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Differentiation of chiral phosphorus enantiomers by ^{31}P and ^{1}H NMR spectroscopy using amino acid derivatives as chemical solvating agents

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Abstract

The ability of commercially available amino acid derivatives, especially Fmoc-Trp(Boc)-OH, to differentiate enantiomers of chiral phosphonates, phosphinates, phosphates, phosphine oxides, and phosphonamidates is demonstrated with ^{31}P, ^{13}C, and ^{1}H NMR spectroscopy. The chiral differentiation provided a rapid and convenient method for measuring the enantiomeric purity of these phosphorus compounds.

1. Introduction

Chiral phosphorus oxide derivatives **1** are a class of compound that have been widely utilized in both chemistry and biology. Chiral phosphine oxides and phosphinate monoesters are important precursors to the corresponding phosphines that are used as chiral ligands for metal catalysts in modern asymmetric transformations.[1–4] The enantiomers of chiral phosphonate and phosphate esters are differentially toxic to multi-cellular organisms and these compounds are used as agricultural pesticides and chemical weapons.[5–7] These applications have stimulated efforts directed at the synthesis, resolution and determination of the enantiomeric purity of chiral phosphorus compounds.[8–12]

Recent advances in the characterization of the catalytic properties of the bacterial phosphotriesterase (PTE) and rationally designed site-directed mutant enzymes have provided a convenient method for the kinetic resolution of racemic phosphinate, phosphonate and phosphate esters through the stereoselective hydrolysis of a single enantiomer.[13–15] These enzyme libraries have facilitated the isolation of enantiomerically pure substrates of either stereochemistry, since mutant enzymes have been identified where the stereoselectivity is either *enhanced* or *inverted* relative to the wild type enzyme.[16,17] The absolute configurations of the unhydrolyzed products could be reliably predicted based upon the known stereoselectivity of the wild type enzyme and the characterized mutant variants.[17,18] However, experimental determination of the precise enantiomeric purity of the isolated phosphinate, phosphonate and phosphate ester products is not trivial. For example, the enantiomeric purity of the four stereoisomers of pinacolyl 4-nitrophenyl methylphosphonate and the two enantiomers of 4-acetylphenyl methyl phenylphosphonate were resolved with much effort by chiral HPLC methods and chiral electrophoresis, respectively.[13,19] A more rapid and

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convenient method is needed, as more of such enantiomers are routinely obtained by enzymatic methods.

An attractive alternative method for the determination of the chiral purity of these compounds is the use of ^{31}P and ^{1}H NMR spectroscopy with a chiral solvating agent.[20] The potential advantages of using chemical solvating agents for the differentiation of enantiomers, relative to other methods, such as the use of lanthanide complexes or the formation of covalent diastereomer derivatives with chiral reagents, are well recognized.[21] Chiral methyl phenyl phosphinothioic acid **2** and *t*-butyl phenyl phosphinothioic acid **3** have been successfully used to discriminate between the enantiomers of chiral phosphorus compounds including the amide and ester derivatives of phosphonic and phosphinic acids.[21–27] However, these chiral reagents are not commercially available.

Thioic acids **2** and **3** are apparently able to form transient diastereomeric complexes in solution with chiral phosphorus esters but the specific mode of interaction is not precisely known. In an effort to mimic the molecular interactions in these complexes with commercially available chiral acids, we chose *N*-acyl substituted amino acids **4**. Modified amino acids have been used as chemical solvating agents for the enantiodiscrimination of chiral α-arylalkylamines.[28] The rationale for the choice of **4** as a potential complexing agent for the differentiation of enantiomers such as **1** is that a broad range of modified amino acids are readily available and the free carboxylic acid may function in a manner similar to the thioic acid in compounds **2** and **3**. The formation of hydrogen bonded complex **5** might be the origin of the differentiation in the chemical shifts between enantiomers.[21–22] In this respect, chiral carboxylic acids, such as **4**, may form similar complexes in solution as presented in complex **6**. Compounds of the general structure **4** are readily available as inexpensive commercial derivatives of amino acids used in solid phase peptide synthesis.[29] Herein we report that ^{31}P and ^{1}H spectroscopy in the presence of protected L-amino acid derivatives can differentiate between the enantiomeric forms of chiral phosphorus oxide derivatives.

2. Results and discussion

To test the potential of *N*-acyl L-amino acid derivatives as chiral solvating agents for the differentiation of the chiral phosphorus compounds, we added *N*-Fmoc-L-phenylalanine to a solution of racemic 4-acetylphenyl cyclohexyl methylphosphonate **7** (see Table 1). Two signals of moderate separation without significant line broadening were observed in the ^{31}P NMR spectrum in $CDCl_3$ at room temperature (Figure 3C). *N*-Fmoc-*N′*-Boc-L-tryptophan (FBTrp), which has a larger side-chain group, was used next and a better resolution between the two enantiomers was obtained. To optimize the conditions for the differentiation of the two enantiomers with this compound, we titrated compound **7** with FBTrp in $CDCl_3$ at room temperature. The observed dependence of the separation in the chemical shift (Δδ in ppb) between the two enantiomers of **7** in the presence of an increasing concentration of FBTrp is presented in Figure 1A. At saturating concentrations of FBTrp the maximum difference in chemical shift is approximately 38 ppb and the data are consistent with the formation of a 1:1 complex between compound **7** and FBTrp.

To determine if the solvating agent preferentially binds one of the two enantiomers over the other, enantiomerically pure (R_P)-**7** and (S_P)-**7** were titrated separately with FBTrp in $CDCl_3$. The values of K_d for the complexes formed between (R_P)-**7** or (S_P)-**7** and FBTrp were calculated by a non-linear least squares analysis to be 21 ± 1 mM and 19 ± 1 mM, respectively, from a fit of the data to equation 1 for the formation of a 1:1 complex (Figure 1B). In equation 1, δ_{ob} is the observed chemical shift for (R_P)-**7** or (S_P)-**7**; δ_A is the chemical shift of (R_P)-**7** or (S_P)-**7** in the absence of FBTrp; δ_{AB} is the chemical shift for the complex of (R_P)-**7** or (S_P)-**7**

with FBTrp; "A" is the total concentration of (R_P)-**7** or (S_P)-**7**; "B" is the total concentration of FBTrp, and K_d is the corresponding dissociation constant.

$$\delta_{ob} = \delta_A + (\delta_{complex} - \delta_A) * \{(A + B + K_d) - [(A + B + K_d)^2 - 4AB)]^{1/2}\} / (2A) \quad \text{(Eq. 1)}$$

These results indicate that the binding strength between FBTrp and either of the two enantiomers of **7** is approximately the same. This conclusion is consistent with the observation that the ^{31}P NMR signals for the two diasteromers in the racemic mixture are of the same linewidth and signal intensity. Therefore, the differentiation of the two enantiomers by FBTrp is attributed to the intrinsic difference in the chemical shift of the two diasteromeric complexes formed between FBTrp and (R_P)-**7** or (S_P)-**7**. The upfield signal in the ^{31}P NMR spectra was assigned to (R_P)-**7** and the downfield resonance to (S_P)-**7** by the addition of the corresponding pure enantiomers separately into the racemic mixture in the presence of FBTrp. The NMR spectra of (R_P)-**7** or (S_P)-**7** alone in the presence of FBTrp demonstrated that the chiral purity of these compounds, prepared via a kinetic enzymatic resolution, exceeded an enantiomeric excess of 98%.

For racemic **7**, the differentiation between the two enantiomers can easily be measured by ^{31}P NMR spectroscopy, but no separation was detectable by ^{1}H NMR spectroscopy. In addition to FBTrp and *N*-Fmoc-L-phenylalanine, other *N*-protected L-amino acids were assessed for the differentiation of racemic **7**. Most of them are unable to discriminate between the two enantiomers and none gave a better separation than FBTrp when measured by ^{31}P NMR or ^{1}H NMR spectroscopy. A change in solvent from chloroform-D to benzene did not improve the resolution.

The ability of FBTrp to differentiate between the enantiomers of compounds which are chiral at a phosphorus center was further tested with the diasteromeric mixture of the methyl phosphonate diester **8** (Figure 2F). In the presence of FBTrp, each of the four stereoisomers of **8** exhibited nearly baseline resolved ^{31}P NMR resonances as shown in Figure 2A. For the assignment of these resonances to specific diastereomers, each of the four isomers was added separately into the complete mixture of stereoisomers in the presence of FBTrp (Figures 2B–E). The two most downfield signals were assigned to enantiomeric pair of (S_PS_C)-**8** and (R_PR_C)-**8** with the (S_PS_C)-**8** isomer as the most downfield resonance. The two upfield resonances were assigned to (S_PR_C)-**8** and (R_PS_C)-8 with (R_PS_C)-**8** as the most upfield resonance. Upon complexation with FBTrp, the chemical shifts of the two diasteromers moved about 0.65 ppm. The separation between the NMR signals of the enantiomeric pair, (S_PS_C)-**8** and (R_PR_C)-**8,** is 46 ppb, which is significantly larger than the 17 ppb separation between the enantiomeric pair of (S_PR_C)-**8** and (R_PS_C)-**8**. This example demonstrates that the separation of the NMR signals upon the addition of FBTrp is dependent upon the identity of the functional groups and stereochemical orientation distant from the phosphorus center.

The generality of using FBTrp to differentiate between chiral phosphorus compounds was tested in the resolution of phosphonates **7–11**, phosphinates **12–14**, phosphates **15–17**, phosphonamidates **18–19** and phosphine oxides **20–21** by both ^{31}P and ^{1}H NMR spectroscopy. The results are summarized in Table 1. Racemic phosphonate compounds with alkoxy groups smaller than cyclohexyl and pinacolyl, **9–11**, were utilized. For racemic compounds **9** and **11**, the ^{31}P resonances for the two enantiomers were distinguishable from one another with differences in chemical shift of 20 and 13 ppb, respectively. However, there was no observable difference in the chemical shifts for the individual enantiomers of **10** by ^{31}P NMR spectroscopy. Nevertheless, for the three phosphonate esters **9–11**, the ^{1}H NMR spectra revealed distinct resonances for the methyl group directly attached to the phosphorus core. In the absence of FBTrp, the ^{1}H NMR signals for these protons were observed as a doublet with a separation 16 Hz due to spin coupling with the adjacent phosphorus. In the presence of FBTrp, each of the

resonances for the methyl phosphonate group in **9**, **10**, and **11** was further separated into resonances for each of the enantiomers with separations of 3, 5, and 3 ppb, respectively.

Compounds **12–14** were assessed as examples of racemic phosphinate esters. For compounds **12** (Figure 3B) and **13**, the differences in the ^{31}P NMR spectra for these resonances are 54 and 51 ppb in the presence of FBTrp, about twice that observed for the phosphonate esters. However, no differentiation was observed in the ^{1}H NMR spectra of either **12** or **13**. For compound **14**, no distinction between the two enantiomers was observed in the ^{31}P NMR spectrum but a separation of 7 ppb was measured in the ^{1}H NMR spectrum of compound **14**. The protons from the methyl group of the ethoxy substituent, which is a triplet in the absence of FBTrp, are further separated from one another with a separation of 7 ppb.

Fully esterified phosphates were examined with compounds **15–17**. These compounds showed the weakest separation in the ^{31}P NMR spectra in the presence of FBTrp. For compounds **15** (Figure 3D) and **16**, the separation in chemical shift values for the individual enantiomers are 8 and 5 ppb, respectively. For compound **15**, the protons of the methoxy group are separated by 2 ppb in the ^{1}H NMR spectrum but for compound **16** no separation was observed in the presence of FBTrp. For compound **17**, there was no separation in either the ^{31}P or ^{1}H NMR spectra in the presence of FBTrp. To determine if other amino acid derivatives could differentiate between the two enantiomers of compound **17**, we tested 18 other protected amino acids. We found that Fmoc-serine (trityl)-OH (FTSer) gave a moderate separation of 3 ppb for the proton resonances of the methoxy group, while no separation of the ^{31}P NMR signal was observed.

Compounds **18** and **19** were examined as examples for phosphonamidates. In the presence of FBTrp in $CDCl_3$, both of these chiral compounds exhibited good separation of the ^{31}P NMR signals. The separation for compound **18** is 46 ppb for ^{31}P (Figure 3A) and 10 ppb for the hydrogens of the methyl group. In the ^{31}P NMR spectrum of the diastereomeric mixture of **19**, two pairs of signals centered at 36.70 and 36.55 ppm were separated by 80 and 17 ppb respectively. In the ^{1}H NMR spectrum, the differences in the chemical shifts for the protons of the methyl group are 6 and 4 ppb for each enantiomeric pair. The absolute configurations corresponding to these resonances have not been determined.

The final chiral phosphorus compounds tested were the phosphine oxides **20** and **21** (Chart 3). Neither compound showed separation in their ^{31}P NMR spectrum in the presence of FBTrp, although complexation of the two compounds with FBTrp was indicated by a 4 ppm downfield change in the chemical shift. In the ^{1}H NMR spectrum of compound **20**, a separation of 6 ppb was observed for the protons of the methoxy group, but no separation was observed for the protons of the methyl group which is attached directly to the phosphorus center. In the ^{1}H NMR spectrum of compound **21** (Figures 4A–D), the resonances for the proton attached to C2, centered at ~5.91 ppm and coupled to the phosphorus core with $J_{P\text{-}H}$ of 25 Hz (Figure 4A), were further split with a separation of 19 ppb in the presence of FBTrp (Figure 4B). The resonance for the methyl group of **21** is a singlet at 2.04 ppm (Figure 4C) in the absence of FBTrp but is a doublet with a separation of 9 ppb in the presence of FBTrp (Figure 4D). The two protons attached to C5 gave a complex set of resonances in the range of 2.26-2.13 ppm (Figure 4C) that are split into two sets of resonances at 2.40-2.29 ppm and 2.25-2.16 ppm in the presence of FBTrp (Figure 4D). No separation for the resonances of the protons attached to C4 was observed.

For compound **21**, the ^{13}C NMR spectra were also recorded in the presence and absence of FBTrp (Figures 5A–D). The ^{13}C NMR resonance for C3 of compound **21**, centered at 164.96 ppm with $J_{P\text{-}C}$ = 25.3 Hz as a doublet (Figure 5A), divided into two pairs of doublets with a separation of 42 ppb in the presence of FBTrp (Figure 5B). The ^{13}C resonances for the methyl

group, centered at 21.22 ppm with $J_{P\text{-}C}$ = 17.2 Hz as a doublet (Figure 5C), split into two pairs of doublets with a separation of 25 ppb in the presence of FBTrp (Figure 5D). However, no separation was observed for the ^{13}C resonances of C2, C4 or C5.

A comparison of atypical separation in the ^{31}P NMR spectra induced by FBTrp for the five types of chiral phosphorus compounds tested in this investigation is presented in Table 1. The magnitude of the chemical shift differences between the pairs of enantiomers are: phosphinates ~ phosphonamidates > phosphonates > phosphates>phosphine oxides. The downfield chemical shift changes in the ^{31}P NMR spectra suggest the complexation of amino acid derivatives with the phosphorus compounds. The discrimination between enantiomers by the modified amino acids is attributed to the chemical shift differences of the diasteromeric complexes because the dissociation constants for the binding of the (R_P)- and (S_P)-enantiomers of compound **7** to FBTrp are essentially identical. However, we do not understand why some enantiomers can be differentiated by ^{31}P NMR and others by ^{1}H or ^{13}C NMR spectroscopy. A direct correlation of the absolute configuration at phosphorus relative to the chemical shift of the diastereomeric complex formed between each enantiomer and FBTrp has not been determined. The direct interaction between these phosphorus compounds and FBTrp is expected to be facilitated by hydrogen bonding. In this respect, FBTrp might function as a versatile chemical solvating agent in the ^{1}H NMR spectroscopy for the enantio-differentiation of chiral compounds, such as alcohols and amines.

3. Conclusion

In conclusion, we have found that FBTrp is a versatile chemical solvating agent that can be used for the differentiation of chiral phosphorus centers in compounds that include phosphine oxides, phosphinates, phosphonates, phosphates and phosphonamidates by ^{31}P, ^{13}C and ^{1}H NMR spectroscopy. The separations of the resonances that are induced by FBTrp in the ^{31}P NMR spectra are substantial except for the chiral phosphates and phosphine oxides, for which the separation is relatively small. This observation has allowed the determination of the enantiomeric purity for chirally enriched phosphorus compounds. Since FBTrp is commercially available and relatively inexpensive, it might be used as a convenient new chemical solvating agent for other chiral compounds that may include chiral sulfoxides.[30] FBTrp or other amino acid derivatives may also be applied in development of new methods for the rapid and convenient determination of enantiomeric excess.[31]

4. Experimental

4.1. General

All of the ^{31}P and ^{1}H NMR experiments were carried out with a Varian Inova-400 Broad Band Spectrometer unless mentioned otherwise. For the ^{31}P NMR spectra, the acquisition time was set to 5–8 seconds with 2 second delay. Aqueous phosphoric acid (85%) was used as an external reference. For titration of the single enantiomers of (R_P)-**7** or (S_P)-**7**, aqueous phosphoric acid (85%) in a sealed capillary was inserted into the NMR tube as an internal reference. For collection of ^{1}H and ^{13}C NMR spectra, standard parameters were used. The amino acid derivatives Fmoc-Trp(Boc)-OH, Fmoc-Ser(Trityl)-OH were used as purchased from EMD Biosciences. (4-Methoxyphenyl)methylphenylphosphine oxide **20** was purchased from ASDI Inc. 3-Methyl-1-phenyl-2-phospholene 1-oxide **21** was purchased from TCI America. The racemic phosphinates, phosphonates and phosphates ester were prepared following known procedures.[13] Phosphonamidates **18** and **19** were made and characterized as described below. Enantiomeric phosphonates were obtained by enzymatic resolution of their racemic mixtures.[19]

4.2. Cyclohexyl methylphosphonamidate 18

To a solution of cyclohexanol (10 mmol) in ethyl ether (20 mL) in a dry ice/acetone bath was added butyl lithium (10 mmol, 2.5 M in hexanes). To the suspension was added a solution of methylphosphonic dichloride (10 mmol) in ethyl ether (40 mL). The mixture was stirred for 20 minutes in a dry ice/acetone bath and then at room temperature for 1 hour. After removal of the solvent, concentrated aqueous ammonia (20 equivalents) was added to the residue and the mixture was stirred at room temperature for 30 minutes. After the ammonia and water were removed under reduced pressure, the residue was resuspended in ethyl ether, filtered and washed with ethyl ether. A solution of ethyl ether was collected and condensed to dryness. Recrystalization from ethyl ether yielded the desired compound **18** as colorless crystals in 80% yield. ^{1}H NMR ($CDCl_3$, δ in ppm): 4.50-4.35 (2H, m); 2.83 (2H, broad); 2.05–2.14 (14H, m). ^{31}P ($CDCl_3$, δ in ppm, 85% aqueous H_3PO_4 as external reference): 32.73.

4.3. Pinacolyl methylphosphonamidate 19

The diasteromeric mixture was prepared in an 85% yield following the procedure described for **18**. ^{1}H NMR ($CDCl_3$, δ in ppm): 4.25-4.17 (1H, CH, m); 2.96, 2.91 (2H, NH_2, s, broad), 1.49, 1.51 (3H, CH_3-P, two doublets, $J_{P\text{-}H}$ = 16.7 Hz); 1.27, 1.24 (3H CH_3-C, two doublets, $J_{H\text{-}H}$ = 6.70 Hz); 0.87, 0.88 (9H, t-butyl, two singlets). ^{31}P ($CDCl_3$, δ in ppm, 85% aqueous H_3PO_4 as external reference): 33.11; 32.77.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

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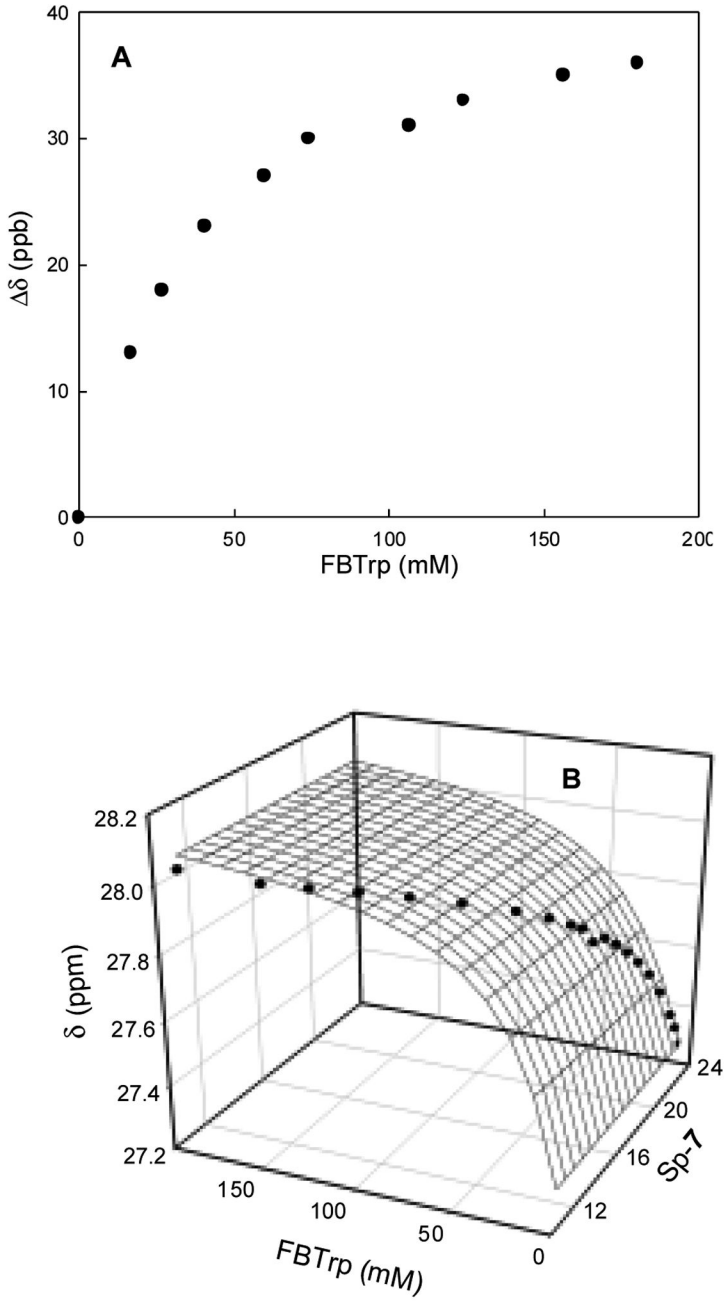


Figure 1.

(**A**) Separation of the ^{31}P NMR signals (Δδ in ppb) for racemic compound **7** (47.5 mM in 0.6 mL $CDCl_3$) after the addition of FBTrp in $CDCl_3$ at 25 °C. (**B**). Binding of variable amounts of (S_P)-**7** with variable amounts of FBTrp in $CDCl_3$ at 25 °C. The data were fit to equation 1 with a K_d of 19 mM.

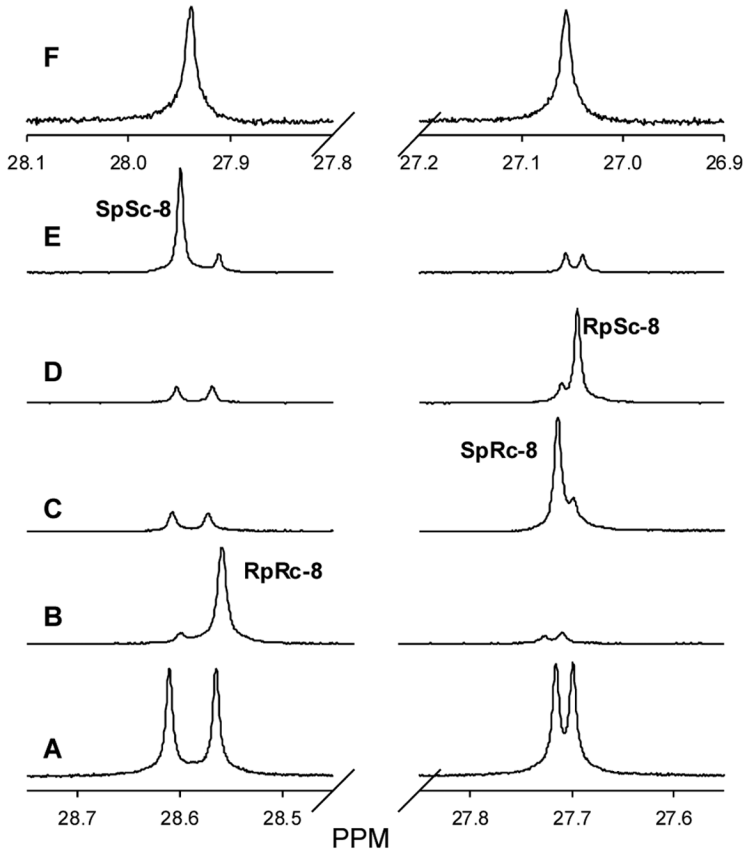


Figure 2.

Assignment of the ^{31}P NMR signals for the individual enantiomers within a racemic mixture of compound **8**. (**A)** Spectrum of diastereomeric **8** in the presence of FBTrp. (**B)** Addition of authentic (R_PR_C)-**8** enantiomer into diastereomeric **8**. (**C)** Addition of (S_PR_C)-**8** enantiomer into diastereomeric **8**. (**D)** Addition of (R_PS_C)-**8** enantiomer into diastereomeric **8**. (**E)** Addition of (S_PS_C)-**8** enantiomer into diastereomeric **8**. (**F)** Spectrum of diastereomeric **8** in the absence of FBTrp.

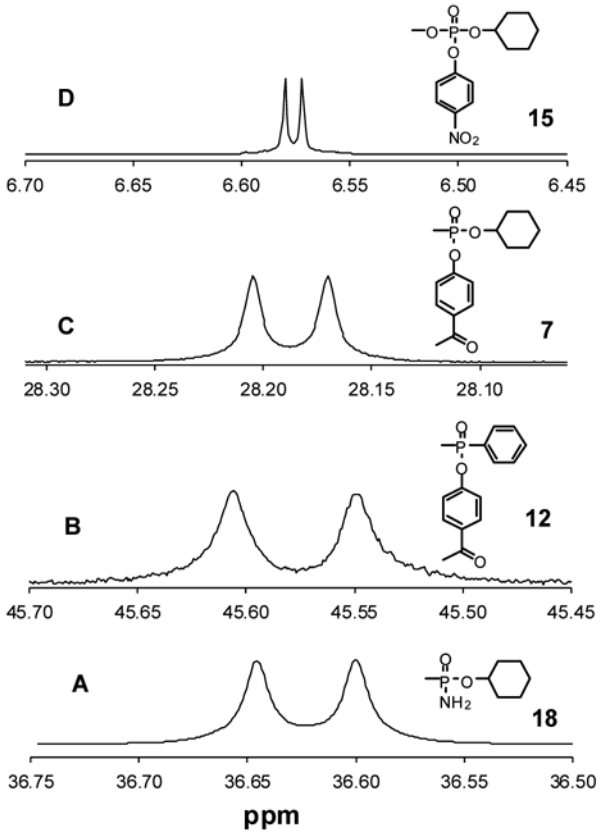


Figure 3.

^{31}P NMR spectra for racemic mixtures of four types of chiral phosphorus compounds utilized in this investigation in the presence of FBTrp. (**A**) Phosphonamidate **18**. (**B**) phosphinate ester **12**. (**C**) methyl phosphonate ester **7**. (**D**) phosphate triester **15**.

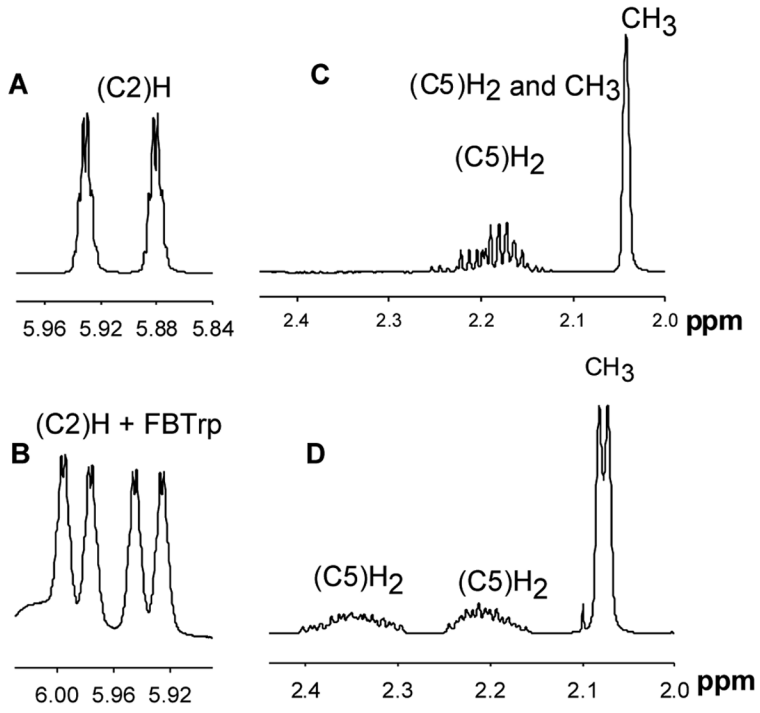


Figure 4.

^{1}H NMR spectra for the protons at C2, C5 and methyl group of compound **21**. (**A**) ^{1}H NMR signal for the proton at C2 in the absence of FBTrp. (**B**) ^{1}H NMR resonance for the proton at C2 in the presence of FBTrp. (**C**) ^{1}H NMR signal for the protons at C5 and the methyl group of **21** in the absence of FBTrp. (**D**) Same as in Figure C but in the presence of FBTrp.

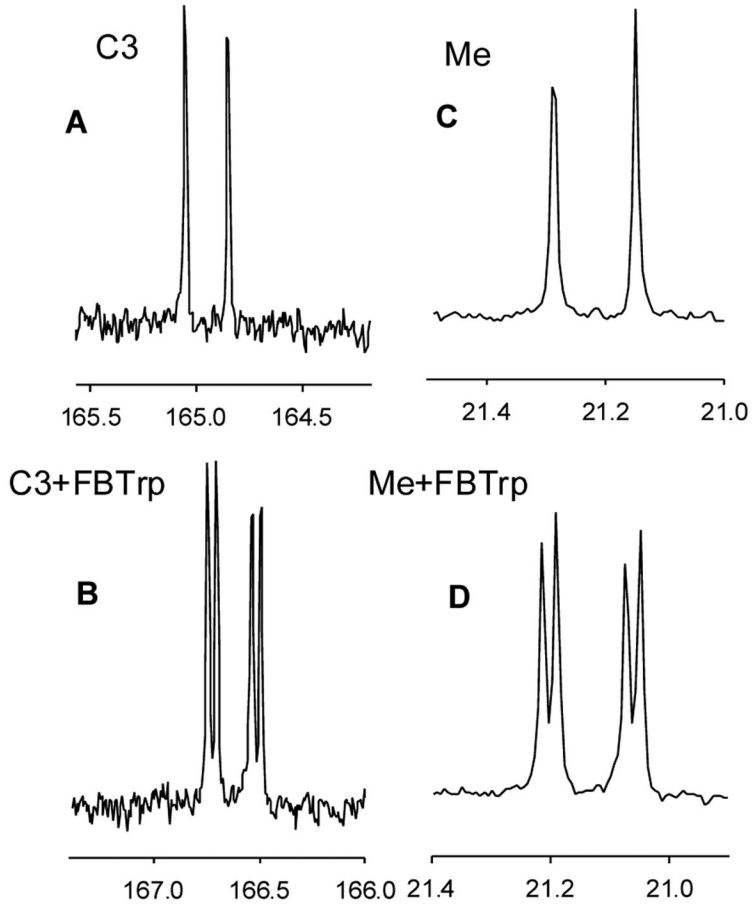
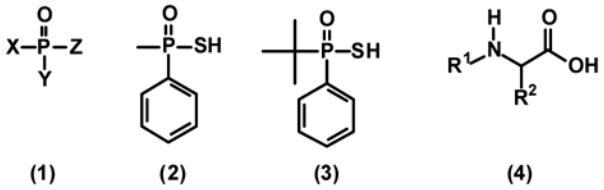
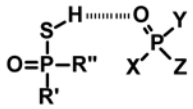


Figure 5.
^{13}C NMR spectra for C3 and the methyl group of compound **21**. (**A**) ^{13}C NMR signal for C2 in the absence of FBTrp. (**B**) Same as in Figure A but in the presence of FBTrp. (**C**) ^{13}C NMR signal of the methyl group in the absence of FBTrp. (**D**) Same as in Figure C but in the presence of FBTrp.

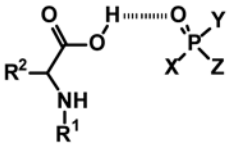


(1) (2) (3) (4)

Chart 1.

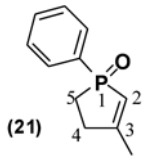


(5)



(6)

Chart 2.

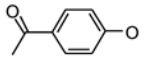
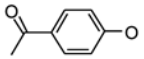
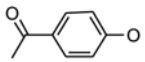
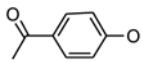
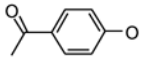
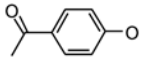
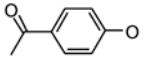
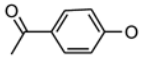
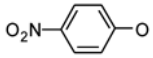
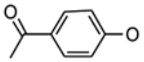
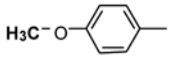


(21)

Chart 3.

Table 1

Differences in the chemical shifts between enantiomers induced upon the addition of 50 mM FBTrp in $CDCl_3$ at 25°*C*.

Entry	X	Y	Z	Δδ (ppb) ^{31}P (NMR)	Δδ (ppb)[a] ^{1}H (NMR)
7	CH_3-			28	0
8 (S_pS_c, R_pR_c)	CH_3-			46	0
8 (R_pS_c, S_pR_c)	CH_3-			17	0
9	**CH_3-**			20	3
10	**CH_3-**			0	5
11	**CH_3-**			13	3
12	CH_3-			54	0
13	CH_3CH_2-			51	0
14	CH_3CH_2-		**CH_3CH_2O-**	0	7
15	**CH_3O-**			8	2
16	CH_3O-			5	0
17	**CH_3O-**			0	3[b]
18	**CH_3-**		H_2N—	46	10
19	**CH_3-**		H_2N—	80, 17	6, 4
20	CH_3-			0	6

[a] When the separation of the proton resonance are indicated, the specific hydrogens are highlighted in bold font.

[b] In the presence of Fmoc-serine(trityl)-OH.