A Common Structural Basis for the Inhibition of Ribulose 1,5-Bisphosphate Carboxylase by 4-Carboxyarabinitol 1,5-Bisphosphate and Xylulose 1,5-Bisphosphate

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Ribulose 1,5-bisphosphate carboxylase/oxygenase (Rubisco) catalyzes the carboxylation of ribulose 1,5-bisphosphate. The reaction catalyzed by Rubisco involves several steps, some of which can occur as partial reactions, forming intermediates that can be isolated. Analogues of these intermediates are potent inhibitors of the enzyme. We have studied the interactions with the enzyme of two inhibitors, xylulose 1,5-bisphosphate and 4-carboxyarabinitol 1,5-bisphosphate, by x-ray crystallography. Crystals of the complexes were formed by co-crystallization under activating conditions. In addition, 4-carboxyarabinitol 1,5-bisphosphate was soaked into preformed activated crystals of the enzyme. The result of these experiments was the release of the activating CO₂ molecule as well as the metal ion from the active site when the inhibitors bound to the enzyme. Comparison with the structure of an activated complex of the enzyme indicates that the structural basis for the release of the activator groups is a distortion of the metal binding site due to the different geometry of the C-3 hydroxyl of the inhibitors. Both inhibitors induce closure of active site loops despite the inactivated state of the enzyme. Xylulose 1,5-bisphosphate binds in a hydrated form at the active site.

The first step in the assimilation of atmospheric CO₂ into biomass, carboxylation of D-ribulose 1,5-bisphosphate (RuBP) is catalyzed by ribulose-1,5-bisphosphate carboxylase (Rubisco, EC 4.1.1.39) and yields two molecules of 3-phospho-D-glycerate (3-PGA). The reaction involves several enzyme-bound intermediates, illustrated in Fig. 1 (for reviews see Refs. 1 and 2). The abstraction of a proton from C-3 of RuBP results in the first intermediate, a 5-carbon enediol (compound II in Fig. 1) to which CO₂ is added. The enediol is unstable and can undergo various unwanted side reactions from the point of view of carbon assimilation. The most notable of the side reactions is the oxygenation of the enediolate by atmospheric O₂, as catalyzed by Rubisco (3–5). This reaction yields equal amounts of 3-PGA (compound V) and phosphoglycolate (compound VI); further metabolism of phosphoglycolate by photorespiration results in a vast loss of biomass. Rubisco also catalyzes the epimerization of RuBP via the enediolate to xylulose 1,5-bisphosphate (XuBP, compound VII) as a result of incorrect stereochemical reorientation of the enediol at C-3 (6–9). If misprotonation is instead directed at C-2, 3-ketoarabinitol 1,5-bisphosphate (compound VIII) or 3-ketoribitol 1,5-bisphosphate may be produced (10, 11). The epimerization products are all potent inhibitors of Rubisco.

Both carboxylation and oxygenation reactions are dependent on prior activation of the enzyme. Activation involves the reversible derivatization of a lysine residue by non-substrate CO₂ in the active site to form a labile carbamate that is subsequently stabilized by a magnesium ion (12, 13).

In the physiological reaction, substrate CO₂ reacts with the enediol to form a 6-carbon intermediate, 2-carboxy-3-keto-D-arabinitol 1,5-bisphosphate (compound III) or its hydrated gemdiol form (compound IV). This intermediate is sufficiently stable that it can be isolated by acid quenching of the enzyme (14). Two stereoisomers of an analogue of this intermediate, 2-carboxy-L-arabinitol 1,5-bisphosphate (2-CABP) and 4-carboxy-D-arabinitol 1,5-bisphosphate (4-CABP, Fig. 4) are exceptionally tight binding and virtually irreversible inhibitors of spinach Rubisco. The overall dissociation constants for 2-CABP and 4-CABP are 1.9 × 10⁻¹³ and 2.8 × 10⁻¹¹ M, respectively (15). Because cleavage of the gemdiol form of the 6-carbon intermediate is assumed to involve the abstraction of a proton from one of the hydroxyls, comparative affinities of 2-CABP and 4-CABP could reflect differences in the interaction of the enzyme with the hydroxyls of the intermediate that ultimately leads to cleavage (15).

We present here the structure of two inhibitor complexes with Rubisco from spinach, one with 4-CABP and the other with XuBP. The complex with XuBP, the C-3 epimer of the substrate RuBP, is meant to mimic the state of the enzyme before the entry of the gaseous substrates CO₂ or O₂. 4-CABP is the C-3 epimer of 2-CABP with a close similarity to the predominant form of the 6-carbon intermediate, the gemdiol form of 2-carboxy-3-keto-D-arabinitol 1,5-bisphosphate (16).

The structure of the 4-CABP complex therefore is expected to give information on this particular step in catalysis. XuBP and 4-CABP share the same configuration at the C-3 center (Fig. 4), a configuration different from the true substrate RuBP and intermediates resulting from it in the reaction on the enzyme. The structures could therefore give information on the stereochemical guidance of the reaction by the enzyme.

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The atomic coordinates and structure factors (codes 1RBO/1RBOSF and IRCO/IRCOSF) have been deposited in the Protein Data Bank, Brookhaven National Laboratory, Upton, NY.

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The abbreviations used are: RuBP, D-ribulose 1,5-bisphosphate; 3-PGA, 3-phospho-D-glycerate; Rubisco, ribulose 1,5-bisphosphate carboxylase/oxygenase; XuBP, D-xylulose 1,5-bisphosphate; 2-CABP, 2-carboxy-D-arabinitol 1,5-bisphosphate; 4-CABP, 4-carboxy-D-arabinitol 1,5-bisphosphate; r.m.s., root mean square.
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**EXPERIMENTAL PROCEDURES**

Both complexes were crystallized using spinach Rubisco purified as described earlier (17). XuBP and 4-CABP were obtained as a gift from G. Lorimer, DuPont.

### 4-CABP Complex

**Cocrystallization Experiment**—Crystals of Rubisco complexed with 4-CABP could be obtained by vapor diffusion from ammonium sulfate solutions under activating conditions in a way similar to procedures described for the 2-CABP complex (18). The crystallization mixture contained 100 mg/ml spinach Rubisco, 3 mM MgCl₂, 25 mM NaHCO₃, and 10 mM 4-CABP in 50 mM phosphate at pH 7.4. This represents a 5-fold increase in the concentrations of HCO₃⁻ and 10 mM 4-CABP in 50 mM phosphate at pH 7.4. This represents a significantly higher concentration of activating agent compared to previous studies (18). The inhibitor complex was formed by incubating a 60 mg/ml protein solution buffered at pH 8.0 with 25 mM HEPES, 10 mM MgCl₂, 50 mM NaHCO₃, and 10 mM 4-CABP. Crystals grew overnight at 4 °C, and final dimensions were 0.3 mm x 0.6 mm x 0.05 mm. Details of data collection, data reduction, and refinement are summarized in Tables I and II.

**Soaking Experiment**—As an alternative to cocrystallization, 4-CABP was soaked into preactivated crystals according to published procedures (19). The activated carbamylated enzyme was stabilized in the crystal by the presence of the product 3-PGA, 3-PGA, which binds relatively weakly to the enzyme (Kᵱ in the millimolar range) (20), can then be replaced by a tighter binding ligand by soaking in the compound into the preformed crystals. 4-CABP was added to crystals of activated Rubisco at a concentration of 8 mM, with the concentrations of NaHCO₃ and MgCl₂ maintained at 50 and 10 mM, respectively, throughout the soak. Soaking was continued for about 4 days prior to data collection. The crystals are isomorphous to cocrystallized 4-CABP crystals and diffract to a resolution beyond 2.1 Å. Refinement of the various complexes was performed similarly as for the cocrystallized complex, but the level of agreement could never be brought under 0.30. To verify this unexpected result, the experiment was repeated a second time, but this time data collection was performed at 100 K where a complete data set can be collected using a single crystal. Details of data collection, data reduction, and refinement are summarized in Tables I and II.

### XuBP Complex

Crystals of the complex with XuBP were grown by cocrystallization under activating conditions and vapor diffusion. An earlier report indicated that the XuBP complex might turn over at a slow rate if magnesium is used as an activator metal (8). Calcium that seems to form only non-productive complexes (21) was therefore used as a precaution. The complex was formed by incubating a 60 mg/ml protein solution buffered at pH 7.8 with 25 mM HEPES, 10 mM CaCl₂, 50 mM NaHCO₃, and approximately 20 mM XuBP for 10 min at room temperature. The protein solution was mixed in a 1:1 ratio with a well solution containing 15–16% polyethylene glycol 4000, 25 mM HEPES, pH 7.8, 0.2 mM NaCl, 50 mM NaHCO₃, and 10 mM CaCl₂. Crystals grew overnight at 4 °C, and final dimensions were 0.3 mm x 0.6 mm x 0.05 mm. Details of data collection, data reduction, and refinement are summarized in Tables I and II.

**RESULTS**

**4-CABP Complex**—Although the crystals were obtained under presumed activating conditions, the resulting complex did not have density either for the carbamate on Lys-201 or for the activator magnesium ion (Fig. 2A). The refined coordinates of the spinach Rubisco 2-CABP complex (Protein Data Bank entry 8ruc) (24) were used as a search model. For the rotation-function calculations using the program TFFC (22) and X-PLOR (23). The refined models of the activator carbamate and the inhibitor complex were similar in quality to the previously determined 2-CABP complex. The inhibitor is bound to the enzyme such that the carboxylate group on the compound is in the same position as that on 2-CABP in complex with the activated enzyme. In effect the only difference is the stereochemistry around C-3 (Fig. 4). Except for the lack of activator carbamate and metal in the 4-CABP structure, the 4-CABP and 2-CABP structures are extremely similar overall, the C⁺ atoms superimposing with a root mean square (r.m.s.) deviation of 0.15 Å on all C⁺ atoms. Differences at the active site consist of relatively few side chain movements. His-294 has moved around such that it forms a contact with the terminal amino group of Lys-201. Glu-60 from the symmetry-related large subunit that reaches into the active site is also in a clearly different conformation and interacts with the hydroxyl group on C-4 of 4-CABP (the equivalent of the C-2 hydroxyl of 2-CABP). Phe-127 is rotated, a change that seems consistent to the movement of Glu-60 (19). The remaining residues are not significantly different between the 4-CABP and 2-CABP complexes.

Data from crystals where 4-CABP was soaked into preformed crystals of Rubisco containing the activator carbamate, the magnesium ion, and 3-PGA were refined using a model derived from the data from the cocrystallized 4-CABP crystals (Table I). Despite extensive trials at refining the complex, it was not possible to reduce the R-factor below 0.30. Electron density maps calcu-
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**TABLE I**

Statistics for data collection and reduction for 4-CABP and XuBP complexes

|                  | 4-CABP cocrystallized | 4-CABP soaked | 4-CABP soaked | XuBP cocrystallized |
|------------------|------------------------|---------------|---------------|---------------------|
| Temperature of data collection (K) | 293                    | 100           | 283           | 283                 |
| Number of crystals | 1                      | 1             | 3             | 11                  |
| Maximum resolution (Å) | 2.3                    | 2.2           | 2.1           | 2.3                 |
| Space group       | C222                   | C222          | C222          | C222, P2_1,2         |
| Cell dimensions a, b, c (Å) | 157.2, 157.2, 201.3    | 155.8, 155.9, 199.6 | 157.4, 157.4, 201.5 | 220.6, 221.7, 115.3 |
| Number of measurements | 688,533               | 967,645       | 958,335       | 1,519,068           |
| Number of unique reflections | 108,532             | 101,538       | 123,599       | 217,862             |
| Completeness of data (%) | 90.7                   | 82.41         | 85.61         | 62.5                |
| Rmerge             | 0.059                   | 0.121         | 0.106         | 0.147               |

* Rmerge = \sum |Fo -Fc| / |Fo| (\sum |Fo|).

**DISCUSSION**

XuBP and 4-CABP Are C-3 Epimers of RuBP and 2-CABP—In the activated 2-CABP complex of spinach Rubisco, the C-3 hydroxyl and one of the oxygens of the carbohydrate are in contact with the magnesium ion. The way XuBP and 4-CABP are bound at the active site means that their C-3 hydroxyls come close (0.4 Å in the case of XuBP and 1.3 Å in the case of 4-CABP) to the position of the C-3 hydroxyl in the 2-CABP complex. Because of the different stereochemistry of the C-3 hydroxyl (Fig. 4) compared with 2-CABP (or the true substrate), this can only be achieved at the cost of a substantial distortion of the metal binding site.

Cocrystallization with XuBP and 4-CABP Results in Non-activated Complexes of the Enzyme with the Inhibitors—Catalytic activity of Rubisco from all species requires the presence of the metal in the active site. Both inhibitor complexes described here were prepared under conditions that produce activated enzyme forms (19). What crystallized in both cases were, however, inactivated complexes with only the inhibitors bound in the active site. The two inhibitors induced closures of flexible loops around the active site, identical to those observed in the activated 2-CABP complex (loop six of the αβ-barrel, a C-terminal segment, and a segment from the N-terminal region of the 2-fold related subunit), but the activating magnesium ion and the lysyl-bound CO₂ were lost, indicating that loop movements are independent of carbamylation or the presence of divalent metal (for further description of movements during Rubisco catalysis, see Ref. 19).

**TABLE II**

Statistics for refinement for the 4-CABP and XuBP complexes

|                  | 4-CABP cocrystallized | 4-CABP soaked | XuBP cocrystallized |
|------------------|------------------------|---------------|---------------------|
| Number of protein non-hydrogen atoms | 4939                   | 4939          |                     |
| Number of solvent molecules | 290                    | 238           |                     |
| Rfree             | 0.015                   | 0.011         |                     |
| Rfree             | 0.171                   | 0.171         |                     |
| r.m.s. deviation  | Bond lengths (Å) 0.113  | 0.113         |                     |
|                   | Bond angles (degrees) 1.886 | 1.822         |                     |

* R = \sum |Fo -Fc| / |Fo| (\sum |Fo|).
of the enzyme or by an initial binding to the activated enzyme with subsequent loss of the metal and the activator CO₂. Inhibition studies indicate that XuBP initially binds to the activated enzyme with weak affinity, as shown by an initial competitive inhibition phase with respect to the substrate RuBP (6). After 20 min the inhibition is essentially non-competitive, implying inactivation of the enzyme. The end product of this reaction is a complex between XuBP and the non-activated form of the enzyme (9, 11). Loss of either the carbamate or the metal will lead to deactivation, leaving the metal ligands free to find other binding partners. In the XuBP structure, O-3 and one of the C-2 diol oxygens form polar interactions with Glu-204 and Asp-203, respectively, interactions that would not be possible in the presence of the metal ion or with a different stereochemistry at C-3.

XuBP Binds as a Diol in the Active Site of Spinach Rubisco—

The electron density of XuBP is consistent with the inhibitor binding as a C-2 diol. This was also found in the XuBP complex of the enzyme from Synechococcus (25). It could be that binding of the diol is favored in this particular structure. Alternatively, XuBP could bind as a ketone to the enzyme, which then assists the hydration of XuBP to a diol. A number of residues are close enough to act as potential acids and bases, thereby facilitating diol formation. Lys-175 and Lys-177 both approach the area which the carbonyl oxygen of the keto-form might be expected to occupy in such a complex and may polarize the carbonyl bond for a nucleophilic attack by a water to form a diol. Asp-203 and Glu-60 are sufficiently close to act as proton acceptors. Protonation of Asp-203 would destabilize the magnesium coordination and favor loss of the metal, but the conspicuous movement of Glu-60 with respect to the activated form of the enzyme might suggest this as a potential base. A curious difference between the Synechococcus and the spinach enzymes is that the latter displays “fallover” (7), the gradual loss of activity with time accompanied by production of XuBP in vitro. This phenomenon has not been detected in the Synechococcus enzyme (10). Given the very high degree of structural similarity of the XuBP complexes from spinach and Synechococcus (r.m.s. difference for all Cα atoms of the L subunits was 0.38 Å), the explanation for the different kinetic behavior must be linked to other differences in these enzymes. An obvious possibility is to consider differences in the stability of the enediolate on the enzyme (10) as well as differences in affinities for RuBP and XuBP. Such differences could result in different likelihoods for
expulsion of the activator groups from the Michaelis complex and for misprotonation of the enediol to form XuBP.

\[ \text{4-CABP Complex} - \text{The binding of this inhibitor to Rubisco is not as extensively studied, but it has so far been assumed that it can form a stable complex with the magnesium-containing, carbamylated form of the enzyme (15).} \]

From the present x-ray diffraction study, it is evident that 4-CABP forms a very stable binary complex with the non-carbamylated metal-free enzyme, a stability that is reflected by the well ordered crystals that can be grown from the complex (the limit of diffraction is about 1.5 Å). In contrast to cocrystallization studies, the soaking experiments with activated crystals are inconclusive in structural

\[ \text{G. Lorimer, personal communication.} \]

**Fig. 3. Comparison of the active site structures in the 2-CABP complex and the 4-CABP complex.** The magnesium ion and the carbon atoms in the 2-CABP complex are colored gray and carbon atoms in the 4-CABP complex are colored yellow. The rest of the atoms have identical colors in both complexes. Coordinates for the 2-CABP complex of spinach Rubisco are from the 1.6-Å resolution refined structure (Protein Data Bank entry 8RUC) (24). Superposition of the structures was carried out using the graphics program O (29). The picture was rendered with RASTER3D (30).

**Fig. 4. Stereochemistry of the substrate RuBP and various inhibitors of Rubisco.**

**Fig. 5. Comparison of the active site structures in the 4-CABP complex and the XuBP complex.** Carbon atoms in the 4-CABP complex are colored yellow and carbon atoms in the XuBP complex are colored green. The rest of the atoms have identical colors in both complexes. Superposition of the structures was carried out using the graphics program O (29). The picture was rendered with RASTER3D (30).
terms but in line with the notion (15) that initially an activated metastable quaternary E-CO$_3$-Mg-4-CABP complex is formed but that this complex slowly rearranges to a more stable non-activated E-4-CABP binary complex, thus expelling the carbamate and the metal from the active site. The data set obtained can be interpreted as a mixture of the two states. Similar soaking experiments using 2-CABP or RuBP did not show this behavior, indicating that there is no inherent problem with the soaking procedure. In the co-crystallization experiment, there is ample time for the expulsion of the metal from the complex to happen before the onset of crystallization. In the soaking experiment, this process is much slower due to a restriction in conformational flexibility of the molecules imposed by the crystal lattice. A similar behavior could hold for the XuBP complex, i.e. initial binding to the activated enzyme with subsequent expulsion of the carbamate and the metal due to the different stereochemistry of the C-3 hydroxyl, and this is corroborated by the inhibition studies by McCurry and Tolbert (6).

The Metal May Be Important in Guiding the Ligand into the Active Site—This work shows that XuBP and 4-CABP bind in the same orientation as the activated complex of 2-CABP. Earlier soaking experiments with 2-CABP into crystals of non-activated Rubisco from *Rhodospirillum rubrum* (26) and tobacco (27) showed the ligand binding with the P1 phosphate in the P5 binding site and vice versa. These findings could be explained with the metal directing the ligand in the active site in the early stages of the binding in the present study. No such guidance was possible in the earlier soaking studies (26, 27).

2-CABP and 4-CABP (Fig. 4) are both close mimics of the gemdiol form of the 6-carbon intermediate, 2-carboxy-3-keto-1,5-arabinitol 1,5-bisphosphate (Fig. 1, compound JV). In the case of 2-CABP a stable activated complex can be formed whereas the configuration of 4-CABP seems to induce strain, which leads to the expulsion of the activator metal and carbamate on Lys-201 from the active site. This strain could be used in catalysis to drive the breakage of the C-2-C-3 bond in the non-productive complexes it leads to deactivation. The hydration at C-2 of XuBP could be a consequence of the loss of the metal and not the cause of it.

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REFERENCES

1. Andrews, T. J., and Lorimer, G. H. (1987) in *The Biochemistry of Plants: A Comprehensive Treatise* (Hatch, M. D., and Boardman, N. K., eds) Vol. 10, pp. 131–218, Academic Press, New York
2. Hartman, F. C., and Harpel, M. R. (1984) *Annu. Rev. Biochem.* 63, 197–234
3. Bowes, G., Ogren, W. L., and Hageman, R. H. (1971) *Biochem. Biophys. Res. Commun.* 45, 716–722
4. Andrews, T. J., Lorimer, G. H., and Tolbert, N. E. (1973) *Biochemistry* 12, 11–18
5. Lorimer, G. H., and Andrews, T. J. (1973) *Nature* 243, 359–360
6. McCurry, S. D., and Tolbert, N. E. (1977) *J. Biol. Chem.* 252, 8344–8346
7. Edmondson, D. L., Badger, M. R., and Andrews, T. J. (1990) *Plant Physiol.* (Bethesda) 93, 1390–1397
8. Yokota, A. (1991) *Plant Cell Physiol.* 32, 755–762
9. Zhu, G., and Jensen, R. G. (1991a) *Plant Physiol.* (Bethesda) 97, 1348–1353
10. Edmondson, D. L., Kane, H. J., and Andrews, T. J. (1990) *FEBS Lett.* 269, 62–66
11. Zhu, G., and Jensen, R. G. (1991b) *Plant Physiol.* (Bethesda) 97, 1354–1358
12. Laing, W. A., and Christeller, J. T. (1976) *Biochem. J.* 159, 563–570
13. Lorimer, G. H., Badger, M. R., and Andrews, T. J. (1976) *Biochemistry* 15, 529–536
14. Lorimer, G. H., Gutteridge, S., and Madden, W. M. (1987) in *Plant Molecular Biology* (von Wettstein, D., and Chua, N.-H., eds), pp. 21–31, Plenum Press, New York
15. Schloss, J. V. (1988) *J. Biol. Chem.* 263, 4145–4150
16. Cleland, W. W. (1990) *Biochemistry* 29, 3194–3197
17. Anderson, I., Tjader, A.-C., Cedergren-Zeppezauer, E., and Brandén, C.-I. (1983) *J. Biol. Chem.* 258, 14088–14090
18. Anderson, I., and Branden, C.-I. (1984) *J. Mol. Biol.* 172, 363–366
19. Taylor, T. C., and Anderson, I. (1996) *Nat. Struct. Biol.* 3, 95–101
20. Borden, T. D., and Müller, D. D. (1983) *Biochem. Int.* 6, 93–99
21. Barcena, J. A. (1985) *Biochem. Int.* 7, 755–760
22. Collaborative Computing Project 4 (1994) *Acta Crystallogr.* Sec. A 50, 760–763
23. Brüning, A. T. (1992) *X-PLOR*, Version 3.1, Yale University Press, New Haven, CT
24. Anderson, I. (1996) *J. Mol. Biol.* 259, 160–174
25. Newman, J., and Gutteridge, S. (1994) *Structure* 2, 495–502
26. Lundqvist, T., and Schneider, G. (1989) *J. Mol. Biol.* 204, 7078–7083
27. Zhu, K. Y. J., Cascio, D., and Eisenberg, D. (1994) *Protein Sci.* 3, 64–69
28. Kraulis, P. J. (1991) *J. Appl. Crystallogr.* 24, 946–950
29. Jones, T. A., Zou, J.-Y., Cowan, S. W., and Kjeldgaard, M. (1991) *Acta Crystallogr.* Sec. A 47, 110–119
30. Merritt, E. A., and Murphy, M. E. P. (1994) *Acta Crystallogr.* Sec. D 50, 869–973
31. Otwinowski, Z. (1990) in *Proceedings of the CCP4 Study Weekend*, pp. 56–62, Daresbury Laboratory, Warrington, UK
32. Engh, R. A., and Huber, R. (1991) *Acta Crystallogr.* Sec. A 47, 392–400
33. Brüning, A. T. (1992) *Nature* 359, 472–474
34. Laskowski, R. A., MacArthur, M. W., Moss, D. S., and Thornton, J. M. (1993) *J. Appl. Crystallogr.* 26, 283–291

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3 T. Taylor and I. Andersson, unpublished results.

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| XUBP atom | Protein/water atom distance | 4CABP atom | Protein/water atom distance |
|-----------|-----------------------------|-------------|-----------------------------|
| O1P       | G404 N 2.7 O4P G404 N 2.8  | T65 O1G1 2.7 T65 O1G1 2.8 |
| O2P       | G403 N 2.8 O5P G403 N 2.9  | WAT O 2.5 WAT O 2.8 |
| O3P       | G381 N 2.9 O6P G381 N 2.8  | K334 NZ 3.1 K334 NZ 2.8 |
| O4        | O5 K175 NZ 3.2              | O4 D203 OD2 3.1 WAT O 3.0 |
| O52       | K334 NZ 3.2                 | E60 OE2 2.6 |
| O22       | D203 OD2 3.1 K175 NZ 3.0   | O6 N123 ND2 3.1 E60 OE1 3.1 |
| O3        | D204 OE1 2.9 S379 OG 3.0   | O3 S379 OG 3.0 |
| O4        | S379 OG 2.8 O7 S379 OG 3.0 | O7 E60 OE1 2.5 K334 NZ 2.9 |
| O5P       | R295 NH2 2.8 O1P R295 NH2 3.0 | WAT O 2.7 WAT O 2.7 |
| O5P       | H327 ND1 2.7 O2P R327 ND1 2.7 | WAT O 2.9 WAT O 2.6 |
| O6P       | R295 NE 3.2 O3P R295 NE 2.9 | WAT O 2.9 WAT O 2.6 |
|           | O6 WAT O 2.6                | O6 WAT O 2.6 |

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a See Fig. 4 for numbering convention of atoms.