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Absolute structure determination as a reference for the enantiomeric resolution of racemic mixtures of cyclophosphazenes *via* chiral high-performance liquid chromatography

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Reversed-phase chiral high-performance liquid chromatography (HPLC) is a potentially powerful technique for the enantiomeric resolution of racemic mixtures, although the elution order of enantiomers is only relative and it is necessary to fully characterize reference systems for this method to provide absolute configurational information. The enantiomeric resolution of a series of racemic di-spiro cyclotriphosphazene derivatives, $N_3P_3X_2[O(CH_2)_3NH]_2$ (X = Cl, Ph, SPh, NHPh, OPh) [(1)–(5), respectively] was carried out by reversed-phase chiral HPLC on a commercially available Pirkle-type chiral stationary phase (R,R)-Whelk-01 using 85:15 (v/v) hexane–thf as the mobile phase. The absolute configurations of the resulting enantiomers of compounds (3) (X = SPh) and (5) (X = OPh) were determined unambiguously by X-ray crystallography. For both (3) and (5) it was found that the *SS* enantiomer eluted before the *RR* enantiomer, indicating a convenient method to determine the absolute configurations of enantiomers of this series of cyclophosphazene derivatives and providing the first set of enantiomeric reference compounds for cyclophosphazene derivatives. These structures demonstrate an interesting anomaly in that the pair of enantiomers of (3) crystallize in enantiomorphically paired space groups whilst, under the same conditions, the solid-state forms of the enantiomers of (5) form structures in Sohncke space groups that are not enantiomorphous.

1. Introduction

Chiral phosphorus compounds (*P*-chiral) have been attracting great interest (Dzygiel *et al.*, 2000; Koval *et al.*, 2003; Lammerhofer *et al.*, 2003) because of their importance as chiral solvating agents (Schmulbach *et al.*, 1971; Lacour *et al.*, 2002), chiral ligands in solution (Shi & Zhang, 2004 and references therein) and enzyme inhibitors (Collinsova & Jiracek, 2000). Cyclophosphazenes are an important class of organophosphorus chemistry and their derivatives have been of considerable interest because, for example, it is possible to design materials with special properties such as thermal stability, catalytic properties, electrical conductivity, liquid crystal and biomedical activity (Allcock, 2003; Allcock & Klingenberg, 1995; Singler *et al.*, 1991; Siwy *et al.*, 2006).

Tetracoordinated P atoms in cyclophosphazenes are pentavalent and potential stereocentres. Although the possibility of optical isomerism in cyclophosphazene derivatives was first discussed over 40 years ago (Shaw *et al.*, 1962), the stereogenic properties of substituted phosphazene compounds have only been investigated systematically in recent years (Davies *et al.*, 2000; Coles *et al.*, 2001; Porwolik-Czomperlik *et al.*, 2002; Beşli *et al.*, 2003; Coles, Davies, Eaton, Hursthouse, Kılıç, Shaw, Şahin, Uslu & Yeşilot, 2004; Coles, Davies, Eaton,

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Hursthouse, Kılıç, Shaw & Uslu, 2004). Such work concentrated on cyclophosphazene derivatives with two centres of chirality giving diastereoisomers whose *meso* and *racemic* forms were characterized by X-ray crystallography and ^{31}P NMR spectroscopy on addition of a chiral solvating agent (CSA) or a chiral shift reagent (CSR). Optically active derivatives of cyclophosphazenes have been reported, but these were based either on cyclization of an optically active acyclic precursor (Schmulbach *et al.*, 1971) or on the optical activity of the substituent such as binaphthoxy derivatives of cyclotriphosphazenes (Dez *et al.*, 1999; Kumar & Kumara Swamy, 2004). There is one report of the separation of enantiomers of cyclophosphazene derivatives by chiral high-performance liquid chromatography (HPLC) and their characterization by circular dichroism (CD) spectroscopy (Bui *et al.*, 2005). For these compounds, in which the cyclophosphazene ring is at the chiral centre, it was found that the CD spectra are very weak and near the limit of detection, indicating that optical rotation at 589 nm is likely to be vanishingly small. It was suggested that X-ray crystallography is likely to be the most reliable method to assign absolute stereochemistry in such cyclophosphazene derivatives (Bui *et al.*, 2005), but once absolute stereochemistry has been assigned by X-ray crystallography, it would be useful to develop a more routine method such as chiral HPLC for assigning the absolute stereochemistry of cyclophosphazene derivatives.

HPLC with chiral stationary phases has been used extensively to determine enantiomeric composition (Pirkle & Pochapsky, 1989) and the contribution of chiral chromatography to the determination of the absolute configuration of enantiomers has recently been reviewed (Roussel *et al.*, 2004). It is found that HPLC with chiral stationary phases may be used to identify the absolute configurations of closely related series of compounds, because the elution order of enantiomers may be correlated with the known enantiomer of the analogues. The method is obviously limited to analytes where enantiomeric standards of structural analogues are known. This study therefore sets out to characterize the relative elution order of enantiomers from a racemic mixture and use the unambiguous assignment of absolute structure from single-crystal X-ray diffraction to generate a reference set of compounds for the enantiomeric resolution of cyclophosphazenes by chiral HPLC.

HPLC methods for separation of enantiomers have been extensively developed and are mainly based on chiral stationary phases (CSPs; Gubitz, 1990; Welch, 1994; Franco *et al.*, 2001; Ganapathy, 2001; Yashima, 2001). Reversed-phase HPLC enantioseparations of organophosphorus compounds with a chiral P atom have already been reported (Pirkle *et al.*, 1996; Gao *et al.*, 1997; Du *et al.*, 2003) using a Pirkle Model CSP (Whelk 01 column) and enantioseparations of organophosphorus pesticides with a chiral P atom have been achieved using cellulose-type CSPs (Ellington *et al.*, 2001; Wang *et al.*, 2006). It has been shown (Beşli *et al.*, 2006) that reversed-phase HPLC using a chiral stationary phase (Whelk 01 column) may be used to characterize racemic cyclophosphazene derivatives with one or two centres of chirality and that the HPLC method was more reliable than using ^{31}P NMR with a chiral solvating agent for determining the stereogenic properties of cyclophosphazenes. A similar conclusion resulted from an analogous investigation of the stereogenic properties of di-spiro cyclotriphosphazene derivatives containing two equivalent centres of chirality, *viz*. the *cis* (*meso*) and *trans* (*racemic*) isomers, $N_3P_3X_2[O(CH_2)_3NH]_2$ (X = Cl, Ph, SPh, OPh, NHPh; Yeşilot & Çoşut, 2007). In that work it was found that chiral HPLC gave a good resolution of enantiomers of the racemic compounds [Fig. 1, (1)–(5)] with resolution factors between 2.49 and 7.40, making them good candidates for enantiomeric separations and determination of absolute configuration. In this work the absolute configurations of di-spiro racemic derivatives of cyclotriphosphazenes, $N_3P_3X_2[O(CH_2)_3NH]_2$ [X = SPh (3); X = OPh (5)] were determined by X-ray crystallography, which gives support to the use of chiral HPLC for determining the absolute configuration of (1)–(5), and as a general method in cyclophosphazene chemistry.

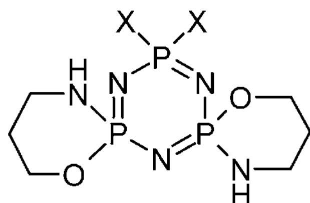

Figure 1
The racemic cyclotriphosphazene compounds studied, where X = (1) Cl; (2) Ph; (3) SPh; (4) NHPh; (5) OPh.

2. Enantiomorph separation by HPLC

Compounds (1)–(5) (Fig. 1) comprise a series of closely related cyclophosphazene derivatives, in which the structural variation is given by gem disubstitution on the ring P atom that is remote from the two P atoms providing the two constant centres of chirality. These compounds have been shown to have resolution factors between 2.49 and 7.40 using a Whelk-01 chiral stationary phase (Yeşilot & Çoşut, 2007), making them favourable for complete separation of enantiomers.

HPLC was performed with an Agilent 1100 series HPLC system (Chemstation software) equipped with a G 1311A pump and G1315B diode array detector monitoring the range 254–360 nm. The HPLC column used was a reversible chiral column (R,R)-Whelk-01 (250 × 4.6 mm) from Regis Tech. Inc. The Whelk 01-chiral stationary phase (CSP) was derived from 4-(3.5-dinitrobenzamido) tetrahydrophenanthrene covalently bound to silica. The mobile phase was a 85:15 (v/v) mixture of hexane–thf, respectively. The sample was dissolved in hexane–dichloromethane (1:1) at a concentration of 20 μg ml^{-1} for the chiral column. HPLC separations using the Whelk-01 CSP were carried out at room temperature on the racemic forms of (1)–(5) using the same flow rate and the same mobile phase, where the mobile phase was a 85:15 (v/v) mixture of hexane–thf and the flow rate was set at 2 ml min^{-1}. A semi-preparative separation of the enantiomeric forms of the racemic

Table 1
Assignment of absolute configurations of enantiomeric cyclotriphosphazene derivatives [(3*a*), (3*b*)] and [(5*a*), (5*b*)] by X-ray crystallography and, by analogy of HPLC profiles, compounds (1*a*), (1*b*), (2*a*), (2*b*), and (4*a*), (4*b*).

Compound	Absolute configuration from structure determination	Assignment of absolute configuration by HPLC	
		Elution time, *t* (min)	Configuration by analogy
(1*a*)	–	8.3	*SS*
(1*b*)	–	10.1	*RR*
(2*a*)	–	8.1	*SS*
(2*b*)	–	12.2	*RR*
(3*a*)	*SS*	8.7	–
(3*b*)	*RR*	13.0	–
(4*a*)	–	12.1	*SS*
(4*b*)	–	16.8	*RR*
(5*a*)	*SS*	8.1	–
(5*b*)	*RR*	12.4	–

compounds (1)–(5) was achieved by repeated injection and collection of the respective fractions from the analytical HPLC column to give approximately 40–50 mg of each enantiomer as an oil phase. The retention times of each enantiomer are summarized in Table 1. These times show a clear difference between each of the two enantiomers for all the compounds studied and therefore the technique can be assumed to differentiate between the two enantiomeric forms of each of these pairs of compounds.

The purities of all individual enantiomers were confirmed by chiral HPLC. An example of the HPLC chromatograms is shown in Fig. 2 for the racemic mixture of (3) and (5), and their enantiomers (3*a*), (3*b*), (5*a*) and (5*b*) (later characterized as the *SS* and *RR* enantiomers, respectively). A similar effective separation of enantiomers was carried out for all compounds (1)–(5) and the crystallization of the enantiomers was achieved using the saturated solution, slow evaporation method from various solvent systems at room temperature.

3. Absolute structure determination of reference systems

3.1. Description of molecular and crystal structures

Suitable crystals for X-ray crystallography resulted for enantiomers of (3) and (5) [designated (3*a*), (3*b*) and (5*a*), (5*b*), respectively] and the molecular structures are shown in Figs. 3 and 4, respectively; the other compounds did not give crystals of the enantiomers that were suitable for X-ray crystallography.

Unambiguous determination of the absolute configuration of all the structures presented was confirmed by means of refinement of the Flack parameter (Flack, 1983). It was therefore confirmed that (3*a*) is the *SS* enantiomer and (3*b*) is the *RR* enantiomer, which have melting points of 443–444 and 445–446 K, respectively. It was also found that (5) is the racemic form, (5*a*) is the *SS* enantiomer and (5*b*) is the *RR* enantiomer, with melting points of 386–387 and 384–385 K for (5*a*) and (5*b*), respectively. Theoretically the melting points of these enantiomers should be identical, however, the broad literature contains many examples where this is not precisely the case, *e.g.* D-(−)-tartaric acid = 445–447 K and L-(+)-tartaric acid = 443–445 K and may be attributed to the presence of slight impurities or polymorphism. Our values have been rechecked for consistency and are completely reproducible.

The bond lengths and angles of the molecular structures conform to expected values, however, the OPh and SPh groups differ in orientation and hence only selected geometric parameters (torsion and dihedral angles between aromatic ring substituents) are provided in Table 2. For the racemic structure of (5) these geometric parameters indicate that the phenyl substituents are in a similar orientation to each other. However, the same measurements for the enantiomers of structures (5*a*) and (5*b*) indicate that the conformations of these groups are quite different when comparing between the structures and the rings are roughly in the same orientation in the structure of (5*a*), but quite different with respect to each other in (5*b*). In structures (5*a*) and (5*b*) one of the N—H groups of the heterocycle is orientated equatorially and the other axially, whilst in the remaining structures all of these groups are arranged equatorially in the ring.

Intermolecular interactions of the N—H···N type are clearly prevalent in this family of compounds and can be

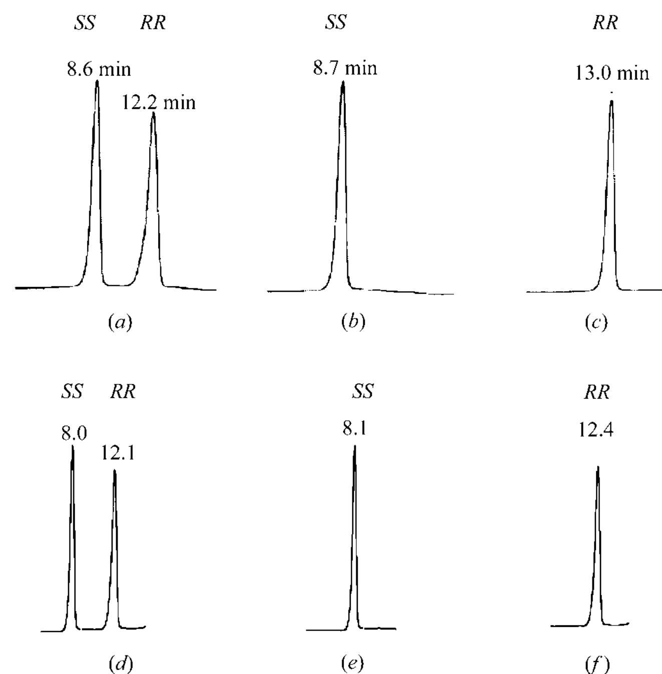

Figure 2
HPLC profiles using a Whelk-01 chiral column (250 × 4.6 mm) and a solvent system of 85% hexane–15% tetrahydrofuran (v/v) at a flow rate of 2 ml min^{-1}. (*a*) Compound (3), racemic form; (*b*) compound (3*a*) (*SS* enantiomeric form); (*c*) compound (3*b*) (*RR* form); (*d*) compound (5), racemic form; (*e*) compound (5*a*) (*SS* enantiomeric form); (*f*) compound (5*b*), *RR* form.

considered to be structure defining, exhibiting interactions between heterocyclic ring amino groups and a phosphazene ring nitrogen or another amino group. In contrasting these interactions between compounds (3) and (5) the structures of (3*a*) and (3*b*) are N3···N5 = 2.281 (13) and N1···N4 = 2.288 (18) Å, respectively, while they are generally slightly weaker and more variable for (5), (5*a*) and (5*b*) [N1···N5 = 2.44 (2)/N2···N4 = 2.17 (4), N4···N5 = 2.20 (4) and N4···N5 = 2.30 (5) Å, respectively]. In all cases this feature results in a one-dimensional chain structure parallel to the *c* axis, except in (5*b*) where the chain runs along the *a* axis of the unit cell. The other (weaker) interaction that prevails throughout the structural family is that of N—H···Pi in the case of (3*a*) and (3*b*) [N4—H4N···Cg = 2.733 (14) Å, 148.0 (11)°; N5—H5N···Cg = 2.73 (2) Å, 148.7 (16)° respectively] and C—H···π in the case of (5), (5*a*) and (5*b*) [C11—H11···Cg = 2.95 (4) Å, 157.00 (8)°, C16—H16···Cg = 2.94 (5)°, 139.01 (9) Å, C14—H14···Cg = 2.64 (16) Å, 157.0 (1)°/C17—

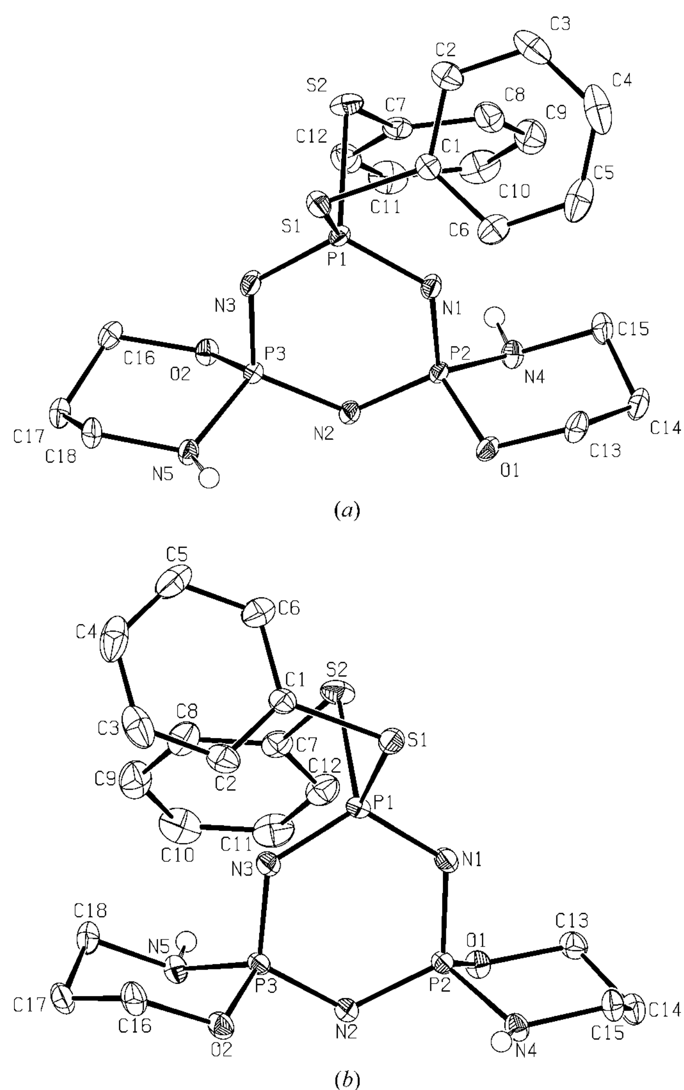

Figure 3

Molecular structures of (*a*) (3*a*), *SS* enantiomer; (*b*) (3*b*), *RR* enantiomer.

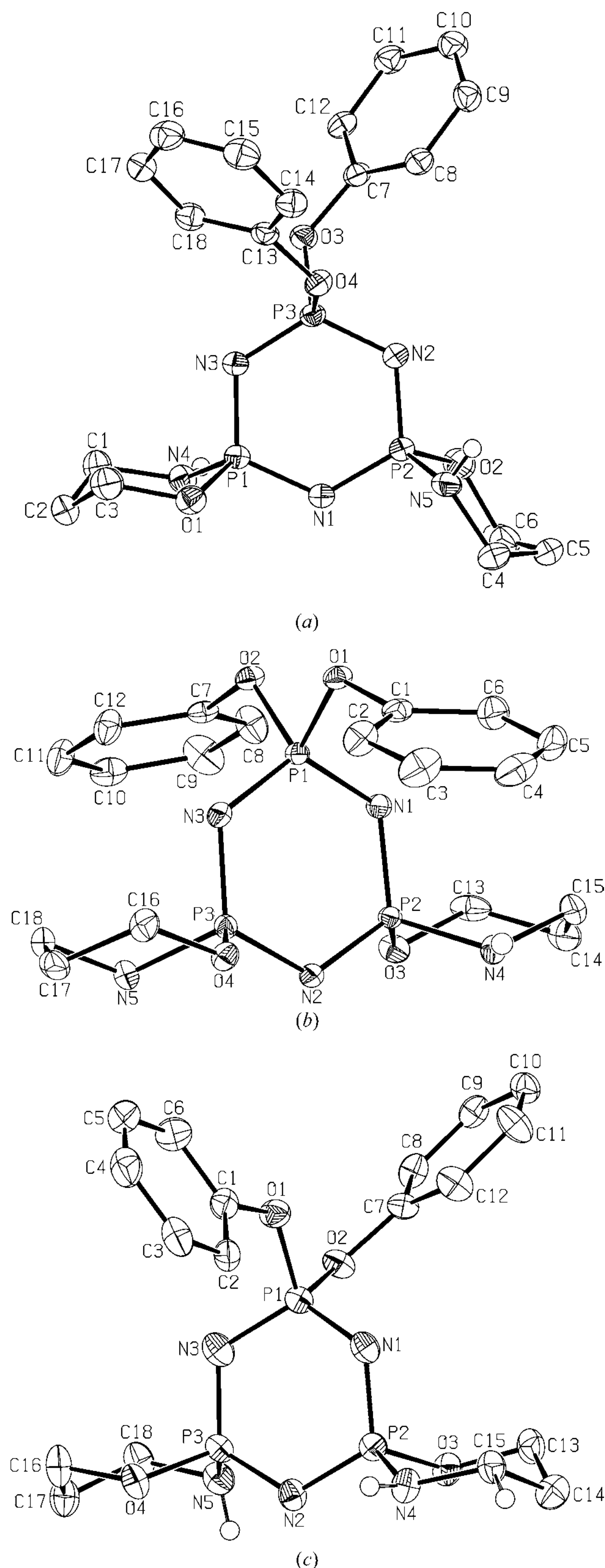

Figure 4

Molecular structures of (*a*) (5), racemic form; (*b*) (5*a*), *SS* enantiomer; (*c*) (5*b*), *RR* enantiomer.

Table 2
Selected torsion angles and dihedral angles (°) between SPh and OPh groups for (3*a*), (3*b*), (5), (5*a*) and (5*b*).

	N—P—O/S—C torsion angle		Dihedral angle between aromatic rings		
	Ring *A*	Ring *B*	Ph1/Ph2	N_3P_3/Ph1	N_3P_3/Ph1
(3*a*)	42.76 (6)	47.04 (6)	77.60 (7)	37.19 (6)	40.40 (6)
	172.66 (6)	−85.20 (5)			
(3*b*)	−172.60 (8)	85.21 (9)	77.50 (11)	37.14 (9)	40.36 (9)
	−42.79 (9)	−46.99 (10)			
(5)	−50.14 (11)	−58.70 (14)	86.06 (15)	62.18 (12)	50.43 (13)
	−179.90 (14)	170.86 (13)			
(5*a*)	58.33 (14)	−65.11 (15)	37.4 (17)	68.60 (15)	74.70 (11)
	−73.67 (16)	66.60 (14)			
(5*b*)	−61.04 (15)	−74.29 (10)	89.3 (3)	88.4 (2)	55.7 (2)
	169.47 (17)	57.27 (16)			

H17*a*···Cg = 2.83 (14) Å, 142.5 (4)°/C17—H17···Cg = 2.76 (17) Å, 160.2 (5)°].

3.2. Comparison of enantiomeric pairs of crystal structures

Out of the 230 total possible space groups it is known that 65 may contain chiral crystal structures and, of these, 22 form the 11 pairs of enantiomorphous space groups, *i.e.* these are the **true** chiral space groups. The remaining 43 space groups do not form enantiomorphic pairs and therefore are actually **achiral**, despite commonly being referred to as part of the 'chiral' family of space groups by most small molecule crystallographers (Flack, 2003). We adopt here the terminology that the 65 space groups that may contain a chiral crystal structure are the **Sohncke** space groups and the 22 true chiral space groups form 11 **enantiomeric** pairs.

Both enantiomers of (3) were crystallized in dichloromethane–hexane (1:1). The *SS* enantiomer of (3) crystallizes in the chiral space group $P4_1$ (3*a*), whilst the *RR* enantiomer crystallizes in its enantiomorphically paired space group $P4_3$ (3*b*). The crystal structures of (3*a*) and (3*b*) are compared in Fig. 5, where the opposite helical forms that the molecules adopt with respect to each other can clearly be seen.

Table 3
Crystallization trials for the enantiomers (5*a*) and (5*b*).

Solvent mixture	(5*a*)	(5*b*)
Hexane:dichloromethane (1:1)	Oil	Oil
Hexane:tetrahydrofuran (2:1)	Block morphology	Prism morphology
Hexane:ethyl acetate (2:1)	Oil	Prism morphology
Benzene:petroleum ether (1:1)	Oil	Oil
Hexane:petroleum ether (1:1)	Oil	Oil

We have been able to obtain the crystal structures of the racemic and both enantiomeric forms of (5). The racemic form of this compound crystallizes in the centrosymmetric space group *Pbcn*. Owing to the inversion symmetry of this space group the opposite enantiomer is generated within the same crystal structure to produce the non-superimposable mirror-image molecule that is the second of the pair of the racemic mixture. Unlike the enantiomorphous pair of space groups observed for (3), which might be considered as the normal or expected crystalization behaviour, the *SS* form (5*a*) crystallizes in the space group $P3_2$, whilst the *RR* form (5*b*) crystallizes in $P2_1$. This observation is somewhat unusual in that these space groups are not enantiomorphous pairs, *i.e.* one is not the inversion of the other, and the crystal structures do not exhibit identical unit cells. Accordingly, despite being the molecular structures containing opposing chiral centres in the same compound, they are not mirror images of one another as highlighted in the overlay plot of Fig. 6.

In this case, in the solid state (5*a*) and (5*b*) are considered to be enantiomers with differences in molecular conformation and therefore are not enantiomorphous (for a full review and definition of terminology see Flack, 2003).

There are two possible sources of pairwise conformational differences in this compound; the exocyclic *X*Ph groups and the six-membered propanolamine rings. As previously found for the enantiomers of (3), their conformations are identical mirror images of each other. However, the conformational features in the structures of (5) are different. The six-membered propanolamine rings may theoretically adopt 'in–in', 'in–out' or 'out–out' chair conformations with respect to

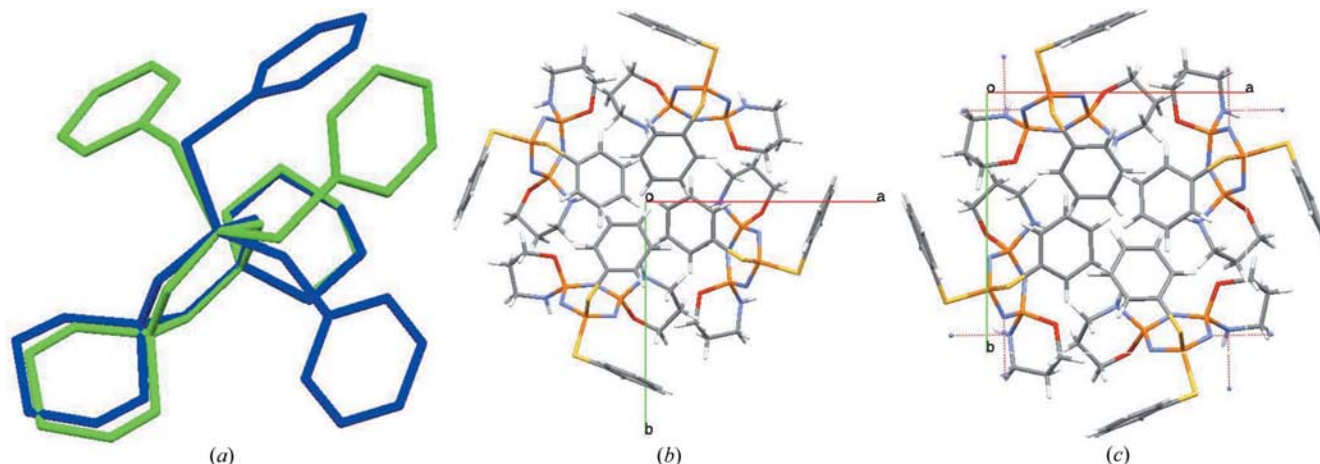

Figure 5
The helical nature of the crystal structures of (3*a*) and (3*b*).

Table 4
Data collection and refinement parameters for structures (3*a*), (3*b*), 5, (5*a*) and (5*b*).

	(3*a*) (*SS* enantiomer)	(3*b*) (*RR* enantiomer)	(5)	(5*a*) (*SS* enantiomer)	(5*b*) (*RR* enantiomer)
Crystal data					
Chemical formula	$C_{18}H_{24}N_5O_2P_3S_2$	$C_{18}H_{24}N_5O_2P_3S_2$	$C_{18}H_{24}N_5O_4P_3$	$C_{18}H_{24}N_5O_4P_3$	$C_{18}H_{24}N_5O_4P_3$
M_r	499.45	499.45	467.33	467.33	467.33
Cell setting, space group	Tetragonal, *P*4(1)	Tetragonal, *P*4(3)	Orthorhombic, *Pbcn*	Trigonal, *P*3(2)	Monoclinic, *P*2(1)
Temperature (K)	120	120	120	120	120
a, *b*, *c* (Å)	11.49120 (10), 11.49120 (10), 16.7890 (2)	11.48990 (10), 11.48990 (10), 16.7827 (2)	38.7541 (16), 10.1505 (4), 10.5550 (5)	10.49200 (10), 10.49200 (10), 16.6645 (3)	6.30730 (10), 17.6438 (4), 9.5481 (2)
β (°)	90	90	90	90	96.5520 (10)
V (Å^3)	2216.95 (4)	2215.62 (4)	4152.1 (3)	1588.69 (4)	1055.62 (4)
Z	4	4	8	3	2
Radiation type	Mo $K\alpha$	Mo $K\alpha$	Mo $K\alpha$	Mo $K\alpha$	Mo $K\alpha$
μ (mm^{-1})	0.48	0.48	0.32	0.32	0.32
Crystal form, size (mm)	Prism, 0.45 × 0.45 × 0.40	Block, 0.55 × 0.20 × 0.20	Needle, 0.65 × 0.02 × 0.01	Block, 0.50 × 0.40 × 0.34	Plate, 0.22 × 0.18 × 0.04
Data collection					
Diffractometer	Bruker–Nonius KappaCCD area detector	Bruker–Nonius KappaCCD area detector	Bruker APEX2	Bruker–Nonius KappaCCD area detector	Bruker–Nonius APEX2 area detector
Data collection method	φ and ω scans	φ and ω scans	φ and ω scans	φ and ω scans	φ and ω scans
Absorption correction	Multi-scan†	Multi-scan†	Multi-scan†	Multi-scan†	Multi-scan†
T_{min}	0.812	0.777	0.817	0.858	0.933
T_{max}	0.830	0.909	0.997	0.900	0.987
No. of measured, independent and observed reflections	41 619, 5057, 4976	15 809, 4532, 4330	19 961, 4703, 3961	4871, 4848, 4802	12 391, 4543, 4430
Criterion for observed reflections	$I > 2\sigma(I)$	$I > 2\sigma(I)$	$I > 2\sigma(I)$	$I > 2\sigma(I)$	$I > 2\sigma(I)$
R_{int}	0.031	0.035	0.055	0.048	0.030
θ_{max} (°)	27.5	27.5	27.5	27.5	27.5
Refinement					
Refinement on	F^2	F^2	F^2	F^2	F^2
$R[F^2 > 2\sigma(F^2)]$, $wR(F^2)$, S	0.019, 0.049, 1.04	0.028, 0.066, 1.03	0.062, 0.135, 1.08	0.029, 0.071, 1.02	0.028, 0.065, 1.01
No. of reflections	5057	4532	4703	4848	4543
No. of parameters	279	279	279	280	279
H-atom treatment	Mixture‡	Mixture‡	Mixture‡	Mixture‡	Mixture‡
$(\Delta/\sigma)_{max}$	0.028	0.004	< 0.0001	0.057	0.002
$\Delta\rho_{max}$, $\Delta\rho_{min}$ (e Å^{-3})	0.16, −0.27	0.19, −0.33	0.55, −0.43	0.18, −0.38	0.26, −0.28
Absolute structure	Flack (1983)	Flack (1983)	–	Flack (1983)	Flack (1983)
Flack parameter	0.01 (4)	−0.01 (5)	–	−0.06 (8)	0.02 (6)

Computer programs used: *DENZO* (Otwinowski & Minor, 1997), *Collect* (Hooft, 1998), *SHELXS*97, *SHELXL*97 (Sheldrick, 2008), *PLATON* (Spek, 2003). † Based on symmetry-related measurements. ‡ Mixture of independent and constrained refinement.

the cyclophosphazene ring. It can be seen from Fig. 4 that these chair conformations are the same ('out–out') in both enantiomers [(5*a*) and (5*b*)], however, the racemic structure has these rings in differing ('in–out') conformations. When viewed perpendicular to the plane of the cyclophosphazene ring the OPh groups adopt the following conformations: (5) – opposing twist with Ph groups facing each other; (5*a*) – OPh groups oriented directly away from each other with Ph rings facing down; (5*b*) – opposing twist with Ph in an edge-to-face arrangement with respect to each other. With the conformations of the propanolamine rings being essentially the same, the difference between the molecular structures of enantiomers (5*a*) and (5*b*) is in the conformations of the OPh moieties.

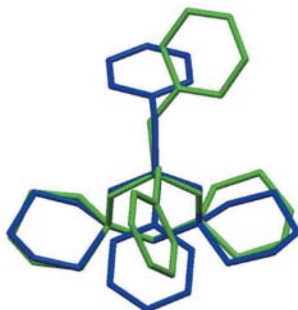

Figure 6
Overlay of the molecular structures showing the lack of mirror symmetry in the *SS* and *RR* enantiomers of (5*a*) and (5*b*).

A number of batches of samples of (5*a*) and (5*b*) were examined as part of an exercise to determine whether or not the second half of the enantiomorphous pair of structures, which theoretically should exist, is present. The crystal morphology of these batches and samples is very homogeneous and each batch had numerous unit-cell determinations and at least one full data collection performed to unambiguously determine the space group. To fully probe the

crystallization space to ensure the absence of these enantiomorphous pairs a crystallization screen was performed and the results are summarized in Table 3. It is proposed that the different crystallization behaviour of enantiomers (5*a*) and (5*b*) might depend on the solvents and conditions under which the crystals are grown. Experience of crystallization of cyclophosphazene compounds has found that the use of a single solvent only rarely produces crystals suitable for crystallography, whereas binary mixtures of solvents have been found to be more successful. Although a number of the most reliable combinations of these solvents was used to screen the crystallization behaviour of (5*a*) and (5*b*), it can be seen that hexane–tetrahydrofuran (2:1) was the only combination of solvents providing crystals of both enantiomers and only hexane–ethyl acetate (2:1) provided crystals of one of the enantiomers, *viz*. (5*b*), which was found to have the identical space group and molecular conformation as that already determined.

The reason for the crystallization of the enantiomers (5*a*) and (5*b*) in non-enantiomorphous space groups can only be a matter of speculation given the data available: a small solvent screen has failed to produce the expected paired space groups and the resulting structures are consistently reproducible. It is noted here that, under the same separation conditions, the crystallization behaviour of (3*a*) and (3*b*) produces the expected enantiomorphic pairing. Although trace amounts of undetected impurity in the chiral stationary phase of the HPLC column could potentially give rise to the non-enantiomorphous space groups of enantiomers (5*a*) and (5*b*), particular attention has been paid to ensure that the individual enantiomers are extremely pure as confirmed by chiral HPLC (Fig. 2). This is demonstrated by the extremely small enantiomeric excess (e.e.) in this experiment, which is derived using the following standard equation: % e.e. = $(A - B) \times 100/(A + B)$, where A is the area of the peak of the major enantiomer and B the area for the minor enantiomer (Eliel *et al.*, 1994). For example, for (3*a*) and (3*b*) the e.e. is 0.0014% (area ratio 49.929:50.071) and for (5*a*) and (5*b*) the e.e. is 0.0008% (area ratio 49.960:50.040). It is noted here that the measurement of uncertainty in HPLC peak areas is complex and not routinely estimated, however, in recent work Hibbert *et al.* (2001) demonstrate it to be < 1%.

The observation described herein whereby structures of enantiomorphous and non-enantiomorphous pairs of racemates of analogous compounds are formed from identical separation and crystallization conditions merits further systematic investigations into the predominance and driving force behind this phenomenon. These investigations are currently underway in our laboratory.

3.3. Crystallographic experimental

Details of the data collection and refinement of structures are given in Table 4.[1] Crystallographic data were collected by means of combined φ and ω scans on a Bruker–Nonius KappaCCD area detector situated at the window of a rotating anode (λMo $K\alpha$ = 0.71073 Å). The structures were solved by direct methods, *SHELXS*97 and refined using *SHELXL*97 (Sheldrick, 2008). H atoms were included in the refinement with those for the amide groups located from the difference map and refined at idealized bond lengths, but displacement parameters and geometry for all others constrained to ride on the atom to which they are bonded. The data were corrected for absorption effects using *SADABS* (Sheldrick, 2003). For structures that solved in one of a pair of enantiomorphic subgroups the related conformation was tested against a refinement of the Flack parameter (Flack, 1983) to ensure the correct enantiomorph had been selected.

[1] Supplementary data for this paper are available from the IUCr electronic archives (Reference: RY5024). Services for accessing these data are described at the back of the journal.

4. Conclusion

For those compounds [(3*a*)/(3*b*) and (5*a*)/(5*b*)] whose absolute configuration of di-spiro racemic derivatives of cyclotriphosphazenes, $N_3P_3X_2[O(CH_2)_3NH]_2$ [X = SPh (3); X = OPh (5)], is known from X-ray crystallographic studies, it is found that the *SS* enantiomers eluted before the *RR* enantiomers. This suggests that the enantiomers of these cyclophosphazene derivatives conform to the general trend that the elution order of enantiomers is correlated with configurations in a series of analogous compounds. Hence it is proposed that for the series of cyclophosphazene derivatives (1)–(5) the *SS* enantiomer elutes before the *RR* enantiomer using identical chiral HPLC and elution conditions. The assignment of absolute configuration for each of the compounds (1)–(5) in Table 2 has then been made on the basis of the elution order of enantiomers. The enantiomers of (3) and (5) crystallize in enantiomorphous and non-enantiomorphous pairings, respectively, and the predominance and driving force behind this behaviour is the subject of further study.

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