relationships between intrinsic tryptophan fluorophores and metal ion binding sites in proteins.

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Communications to the Editor

Crystallographic Assessment of Absolute Configuration in 2′-Deoxyadenosine Cyclic 3′,5′-(R_p)-Phosphoranilidate. Direct ^{31}P-^{15}N Spin-Spin Coupling as a Probe for Configurational Assessment

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Recent works in this laboratory have shown that the diastereomeric nucleoside cyclic 3′,5′-phosphoranilidates and 3′,5′-phosphoranilidothioates are valuable intermediates in the stereospecific synthesis of P-chiral nucleoside cyclic 3′,5′-[^{18}O]-monophosphates,[1] cyclic 3′,5′-phosphorothioates,[2] and 3′,5′-phosphoroselenothioates,[3] which are important tools for mechanistic investigations of phosphotransferase reactions.[4] Because the PN → PX (X = ^{16}O, ^{18}O, S, Se) conversion proceeds with retention of configuration,[1,5] assignment of absolute configuration at the phosphorus atom in anilidates solves the problem of the absolute configuration of the corresponding nucleoside cyclic 3′,5′-[^{18}O]monophosphates, -phosphorothioates, and -phosphoroselenothioates.

Two criteria of configurational assessment at the phosphorus atom in nucleoside cyclic 3′,5′-phosphoranilidates were applied: (i) chemical-shift values in the ^{31}P NMR spectra of diastereomers,[2a,b] (ii) direct spin–spin coupling between phosphorus and exocyclic nitrogen-15 of the anilido moiety.[2d] Both criteria were adopted to nucleoside cyclic 3′,5′-phosphoranilidates from former works on diastereomeric 2-anilido-2-oxo-4-methyl-1,3,2-dioxaphosphorinanes[2d,6,7] and based on the assumption that the dioxaphosphorinanyl part of nucleoside cyclic 3′,5′-phosphoranilidates exists in the chair conformation. The results presented in this communication add proof to the validity of our assumption and deliver an additional example that direct spin–spin coupling constants between phosphorus and exocyclic nitrogen-15 nuclei can be applied as a certain criterion for distinguishing between axial and equatorial positions of the exocyclic arylamino group within the pair of diastereomeric 2-amido-2-oxo-1,3,2-dioxaphosphorinans. Both diastereomers of 2′-deoxyadenosine cyclic 3′,5′-phosphoranilidate (**1**) were obtained by reacting 2′-deoxyadenosine cyclic 3′,5′-phosphate (cdAMP) with triphenylphosphine–carbon tetrachloride–aniline.[2d] The reaction was performed in pyridine solution and both isomers of **1** were obtained in 46% yield. The products **1** were isolated and separated by chromatography on preparative TLC plates.[8] They were identical with authentic samples of **1** by using the criteria of TLC, mass spectrometry and ^{31}P NMR.[2b]

Compound (R_p)-**1** [R_f 0.27, ^{31}P NMR ($CHCl_3$, upfield from H_3PO_4) δ −2.88] crystallizes from methanolic solution in the orthorhombic space group $P2_12_12_1$ with cell dimensions a = 12.094 (3), b 22.409 (4), and c = 6.806 (2) Å; Z = 4. Intensities were recorded with an automatic Stoe four-circle diffractometer operated in the $\theta/2\theta$ scan mode (Ni-filtered, Cu Kα radiation) and corrected for Lorentz and polarization factors but not for absorption. The structure was solved by the multisolution method MULTAN[9] and refined by least-squares methods to the final disagreement index R = 7.09% including all 1550 reflections ($R = \sum||F_o| - |F_c||/\sum F_o$). On the basis of the ribose configuration we could choose the proper enantiomorph ($\bar{x}$, $\bar{y}$, $\bar{z}$) to establish the configuration around the phosphorus atom as R_p.

The structure of (R_p)-**1** is presented in Figure 1. The $O^{3'}$,-P,$O^{5'}$-dioxaphosphorinanyl ring of **1** exists in a chair conformation while the phenylamino group occupies the axial position. The bond lengths and the bond angles [P–NP 1.614 (7), NP–C_{10} 1.439 (9), NP–HNP 1.10 (7) Å; ∠P–NP–C_{10} 124.0 (5)°, ∠P–NP–HNP 119.0 (5)°, ∠C_{10}–NP–HNP 117.0 (6)°] indicate that hybridization of the phenylamino nitrogen atom is closer to sp^2.

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(1) Baraniak, J.; Lesiak, K.; Sochacki, M.; Stec, W. J. *J. Am. Chem. Soc.* **1980**, *102*, 4533.
(2) (a) Zielinski, W. S.; Stec, W. J. *J. Am. Chem. Soc.* **1977**, *99*, 8365. (b) Lesnikowski, Z. J.; Stec, W. J.; Zielinski, W. S. *Nucleic Acids Res., Spec. Publ.* **1978**, *4*, S45. (c) Baraniak, J.; Lesiak, K.; Stec, W. J. *Pol. J. Chem.* **1979**, *53*, 1387. (d) Baraniak, J.; Kinas, R. W.; Lesiak, K.; Stec, W. J. *J. Chem. Soc., Chem. Commun.* **1979**, 940.
(3) Lesiak, K. Lesnikowski, Z. J.; Stec, W. J.; Zielinska, B. *Pol. J. Chem.* **1979**, *53*, 2041.
(4) Eckstein, F. *Acc. Chem. Res.* **1979**, *12*, 204. Knowles, J. R. *Annu. Rev. Biochem.* **1980**, *49*, 877.
(5) (a) Stec, W. J.; Okruszek, A.; Lesiak, K.; Uznanski, B.; Michalski, J. *J. Org. Chem.* **1976**, *41*, 227. (b) Lesiak, K.; Stec, W. J. *Z. Naturforsch. B* **1978**, *33B*, 782. (c) Gerlt, J. A.; Coderre, J. A. *J. Am. Chem. Soc.* **1980**, *102*, 4531.
(6) (a) Stec, W. J.; Lopusinski, A. *Tetrahedron* **1973**, *29*, 547. (b) Stec, W. J.; Okruszek, A. *J. Chem. Soc., Perkin Trans. 1* **1975**, 1828.
(7) Kinas, R. W. Ph.D. Thesis, 1979.
(8) Triethylammonium salt of cyclic 2′-dAMP (0.8 mmol) and triphenylphosphine (630 mg, 2.4 mmol) were dissolved in pyridine (10 mL) freshly distilled from CaH_2. To this mixture carbon tetrachloride (0.23 mL, 2.4 mmol) and aniline (465 mg, 5 mmol) were added, and the reaction mixture was stirred at room temperature for 12 h. The reaction was quenched with water. Solvents were evaporated and the residue was coevaporated with toluene. The solid residue was extracted with ether and after extraction dissolved in methanol (10 mL). Methanolic solution was added to ether (800 mL) and the precipitate was filtered off. This solid product, containing mainly both diastereomers of **1**, was dissolved in metanol/chloroform and purified on preparative TLC plates [2 mm, PSC E. Merck, developing system chloroform–ethanol (85:15)]. Both isomers of **1** (196 mg) were eluted by means of chloroform–methanol (3:2). Separation of the diastereomers of **1** was achieved on preparative plates, developing system as above. (R_p)-**1** (44%): R_f 0.27, $M^{+\cdot}$ m/z 338; ^{31}P NMR (pyridine, upfield from external H_3PO_4); δ −3.21 (S_p)-**1** (56%): R_f 0.37, $M^{+\cdot}$ m/z 338, ^{31}P NMR (downfield from external H_3PO_4) δ +0.73.
(9) Main, P.; Germain, G.; Woolfson, M. M. MULTAN, a system from computer progams for automatic solution of noncentric crystal structures, York, Louvain, 1974.
(10) Johnson, C. K.; ORTEP, Report ORNL-3894, Oak Ridge National Laboratory, Oak Ridge, TN, 1965.

0002-7863/81/1503-2862$01.25/0

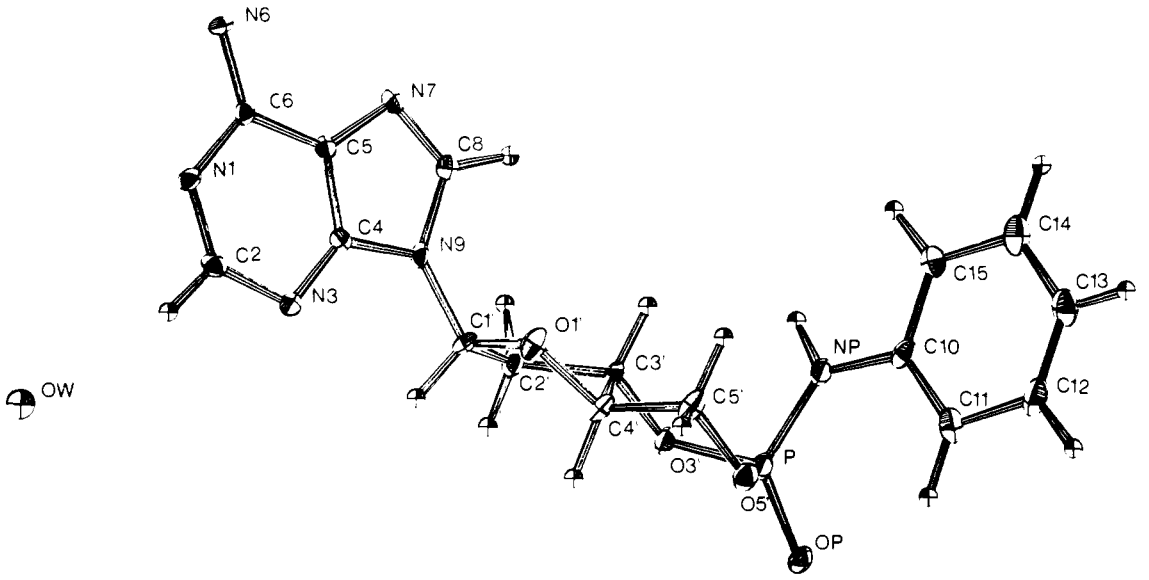

Figure 1. A plot of (R_p)-**1** showing thermal ellipsoids of the atoms at the 50% probability level.[10]

The adenin-9-yl residue is fairly planar and exists in an anti conformation relative to the ribose [κ_{CN} = O(1′)–C(1′)–N(9)–C(4) = −98.1 (8)°].[11] Ribose puckering is a characteristic C(4′)-*exo*,C(3′)-*endo*.[12] The orientation of the C(5′)–O(5′) bond is trans, gauche [∠O(5′)–C(5′)–C(4′)–O(1′) = 179.7 (6)°].[11] Due to the presence of a hydrating water in the crystal lattice, the molecules of **1** are linked via hydrogen bonds: OP–OW^{i}, 2.722 Å; NP–OW^{ii}, 2.89 Å; N(1)–OW^{iii}, 2.87 Å; N(6)–OW, 3.20 Å.

The unambiguous assignment of absolute configuration at phosphorus as (R_p)–**1** prompted us to synthesize the diastereomers of 2′-deoxyadenosine cyclic 3′,5′-[^{15}N]phosphoranilidate. Their synthesis was achieved by treatment of the triethylammonium salt of cdAMP with Ph_3P–CCl_4–[^{15}N]aniline.[13] (S_p)-**1**: R_f 0.33 [$CHCl_3$–EtOH (85:15)], ^{31}P NMR (C_5H_5N, downfield from H_3PO_4) δ +0.74, $^1J_{P-^{15}N}$ = 47.4 Hz; (R_p)-**1**: R_f 0.27, ^{31}P NMR (C_5H_5N, upfield from H_3PO_4) δ −3.21, $^1J_{P-^{15}N}$ = 36.7 Hz. The values of the direct spin–spin coupling constant $^1J_{P-^{15}N}$ fulfill the empirical rule: "For the pair of diastereomeric chair-shaped 1,3,2-dioxaphosphorinanes the direct spin–spin coupling constant between phosphorus and exocyclic magnetically active X (I = $^1/_2$; ^{1}H, ^{13}C, ^{15}N, ^{19}F, ^{77}Se) depends on the spatial orientation of X. $^1J_{P-X}$ acquires a lower absolute value for the isomer with the axially oriented X than for the one having X in the equatorial position".[2d,3,14]

$$|^1J_{P-X}|_{ax} < |^1J_{P-X}|_{eq}$$

In the light of recent work by Bentrude and Sopchik,[15] the correlation of the phosphorus configuration with $|^1J_{^{15}NP}|$ appears also valid even through one of diastereomeric forms of thymidine cyclic 3′,5′-phosphordimethylamidate[16] exists in solution largely in the nonchair dioxaphosphorinane conformation.

Other than the fundamental information about the solid-state structure of (R_p)-**1**, the precursor of 2′-deoxyadenosine cyclic 3′,5′-[^{18}O]phosphate[5c] and -phosphorothioate,[2b] this work clearly demonstrates the applicability of heteronuclear magnetic resonance for the unambiguous configurational assignments at the phosphorus atom in the diastereomeric 2-arylamino-2-oxo-1,3,2-dioxaphosphorinanes.

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(11) Saenger, W. *Angew. Chem. Int. Ed. Engl.* **1973**, *12*, 591.
(12) Chwang, A. K.; Sundaralingam, M. *Acta Crystallogr., Sect. B* **1974**, *B30*, 1233.
(13) Essentially the same procedure as described under ref 8 was applied by using [^{15}N]aniline, isotopic enrichment 52%.
(14) (a) Stec, W. J.; *Z. Naturforsch. B* **1974**, 29*B*, 109. (b) Stec, W. J.; Kinas, R. W.; Okruszek, A. *Ibid.* **1976**, *31B*, 393.
(15) Sopchik, A. E.; Bentrude, W. G. *Tetrahedron Lett.* **1980**, 4679.
(16) Stec, W. J.; Zielinski, W. S. *Tetrahedron Lett.* **1980**, 1361.

Molecular Structure of 10-Bromo-1,6-methano-2-aza[10]annulene[1]

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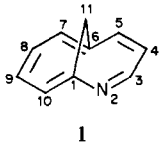
The new heterocyclic system 1,6-methano-2-aza[10]annulene (**1**), recently synthesized by Vogel et al.,[2] qualifies as an aromatic 10π analogue of pyridine in spectroscopic and—to some extent—chemical respects. A crystalline bromo substitution product of **1**, tentatively assigned the structure of 10-bromo-



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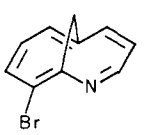

2

1,6-methano-2-aza[10]annulene (**2**), is obtained by successive treatment of **1** with bromine and the base 1,5-diazabicyclo-[4.3.0]non-5-ene (addition–elimination mechanism).[3]

As reported here, the X-ray structure analysis of this compound not only confirmed the formation of **2** but also allowed the full

(1) Systematic name 3-bromo-2,7-methanoaza[10]annulene; the above nomenclature is used in order to avoid confusion in structural comparisons with bridged [10]annulenes.
(2) Schäfer-Ridder, M.; Wagner, A.; Schwamborn, M.; Schreiner, H.; Devrout, E.; Vogel, E. *Angew. Chem.* **1978**, *90*, 894.
(3) Vogel, E.; Schwamborn, M.; Hilken, G., unpublished results.

0002-7863/81/1503-2863$01.25/0