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Conformational Transmission in Pentacoordinated Phosphorus Systems, Modelling the Activated State of Cyclic AMP

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ABSTRACT

The proposed activated state of cyclic adenosine 3',5'-monophosphate (cAMP) is modelled by two nucleoside cyclic 3',5' P'-TBP compounds 3 and 4. The reason for the design of compound 3, in which a probe fragment (O--CH2-CH2-OCH3 group) is linked to phosphorus, was reflected in a conformational transmission effect, which occurs when the probe is located in the axis of a P'-TBP. This means that the six-membered 3',5'-dioxaphosphorinane ring predominantly remains in a diequatorial (e,e) orientation. In the absence of conformational transmission, as in compound 4, the 3',5'-dioxaphosphorinane ring favors an equatorial-axial (e,a) orientation. From this we conclude that the occurrence of conformational transmission can stabilize the (e,e) orientation of the 3',5'-ring. This can be of relevance to the activation of protein kinase by cAMP. In order to obtain more structural information from 3 and 4, we performed MNDO calculations on the models 8-10. These calculations revealed that the (e,e) orientation of the 3',5'-ring is destabilized by 3-4 kcal/mol compared to the (e,a) orientation. For the (e,a) geometry, the 3',5'-ring adopts a twist conformation, whereas the (e,e) oriented 3',5'-ring shows a half-chair geometry.

INTRODUCTION

In the past decade, we have extensively studied conformational transmission in stabilized five-coordinated phosphorus (P') model compounds [1, 2]. A clear visualization of the conformational transmission effect is obtained by comparing the trigonal bipyramidal (TBP) P' system 1 with its four-coordinated phosphorus (PIV) counterpart 2 (Scheme 1).

Variable temperature 1H NMR experiments on compound 1 have revealed that trans orientation of O5 and O1', clearly predominates for the axially located tetrahydrofurfuryl group in 1, as a result of enhanced electrostatic repulsion between O5 (axial in the TBP), and O1' [3]. The two equatorial tetra-
hydrofurfuryl groups in 1, as well as the three equivalent tetrahydrofurfuryl groups in 2, showed a highly comparable preference for a O₅-O₇ gauche conformation [4]. The results on 1 unequivocally revealed that the intrinsic bonding properties of pentacoordinated phosphorus in the TBP geometry (leading to increased electron density for the axial sites, in comparison with the equatorial sites [1d]) are actually reflected in the conformational properties of the three tetrahydrofurfuryl substituents. Subsequent experiments have shown that the conformational transmission effect is not restricted to tetrahydrofurfuryl groups; e.g., the occurrence of conformational transmission was also clearly established for PV-TBP systems that entail one or more O-CH₂-CH₂-X substituents (X = OR, SR, NR₂) [1j], as well as for P⁴-TBP compounds modelling an activated state of phospholipids [1g, n] and phosphorylated sugars [1f, i].

In this paper, we focus on the P⁴-TBP compounds 3 and 4, which are model systems for the P⁴-TBP activated state of 3',5'-cyclic adenosine monophosphate (cAMP) [2, 5] (Scheme 2).

![Scheme 2](image)

We observed conformational transmission within the O-CH₂-CH₂-OCH₃ group in 3 (vide infra), from which it can be concluded that the O-CH₂-CH₂-OCH₃ group resides mostly in the axis of the TBP. Bearing in mind the ring strain rule [6] (stating that the tetrachloro-1,2-benzoquinone ring in 3 is in equatorial-axial (e,a) location), it then follows that the 3',5'-dioxaphosphorinane ring part of the time occupies a diequatorial (e,e) location in the TBP [2]. We feel that this application of the conformational transmission effect provides a unique clue in order to establish the location of the 3',5'-ring. For compound 4, in which evidently no conformational transmission can occur, we derived from the three-bond phosphorus-proton J-coupling measured for the methoxy protons that the 3',5'-ring is predominantly in equatorial-axial (e,a) location [1j, 2].

These experimental results are of relevance with respect to the original model description for the mechanism of activation of cAMP, as proposed by van Ool and Buck [5b]. It was proposed that activation of protein kinases by cAMP occurs via a diequatorial location of the 3',5'-dioxaphosphorinane ring (compare 3), whereas hydrolysis of cAMP by phosphodiesterases proceeds through a P⁴-TBP intermediate with (e,a) orientation of the 3',5'-ring (compare 4). Our present results on 3 and 4 confirm the idea that formation of a P⁴-TBP structure by a P⁴ → P⁴ activation within a 3',5'-cyclic nucleotide can result in two isomeric structures, i.e. (e,a) and (e,e), respectively.

In the following, we will briefly outline the experimental data that were obtained for 3 and 4. Moreover, the results of a set of MNDO model calculations on (e,a) and (e,e) P⁴-TBP systems will be described.

CONFORMATION OF THE P⁴-TBPs 3 AND 4

Location of the 3',5'-Dioxaphosphorinane Ring

The conformational analysis of the C₉-C₉ bond in 3 was based on the four vicinal H-H J-couplings (J₉₉₉₉, J₉₉₅₉, J₉₅₅₉, and J₅₅₅₉), obtained from the 400 MHz ¹H NMR spectrum of 3 in CD₂Cl₂ at -41°C. Iterative simulation of the subspectra was performed with a standard computer program (see Figure 1). The conformation around C₉-C₉ can be described as an equilibrium between one O₅-O₇ trans and two approximately degenerate O₅-O₇ gauche rotamers (+, -) (see Figure 2).

The value of ΣJ = J₉₉₅₉ + J₉₅₅₉ + J₅₅₅₉ can be calculated as a function of the O₅-O₇ torsion angle, using the empirically parametrized Karplus equation developed by Altona et al. [7]. The ΣJ values calculated for the trans rotamer and for the gauche(+) and gauche(-) rotamers are 30.0 Hz and 16.4 Hz respectively, leading to the formula:

\[ \alpha(O₅-O₇ \text{ trans}) = \frac{\Sigma J_{\text{exp}} - 16.4}{30.0 - 16.4} \times 100\% . \]

The conformational analysis of the C₉-C₉ bond in 3 showed a 51% population of the O₅-O₇ trans rotamer, and a total of 49% population of the O₅-O₇
I

FIGURE 1 Computer Simulated (upper trace) and Experimental (lower trace) Expansion of the H$_4$/H$_2$ Pattern in the 400 MHz $^1$H NMR Spectrum of Compound 3 at -41°C.

gauche rotamers. For comparison, an analogous conformational analysis was performed for the cis-phosphate and cis-phosphite counterparts of 3 (i.e., compounds 5 and 6, respectively) (Scheme 3).

In comparison with 3, these data (see Table 1) show considerably diminished O$_a$-O$_b$ trans populations (10% and 9% O-O trans for 5 and 6, respectively).

The location of the 3',5'-ring in compound 4 was determined qualitatively on the basis of the NMR coupling constant between phosphorus and the methoxy protons ($^3$J$_{POMe}$[1]j]. As a reference system for 4, we used compound 7 (Scheme 4). Due to pseudorotation around the P$^\pi$ center the experimental $^3$J$_{POMe}(7)$ represents a time-averaged value, i.e., the three methoxy groups are rapidly exchanged over

one axial and two equatorial sites in the TBP. Thus,

$$^3$J$_{POMe}(7) = \frac{1}{3}[^3$J$_{POMe}(ax) + 2^3$J$_{POMe}(eq)]$$

For the equatorial bonds the hybridization of phosphorus in a TBP results in an enlarged s-character in comparison with the axial ones [8], i.e., $^3$J$_{POMe}(equatorial) > ^3$J$_{POMe}(axial)$. We measured $^3$J$_{POMe}(4) = 14.3$ Hz, and $^3$J$_{POMe}(7) = 13.6$ Hz (400 MHz $^1$H NMR at 20°C; solvent CD$_2$Cl$_2$); i.e., the methoxy group in 4 has a preference for equatorial location. Combining this with the well-known ring strain rule, which states that five-membered rings preferentially adopt an (e,a) location in the TBP [6], reveals that the 3',5'-ring of 4 predominantly remains in an (e,a) location. Our experimental data on 4 do not discriminate between the possibilities of either rapid pseudorotation (on the NMR timescale), or complete absence of pseudorotation around the P$^\pi$-TBP. However, if pseudorotation does occur according to the well-known Berry mechanism [9], it is the methoxy group that will be acting as the pivot. In that case, the methoxy group will remain equatorial during the dynamic exchange of the other four ligands over the axial and equatorial sites in the TBP.

The conformational analysis of 3, 5, and 6 strongly indicates that conformational transmission occurs in 3. From this, we conclude that the dynamic equilibrium of phosphorus pseudorota-

FIGURE 2 Newman Projections of the Rotamers around the C$_3$-C$_4$ Bond.
Combining this with the ring rule (vide supra) [6], it follows that the 3',5'-ring in 3 is engaged in a pseudorotation mechanism [9], using either O₃ or O₅ as the pivot.

**Conformation of the 2'-Deoxyribose Ring**

The conformation of the sugar ring of 3–6 was performed with use of the PSEUROT program [10]. As input data, the sets of vicinal H–H coupling constants (J_H,H_P, J_H,H_C, J_H,H_O, and J_H,H_X) measured for each compound (see Table 2) were used. The best-fit conformational parameters of two sugar structures participating in a rapid conformational equilibrium was calculated, as well as the equilibrium composition.

Using the present data as basis, PSEUROT rapidly converged toward a single conformation that is characterized by a phase angle (P) of 35.9°, and a maximum puckering amplitude (ν_max) of 39.9° [11]. The five endocyclic torsion angles ν₀...ν₄ can be calculated from P and ν_max according to the formula ν = ν_max · cos(P + (j-2)·144°) [12], i.e., ν₀[Co₄–O₄–C₁–C₂] = -12.2°; ν₁[O₄–C₁–C₂–C₃] = -12.4°; ν₂[C₁–C₂–C₃–C₄] = 32.3°; ν₃[C₂–C₃–C₄–O₄] = -39.9°; ν₄[C₃–C₄–O₄–C₁] = 32.2°. From these results, we conclude that the structure of the 2'-deoxyribose ring in 3–6 resides in a twist (tíT) geometry (vide infra), which perfectly agrees with X-ray crystallographic studies on 3',5'-cyclic phosphites and phosphates [13].

**Conformation of the 3',5'-Dioxaphosphorinane Ring**

There is substantial interest in the conformational properties of dioxaphosphorinane rings attached to a P'-TBP structure, especially since the 3',5'-ring in CAMP is likely to undergo a chair into nonchair (boat and/or twist) conformation prior to, or concerted with, the formation of a P'-TBP intermediate [14]. The conformational analysis of the 3',5'-ring in 5 and 6 is based on the work of Bentrude et al. [15]. These studies showed that cis 3',5'-cyclic nucleotides normally have a chair geometry in which phosphorus and H₂ are anti-periplanar, while phosphorus and H₅ are in a gauche orientation. This conformation results in J_P₇.H₅ = 2.5 Hz, J_P₅.H₆ = 10.7 Hz for cis-phosphites [16] and J_P₅.H₆ = 22.5 Hz for cis-phosphates [17]. The vicinal H–P coupling constants fit into classical Karplus type relations with quite different parameterizations for phosphate and phosphite structures [15, 16]. Table 2 shows that J_P₇.H₅ and J_P₅.H₆ measured for the cis-phosphate 5 closely resembles the standard values for a chair conformation. Also the J_P₇.H₅, J_P₅.H₆ data as obtained for the cis-phosphate 6 corresponds to a chair geometry.

Recent studies of Bentrude et al. [14] are the basis for our conformational analysis of the 3',5'-ring in 3 and 4. The validity of a Karplus type equation for J_P₇ scalar couplings in P'-TBP systems is strongly suggested by these studies [14]. Antiperiplanar orientation of H and P across H–C–O–P and H–C–N–P coupling paths was found to result in large J_P₇ couplings (26.1 and 25.0 Hz, respectively), while much smaller values of J_P₇ were found for gauche orientations (8.7 and 5.5 Hz for H–C–O–P and H–C–N–P respectively). Large J_P₇ coupling constants (≈27 Hz) observed for 3 and 4 were interpreted as evidence for antiperiplanar orientation of P and H. We conclude from the data in Table 2 that phosphorus and H₂ are antiperiplanar in 3 and 4, while phosphorus and H₅ exist in a gauche conformation. It is clearly demonstrated that 3 and 4 populate a nonchair structure for the 3',5'-dioxaphosphorinane ring.

**MNDO CALCULATIONS ON (e,a) AND (e,e) P'-TBPs**

A better insight could be obtained into the molecular conformation of the P'-TBPs 3 and 4 by per-

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**TABLE 1 Values of J_H,H_P, J_H,H_C, J_H,H_O, and J_H,H_X for each compound (see Table 2).**

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<td>5.3</td>
<td>17.6</td>
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</table>

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**Scheme 4**

![Scheme 4](image-url)
forming a set of MNDO semi-empirical calculations [18] on the isomeric model systems 8, 9 (both (e,a)), and 10 (e,e). The experimental data describing the structure of the 2'-deoxyribose ring, the torsion angle [P–O5–C5–H5], and the torsion angle [O5–C5–C4–O1] (vide supra) were used as input values for the MNDO calculations. During the calculations, only the bond angles and torsion angles defining the TBP geometry were fixed. The obtained structures are illustrated in Figure 3; the calculated heats of formation of 8–10 are $-263.7$, $-262.6$, and $-259.8$ kcal/mol, respectively. From this, it should be noted that both (e,a) forms are almost equally stable, while the (e,e)-isomer is destabilized by approximately 3–4 kcal/mol.

In all three calculations the resulting optimized

<table>
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<th>Compound</th>
<th>$J_{H_1,H_2}$</th>
<th>$J_{H_1,H_2}$</th>
<th>$J_{H_1,H_3}$</th>
<th>$J_{H_1,H_4}$</th>
<th>$J_{H_2,H_3}$</th>
<th>$J_{H_2,H_4}$</th>
<th>$J_{H_3,H_4}$</th>
<th>$J_{H_5,P}$</th>
<th>$J_{H_6,P}$</th>
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<td>3</td>
<td>2.6</td>
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<td>9.1</td>
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<td>27.4</td>
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<td>3.3</td>
<td>8.4</td>
<td>8.4</td>
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<td>9.2</td>
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<tr>
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<td>9.2</td>
<td>8.3</td>
<td>10.4</td>
<td>9.2</td>
<td>10.6</td>
<td>4.7</td>
<td>&lt;1</td>
<td>22.4</td>
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conformations showed that the 2'-deoxyribose ring remained almost unchanged. Most probably, the limited structural freedom of the 2'-deoxyribose ring can be attributed to the trans fusion in our systems. However, the 3',5'-dioxaphosphorinane ring showed slight structural variations. For the (e,a)-isomers 8 and 9, it is found that the ring predominantly remains in a twist structure in which O₅, C₅, C₄, and O₃ are approximately in the same plane; the P atom is bent toward the exo side of this plane, while C₃ is located on the endo side. At the phosphorus end the dioxaphosphorinane ring of the (e,e) system 10 is flattened, as is obvious from the low values of the torsion angles [C₄-C₅-O₅-P] (−5.0° for 10, −31.8° and −27.3° for 8 and 9, respectively), and [C₅-O₅-P-O₃] (19.8° for 10, 50.6° and 40.6° for 8 and 9, respectively) (see Table 3) [19]. The conformation of the dioxaphosphorinane ring in 10 seems to fit best with a half-chair (chaise longue) in which C₃ acts as the back.

The present experimental and theoretical data clearly show that activation of cAMP via formation of a PV-TBP intermediate will induce a nonchair conformation of the 3',5'-dioxaphosphorinane ring, which is in complete agreement with the results of Bentrude et al. [14]. Furthermore, our experimental data show that formation of a PV-TBP structure may lead to (e,a) or (e,e) orientation of the 3',5'-dioxaphosphorinane ring. Although the MNDO calculations reveal that an (e,a) orientation is slightly energetically favorable, it appears from the data on 3 that conformational transmission in one of the ligands will help to stabilize the (e,e) isomer. This effect may be of importance with respect to the binding of cAMP to the regulatory subunit of protein kinases.

Based on the X-ray studies of the cAMP binding domain in the crystal structure of the bacterial catabolite gene activator protein (CAP) dimer [20], we have synthesized the PV-TBP model system 11, in which a serine group is linked to the PV-TBP (Scheme 5). The design of compound 11 was based on the close proximity of the CH₂-OH side-chain of serine-83 to the phosphorus atom of cAMP, i.e. the formation of a PV-TBP activated state in which the CH₂-OH of serine serves as the additional fifth ligand appears to be plausible. The resulting PV-TBP may show conformational transmission by virtue of enhanced charge repulsion between O₅ and N [11]; i.e., (e,e) orientation of the 3',5'-ring could be stabilized in this way. At present, the conformation of the PV-TBP 11 is not solved completely, but we succeeded in synthesizing compound 11 (R = H), as was evident from the 3¹P NMR spectrum (162 MHz; −20°C; solvent CD₂Cl₂; δ = −44.2).

**REFERENCES AND NOTES**

The authors wish to thank Mr. L. J. M. van de Ven for his help in the analysis of the ¹H NMR spectra and Mr. H. Eding for his assistance in the layout.

Five-membered dioxaphospholene rings have a pronounced preference for (e,a) location in a P^-TBP. (R. R. Holmes, J. Am. Chem. Soc., 100, 1978, 433; Pentacoordinated Phosphorus; American Chemical Society: Washington, D.C., 1980; Vols I, II; ACS Monograph No. 175, 176).


A half chair conformation of the 3',5'-dioxaphosphorinane ring in the case of (e,e)-orientation in the P^-TBP was also predicted by Yu and Bentrude. See ref. 14a.