USE OF 2D NMR FOR THE ASSIGNMENT OF STRUCTURE OF 1,3,2-OXAZAPHOSPHOLIDIN-2-ONES[1]

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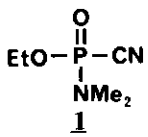
Abstract - Reaction of a chiral amino alcohol with an alkyl phosphorodichloridate gives a mixture of diastereomeric 1,3,2-oxazaphospholidin-2-ones which are epimeric at phosphorus. Analysis by 1D and 2D nmr has shown that it is possible to establish the absolute configuration at phosphorus by nmr techniques. Specifically, stereospecific differences in chemical shifts and NOESY spectra for each isomer are used to establish the absolute stereochemistry at phosphorus for (4*R*,5*S*)-2-ethoxy-3,4-dimethyl-5-phenyl-1,3,2-oxazaphospholidin-2-one diastereomers.

Organophosphorus nerve agents, by virtue of a tetrahedral phosphorus, exist as mixtures of enantiomers. The isomers of soman, for example, have stereospecific differences in their rates of acetylcholinesterase (AChE) inhibition, detoxification, reactivation and toxicological properties.[2] The C(±)P(-) isomers are 1 x 10^5 times more active AChE inhibitors and 100 fold more toxic[3] than the C(±)P(+) isomers and the (-)-isomer of tabun (1), a phosphoramidate nerve agent, is approximately 8 times more toxic than the (+)-isomer.[4] These differences in AChE inhibitory activity and toxicological properties are mainly due to differences in the absolute configuration at phosphorus. Because of the differences in reactivation rates of soman inhibited human brain and erythrocyte AChE by therapeutic oximes,[5] and this laboratory's ongoing investigation into the medicinal chemistry of HI-6,[6] a facile, stereospecific synthesis of chiral inhibitors of known configuration would be valuable for further AChE inhibition and reactivation studies.[7]



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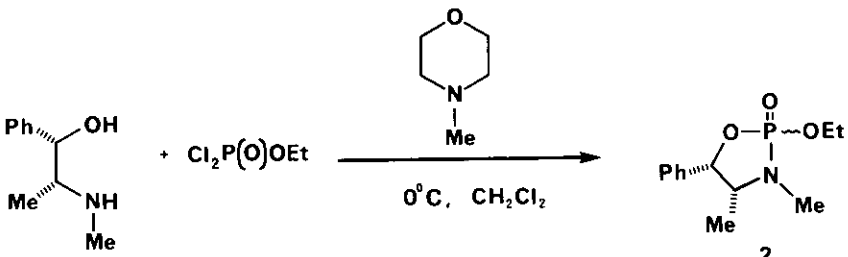
Cyclic phosphoramidates, such as the 1,3,2-oxazaphospholidin-2-ones, were chosen as analogues for the nerve agent tabun. In the reaction of ephedrine and an alkyl phosphorodichloridate, the stereochemistry at the C-4 and C-5 centres is retained, giving a pair of diastereomers differing only in their absolute configuration at phosphorus which are separable by conventional chromatographic methods.[8] (4*R*,5*S*)-2-Ethoxy-3,4-dimethyl-5-phenyl-1,3,2-oxazaphospholidin-2-one (2) was prepared from (1*S*,2*R*)-(+)-ephedrine and ethyl phosphorodichloridate in moderate yield (Scheme 1).[9] The diastereomers were separated by preparative layer chromatography. Assignments of the ^{1}H chemical shifts were straightforward and are given in the experimental section. The 1,3,2-oxazaphospholidin-2-ones possess several structural and stereochemical features which make the use of the NOESY experiment attractive for establishing the absolute configuration at phosphorus. Although X-ray analysis has been used to unambiguosly assign the solid-state configuration of one diastereomer[10] of (4*R*,5*S*)-2-phenoxy-3,4-dimethyl-5-phenyl-1,3,2-oxazaphospholidin-2-one, not all 1,3,2-oxazaphospholidines are solids, making the NOESY experiment particularily attractive for studying solution-state configurations.



Scheme 1

The diastereomers are epimeric at phosphorus with the 2-ethoxy substituent either *cis* or *trans* with respect to H-4 and H-5 thus NOE effects between the P-OEt group and either the H-4,5 or 4-Me and 5-phenyl substituents should be identifiable. The NOESY experiment[11] allows for all NOE effects to be observed at once in a 2D manner.[12] The experimental parameters were optimized based on the measured T_1 values for the 1,3,2-oxazaphospholidines which ranged from 2.5 sec for H-4/5 to 0.5 sec for the 4-Me

group. Experiments showed that variations in the preaquisition delay and the mix times had dramatic changes on the spectrum. With shorter delays, there was little cross-peak information and the diagonal showed a number of artifacts. Values of d1 = 4 and mix = 2.5 were used for the NOESY experiments. NOESY spectra of 2-ethoxy-1,3,2-oxazaphospholidine epimers are shown in Figure 1. For **2a**, where the 2-ethoxy group is *trans* to the 4-Me and 5-phenyl groups, the only cross peak of relevance is that between the 2-ethoxy methylene protons and H-5. In contrast, the NOESY spectrum of the major diastereomer, **2b**, where the 2-ethoxy group is *cis* to the 4-Me and 5-phenyl groups, clearly shows a number of cross-peaks as expected. Cross-peaks are observed between the 2-ethoxy methylene protons and the 5-phenyl, NMe, and 4-Me groups, clearly establishing the configuration at phosphorus. From this analysis, **2a** is thus assigned the *S* absolute configuration and **2b** the *R* configuration at phosphorus.

The 1D ^{1}H nmr data of the 2-ethoxy-1,3,2-oxazaphospholidin-2-one diastereomers supports the assignment of configuration at phosphorus. A distinct downfield shift is observed for the H-5 protons: δ 5.638 (**2b**) and 5.501 (**2a**). By analogy to the previous 2-phenoxy assignments, in **2b**, where H-5 is deshielded relative to the other diastereomer, the P=O is *cis* to H-5 and the 2-ethoxy group is *trans*; in **2a**, the P=O is *trans* to H-5.[8]

Nmr is a powerful method for the analysis of stereochemistry and 1- and 2-D experiments provide complementary data for the unambiguous assignment of absolute stereochemistry. From the 1D and 2D nmr data, the absolute configurations of the diastereomers of (4*R*,5*S*)-2-ethoxy-3,4-dimethyl-5-phenyl-1,3,2-oxazaphospholidin-2-one are: **2a** (minor isomer) 2*S*; **2b** (major isomer) 2*R*. NOESY allows for the analysis of all potential NOE interactions and these experiments provide an independent corroboration of other methods established in the literature for cyclic phosphoramidates .

EXPERIMENTAL

All reactions were performed under a positive pressure of dry N_2. All solvents employed were reagent grade or better. *N*-Methyl morpholine was distilled from NaH and stored over 3 Å molecular sieves. The term *in vacuo* refers to removal of solvent by Buchi Rotavapor at water aspirator vacuum followed by 0.1

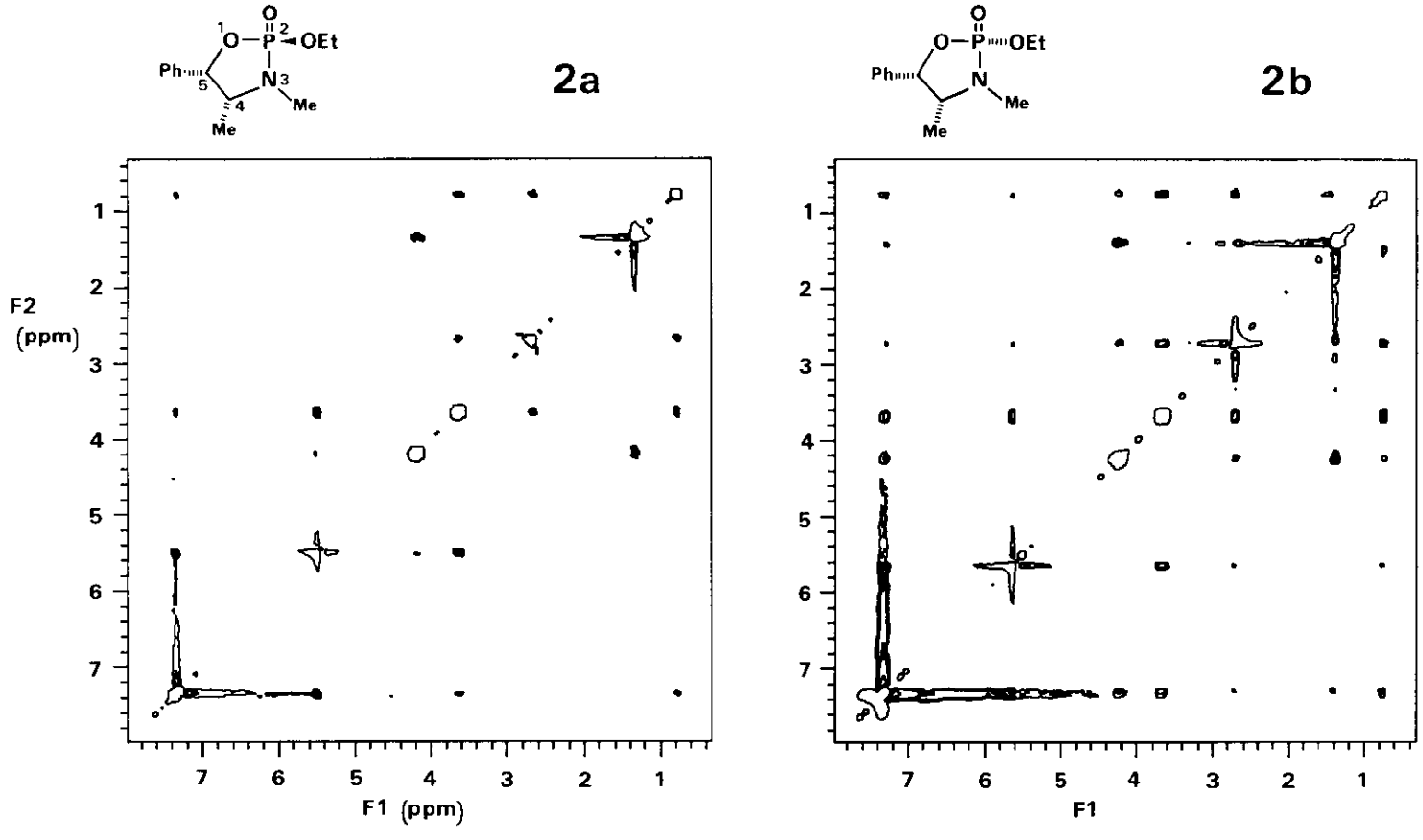


Figure 1. Comparison of the NOESY spectra of the diastereomers of (4*R*,5*S*)-2-ethoxy-3,4-dimethyl-5-phenyl-1,3,2-oxazaphospholidine-2-one. The absolute configurations at phosphorus are: **2a** (minor isomer) *2S*; **2b** (major isomer) *2R*.

Torr vacuum. Preparative layer chromatography was done on E. Merck 1 mm precoated silica gel plates (60 F_{254}). Angular rotatory powers were measured with an Optical Activity AA-10 automatic polarimeter and a 0.5 dm sample tube. Nmr spectra were recorded on a Varian VXR 300S spectrometer using standard pulse sequences. Sample temperature was regulated at 25 °C and 2D nmr experiments were run on non-spinning samples in $CDCl_3$ (9.8 atom % D, 0.03 % v/v TMS internal standard). Chemical shifts are reported in δ ppm and coupling constants in Hz. ^{31}P Nmr spectra were referenced relative to external triethylphosphate (-1.00 ppm). NOESY experimental parameters: spectral width (sw)= 2039 Hz, pulse width (pw) = 14.5 μsec, d1 = 4 sec, mix = 2.5 sec, 512 increments in the second frequency domain (fn = 512) and 16 repetitions at each increment (nt = 16). NOESY data was zero filled (fn1 = fn) before processing and symmetrization. The following abbreviations are used: plc (preparative layer chromatography), *c* (concentration, g per 100 ml), s (singlet), d (doublet), t (triplet), q (quartet), sep (septet), m (multiplet).

(4*R*,5*S*)-2-Ethoxy-3,4-dimethyl-5-phenyl-1,3,2-oxazaphospholidin-2-one (**2**) Ethyl phosphorodichloridate (3.26 g, 0.02 mol) was dissolved in CH_2Cl_2 (35 ml) and cooled to 0° C. Separately mixed (1*S*,2*R*)-(+)-ephedrine (3.83 g, 0.022 mol) and *N*-methylmorpholine (4.84 ml, 0.044 mol) were added dropwise to the phosphorodichloridate solution. The reaction mixture was stirred at 0° C for 2 h. The suspension was filtered, the filtrate was concentrated *in vacuo*, redissolved in petroleum ether, filtered and again concentrated *in vacuo* to give a clear colourless oil (1.00 g, 19.6%, diastereomeric ratio 1.38:1). The major and minor diastereomers were assigned by the ^1H nmr methine signals at δ 5.638 and 5.501 respectively. The oil was chromatographed on plc plates (100 % EtOAc) to give pale yellow liquids. **2a** (minor isomer, 0.29 g, 5.5%, R_f 0.25, 2*S*): $[\alpha]_D^{21.6}$ +93° (c=0.94, CH_2Cl_2). ^{31}P Nmr: δ 19.22. ^1H Nmr: δ 7.41-7.25 (m, 5 H, ArH), 5.501 (dd, *J*=6.3, 3.3, 1 H, H-5), 4.180 (m, 2 H, 2-O*CH*$_2$CH$_3$), 3.648 (sep, *J*=6.3, 1 H, H-4), 2.674 (d, *J*=10.2, 3 H, NCH$_3$), 1.362 (dt, *J*=7.1, 0.6, 3 H, 2-OCH$_2$*CH*$_3$), 0.797 (d, *J*=6.6, 3 H, 4-CH$_3$). ^{13}C Nmr: δ 135.8 (d, *J*=7.6), 128.2 (s), 128.1 (s), 125.9 (s), 80.31 (d, *J*=1.6), 63.66 (d, *J*=1.6), 59.07 (d, *J*=12.8), 28.60 (d, *J*=4.8), 16.30 (d, *J*=6.4), 14.00 (d, *J*=3.2). **2b** (major isomer, 0.36 g, 6.8%, R_f 0.39, 2*R*): $[\alpha]_D^{23.4}$ +75° (c=0.17, CH_2Cl_2). ^{31}P Nmr: δ 19.72. ^1H nmr: 7.41-7.27 (m, 5 H, 5-Ph), 5.638 (dd, *J*=6.3, 2.2, 1 H, H-5),

4.223 (m, 2 H, OCH_2CH_3), 3.672 (dq, J=16.5, 6.6, 1 H, H-4), 2.714 (d, J=10.2, 3 H, NCH_3), 1.400 (dt, J=7.2, 0.6 Hz, 3 H, 2-OCH_2CH_3), 0.759 (d, J=6.6, 3 H, 4-CH_3). ^{13}C Nmr: δ 136.1 (d, J=8.2), 128.3 (s), 128.0 (s), 125.7 (s), 80.30 (d, J=2.4), 64.04 (d, J=6.7), 59.53 (d, J=13.3), 29.01 (d, J=5.2), 16.55 (d, J=5.6), 13.38 (d, J=0.83).

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