Comparison of Dynamic HPLC and Dynamic NMR in the Study of Conformational Stereodynamics: Case of the Enantiomers of a Hindered Secondary Phosphine Oxide[1]

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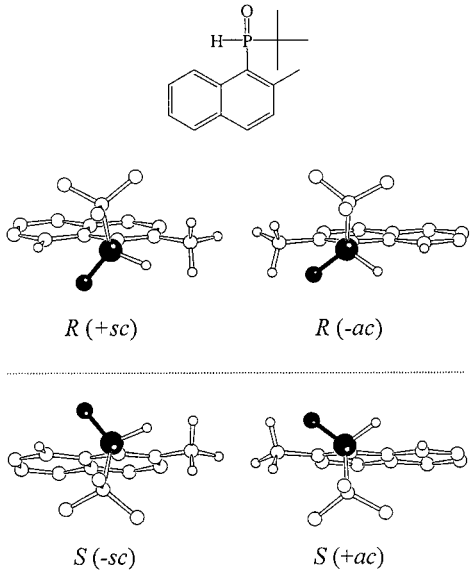
Abstract: Static and dynamic stereochemistry of HP(O)Bu^{t}Ar (Ar = 2-methyl-1-naphthyl) has been studied by a combination of variable-temperature NMR (^{1}H and ^{13} P), HPLC, and CD measurements as well as by MM calculations. Two unequally populated stereolabile isomers for each configurational enantiomer have been detected and their *anticlinal* and *synclinal* structures assigned. All the four species have been physically separated and identified on a cryogenic HPLC enantioselective column at −83 °C. The interconversion barrier measured by dynamic NMR yields essentially the same value as that measured by dynamic HPLC (14.75 and 14.95 kcal mol^{-1}, respectively).

Introduction

It is well established that rapid interconversion of conformational isomers at equilibrium can be investigated by dynamic NMR spectroscopy, which yields quite reliable values for the corresponding free energies of activation ($\Delta G^{\ddagger}$). Variable-temperature chromatography might be also used to measure the free energies of activation, if on-column interconversion of conformers can be observed in an appropriate temperature range. Furthermore, the use of enantioselective columns allows the measurement of the interconversion barriers required to transform one conformational enantiomer into the other. To test whether the discontinuous plate model theory[2a] is sufficiently accurate to properly describe, even at very low temperature, how an exchange process modifies the shape of the chromatographic peaks, we have compared the $\Delta G^{\ddagger}$ value obtained in this way with that derived from an accurate line shape simulation of the dynamic NMR spectra. A compound we found suitable for this purpose, in that it can be studied by both techniques, is that of *tert*-butyl-1-(2-methyl-1-naphthyl)phosphine oxide, **1**, HP(O)Bu^{t}Ar (Ar = 2-methyl-1-naphthyl), which bears two stereogenic elements corresponding to the configurationally stable chiral phosphorus atom and to the stereolabile Ar−P rotation axis. As a consequence, two diastereoisomers of different stability should be, in principle, observable in an appropriate temperature range for each enantiomer at phosphorus. Such a situation, which is somehow similar to the one we had observed for sulfoxides with analogous structures,[2b,c] is illustrated in Chart 1.

Chart 1. MM-Computed Structure of the *sc* (More Stable) and *ac* (Less Stable) Conformational Rotamers of the *R*,*S* Enantiomers of **1**



Results and Discussion

The ambient-temperature ^{1}H NMR spectrum of **1** shows a number of broad lines that are indicative of an equilibrium

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(1) Conformational Studies by Dynamic NMR. 75. For part 74, see: Lunazzi, L.; Mazzanti, A.; Muñoz Alvarez, A. *J. Org. Chem.* **2000**, *65*, in press (Web release date: April 14, 2000).

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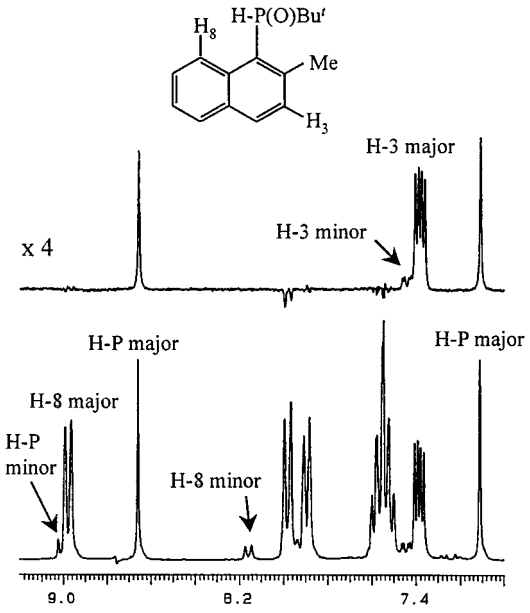

Figure 1. Bottom: Part of the low-field region of the ^{1}H (300 MHz) spectrum of **1** at −65 °C in CD_2Cl_2 showing two sets of signals for the major and minor conformers. Top: Difference NOE spectrum (vertically expanded by a factor of 4) obtained at the same temperature by simultaneous irradiation of the minor and major signals (at 2.95 and 2.65 ppm, respectively) of the Me group in the position 2 of the naphthalene ring.

between two conformational isomers (rotamers). When the sample is cooled at −65 °C (in CD_2Cl_2) the spectral lines sharpen considerably and two sets of signals, corresponding to a pair of species in different proportions, are observed. This is particularly evident for the methyl group in position 2 of the naphthalene ring which displays two well-separated singlets at 2.95 and 2.65 ppm, with an intensity ratio of 9:91, respectively. The phosphorus-bonded hydrogen signal of the major conformer appears as a doublet (J_{HP} = 465 Hz) centered at 7.84 ppm whereas only one of the two lines of the minor doublet is observed at 9.1 ppm (arrowed line in the bottom spectrum of Figure 1), the other one (expected at higher field) being overlapped by the aromatic signals.

To assign the structure of the two conformational stereoisomers a NOE experiment was carried out at −65 °C, by simultaneously irradiating both the major and minor lines of the 2-methyl group and recording the difference spectrum (i.e. that obtained by subtracting from the irradiated spectrum the one obtained in the same conditions without irradiation). As expected, the major and minor multiplets of the hydrogen in position 3 on the naphthalene ring both experience a NOE effect (Figure 1, top) whereas *only the major* doublet due to the H−P hydrogen is enhanced (about 11%), that of the minor doublet being, on the contrary, canceled (Figure 1, top).[3] Accordingly, the more stable conformer must be the one having the H−P hydrogen close to the 2-methyl substituent (synclinal, *sc*)[4] as in Chart 1.

This result also agrees with the chemical shift sequence of the hydrogen in position 8 of the naphthalene ring (Figure 1, bottom), in that the major conformer (*sc*) has the H-8 signal appearing at a field (8.98 ppm) much lower than that (8.15 ppm) of the minor one (anticlinal, *ac*).[4] This is because in the *sc* conformer the hydrogen in position 8 experiences the deshielding effect of the P═O double bond in a syn relationship. The opposite trend occurs, accordingly, for the 2-Me signal (minor and major conformers having their shifts at 2.95 and 2.65 ppm, respectively) since, in this case, it is the methyl group of the *ac* conformer (minor) which experiences the deshielding effect mentioned above. The ratio of the two conformers of Chart 1 seems quite independent of the polarity of the solvent: the same ratio was in fact observed both in CD_2Cl_2 and in CD_3OD.

Molecular mechanics calculations (MMX force field[5]) confirm that the averaged distance between the hydrogens of the 2-Me group and the hydrogen bonded to phosphorus in the *ac* conformer is too large (4.74 Å, as opposed to 2.83 Å of the *sc* conformer) to yield noticeable NOE effects.[6] The same calculations also indicate that the theoretical minimum corresponding to the *sc* conformer has an energy level 0.67 kcal mol^{-1} lower than that of the *ac* conformer: if a negligible $\Delta S°$ value is assumed, the corresponding ratio at −65 °C should be 83:17, a value not far from the one experimentally observed (91:9) at this temperature. The computed dipole moments of the two conformers are quite similar (1.4 D for *sc* and 1.2 D for *ac*), thus accounting for the independence of the conformer ratio upon the solvent polarity.

The ^{31}P spectrum, taken at −45 °C, comprises a doublet (J_{HP} = 465 Hz) for the major conformer with a shift of 74.6 ppm and a further fine structure (J = 14.8 Hz) due to the coupling with the *tert*-butyl hydrogens. The minor conformer shows an analogous doublet, with the same J values, at δ = 70.7 ppm. The integrated intensities yield essentially the same ratio as observed in the ^{1}H spectrum. The ^{1}H-decoupled ^{31}P spectrum consequently displays two sharp lines at low temperature that broaden and eventually coalesce in a reversible manner on warming the sample: the temperature dependence is reported in Figure 2 together with the computer simulation. The corresponding interconversion barrier of the more into the less stable species was determined as $\Delta G^{\ddagger} = 14.7_5 \pm 0.15$ kcal mol^{-1}, a value independent of temperature within the experimental errors. From this measurement it can be inferred that at −65 °C the conformational stereoisomers have a half-life time of about 7−8 min, thus are sufficiently long living to allow, in principle, a physical separation at that temperature.

To achieve the *sc* to *ac* interconversion, the rotation about the Ar−P bond moves the *tert*-butyl group across the naphthalene ring, and this can occur according to two possible pathways: either the *tert*-butyl group overtakes the hydrogen in position 8 or the methyl in position 2. Molecular mechanics calculations were used to estimate the barriers involved in the two possible pathways. The energy surface, computed as function of the torsion angles (driven in steps of 6°) about the Ar−P bond (ϕ) and about the P−Bu^t bond (θ), yields two maxima corresponding to the transition states indicated as no. 1 and no. 2 in Figure 3. When the *tert*-butyl group overtakes

(3) A small negative NOE effect is observed for the doublet of the major conformer at 7.95 ppm in Figure 1. This signal corresponds to that of the hydrogen in position 4 of the naphthalene ring, and its small negative NOE is a consequence of the large positive NOE experienced by the nearby hydrogen in position 3. This indirect negative NOE is known to occur quite often when two hydrogens are in a ortho relationship (see: Kruse, L. I.; DeBrosse, C. W.; Kruse, C. H. *J. Am. Chem. Soc.* **1985**, *107*, 5435).

(4) As the MM-computed dihedral angle C-8a,C-1,P,O in Chart 1 has a value (56°) exceeding 30°, the term synclinal (*sc*) has been preferred to the term synperiplanar (suggested by: Baker, R. W.; Kyasnor, R. V.; Sargent, M. V. *J. Chem. Soc., Perkin Trans 2* **1998**, 1333) for the more stable conformer. Likewise the term anticlinal (*ac*) has been preferred to antiperiplanar for the less stable conformer, since the corresponding angle (146°) is less than 150°.

(5) Computer package PC Model 4.0, Serena Software, Bloomington, IN.

(6) On the basis of these distances, the NOE expected to occur in the less stable *ac* rotamer should be 22 times smaller than that determined in the *sc* rotamer, i.e. 0.11/22 = 0.005. A NOE value of 0.5% is too small to be observed in our experimental conditions.

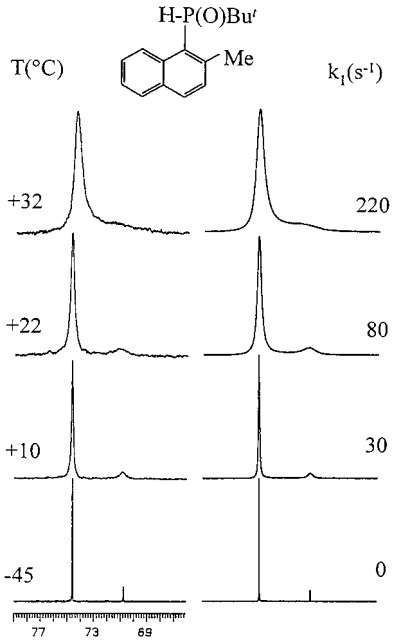

Figure 2. ^{1}H-decoupled ^{31}P spectrum (121.45 MHz) of **1** as function of temperature in CD_2Cl_2 (left). On the right are reported the computer simulations obtained with the rate constants (k_1, in s^{-1}) for the interconversion of *sc* into *ac*.

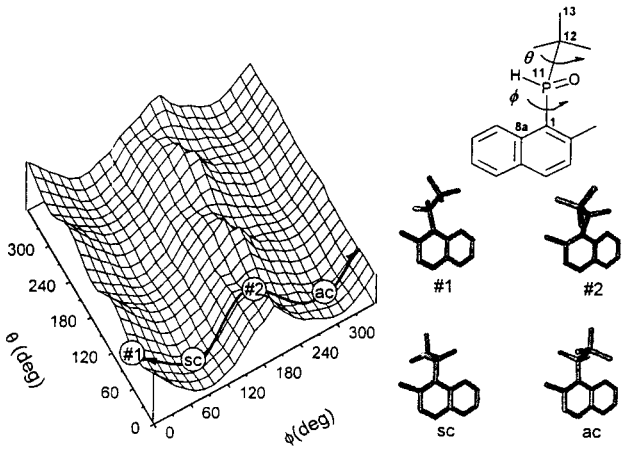

Figure 3. MM-computed potential energy surface of **1** as function of the torsion angle Ar−P (ϕ), corresponding to the dihedral defined by atoms 8a-1−11−12 and of the torsion angle P−Bu^t (θ), corresponding to the dihedral defined by the atoms 1−11−12−13. The values for ϕ (°), θ (°), and energy (kcal mol^{-1}) are the following: *sc*, 84, 60, 28.66; *ac*, 276, 60, 29.33; no. 1, 6, 36, 43.48; no. 2, 186, 54, 44.29.

the 2-Me substituent ($\phi = 186°$ and $\theta = 54°$), we have the transition state no. 2, which has an energy 15.6 kcal mol^{-1} higher than the ground state. When the *tert*-butyl group overtakes H-8 ($\phi = 6°$ and $\theta = 36°$), we have the transition state no. 1, which has an energy 14.8 kcal mol^{-1} higher than the ground state. Since the latter value is the lowest one and, in addition, is equal to the barrier experimentally determined, its related pathway should correspond to the preferred rotation mode for **1**.

The low-temperature ^{1}H-decoupled ^{31}P spectrum was also recorded in the presence of an excess (molar ratio 10:1) of an enantiomerically pure chiral solvating agent (*l*-TFAE, i.e. 1,1,1-trifluoro-9-anthrylethanol). As shown in Figure 4 the single signals of both the major and minor conformers are now split into 1:1 doublets, each displaying a separation of about 3.33 ppm. This is a consequence of the chirality of the phosphorus

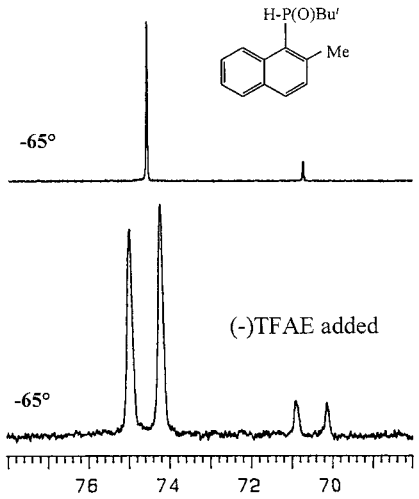

Figure 4. 121.45 MHz ^{1}H-decoupled ^{31}P spectrum of **1** at −65 °C in CD_2Cl_2 before (top) and after (bottom) the addition of a 10:1 molar excess of the chiral solvating agent *l*-TFAE (see text).

atom which, in these conditions, yields distinguishable spectra for the *R* and *S* enantiomers, both in the *sc* and *ac* conformational disposition.

The two residual[7] enantiomers of **1** were resolved at room temperature on a brush-type chiral stationary phase (see Experimental Section) to yield equally intense peaks when UV detected (325 nm) and two oppositely signed peaks when CD detected at the same wavelength (Figure 5, bottom): their complete CD spectra are shown on the top of Figure 5. As the column temperature is lowered to about −50 °C the two peaks showed considerable broadening due to on-column exchange. An interconverting region preceding the peaks appears between −60 and −70 °C, indicating that the minor conformer has a lower affinity for the CSP than the major one. Further cooling of the column to −83 °C allowed a complete separation of the four species which no longer interconvert during their passage trough the column, as evident form the absence of any interconverting region between the resharpened peaks (Figure 6b). The same low-temperature experiment performed on each individual enantiomer (that had been resolved by semipreparative enantioselective HPLC at ambient temperature, as shown in Figure 5) yielded two separate sets of peaks, due to the *ac* and *sc* conformers, which appeared (see Experimental Section) in the same positions of the racemic mixture (Figure 6c,d). When a CD detector is employed to monitor the racemic mixture at −83 °C, the first and third peaks appear positive and the second and fourth negative (Figure 6a): as expected, the positive pair corresponds to the rotamers of one enantiomer and the negative pair to the rotamers of the other enantiomer, in agreement with the results of Figure 6c,d. Relative peak areas were measured by digital integration of UV and CD signals of **1** by injecting the solution prepared at ambient temperature into the cooled column, kept at −83 °C. In this conditions the measured areas yield the ratio existing at ambient temperature (+25 °C), since no time was available for readjusting the equilibrium to the lower temperature. If $\Delta G°$ value is assumed to be temperature invariant, the ratio measured in this way (i.e. 16:84 at +25 °C) translates into 8:92 at −65 °C, in excellent agreement with the NMR determination at the same temperature (9:91). Because of this coincidence the chromatographic peaks were assigned on the basis of the NMR results, the more abundant being thus attributed to the *sc* conformer.

(7) Eliel, E. L.; Wilen, S. H. *Stereochemistry of Organic Compounds*; J. Wiley: New York, 1994; p 55.

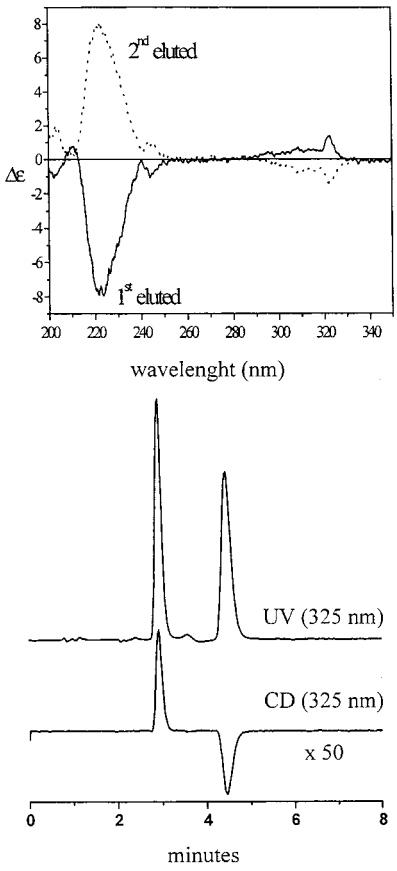

Figure 5. Bottom: HPLC resolution of the two residual enantiomers of **1** at 20 °C. A 100 × 4 mm i.d. colmun packed with (*R*,*R*)-DACH-DNB as chiral stationary phase has been employed. Eluent: 1% MeOH in CH_2Cl_2, flow rate 1.50 mL/min. Top: CD spectra of the two residual enantiomers of **1** in MeOH at 25 °C.

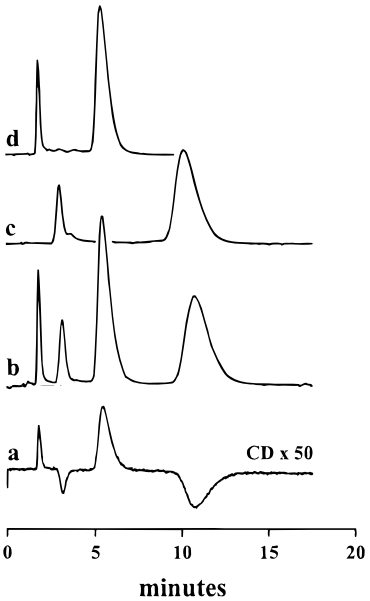

Figure 6. HPLC resolution of the four stereoisomers in a racemic mixture of **1** at −83 °C (trace a, CD detection, trace b, UV detection). The two top traces (UV detection at −83 °C) display the resolution of the pair of rotamers carried out for each individual enantiomer of **1**, obtained by semipreparative HPLC (traces c and d correspond respectively to the second and first eluted enantiomer of Figure 5).

In analogy with the dynamic NMR analysis, peak shape analysis in chromatography gives quantitative kinetic information for on-column interconversion process. Computer simulations of the chromatographic profiles were carried out on the individual resolved enantiomer of **1** using the discontinuous plate model.[8] This model treats the on-column interconversion as the sums of two independent sets of equilibria occurring in the mobile and stationary phase and yields apparent rate constants [$k_{1(app)}$ and $k_{2(app)}$ as in Figure 7]. They correspond to the sums of the rate constants in the two phases weighted by the time each rotamer is present in each phase. It should be stressed that, in the presence of the chiral stationary phase, each pair of rotamers of the *R* and *S* enantiomers may interconvert, in principle, at a different rate as a result of the specific interactions with the stationary phase. Indeed we obtained different values for both the forward and backward rate constants for the *ac* to *sc* on-column interconversion (Figure 7). The isomerization barrier of the more (*sc*) into the less (*ac*) stable species for the pair of rotamers deriving from the first eluted enantiomer (i.e. the one with a positive CD peak in Figure 5) amounts to 14.8 ± 0.3 kcal mol^{-1} whereas that for the second pair of rotamers amounts to 15.1 ± 0.3 kcal mol^{-1}. In the first case the value is identical to that obtained by NMR technique, whereas in the second case the stationary phase seems to display a small deactivating effect, compared to the same process in a free solution.[9] The difference, however, is too small to be significant and the averaged value of 14.9_5 kcal mol^{-1} should be taken as the result of the HPLC determination for the interconversion of the *sc* into the *ac* species. In any case the agreement with the barrier measured independently by NMR is very satisfactory.

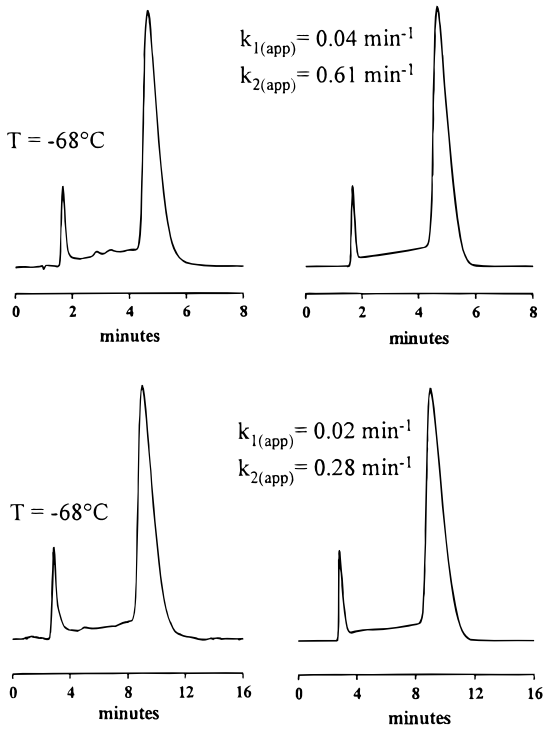

Figure 7. Experimental (left) and computer-simulated (right) DHPLC traces of the two residual enantiomers of **1** that had been isolated by semipreparative enantioselective HPLC at 20 °C. The top left and bottom left traces (UV detected at 325 nm) correspond respectively to the first and the second eluted enantiomer of Figure 5, as well as to the traces d and c of Figure 6. The *k* values reported on the top right and on the bottom right of the picture are the apparent rate constants for the *sc* to *ac* (k_1) and *ac* to *sc* (k_2) conversions.

(8) Jung, M. *QCPE Bull.* **1992**, *12*, 52.
(9) Gasparrini, F.; Misiti, D.; Pierini, M.; Villani, C. *Tetrahedron: Asymmetry* **1997**, *8*, 2069.

The assignment of the absolute configuration (*R* or *S*) is not straightforward but could be attempted by comparing the CD spectrum of **1** with that of the analogous sulfoxide, where the HP═O had been replaced by the S═O moiety. The absolute configuration of the latter had been independently obtained by means of a reliable enantiospecific synthesis.[2] The CD spectrum of such a sulfoxide bears a certain analogy with that reported on the top of Figure 5. In the sulfoxide spectrum there are, in fact, three peaks of the same sign (at 215, 245, and 270 nm) that approximately correspond to the three peaks (at 205, 224, and 244 nm) observed in **1**. The sulfoxide also exhibit two peaks of opposite sign at 300 and 323 nm, analogous to the opposite signed peaks at 310 and 325 nm in the spectrum of **1**. To the enantiomer of sulfoxide displaying a positive sign for the three lower wavelength peaks and a negative sign for the two higher wavelength peaks, the configuration *S* had been assigned.[2b] If the analogy between the spectra of the two molecules is accepted, then the second eluted enantiomer of **1** (Figure 5) should have the *S* configuration. It is noteworthy to recall that when using the same type of *RR* chiral column (see Experimental Section) also the second eluted enantiomer of the analogous sulfoxide had the *S* configuration.[2b] Of course one cannot exclude that the HP═O and S═O moieties might exhibit opposite CD and chromatographic behavior, so that the present assignment remains rather speculative.

Conclusions

The two conformational isomers (rotamers) of **1** have been detected by low-temperature NMR spectroscopy and the synclinal (*sc*) and anticlinal (*ac*) structures assigned, by NOE experiments, to the more and to the less stable form, respectively. Their interconversion barrier was determined by analysis of the dynamic ^{31}P NMR spectra. Molecular mechanics calculations gave results in agreement with experiment and indicated that the interconversion pathway, corresponding to the passage of the *tert*-butyl group by H-8, is preferred to the passage of the same group by the 2-methyl substituent. Each rotamer comprises a pair of configurationally stable enantiomers that became NMR detectable in a chiral environment. The two enantiomers at phosphorus were also physically separated at ambient temperature by enantioselective HPLC, and each of them was further resolved into the *ac* and *sc* rotamers on a cryogenic highly stereoselective HPLC column at −83 °C. The interconversion barrier of the *sc* into the *ac* rotamer, determined by computer simulations of the chromatographic profiles, yielded an average value (14.9_5 kcal mol^{-1}) which matched very satisfactorily that obtained by NMR for the same interconversion process (14.7_5 kcal mol^{-1}).

Experimental Section

Material. *tert*-Butyl-1-(2-methylnaphthyl)phosphine oxide (**1**) was prepared as follows. To a solution of 1-(2-methylnaphthyl)magnesium bromide [prepared from 1.2 g (0.05 mol) of magnesium and 11.12 g (0.05 mol) of 1-bromo-2-methylnaphthalene in 15 mL of dry diethyl ether] cooled to −5 °C was added dropwise 8 g of *tert*-butyldichlorophosphine (Strem) dissolved in 35 mL of dry ether. The reaction mixture was kept at −5 °C for 1 h and was then heated to reflux for additional 15 h. The reaction was quenched with 25% H_2SO_4, the ethereal layer was separated, and the water phase extracted with chloroform (3 × 50 mL). The combined organic layers were dried ($MgSO_4$) and evaporated under reduced pressure. The use of a good fumehood is recommended throughout these operations. The resulting oily residue was subjected to a short column chromatography on silica gel using 10:1 chloroform−acetone as eluent and yielded 1 g (8%) of **1** as white solid which was finally purified by recrystallization from 2:1 toluene−hexane. Mp: 132 °C (uncorrected). ^{1}H NMR (CD_2Cl_2, 300 MHz): δ 1.20 (9H, d, J_{P-H} = 16.6 Hz), 2.70 (3H, bs), 7.30−7.40 (1H, m), 7.45−7.60 (2H, m), 7.80−7.95 (2H, m), 7.90 (1H, d, J_{P-H} = 465 Hz) 9.04 (1H, bs). ^{13}C NMR (CD_2Cl_2, 75.5 MHz): δ 23.43 (CH_3, d, J = 8.6 Hz) 25.56 (CH_3, d, J = 2.5 Hz), 35.63 (q, d, J = 68.6 Hz), 126.40 (CH), 127.60 (CH), 127.50 (CH, bs); 129.25 (CH, bs), 130.57 (CH, d, J = 9.7 Hz), 133.39 (CH, d, J = 2.7 Hz). ^{31}P NMR (CD_2Cl_2, 121.4 MHz): δ 70.67 (minor),74.22 (major). Anal. Calcd for $C_{15}H_{19}OP$: C, 73.15; H, 7.77. Found (Perkin-Elmer 2400 CHNS/O analyzer): C, 72.95; H, 7.80. Chromatographic isolation of the residual enantiomers of **1** at 25 °C was performed on a 250 × 10 mm chiral column packed with (*R*,*R*)-DACH-DNB CSP[10] (0.5% methanol in dichloromethane as mobile phase; flow rate 6.0 mL/min, refractive index detection; 10 mg per run loading): 1st eluted, CD (MeOH) 322.6 (+1.64), 244.2 (−0.94), 223.6 (−7.84); 2nd eluted, CD (MeOH) 322.5 (−1.34), 243.4 (+0.96), 222.4 (+7.92).

NMR and CD measurements. NMR spectra were recorded on a Varian Gemini 300 provided with a standard variable temperature unit. The ^{1}H shifts were related to TMS; the ^{31}P shifts to aqueous 85% H_3PO_4. The temperatures were calibrated by means of a Ni/Cu thermocouple inserted into the probe before the measurements. Line shape simulations were performed by making use of a PC version of the DNMR 6 Program.[11] Circular dichroism (CD) spectra were obtained with a JASCO J710 dichrograph.

Chromatography. Analytical chromatography was performed on a HPLC system composed by a Waters model M510 pump, a Rheodyne model 7725i 20 mL injector, and a Jasco model CD 995 UV/CD detector. Preparative chromatographic was performed on a Waters model Delta Prep. 3000 apparatus equipped with a Knauer differential refractometer. Chromatographic data were collected and processed using the Millenium 2010 chromatography manager software (Waters Chromatography).

Low-Temperature and Dynamic HPLC. Cryogenic HPLC was performed placing the column [100 × 4 mm i.d., packed with (*R*,*R*)-DACH-DNB CSP] in a dry-ice/2-propanol cooling bath, with a 1 m long inlet capillary wrapped around the column to ensure thermal equilibration of the mobile phase. Temperatures were maintained within ±0.5 °C for at least two consecutive, replicate analysis.

Simulations of Dynamic Chromatograms. The experimental chromatograms were simulated using a modified version of the SIMUL package,[8] which is based on the discontinuous plate model. The upgraded version can simulate on-column interconversions between nonenantiomeric species (i.e. it can model a system in which the direct and reverse isomerization processes occur at different rates in the achiral mobile phase, where the two interconverting species are present in differing amounts). Additionally, it can handle asymmetric, non-Gaussian-shaped peaks. Experimental chromatograms in ASCII format are uploaded in the program, and simulations are carried out automatically starting from a set of input parameters (column holdup volume, retention times). Other parameters (number of theoretical plates, direct and reverse rate constants in the mobile and stationary phases, relative amount of the two interconverting species, peak asymmetry factors) are left free floating and optimized by a simplex routine until a given rms is obtained between the simulated and experimental chromatograms.

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(11) QCPE program No. 633, Indiana University, Bloomington, IN.