Crystal Structure of Carbapenem Synthase (CarC)*

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The proposed biosynthetic pathway to the carbapenem antibiotics proceeds via epimerization/desaturation of a carbapenam in an unusual process catalyzed by an iron- and 2-oxoglutarate-dependent oxygenase, CarC. Crystal structures of CarC complexed with Fe(II) and 2-oxoglutarate reveal it to be hexameric (space group C222₁), consistent with solution studies. CarC monomers contain a double-stranded β-helix core that supports ligands binding a single Fe(II) to which 2-oxoglutarate complexes in a bi-dentate manner. A structure was obtained with L-N-acetylproline acting as a substrate analogue. Quantum mechanical/molecular mechanical modeling studies with stereoisomers of carbapenams and carbapenems were used to investigate substrate binding. The combined work will stimulate further mechanistic studies and aid in the engineering of carbapenem biosynthesis.

Carbapenems possess a broad spectrum of antibacterial activity and are relatively stable to serine β-lactamases that are a major cause of resistance to penicillins and cephalosporins (1, 2). Carbapenems, such as thienamycin, were first isolated from bacterial extracts, including those from Serratia marcescens, Erwinia carotovora, and Streptomyces cattleya (3, 4). Early attempts to improve the fermentation titers to commercially useful levels were unsuccessful (5), and carbapenem use is, in attempts to improve the fermentation titers to commercially useful levels were unsuccessful (5), and carbapenem use is, in

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mediated production of an active carbapenem antibiotic and describe crystal structures of CarC that define its active site structure thus enabling mechanistic and engineering studies aimed at altering its selectivity.

**EXPERIMENTAL PROCEDURES**

**CarC Cloning, Expression, and Purification**—A PCR-amplified DNA product corresponding to the *E. carotovora* carC gene (8) was engineered as an Ndel-BamHI fragment into the pET24a expression vector (Novagen) and transformed into *E. coli* BL21(DE3) supercompetent cells. Cells were grown in shake flasks at 37 °C using 2YT medium containing 50 μg/ml kanamycin. At A_{600} 0.8, cells were induced with 0.5 mM isopropyl-1-thio-

**Bioassay**—Assay mixtures were transferred into holes (11-mm diameter) bioassay plates (*E. coli* X580) and incubated at 37 °C overnight. A typical assay mixture consisted of Tris-HCl, pH 9 (10 mM), 2-oxoglutarate (200) (10 mM), MgCl2 (2 mM), ATP (3 mM), CMP (3 mM), CarA (2.5 mg/ml), CarC (1.6 mg/ml), and FeSO4 (1 mM). The assay was incubated at 37 °C for 30 min. Supernatant was filtered (0.22-/m, Millipore), then loaded onto a DEAE-Sepharose FF column (30 ml) (Amersham Biosciences) at 20°C using 2TY medium (0.2 M Tris-HCl, pH 8.0, and 1 mM EDTA). Fractions containing CarC were pooled, concentrated, and applied to an S75-Superdex column equili-
There was one Ramachandran outlier whose conformation appeared to be supported by the electron density (Leu-108 in subunit B) (92.8%, 0.0%, and 0.2% in the core, allowed, generously allowed, and disallowed regions, respectively). The electron density maps indicated that t-NAP was present in subunits B and C but at a much lower level, 7.0%, 0.0%, and 0.2% in the core, allowed, generously allowed, and disordered regions, respectively. Note that, although the submitted PDB file (1NX8) indicates one orientation (orientation I, see "Results and Discussion"), the orientation of t-NAP within the active site could not be unequivocally inferred from the electron density maps and a second orientation (II) is possible.

Molecular Modeling—Hydrogens were added to the complex using the HBUILD routine of CHARMM (version 27) (29). The final model included the protein atoms, the t-NAP ligand, 2OG, Fe(II), and 39 crystallographic waters, 4119 atoms in total. Models of the structure complexed with (3S,5R)- and (3S,5R)+carbapenems were prepared using QUANTA (30). Modeling studies were carried out with both carbapenems, using orientations I and II of t-NAP as initial "templates." In each case, a full occupancy oxygen atom was added (at a distance of 2.2 Å from Fe(II)) in the ligation position opposite to His-251 (the proposed catalytic cycle involves an Fe(IV)═O intermediate). Without this oxygen, the minimization resulted in the substrate carboxylate ligating to Fe(II). The involvement of such an iron-substrate carboxylate complex in catalysis seems unlikely both with respect to precedent (31) and on mechanistic grounds but cannot be ruled out. Each of these models contained 4119 atoms. A combined quantum mechanical/molecular mechanical (32, 33) (QM/MM) potential was used to perform minimizations of the model systems, while keeping Fe(II) and the atoms that are bound to it (His-101-NH2, Asp-103-OH, His-251-NH2, 2-keto, and 1-carboxylate oxygens of 2OG, and the additional oxygen atom in the two carbapenam systems) fixed in their original positions (Table II). In each case the ligand was described by a QM potential and the rest of the system by a coupled MM potential. The CHARMM standard all-atom parameters (34) were used in the MM region, except for the oxygen in the empty ligation position on Fe(II), which was given a charge of –1, and for the 2OG, whose charges were calculated by fitting them to the B3LYP/6–31G* electrostatic potential in vacuum (35), using Gaussian98 (36). For non-bonded interactions, the electrostatics terms were truncated with a force switch function between 10 and 14 Å and the van der Waals terms with a shift function with a cutoff distance of 14 Å (37). The QM region was treated with the semi-empirical quantum mechanical method AM1 (38) implemented within CHARMM (33). The QM/MM minimizations included the steepest descent method followed by the Adopted Basis Newton-Raphson method implemented in CHARMM, until the average r.m.s. gradient was less than 0.01 kcal mol⁻¹ Å⁻¹.

### Table I

| Data collection, phasing, and refinement statistics |
|----------------------------------------|
| Numbers refer to residues. Rmerge = Σh | I(h) – Σh | I(h) / Σh | × 100, where I(h) are integrals of symmetry-redundant reflections, and | I(h) is the mean intensity for reflection h. |
| | | | |
| Space group | C222₁ |
| Dimension | a (Å) 79.9 | 80.4 |
| b (Å) 163.9 | 164.1 |
| c (Å) 146.5 | 146.3 |
| Molecules per asymmetric unit | 3 |
| Temperature (K) | 100 |
| Wavelength (Å) | 0.9785 |
| Resolution (Å) | 2.40–30 (2.40–2.46) |
| Observations | 173615 |
| Unique reflections | 37852 |
| Completeness (%) | 99.7 (99.4) |
| Rmerge (%) | 6.3 (29.1) |
| (I/σ(I)) | 9.9 (2.5) |
| Rcryst | 0.223 |
| Rfree | 0.286 |
| r.m.s.d. | 0.018 |
| Bond length (Å) | 1.887 |
| Bond angle (°) | 1.725 |
| Disordered residues | |
| A | 67–78/161–172 |
| B | 68–72/161–170 |
| C | 69–79/162–172 |
| Average B-factor | 23, 34 |
| PDB ID code | 1NX4 |

### Table II

| Interatomic distances and relative energy data for QM/MM calculations of the stability of carbapenems modeled into the active site of CarC CHARMM/AM1 |
|----------------------------------------|
| Distance (Å) |
| | |
| Model | Relative energy (kcal/mol) |
| | Fe(C2-H) | Fe(C2-H) | Fe(C3-H) | Fe(C5-H) | Fe(C6-H) | Arg267-H112 | Arg267-H112 | Gly104-H80 | Gly105-H80 | Leu106-H80 | Ala107-H80 | Tyr191-H80 | Gly104-H80 | Gly105-H80 | Gly106-H80 | Leu106-H80 |
| 1. (3S,5S)* | −7028 | 6.7 | 8.1 | 7.7 | 4.2 | 5.4 | 6.9 | 2.0 | 1.9 | 3.3 | 2.3 | 2.1 | 2.1 | 19 | 4.1 | 2.5 | 3.0 | 5.0 |
| 2. (3S,5R)* | −7032 | 6.6 | 8.0 | 8.3 | 7.3 | 4.4 | 5.7 | 2.1 | 1.9 | 1.9 | 2.4 | 3.4 | 4.6 | 2.0 | 3.8 | 2.5 | 2.0 | 4.6 |
| 3. (3S,5S)* | −7028 | 5.2 | 5.0 | 5.8 | 8.7 | 10.0 | 9.0 | 2.0 | 1.8 | 5.4 | 6.5 | 5.9 | 5.8 | 4.8 | 3.4 | 2.1 | 2.4 | 2.1 |
| 4. (3S,5R)* | −7028 | 5.2 | 4.9 | 5.2 | 8.5 | 8.6 | 8.8 | 2.0 | 1.8 | 5.1 | 6.3 | 5.6 | 5.4 | 6.2 | 5.0 | 4.3 | 3.8 | 2.1 |
| 5. (5R) | 10.5 | 6.9 | 5.1 | 6.5 | 9.1 | 8.8 | 4.3 | 1.8 | 2.1 | 1.8 | 3.6 | 2.2 | 2.8 | 2.3 | 4.3 |
| 6. (5S) | 10.7 | 8.6 | 6.3 | 6.3 | 9.2 | 9.1 | 4.4 | 2.0 | 2.0 | 1.8 | 3.9 | 2.0 | 2.4 | 2.6 | 4.3 |

* Values refer to residues. Rmerge = Σh | I(h) – Σh | I(h) / Σh | × 100, where I(h) are integrals of symmetry-redundant reflections, and | I(h) is the mean intensity for reflection h. |

** r.m.s.d., root mean square deviation from ideality. |

* Protein, solvent, and substrate, respectively. 

* All distances are between hydrogens and heavy atoms. The values are in Angstroms. 

** Models built based on t-NAP orientation II. 

* Models built based on t-NAP orientation I.
RESULTS AND DISCUSSION

The CarC Reaction—The proposed carbapenam-3-carboxylic acid intermediate was prepared from the appropriate β-amino acid precursor using CarA.2 The carC and carA genes were cloned from the E. carotovora genomic DNA and expressed in E. coli, and the corresponding proteins were purified by standard techniques. The (5S-carboxymethyl)-S-proline (trans-CMP) putative substrate for CarA was prepared via minor modification of reported methodology (39).

Assays with CarA alone, and combined CarA/CarC assays, containing the appropriate cofactors and potential CarA substrates, were conducted. With the trans-CMP substrate, no antibiotic activity was observed in assays with CarA alone. With trans-CMP in the presence of both CarA and CarC, a clear zone of activity was observed. Liquid chromatography-MS of CarA assay mixtures using trans-CMP led to the observation of a new peak with a mass corresponding to a carbapenam (negative ion electrospray: 154 Da [M-H]−) absent in controls. With trans-CMP in the combined CarA/CarC reactions, peaks were observed for masses corresponding to both a carbapenam and a carbapenem (negative ion electrospray: 152 Da [M-H]−), again absent in controls.

Assuming that CarA does not catalyze epimerization, the results imply that CarA can mediate β-lactam ring formation from trans-CMP to give (3S,5S)-carbapenam (Scheme 1, path a). They also suggest that the (3S,5S)-carbapenam can be converted by CarC to the (5R)-carbapenem. Because it cannot be entirely ruled out that the (3S,5S)-CMP used in this study was contaminated with a low level of its (3R,5R)-enantiomer, on the basis of our data alone the possibility that the natural substrate for CarC is a (3R,5R) carbapenem cannot be discounted (Scheme 1, path b); however, this would be in conflict with the results and conclusions of both Li et al. (12) and Bycroft et al. (14, 21). The level of substrate conversion effected by CarA with the trans-CMP was low compared with that of β-lactam synthetase (from the clavulanic acid biosynthesis pathway) with its natural substrate, possibly indicating an alternative in vivo substrate (see Scheme 1) or that a multiprotein complex is required to effect full activity (11). The organization of β-lactam biosynthesis proteins into a metabolon has also been suggested for clavulanic acid (40).

Crystallization and Oligomerization—Crystals of CarC complexed with Fe(II) and 2OG were obtained under anaerobic conditions. Crystals were also obtained anaerobically for CarC together with Fe(II), 2OG, and a substrate analogue (N-acetyl-L-proline) (see below). The structure was solved by molecular replacement using the model of SeMet-substituted CarC complexed with Fe(II).

Analysis of crystallographic symmetry revealed that CarC crystallizes as a hexamer comprised of two trimers (ABC and DEF in which A = D, B = E, and C = F) (C2221) (Fig. 1). Each asymmetric unit contains three monomers in a trimeric arrangement, with a hexamer being generated by a 2-fold crystallographic symmetry axis. Gel filtration and native gel electrophoresis studies also indicated that the predominant form of CarC in solution is also hexameric with low levels of monomeric and trimeric forms also being observed (molecular mass by analytical gel filtration: ~200 kDa).

Within each trimer the monomers are arranged such that the
active sites are well separated and directed toward the exterior of the hexamer, which possesses a large central channel. Hydrogen bonds and electrostatic interactions form links between the monomers and link the ABC and DEF trimers to form the hexamer; residues from H9251 to H9256 on the A subunit interact with the loop linking the H9252 and H9253 strands on the B-subunit.

With respect to interactions between the ABC and DEF trimers, A interacts most closely with E (the symmetry-equivalent of B), whereas B interacts with D (the symmetry-equivalent of A). C, however, interacts with F, its own symmetry-equivalent. This difference results from a crystallographic 2-fold symmetry axis that runs between A/E and B/D pairs but between C and F monomers (Fig. 1b). In the case of the A/E interactions, residues from the N terminus, H9251 and H9252, from the A subunit interact with their counterparts on the E subunit to form the hexamer.

Overall Structure of the Monomer—The structures of the A, B, and C monomers are similar but not identical (the r.m.s. deviations of the Ca atoms for the AB, BC, and CA pairs were 0.27, 0.26, and 0.17 Å, respectively). In the following discussions, the descriptions refer to the B monomer. The CarC main chain contains 14 β-strands, 8 of which (β1–β4, β6, and β11–β14) combine to form the distorted double-stranded β-helix (DSBH or jellyroll) motif characteristic of the 2OG oxygenase superfamily that includes CAS (41), taurine dioxygenase (TauD) (42), and proline 3-hydroxylase (43) (Fig. 2). A DSBH is found in a wide range of metal binding (including the Cu(II)-utilizing quercetin 2,3-dioxygenase (44)) and non-metal-binding proteins.

The presence of an extended insert (residues 135–225 (α4–α6 and β7–β10)) between the fourth and fifth strands of the DSBH places CarC within a distinct sub-group of 2OG oxygenases that includes CAS (45) but not deacetoxycephalosporin C synthase (DAOCS). The structural similarity between CarC and CAS (23% sequence identity) reinforces proposals that clavam and carbapenem biosynthesis have a close evolutionary relationship (8).

Active Site and 2-Oxoglutarate Binding—The active site is predominantly made up of residues from strands β13, β14, and β6 of the DSBH (His-251, Arg-253, Arg-263, Arg-267, Gln-269, and Thr-130). The remainder of the active site is constructed from two loops: those linking β3/β4 (His-101, Asp-103, Gly-104, Gly-105, Leu-106, and Ala-107) and those linking β9/β10 (Tyr-191, Phe-194, and Trp-202) with the latter on β10. The entrance to the active site is formed by the extended loop linking β3/β4 (residues 85–111) and, probably, by the loops linking α3/β4 and β7/β8, which are disordered.

The 5-carboxylate of 2OG is bound by the side chains of Arg-263 (β14, 3.2 and 2.9 Å), Thr-130 (β6, 2.7 Å), and Arg-253 (β13, 3.0 Å). Arg-263 and Thr-130 are conserved in CAS and
TauD (42), although the presence of Arg-253 is distinct to CarC. The 2-keto- and 1-carboxylate groups of 2OG bind in a bi-dentate manner to the single iron, which is ligated by side chains from His-101, Asp-103, and His-251, all either part of or close to the DSBH (Fig. 3). These residues form a conserved 2-His-1-carboxylate triad of residues (46). CarC differs from CAS, but not TauD, in that its carboxylate ligand comes from an Asp rather than a Glu residue, highlighting the special nature of CAS in this regard. In the CarC-Fe(II)-2OG structure, a water molecule is ligated to the ferrous iron opposite His-101 thus giving a six-coordinate arrangement. In the CarC-Fe(II)-2OG structure, the 2OG is ligated such that its 2-keto-group is opposite Asp-103, consistent with results for other 2OG oxygenases. The relative arrangement of Fe(II) ligands in the structures of anthocyanidin synthase and DAOCS positions the 2OG 1-carboxylate opposite His-251 (using the CarC numbering system); however, in TauD and CAS the 2OG 1-carboxylate is opposite His-101 (using the CarC numbering system). With CarC the 2OG 1-carboxylate appears to be in an intermediate position but is closer to being opposite to His-251.

When a CAS-Fe(II)-2OG-substrate complex was exposed to NO, acting as a dioxygen analogue, a rearrangement occurring placed the 2OG 1-carboxylate opposite His-251 (using the CarC numbering system), suggesting that in all cases dioxygen binding may occur trans to His-101 (31). However, as for related oxygenases, the substrates or substrate analogues (i.e. L-NAP for CarC) may not be correctly positioned to effect substrate oxidation if the ferryl species is formed opposite to His-101. It has been proposed that, following decarboxylation of 2OG, the ferryl species rearranges to be opposite His-251 and adjacent to the substrate (31). Alternatively, it cannot be ruled out that the 1-carboxylate can rearrange such that dioxygen can then bind opposite to His-251.

Substrate Binding—In addition to the Fe(II)-2OG complex and associated residues, the active site cavity comprises a largely hydrophobic region including Leu-98, Leu-106, Phe-194, Tyr-191, and Trp-202. The latter three are arranged such that the indole ring of Trp-202 is sandwiched by the aromatic rings of Phe-194 and Tyr-191 in an approximately orthogonal manner. These residues form a hydrophobic wall on one side of the active site cavity (Figs. 2 and 5). On another face of the active site the backbone amide N-Hs of Gly-104, Gly-105, and Leu-106 and the side chains of Ser-109 and Tyr-191 form a notable sequence of H-bond donors. The phenolic OH of Tyr-191 points toward the proposed substrate binding area.

A 2OG turnover assay was used to screen a limited range of substrate analogues for binding to CarC. L-NAP and β-NAP were found to inhibit 2OG turnover (~60 and 25% of uncoupled turnover respectively). L-NAP was then used in cocrystallization experiments. The occupancy level of L-NAP differs between the three monomers A, B and C, from partial occupancy in B and C to very limited occupancy in A.

The CarC-Fe(II)-2OG-L-NAP structure is very similar to that of the CarC-Fe(II)-2OG complex. A water molecule is probably ligated to the ferrous iron opposite to His-101, possibly reflecting the observation that L-NAP inhibits (uncoupled) 2OG turnover. A strategy for potential inhibition of 2OG oxygenases is thus to hinder oxygen binding via stabilization of octahedral iron coordination chemistry. Due to the similar size of the acetyl and carboxylate groups of the L-NAP and its partial occupancy, it was not possible to unequivocally assign the orientation of L-NAP within the active site. Thus two possible orientations of L-NAP (I and II) related by a rotation of ~180° are equally likely. In both orientations, two of the methylene of L-NAP buttress against the side chain of Trp-202 in a hydrophobic interaction. In orientation I, the acetyl group is directed toward Arg-267 and Gln-269 and the backbone amide N-Hs of Gly-104. The hydrogens on the opposite face of the proline ring to the carboxylate are directed toward the iron. In orientation II, the carboxylate is directed toward the side chains of Arg-267 and Gln-269 and the backbone amide N-Hs of Gly-104. The hydrogens on the same face as the carboxylate are directed toward the iron. The proposed interaction of the carboxylate with Arg-267 in orientation II, is supported by the precedent of CAS, where the equivalent arginine (Arg-297, CAS) forms interactions with both the 1-carboxylate of 2OG and the substrate carboxylate, suggesting that this residue plays a key role in catalysis (Fig. 4).

Because the natural CarC substrate was not available in sufficient quantities for crystallization work, we used QM/MM modeling studies to investigate possible modes of carbapenam binding. Initial minimization of the model based on the CarC-Fe(II)-2OG-L-NAP crystal structure, in which the L-NAP is bound in orientation II resulted in a similar overall structure (r.m.s. deviation for non-hydrogen atoms, without waters: 1.5 Å), except for small changes in the loop between residues Gly-104 and Ser-109. Minimizations were then performed with the (3S,5S)- and the (3S,5R)-carbapenam models using both orientations I and II of L-NAP as initial “templates,” i.e. by overlaying the pyrrolidino-carboxylate rings. Different binding possibilities are observed in the models, which suggest the Gly-104 to Ser-109 loop may be involved in binding a substrate carboxylate or lactam carbonyl. However, in each case, the results indicated similar energies for both the (3S,5S)- and the (3S,5R)-carbapenams (Fig. 5).

Mechanistic Discussion—Studies with other 2OG oxygenases have indicated that conformational changes occur upon
substrate binding (47) such that the substrate binds to an “open” conformation but is isolated at the active site when it is oxidized. Thus the current crystallographic and modeled CarC structures may not precisely represent the active site conformation in which substrate binding and/or oxidation occurs. Nonetheless, they identify key residues involved in catalysis and suggest possible mechanisms for the CarC reaction.

Given the precedents with CAS and TauD, and the observation that the carboxylate of L-NAP may bind to the active site arginine (Arg-267), it is possible that the carboxylate of the (3S,5S)-carbapenam substrate binds to Arg-267 and Gln-269 during its oxidation, in a similar manner to that observed in orientation II of L-NAP and associated models; modeling suggests direct abstraction of a hydrogen at C-5 of the (3S,5S)-carbapenam may occur (Fig. 5). However in this case it would seem that re-orientation of the substrate would be required if a ferryl intermediate was to both abstract a hydrogen and re-hydrogenate “directly” at the C-5 position. The modeling studies indicate that a (3S,5R)-carbapenam, with or without a C-5 radical, could be accommodated in the active site, but there is no clear driving force for such a conformational change.

Instead, orientation I of L-NAP and associated models may represent the productive conformation. In this case epimerization may occur via abstraction of the C-3 or C-2 hydrogens (Scheme 2, a and b) or less likely, the C-1 hydrogen, followed by opening and ring closing of the bicyclic β-lactam system. Desaturation can then occur as in CAS. This proposal is attractive, because re-orientation of the substrate in the active site is not required for a ferryl intermediate to effect both epimerization and desaturation. In this proposal re-hydrogenation of a radical intermediate can lead to the (3S,5R)-carbapenam in a shunt pathway.

Other possibilities can be envisaged. Epimerization via abstraction at C-6 seems unlikely, because it would invoke a high energy primary radical and desaturation would either require significant re-orientation or unprecedented rearrangements/H-shifts. Given the precedent of ribonucleotide reductase (48) and others, the possibility that epimerization occurs via a process involving a protein based radical should be considered. However, analysis of the active site does not reveal clear candidates:

Scheme 2. Possibilities for the CarC-catalyzed epimerization/desaturation process. The proposed 5-endo trig (or 4-exo trig) radical cyclizations in a and b have synthetic precedent (50), including an imine substrate (51). Note the possible intermediacy of dative stabilized radicals. The proposed shunt pathway requires reduction of the ferryl intermediate (Fe(IV)=O ↔ Fe(III)=O) to complete the catalytic cycle in a similar fashion to uncoupled cycles. Epimerization via hydrogen abstraction at C-1 in a process analogous to a is also a possibility, but the modeling studies suggest this is less likely.
Crystal Structure of CarC

Tyr-191 and Trp-202 were considered possibilities for such a role but appear incorrectly positioned. Thus, we favor a mechanism solely mediated via hydrogen transfers to the Fe(IV)=O and Fe(III)−OH intermediates, which are believed to occur with reactions catalyzed by related oxygenases involving rearrangements and desaturations, such as DAOCS (47) and CAS (41, 49).

Acknowledgments—We thank the European Union, the Biotechnology and Biological Sciences Research Council, the Engineering and Physical Sciences Research Council, the Wellcome Trust, and Amura for funding; the staff at the Synchrotron Radiation Source, Daresbury for technical support; Dr. N. J. Oldham for MS analyses; Prof. B. W. Bycroft for a preprint of Ref. 21; and Dr. J. M. Elkins for helpful discussions.

REFERENCES

1. Livermore, D. M., and Woodford, N. (2000) Curr. Opin. Microbiol. 3, 489–495
2. McGowan, S. J., Bycroft, B. W., and Salmond, G. P. C. (1998) Trends Microbiol. 6, 203–208
3. Kahan, J. S., Kahan, F. M., Goegelman, R., Currie, S. A., Jackson, M., Stapley, J. M., Hardie, K. R., Williams, P., Stewart, J., Adlington, R. M., Roach, P. L., and Baldwin, J. E. (1999) J. Am. Chem. Soc. 121, 2039–2045
4. Parker, W. L., Rathnum, M. L., Wells, J. S., Jr., Trejo, W. H., Principe, P. A., and Nicolaou, K. C. (1996) in Classics in Total Synthesis: Targets, Strategies, Methods (2000) Bycroft, B. W., and Salmond, G. P. C. (1996) Microbiology 142, 541–550
5. McRae, B., Merz, K. M., and Kollman, P. A. (1990) J. Comput. Chem. 11, 431–439
6. Frisch, M. J., Trucks, G. W., Schlegel, H. B., Scuseria, G. E., Robb, M. A., Cheeseman, J. R., Zakrzewski, V. G., Montgomery, J. A., Stratmann, R. E., Burant, J. C., Dapprich, S., Millam, J. M., Daniels, A. D., Kudin, K. N., Strain, M. C., Farkas, O., Tomasi, J., Barone, V., Cossi, M., Cammi, R., Mennucci, B., Pomelli, C., Adamo, C., Clifford, S., Ochterski, J., Petersson, G. A., Ayala, P. Y., Cui, Q., Morokuma, K., Malick, D. K., Rabuck, A. D., Bagavath, R. V., Raghavachari, K., Foresman, J. B., Cioslowski, J., Ortiz, J. V., Stefanov, B. B., Liu, G., Liashenko, A., Piskorz, P., Komaromi, I., Gomperts, R., Martin, R. L., Fox, D. J., Keith, T., Al-Laham, M. A., Peng, C. Y., Nanayakkara, A., Gonzalez, C., Challacombe, M., Gill, P. M. W., Johnson, B. G., Chen, W. W., Andrews, J. L., Head-Gordon, M., Rego, E. S., and Pople, J. A. (1998) Gaussian98, Gaussian Inc., Pittsburgh, PA
7. Steinbach, P. J., and Brooks, B. R. (1994) J. Comput. Chem. 15, 667–683
8. Dewar, M. J. S., Zoebisch, E. G., Healy, E. F., and Stewart, J. P. (1985) J. Am. Chem. Soc. 107, 7332–7339
9. Kanno, O., Shimoji, Y., Ohya, S., and Kamamoto, I. (2000) J. Antibiot. 53, 404–414
10. Kershaw, N. J., McNaughton, H. J., Hewitson, K. S., Hernandez, H., Griffin, J., Hughes, C., Greaves, P., Barton, B., Roche, B., and Schofield, C. J. (2002) Eur. J. Biochem. 269, 2052–2059
11. Zhang, Z., Ben, J., Stammers, D. K., Baldwin, J. E., Harlos, K., and Schofield, C. J. (2000) Nat. Struct. Biol. 7, 127–133
12. Klungness, K. R., Chen, W., Weng, M. W., Andrews, J. L., Head-Gordon, M., Rego, E. S., and Pople, J. A. (2002) J. Mol. Biol. 322, 1039–1052
13. Twyman, S. R., Chen, W., Weng, M. W., Andrews, J. L., Head-Gordon, M., Rego, E. S., and Pople, J. A. (1998) J. Comput. Chem. 19, 1111–1134
14. Terwilliger, T. C., and Berendzen, J. (1999) Acta Crystallogr. Sect. D Biol. Crystallogr. 55, 760–763
15. Ruben, T. C., and Berendzen, J. (1999) Acta Crystallogr. Sect. D Biol. Crystallogr. 55, 849–866
16. Cerny, V., and Kuntze, T. (1996) Acta Crystallogr. Sect. D Biol. Crystallogr. 52, 247–255
17. Hogan, D. A., Auchtung, T. A., and Hausinger, R. P. (1999) J. Bacteriol. 181, 415–425
18. Iwata-Reuyl, D., Basak, A., and Townsend, C. A. (1999) Eur. J. Biochem. 266, 5865–5866
19. Pasetti, F., Schroeder, R. H., Beil, S., and Pardi, G., von Noort, P., Pijning, T., Ren, J., Stammers, D. K., Baldwin, J. E., Harlos, K., and Schofield, C. J. (2002) J. Mol. Biol. 322, 1039–1052
20. Que, L., Jr. (2000) Nat. Struct. Biol. 7, 182–184
21. McGowan, S. J., Holden, M. T. G., Bycroft, B. W., and Salmond, G. P. C. (1999) Trends Microbiol. 7, 545–556
22. Bycroft, B. W., and Salmond, G. P. C. (1998) Biochemistry 37, 2684–2696
23. Bycroft, B. W., and Salmond, G. P. C. (1996) J. Mol. Microbiol. 75, 415–426
24. Bycroft, B. W., and Salmond, G. P. C. (1998) Acta Crystallogr. Sect. D Biol. Crystallogr. 54, 271–274
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*J. Biol. Chem. 2003, 278:20843-20850.*
doi: 10.1074/jbc.M213054200 originally published online February 28, 2003

Access the most updated version of this article at doi: 10.1074/jbc.M213054200

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