ M in acid for the subsequent experiments, which corresponds to 0.2 equivalents with respect to the OPV. Furthermore, the high CD intensity observed from the aggregates enabled us to reduce the path length of the cell from one centimetre to one millimetre, thereby reducing the noise in the spectra caused by saturation of the detection device as well as dropping by 10-fold the quantity of guest required (6 nM). In addition, a previous report on A-OPV3 showed the alignment of aggregates linked to convection flow in the one centimetre cell inducing a linear dichroism signal which modifies the spectra observed, while working with a one millimetre width reduces this phenomenon efficiently.[14]

Then, with the new parameters, we performed a temperature dependent study on the helical assemblies obtained in the presence of (+)-phencyphos to check if a thermodynamic control, preferable for a good reproducibility of values, was possible. A solution containing preformed A-OPV3 aggregates was heated at a rate of 60 K $h^{-1}$, previously used in studies on such assemblies.[13] We observed a quick decrease of the CD intensity of the A-OPV3 aggregates whose chirality was induced by (+)-phencyphos above 295 K, with a complete disappearance of the optical activity at approximately 320 K (Fig. 4). For this system, the recovery of the chiral aggregates upon cooling is a

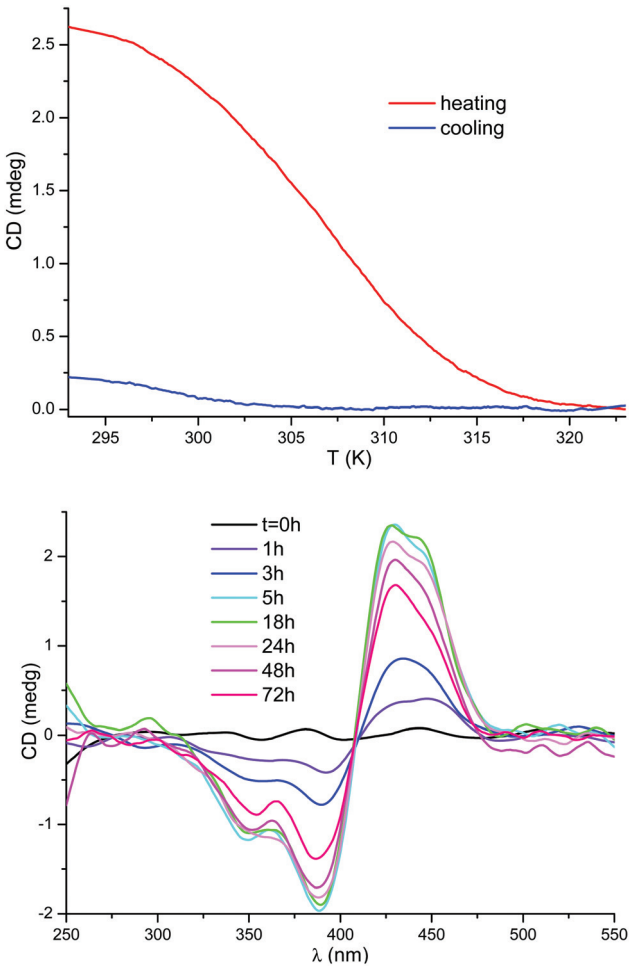


**Fig. 4** Top: temperature dependent CD measurements during heating and cooling (rate of 60 K $h^{-1}$). Bottom: the evolution of CD spectra for an A-OPV3 solution with (+)-phencyphos in methylcyclohexane stored at room temperature.

much slower mechanism than that previously observed with A-OPV4.[13] Even though a CD signal appeared below 305 K, the full intensity was not obtained on reaching 295 K. When the final temperature of the cooling was reduced to 10 °C or 0 °C, no acceleration of the induction process was observed. A perfect control clearly requires a slower rate which is not compatible with our quest for a high throughput routine technique.

Thus, we decided to observe the evolution of the signal in a solution prepared in the same conditions as our previous studies (short heating at 60 °C and storage at RT). Under these conditions, it appears that the maximum intensity is obtained after 5 h. Subsequently, the optical activity remains quite stable even though we observed a slow decrease over the following days which could be attributed to the warm ambient temperature in our laboratory (25–30 °C), sufficient to permit a slow dismantling of helical aggregates. This feature calls our attention to the importance of the storage conditions (controlled temperature) to achieve a good reproducibility of the obtained values.

Having determined suitable parameters for the optimum detection of a chiral acid, we explored induction experiments with non-enantiopure guests, working with mixtures of (+)- and

 

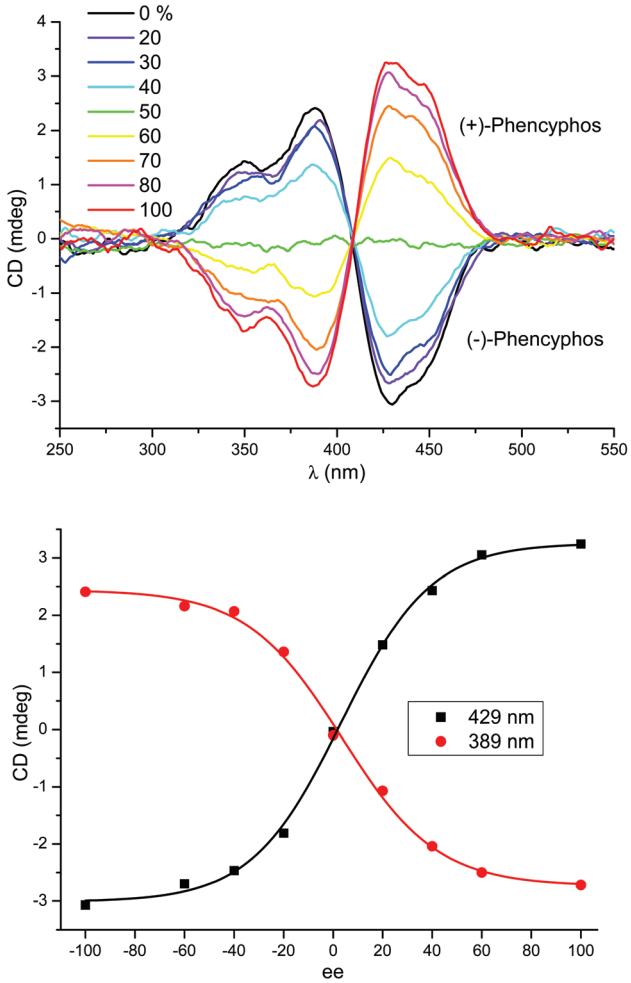


**Fig. 5** Top: an overlay of the CD spectra obtained for different mixtures of (+)- and (−)-phencyphos with constant total concentration (% of phencyphos in the mixture). Bottom: the corresponding intensity at the two maxima as a function of ee (A-OPV3 $10^{-4}$ M, (+/−)-phencyphos: 2 × $10^{-5}$ M in methylcyclohexane, cell: 1 mm path length). The error in the measurements is ±10%, which arises largely because of the poor solubility of the acid in the solvent required for the measurements.

(−)-phencyphos at constant concentration to determine the minimum ee detectable. As the majority enantiomer in the mixture was changed from (−) to (+)-phencyphos, a gradual inversion of the CD signals was obtained (Fig. 5), passing through a flat line at the composition corresponding to a racemic mixture. The major enantiomer of the guest molecules clearly controls the helical sense of the OPV stacks. The plot of CD intensity (Fig. 5) at the 390 and 430 nm maxima clearly shows a non-linear behaviour associated with the chiral amplification similar to that observed for the A-OPV4 assemblies. Even at this concentration we record CD spectra showing traces of helical aggregates even for an ee of 4%. However, our experience shows that a minimum ee of 10% is required (the approximate error) to achieve a reproducible induction. Thus, we consider this value as the reliable limit of detection of this method.

In order to confirm the possible general use of this method we decided to screen different phencyphos derivatives, chlocyphos

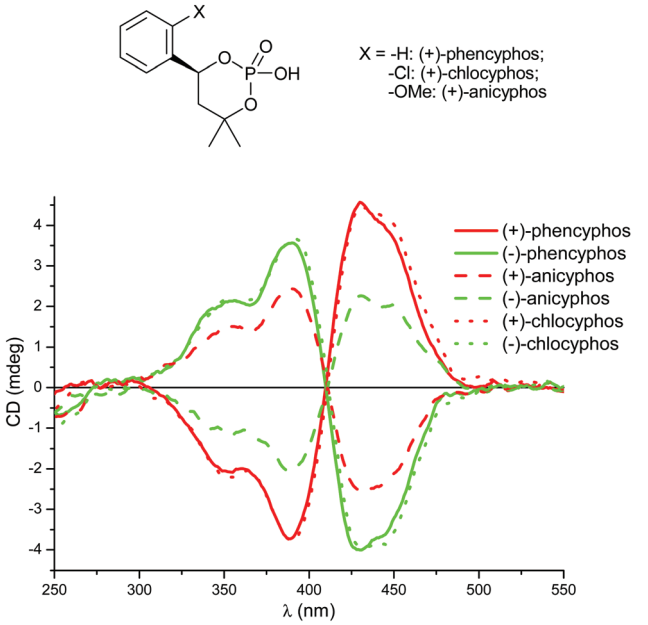


**Fig. 6** Overlay of the CD spectra obtained with different cyclic phosphoric acid derivatives (5 × $10^{-5}$ M, A-OPV3 $10^{-4}$ M in methylcyclohexane, cell: 1 mm path length).

and anicyphos, which differ from phencyphos by substitutions on the phenyl ring with a chlorine or a methoxy group, respectively (Fig. 6). Substitution pattern changes can influence chiral induction phenomena. While the behaviour looks exactly the same as that for phencyphos for the former, in the case of anicyphos the result is different in intensity and sign, although the general shape of the spectrum is maintained, with equal maxima to the related derivatives (Fig. 6). This observation was unexpected, considering that presumably the three compounds have exactly the same geometry in solution. Therefore, it seems unreliable to draw a direct correlation between the preferential helicity in the assemblies and the configuration of the chiral guest. In order to determine the major component in a mixture with this technique, it appears essential to perform calibration measurements with each enantiomer. It should be noted, however, that the OPV derivative can be recovered from these trials using silica and alumina gel chromatography, whereby the achiral compound can be isolated and used anew.

To expand the scope of the induction-amplified chirality sensing, we studied mandelic acid (Fig. 1), which shows a similar behaviour to phencyphos toward the A-OPV3 aggregates (Fig. 7). With the two enantiomers we obtained similar shapes for the CD curves arising from the A-OPV3, although there is a clear difference in the UV part of the spectra—the mandelic acid induces a band at 325 nm. It demonstrates once again the importance of performing a preliminary study with the chiral inducer before any detection experiments. This additional band may be a result of a polymorphic superstructure in the OPV stack. The CD signal at 430 nm is still the more intense one (as with the phencyphos derivatives), so this feature supports the idea of a possible use for detection of enantiomeric excess in a wide range of chiral carboxylic or phosphoric acids.

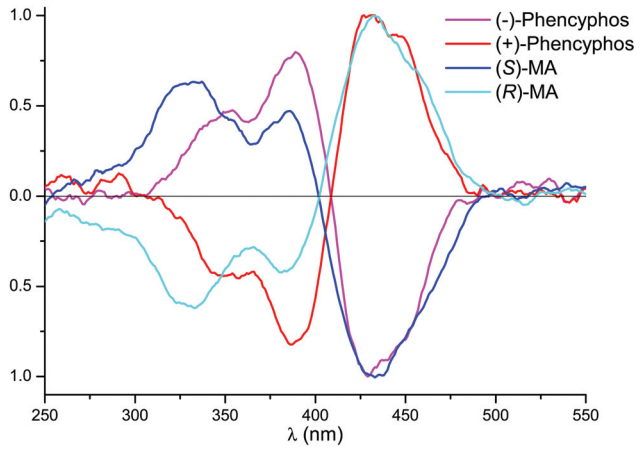


**Fig. 7** Normalized CD spectra of the A-OPV3 stacks obtained with both enantiomers of phencyphos and mandelic acid (respectively $5 \times 10^{-5}$ M and $10^{-4}$ M with A-OPV3 $10^{-4}$ M in methylcyclohexane, cell: 1 mm path length).

## Conclusion

The induction a preferential helicity in A-OPV3 aggregates seems a viable routine technique for the detection of an enantiomeric excess in chiral organic acid samples. This method is particularly suitable for carboxylic acids because of the high selectivity of this system through a two point hydrogen bond with the A-OPV3 dimer. An ee of 10% in mixtures of (+)- and (−)-phencyphos as been clearly evidenced in a series of samples using only 1.5 µg of the guest. Thus, we reached a range of concentrations close to the ones used for chiral-HPLC techniques even if the quantity of solvent required for such optical measurement (0.3 mL) is still much bigger than the few µL necessary for any chromatographic analysis. The use of these supramolecular assemblies appears tricky to implement as a routine technique but it should be considered that only a low concentration is required. Only 10 mg A-OPV3 allowed the preparation of more than 200 samples and then the oligomer can be recovered quantitatively by chromatography. Screening of the pure enantiomers is wise prior to the use of the method to detect scalemic mixtures. This supramolecular approach to evaluation of chiral content in samples could also be applied to other types of aggregates based on achiral molecules which show sensitivity to molecular chiral inducers. It complements very recent fluorescence-detected diastereoselective analysis of acids using chiral amine derivatives of tetraphenylethylene,[15] where the sensitivity is of the same order. In the work described here, detection works with tiny amounts of chiral inducer for the OPV stack helicity. These approaches can surely be extended to the determination of chirality in a wide range of chiral compounds.

The results also infer that the optical activity of the achiral OPV unit—a result of the formation of a preferred average angle between these conformationally quite rigid moieties—can be easily tuned through the proportion and chirality of the chiral acid. This observation is interesting regarding the preparation of chiral molecular materials where changing the twist between π-functional units can lead to varied optical and electronic properties.[16]

## Experimental part

Phencyphos derivatives were a kind gift from Syncom BV. OPV was synthesized according to a literature procedure.[13] All other chemicals were purchased from Sigma-Aldrich. Methylcyclohexane (spectrophotometric grade) was used without further purification. Circular dichroism spectra were recorded with in cells with 1 cm and 1 mm path lengths using a Jasco J-720 spectropolarimeter and were analyzed using the software OriginPro 8.

### Preparation of samples

A series of vials were loaded with an identical amount ($2 \times 10^{-8}$ mol) of a mixture of pure (+)- and (−)-phencyphos with differing ee obtained by a precise weighing (four figure balance) of each quantity introduced. With a microsyringe between 10 and 100 µL of solutions of each guest ($2 \times 10^{-5}$ M in dichloromethane) were transferred to a vial and left open on the bench for evaporation of the solvent. Then, 1 mL of the A-OPV3 solution ($10^{-4}$ M in MCH) was added. Once the vials were sealed, the sample was homogenised with a sonic bath for 20 min to achieve complete dissolution of the guest. All the vials were put in an oil bath at 60 °C for 15 min. After manual stirring and an additional 5 min of heating, the batch was taken out the bath and stored overnight in an air-conditioned room at 20 °C. CD spectra of the series were recorded in sequence over 20 min. This delay is assumed to be short enough for comparing the spectra without the influence of the storage time to consider that the series keep the same "age". Spectra of the first samples were recorded a second time to confirm the absence of any significant variation of intensity during this period.

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