result in part from the fact that the emission and absorption dipoles are not parallel ($\delta \neq 0$). It is possible, however, that the variation in initial values observed reflects differences in mobility among these fluorophores on the picosecond time scale. It is of interest to note that while Munro et al. concluded the tryptophan of Staphylococcus aureus nuclease B is rigid, it had the smallest initial anisotropy (0.18) of the five proteins studied. A similar anomalously low value of $r_e(0) = 0.18$ has been reported for the tryptophans in carbonic anhydrase. Lakowicz and Weber[3] suggest subnanosecond tryptophan motions may be responsible for this small initial fluorescence depolarization. Measurements of fluorescence depolarization of tyrosines in proteins would be of interest both for comparison with experimental results already obtained for the bulkier tryptophan residues and for comparison with the values calculated for the initial fluorescence depolarization of tyrosines obtained from a molecular dynamics simulation. Such measurements on tyrosines in PTI are in progress.[14]

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Registry No. PTI, 50936-63-5.

(14) A. Kasprzak and G. Weber, to be submitted for publication.

The Use of "Enantiopolar" Directions in Centrosymmetric Crystals for Direct Assignment of Absolute Configuration of Chiral Molecules: Application to the System Serine/Threonine

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X-ray analysis does not distinguish between enantiomeric crystal structures $\{R\}_d$ and $\{S\}_l$ [where the enantiomerically resolved molecules R and S pack in the corresponding $\{\}_d$ and $\{\}_l$ chiral crystal] unless the Bijvoet method of anomalous dispersion is applied. On the other hand, X-ray analysis of a centrosymmetric crystal composed of a racemate $\{RS\}$ establishes unambiguously the sites occupied by the R and S moieties with respect to the crystal axes (Figure 1). Consequently, in contradistinction to chiral crystals, the orientation toward all crystal faces of the substituents attached to the R and S chiral molecules is unambiguously assigned.[1] However, because of the coexistence of the two enantiomers in the crystals, this knowledge cannot be directly exploited for the assignment of absolute configuration of chiral resolved molecules. This limitation may be circumvented if the structural information contained in the racemic crystal is transferred to a third chiral molecule, interacting stereospecifically with the racemic system through a well-defined mechanism.

Our studies on inhibition of growth of conglomerates[2] and polar crystals[1] by adsorption of resolved "tailor-made" impurities have led to an understanding of the correlation between the crystal structure of the substrate, molecular structure of the additive, and the affected growth directions, as revealed by specific morphological changes. This correlation has been applied for a direct assignment of the absolute configuration of chiral polar crystals.[1] The same approach is adopted here for the direct assignment of absolute configuration of resolved impurities through morphological changes induced stereoselectively on the enantiotopic faces of centrosymmetric crystals with appropriate packing features. A requirement for application of this method is that within the racemic crystal specific functional groups attached to an R molecule (Scheme I) point toward faces hkl (f1), but not toward $\bar{h}\bar{k}\bar{l}$ (f$\bar{1}$), while the same functional groups attached to an S molecule will emerge at the enantiotopic faces $\bar{h}\bar{k}\bar{l}$, but not toward hkl.

Let us consider the crystallization of a racemate of this type, in the presence of a chiral additive R', appropriately designed so that it will fit in the site of an R molecule on the growing crystal faces f1 or f2 (Scheme I) but not at the enantiotopic faces f$\bar{1}$ or f$\bar{2}$. On the basis of the above mechanism of inhibition previously investigated,[1,2] this adsorbed molecule will hinder growth along the $+b$ direction but not along $-b$. It is therefore expected that either the areas of the f1 and f2 faces will increase relative to their enantiotopic faces or new faces will appear on the $+b$ side of the crystal. By virtue of symmetry, additive S' will inhibit growth of faces f$\bar{1}$ and f$\bar{2}$, but not *f*1 and *f*2, while racemic $R'S'$ will inhibit

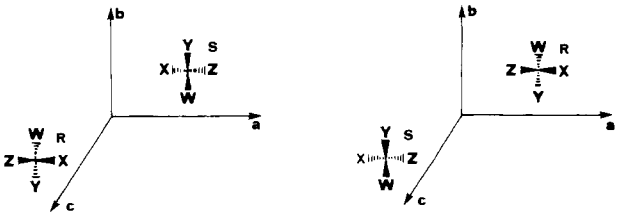


Figure 1. On the left, chiral molecules R and S are related by a center of inversion at the origin; interchanging them leads to the diastereomeric arrangement shown on the right. Therefore the orientation of groups w, x, y, z of one chirality in a given crystal structure is unambiguously fixed with respect to the *a*, *b*, *c* axes.

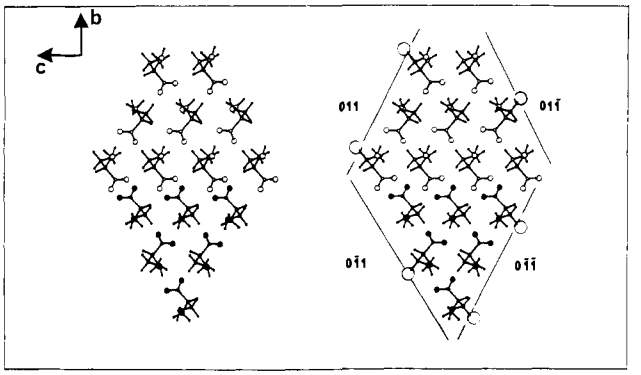
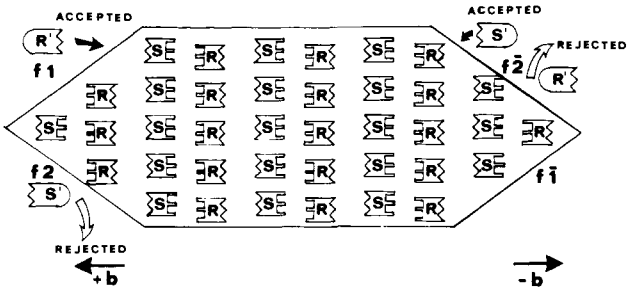


Figure 2. Packing of $\{RS\}$-serine viewed along the *a* axis. Molecules are packed in *b c* layers which are homochiral. For clarity only half of each R (open circles) and S (full circles) layer is shown. The four {011} crystal faces are shown. Threonine impurity molecules (with the $-CH_3$ group indicated by large circles), are inserted stereospecifically.

Scheme I



(1) For part 2, see Z. Berkovitch-Yellin, L. Addadi, M. Idelson, L. Leiserowitz, and M. Lahav, *Nature (London)*, in press.
(2) L. Addadi, Z. Berkovitch-Yellin, N. Domb, E. Gati, M. Lahav, and L. Leiserowitz, *Nature (London)*, in press.

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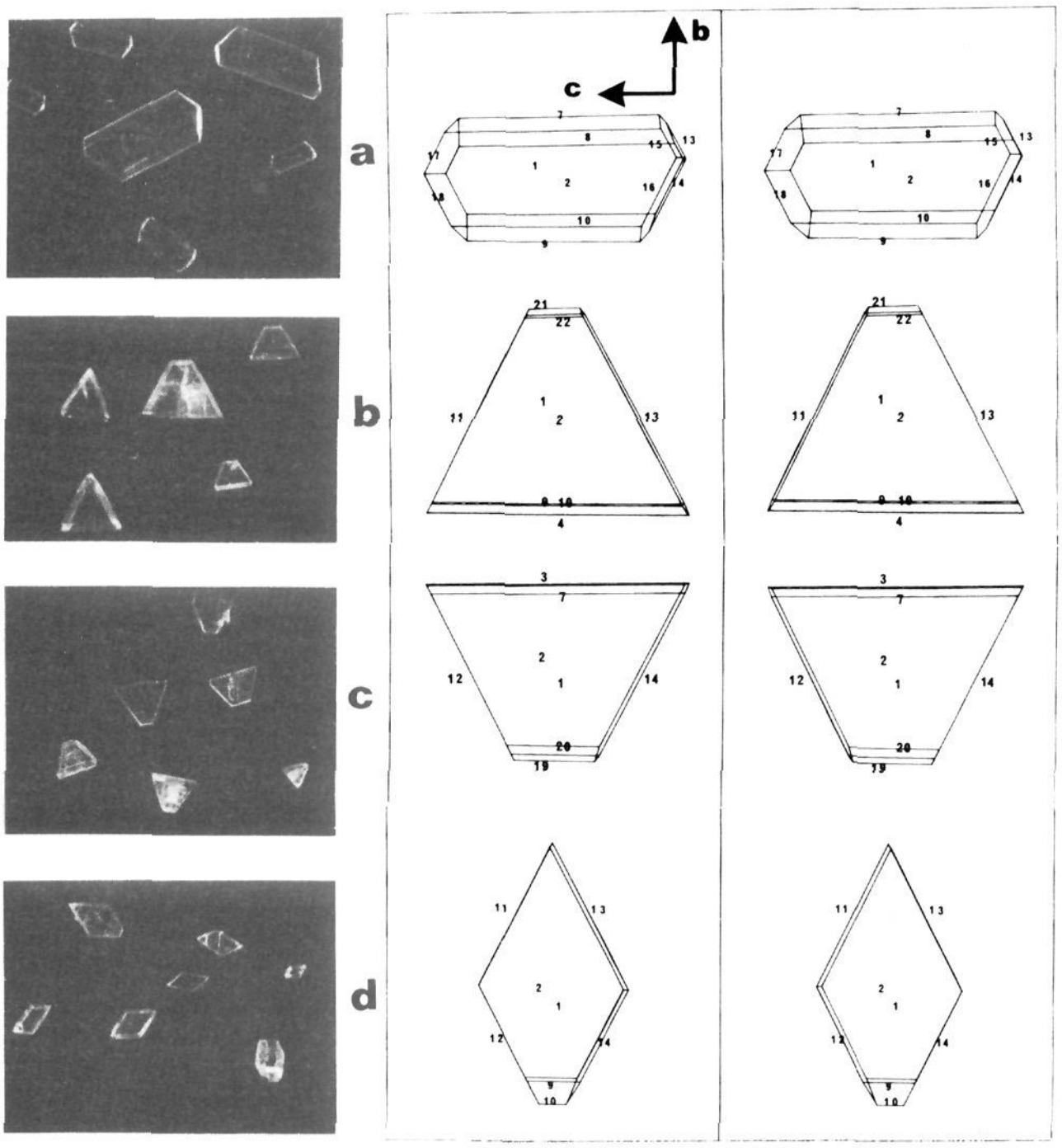

Figure 3. Photographs and stereoviews of pure and affected crystals of {*RS*}-serine. (a) Pure {*RS*}-serine; (b) {*RS*}-Ser + (*R*)-Thr; (c) {*RS*}-Ser + (*S*)-Thr; (d) {*RS*}-Ser + (*RS*)-Thr. Miller indexes of numbered faces are *1* (100), *2* ($\bar{1}$00), *3* (010), *4* (0$\bar{1}$0), 7 (110), *8* ($\bar{1}$10), *9* (1$\bar{1}$0), *10* ($\bar{1}\bar{1}$0), *11* (011), *12* (0$\bar{1}$1), *13* (01$\bar{1}$), *14* (0$\bar{1}\bar{1}$), *15* (11$\bar{1}$), *16* (1$\bar{1}\bar{1}$), *17* ($\bar{1}$11), *18* ($\bar{1}\bar{1}$1), *19* (1$\bar{2}$0), *20* ($\bar{1}\bar{2}$0), *21* (120), and *22* ($\bar{1}$20).

growth along both directions $+b$ and $-b$. From these morphological changes on the crystal {*RS*} coupled with the knowledge of the orientation of the molecules inside the crystal relative to the faces (acquired from the X-ray structure analysis), it is possible to assign the absolute configuration of the impurity.

In principle this method is applicable to any centrosymmetric crystal. However, in practice, triclinic and monoclinic crystals are the most easily amenable to experimental analysis. The triclinic space group $P\bar{1}$ is appropriate, provided all molecules of the same handedness are parallel to each other. All centrosymmetric monoclinic space groups (e.g. $P2/a$, $P2_1/a$, $A2/a$) are also appropriate, provided the specific functional groups to be modified are aligned approximately parallel to the unique b axis.[3,4]

We illustrate the application of this approach to the system serine (Ser)/threonine (Thr). Crystalline {*RS*}-serine has appropriate symmetry and molecular packing (Figure 2).[5] Both C–H bond vectors of the rigid methylene group of serine have major components along the unique b axis. Thus replacement of one of them by a methyl (as in threonine) will inhibit growth along the b direction. In an (*R*)-Thr[6] molecule [with the side-chain carbon of chirality *S*], the methyl group will replace the pro-*S* hydrogen atom of (*R*)-Ser so as to inhibit growth along $+b$. By symmetry the CH_3 group of (*S*)-Thr will replace the pro-*R* hydrogen of (*S*)-Ser and hance inhibit growth along $-b$ (see Figure 2).

A requirement for correct interpretation of the change in crystal habit is that an adsorbed threonine molecule takes the place of a serine molecule with almost the same orientation and conformation. This condition is satisfied because each serine molecule participates in eight hydrogen[7] bonds in the crystal.

{*RS*}-Ser forms tabular crystals with well-developed faces from 1:5 1-propanol/water solution. Crystals were grown by slow evaporation, in the presence of seeds of the pure material, from pure (*RS*)-Ser, (*RS*)-Ser + (*R*)-Thr, (*RS*)-Ser + (*S*)-Thr (10% wt/wt), and (*RS*)-Ser + {*RS*}-Thr (20% wt/wt) (Figure 3). The crystal faces of pure and affected {*RS*}-Ser were characterized and their crystal dimensions measured as described previously.[1,2] Computer-drawn pictures of these crystals are also shown in Figure 3. The morphological symmetry of pure {*RS*}-Ser is $2/m$; the crystals affected by either (*R*)-Thr or (*S*)-Thr exhibit reduced morphological symmetry 2 (the mirror plane is lost) and are enantiomorphous. (*RS*)-Thr leaves the morphological symmetry unchanged at $2/m$. (*R*)-Thr is easily occluded on the growing (01$\bar{1}$) and (011) faces. Upon adsorption the C–CH_3 group

(3) For a noncentrosymmetric space group, e.g., *Pa* or *Aa*, the direction of the *R* and *S* moieties is unambiguously assigned with respect to the unique *b* axis (as for monoclinic centrosymmetric space groups) but not with respect to the *a* and *c* axes.

(4) For a study on morphological changes induced on racemic crystals of $PbCl_2$ by chiral amylose, see F. D. Miles, *Proc. R. Soc. London, Ser A*, **132**, 266 (1931).

(5) M. N. Frey, M. S. Lehman, T. F. Koetzle, and W. C. Hamilton, Acta *Crystallogr., Sect. B*, **B29**, 876 (1973).

(6) (*R*)- and (*S*)-threonine and serine are D- and L-threonine and serine, respectively.

(7) Strong experimental support for this suggestion is obtained from the crystal structure of the solid solution of (*R*)-threonine and (*R*)-*allo*-threonine. P. Swaminathan and R. Srinivasan, *J. Cryst. Mol. Struct.* **5**, 101 (1975).

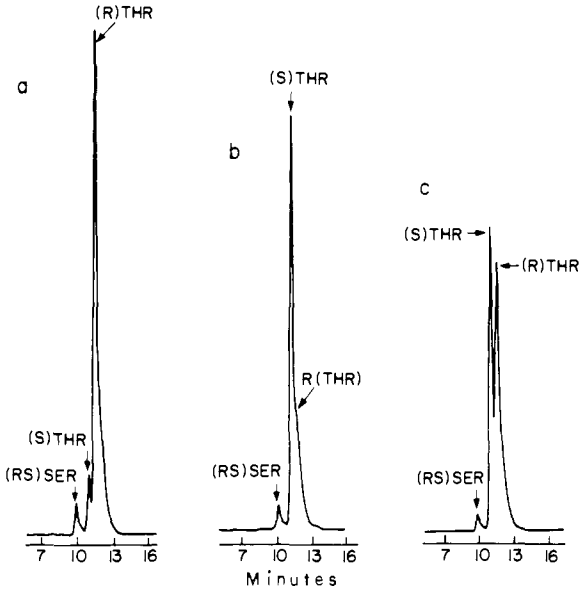

Figure 4. Enantiomeric analyses of threonine in the rhomb-like crystals of (*R*,*S*)-serine: (a) tip of the crystal from the +*b* direction; (b) tip of the same crystal from the −*b* direction; (c) whole crystal. The small amounts of unresolved (*R*,*S*)-serine are residual from the cation exchange separation of threonine from the serine crystal.

emerges away from these faces (as seen in Figure 2), thus hindering the natural growth perpendicular to them with subsequent increase of their areas (Figure 3b). The same impurity cannot be occluded at the symmetry-related $(0\bar{1}\bar{1})$ and $(0\bar{1}1)$ faces; the growth rate perpendicular to these faces is therefore increased with respect to that of the affected faces, thus causing their disappearance. The effect of (*S*)-Thr, which induces the enantiomorphous morphology (Figure 3c) can be explained in an equivalent manner. (*RS*)-Thr induces a morphological change (Figure 3d), which is a simple combination of those induced by each impurity separately, turning the crystals into rhombs.

The morphological changes, and our interpretation thereof, imply that in this last experiment (*RS*)-Thr must segregate along the *b* axis during crystal growth; occluded (*R*)-Thr will prevail at the +*b* half of the crystal (top half Figure 3d) whereas (*S*)-Thr will prevail at the −*b* half of the crystal. Several rhombic crystals (1 × 1 × 0.4 mm) whose +*b* and −*b* directions were characterized by X-rays were either cut in half perpendicular to the *b* axis or small pieces were chipped from the +*b* and −*b* tips of the rhombs. These crystal fragments were examined for an enantiomeric ratio of (*R*)- and (*S*)-Thr by HPLC, on a chiral phase,[8] and the results for a typical sample are illustrated in Figure 4. This analysis shows that (*R*)-Thr is occluded in the +*b* half and (*S*)-Thr in the −*b* half, with an ee of 84% for both tips of a crystal and 60% for crystals cut in two.

Extension of these preliminary studies to other systems, such as serine/*allo*-threonine, proline/hydroxyproline, and *meso*-dimethylsuccinic acid with its chiral resolved monoesters, are under current investigation.

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Registry No. (*RS*)-Ser, 302-84-1; (*R*)-Thr, 632-20-2; (*S*)-Thr, 72-19-5; (*RS*)-Thr, 80-68-2.

(8) The HPLC analysis of (*RS*)-threonine was carried out by a method similar to that described elsewhere.[9] Crystal fragments of serine, containing less than 0.5% threonine, were dissolved in 20 μL of buffer (0.1 N pyridine with glacial acetic acid added to pH 3.06). Threonine, almost free of serine, was collected from a cation exchange column (5 × 0.46 cm) self-packed with 5 μm resin of the polystyrene–divinylbenzene–sulfonic acid type eluted with the above buffer. The fractions containing the threonine were evaporated to dryness under nitrogen and redissolved in 50 μL of the chiral mobile phase, *N*,*N*-dimethyl-(*S*)-valine (8 mM) and cupric acetate (4 mM) in water. Samples of 20 μL were analyzed on a reversed phase column (24 × 0.46 cm) self-packed with 5 μm Nucleosil C_{18} (Machery, Nagel and Co., Duren, G. F.R.) eluted with the above chiral mobile phase, using fluorescence detection.[9]

(9) S. Weinstein, M. H. Engel, and P. E. Hare, *Anal. Biochem.*, in press.

Thermal Tetramerization of 1-Phenyl-3,4-dimethylphosphole. An Access to 2,2′-Biphospholes and to 2,2′-Diphosphafulvalene Complexes

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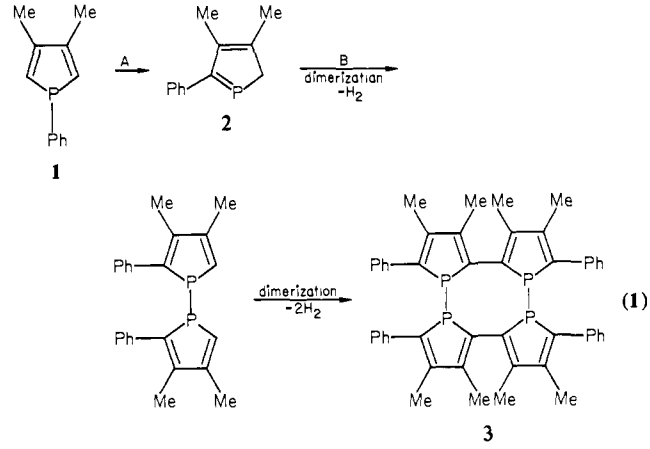
In a preceding communication[1] we demonstrated that 1-phenyl-3,4-dimethylphosphole, **1**, isomerized at high temperature



(1)

to give 2-phenyl-3,4-dimethyl-5*H*-phosphole, **2**, as an unstable two-coordinate species which was trapped by tolane, methanol, and 2,3-dimethylbutadiene. In view of this unexpected and interesting result,[2] we decided to study the thermolysis of **1** alone. When heated at 170 °C for 60 h, **1** yielded a complex mixture of products. When the mixture stood at room temperature, a pure bright red solid precipitated amidst a colorless liquid. This solid, **3**, contained four phosphole units.[3] Its structure was unambiguously deduced from the X-ray crystal structure of its decacarbonyldimolybdenum complex. The mechanism of the formation of **3** is obviously complicated. We propose eq 1).

The occurrence of step A was demonstrated in our previous paper.[1] The production of a transient 1,1′-biphosphole through

(1) Mathey, F.; Mercier, F.; Charrier, C.; Fischer, J.; Mitschler, A. *J. Am. Chem. Soc.* **1981**, *103*, 4595.

(2) In this case, the behavior of phospholes parallels the behavior of siloles; see: Barton, T. J.; Wulff, W. D.; Arnold, E. V.; Clardy, J. *J. Am. Chem. Soc.* **1979**, *101*, 2733. A similarity between siloles and phospholes has already been noted during UV photodimerization experiments: Barton, T. J.; Nelson, A. J. *Tetrahedron Lett.* **1969**, 5037.

(3) The thermolysis is performed in a sealed glass tube on 1.9 g of **1**. After 60 h at 170 °C, the cooled mixture is recovered with CH_2Cl_2. The insoluble red crystals of **3** are removed by filtration: mp > 260 °C; yield 0.38 g (20%); ^{1}H NMR ($CDCl_3$) δ 2.03 (br s, 12 H, Me), 2.19 (m, 12 H, Me), 7.40 (br s, 20 H, Ph); ^{31}P NMR (H_3PO_4, $CDCl_3$, δ positive for downfield shifts): δ −11.6; mass spectrum (70 eV, 240 °C) *m*/*e* 744 (M, 100%), 558 (M−$C_{12}H_{11}P$, 41%), 372 (M/2, 50%).

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