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Intramolecular Cooperativity in the Reaction of Diacyl Phosphates with Serine β-Lactamases†

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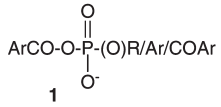
ABSTRACT: Asymmetric diaroyl phosphates ($ArCOOPO_2^-OCOAr'$, where Ar = Ph, Ar′ = 4-biphenyl, 2-benzothiophenyl and 2-benzofuranyl) have been prepared, evaluated as serine (classes A, C, and D) β-lactamase inhibitors, and compared with respect to the latter with their symmetric parents, where Ar = Ar′. The asymmetric compounds, in general, were found to react with the β-lactamases in two modes, corresponding to different orientations with respect to the active site, whereby either of the two aroyl groups may acylate the enzyme to form two different inert acyl-enzymes, E-COAr and E-COAr′ . In all cases, the asymmetric compounds, in one orientation, react more rapidly with the enzymes tested than one symmetrical parent but not both. From comparisons of activation free energy differences, it was found that the changes in free energy on changing from one aryl group to another, in either the acyl group or the leaving group, were not additive, i.e., that the effect of changing one aroyl group to another depended on the leaving group and vice versa. Thus, intramolecular cooperativity between the aroyl groups must exist, arising either from direct interaction between them or from protein-mediated interaction or from a combination of both. Such cooperativity brings fresh opportunities and challenges to the search for novel β-lactamase inhibitors.

Aroyl phosphates, **1**, have proven to be very effective inhibitors of serine β-lactamases (*1*−*4*). The phosphate leaving group appears to interact with polar residues in such a way as to enhance active site acylation by both specific amidoacyl groups and nonclassical aroyl groups (*2*−*6*). Acylation by the latter leads to hydrolytically refractive acyl-enzymes and thus to significant inhibition (Scheme 1). A series of diaroyl phosphates, **2**, have proven to be particularly effective inhibitors (*3*, *4*). Structure−activity studies have shown that hydrophobic substituents enhance inhibition, largely through enhancement of acylation rates, while electron-donating substituents enhance inhibition by depression of deacylation rates (*3*).



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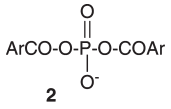

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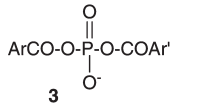

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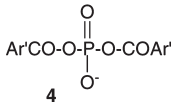

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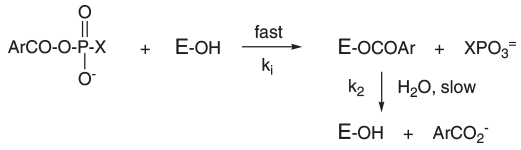
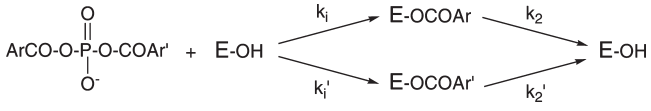
It is obvious that the two aroyl groups of **2** must interact with the enzyme active site differently and thus contribute differently to the inhibitory activity of **2**. In order to understand the way in which the two aroyl groups separately contribute, asymmetric diaroyl phosphates (**3**) were needed. Such compounds would also allow more versatile modulation of pharmacological properties

Scheme 1



Scheme 2



for any practical application of these compounds. β-Lactamase inhibitors are of value in protecting β-lactam antibiotics from β-lactamases and thereby extending the clinically useful lifetime of the latter molecules (*7*).

The functional asymmetry of **3** means that two modes of reaction are possible, acylation by ArCO with an $Ar'COOPO_3^=$ leaving group and vice versa (Scheme 2). In general, the rates of the two possible reactions will be different, and one mode of reaction will lead to more effective inhibition, i.e., to a higher proportion of that acyl-enzyme in the steady state. Another issue of interest stemming from the reactivity of **3** is that of additivity. This can be seen in the comparison of the reactivity of **3** with that of the symmetrical parents **2** and **4** (Scheme 3). In Scheme 3, **3a** and **3b**, which obviously are representations of the same molecule, correspond to the alternative orientations of **3** bound at the active site. The issue then is whether $\delta\Delta G^{\ddagger}$ (the change in activation free energy of enzyme acylation) between **2** and **3a** ($\delta\Delta G_1^{\ddagger}$, the effect of changing the leaving group from Ar to Ar′ with a common acyl group) is equal to that between **3b** and **4**,

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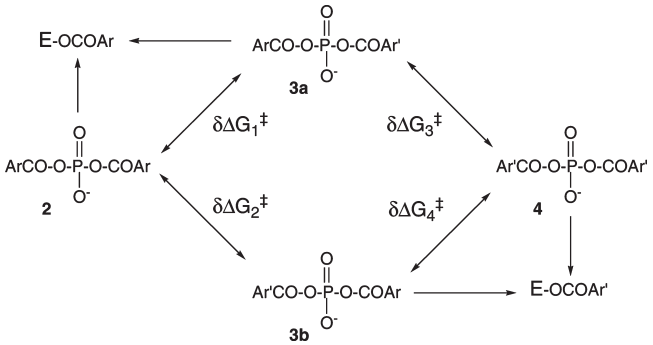
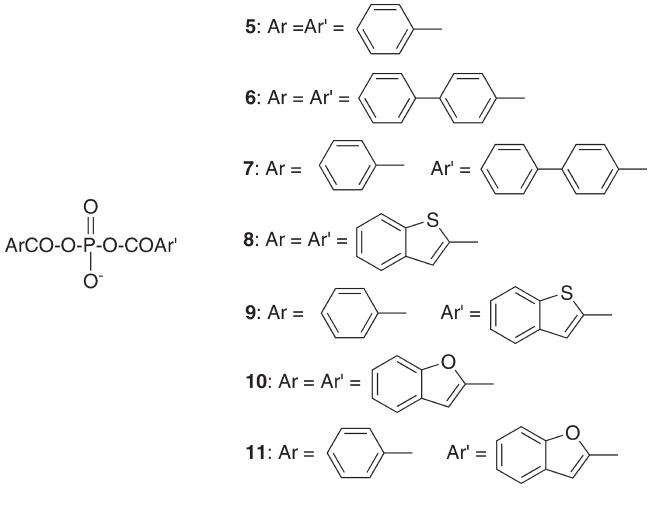
Scheme 3



$\delta\Delta G_4^{\ddagger}$. Similarly, is $\delta\Delta G_2^{\ddagger}$ (the effect of changing the acylating group from Ar to Ar′ with a common leaving group) equal to $\delta\Delta G_3^{\ddagger}$? If not, there must be an intramolecular cooperativity between the acylating group and the leaving group, which could, in principle, be exploited in inhibitor design.

Intramolecular cooperativity is, of course, well known in the reactions of a variety of enzymes with their substrates and inhibitors. The phenomenon is probably best known in proteinases where cooperative interactions between S_n and S_{-n} residues via their interaction with the P_n and P_{-n} sites, respectively, have been clearly demonstrated (*8*−*11*). Similar observations have been made with glycohydrolases (*12*) and nucleases (*13*). Cooperative interactions between aryl substituents have recently been observed in noncovalent phosphonate inhibitors of serine β-lactamases (*14*). In the present article, we describe the synthesis of asymmetric diaroyl phosphates (**3**), an analysis of their reactions with typical class A, C, and D serine β-lactamases in order to determine the dominant mode of reaction (i.e., is k_i or k_i' of Scheme 2 greater?), and an analysis of the degree of cooperativity. The diaroyl phosphates evaluated were **5**−**11**.



MATERIALS AND METHODS

The purified *Enterobacter cloacae* P99 and *Escherichia coli* W3310 TEM-2 β-lactamases were purchased from the Centre for Applied Microbiology and Research (Porton Down, Wiltshire, U.K.) and used as received. The OXA-1 β-lactamase was a generous gift from Dr. Michiyoshi Nukaga of Jyosai University, Japan. Cephalothin was a gift from Eli Lilly and Co. Benzylpenicillin was purchased from Sigma-Aldrich.

Scheme 4

The preparation of the symmetrical diacyl phosphates **5**, **6**, **8**, and **10** was as described in the preceding article (*4*). The asymmetric compounds **7**, **9**, and **11** were prepared in the same way as **6**, **8**, and **10** (*4*), i.e., generated in the same reaction mixtures, and separated from the symmetrical compounds and purified by hplc (Macherey-Nagel SS 250/0.5 in/10-nucleosil 300-7 C18 reverse phase column; **7**, retention time 19.8 min from a 0−100% MeOH/H_2O gradient with flow rate 3 mL/min; **9**, retention time 24.4 min, 20−80% MeOH/H_2O gradient, 3 mL/min; **11**, retention time 14.7 min, 20−80% MeOH/H_2O gradient, 3 mL/min) and characterized as noted below.

Sodium Benzoyl 4-Biphenylcarbonyl Phosphate (*7*). ^{1}H NMR (DMSO[1]-d_6) δ 7.28 (t, J = 6.6 Hz, 1H), 7.32 (t, J = 6.0 Hz, 1H), 7.35 (t, J = 8.1 Hz, 2H), 7.52 (t, J = 9.0 Hz, 2H), 7.55 (d, J = 8.4 Hz, 2H), 7.61 (d, J = 6.9 Hz, 2H), 7.89 (d, J = 9 Hz, 2H), 7.95 (d, J = 7.5 Hz, 2H). ^{31}P NMR (DMSO-d_6) δ −21.12. FTIR (KBr, cm^{-1}) 1725.1. ES(−)MS m/z 381.20.

Sodium Benzoyl Benzo[*b*]*thiophenecarbonyl Phosphate* (*9*). ^{1}H NMR (DMSO-d_6) δ 7.36 (t, J = 7.6 Hz, 1H), 7.43 (t, J = 7.2 Hz, 2H), 7.44 (t, J = 7.8 Hz, 1H), 7.60 (t, J = 7.8 Hz, 1H), 7.86 (d, J = 6.6 Hz, 1H), 7.88 (d, J = 7.2 Hz, 1H), 7.97 (d, J = 7.8 Hz, 2H), 8.14 (s, 1H). ^{31}P NMR (DMSO-d_6) δ −18.37. FTIR (KBr, cm^{-1}) 1719.2. ES(−)MS m/z 361.07.

Sodium Benzoyl Benzo[*b*]*furancarbonyl Phosphate* (***11***). ^{1}H NMR (DMSO-d_6) δ 7.26 (t, J = 7.8 Hz, 2H), 7.41 (d, J = 7.8 Hz, 1H), 7.44 (t, J = 6.9 Hz, 1H), 7.50 (t, J = 8.4 Hz, 1H), 7.59 (t, J = 7.5 Hz, 1H), 7.69 (d, J = 7.8 Hz, 1H), 7.73 (s, 1H), 7.96 (d, J = 7.2 Hz, 2H). ^{31}P NMR (DMSO-d_6) δ −18.4. FTIR (KBr, cm^{-1}) 1731.0. ES(−)MS m/z 345.02.

Inhibition Kinetics. All kinetics experiments were performed at 25 °C in a buffer at pH 7.5 containing 20 mM MOPS. When the OXA-1 β-lactamase was studied, 50 mM $NaHCO_3$ was included in the buffer. Stock solutions of the diacyl phosphates in DMSO were prepared as described in the preceding article (*4*), except for **8** and **9**, which were unstable in this solvent, possibly from oxidation. Stock solutions of **8** and **9** were therefore prepared in DMF, which at concentrations up to 4% v/v, did not affect the activity of the enzymes. Kinetics data were fitted to Scheme 4 in all cases. The slow turnover rate constant k_2 could be determined spectrophotometrically at 300 nm from steady state experiments carried out at saturating values of [I] (*4*). Effective steady state inhibition constants, K_i (= k_2/k_i), or, equivalently, effective K_m values if I is considered a substrate were determined either directly spectrophotometrically at 300 nm from a Henri−Michaelis−Menten analysis or by competitive inhibition of hydrolysis of a suitable substrate (*4*). Experimental details are expanded in Supporting Information (Table S1).

Electrospray Mass Spectroscopy of Enzyme−*Inhibitor Complexes*. Mass spectra of OXA-1 and P99 β-lactamase complexes with **7** were determined as previously described for **5** (*3*). Thus a reaction mixture containing the OXA-1 β-lactamase (25 μM) and **7** (520 μM) was quenched with trichloroacetic acid

[1]Abbreviations: DIEA, di-isopropylethylamine; DMF, dimethyl formamide; DMSO, dimethyl sulfoxide; ESMS, electrospray ionization mass spectroscopy; FTIR, Fourier transform infrared; HATU, *O*-(7-azabenzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate; MOPS, 3-morpholinopropanesulfonic acid; NMR, nuclear magnetic resonance.

after 15 min. A similar mixture of the P99 enzyme (15.7 μM) and **7** (4 mM) was treated in the same way after 5 min. The washed and dried precipitates were subjected to electrospray mass spectroscopy at the Mass Spectroscopy Laboratory, School of Chemical Sciences, University of Illinois.

RESULTS AND DISCUSSION

The synthesis of asymmetric diaroyl phosphates **7**, **9**, and **11** could not be achieved by the method of Chantrenne (*15*) that we had previously employed to prepare most of the symmetrical compounds (*3*, *4*). All attempts by this method to prepare asymmetric compounds appeared to lead only to symmetrical compounds. Synthesis of the required asymmetric diaroyl phosphates was, however, achieved by a one-pot two-step acylation process involving a HATU-mediated first step and a second acylation by aroyl chloride (Scheme 5). This process yielded a mixture of three products, the symmetrical diaroyl phosphates **2** and **4**, and the asymmetric molecule **3** in comparable amounts, as shown clearly in ^{31}P NMR spectra. Equilibration of the three compounds may have occurred via triaroyl phosphates. The three could be separated and isolated by means of HPLC and identified by NMR and mass spectra. The method employed here was also found to be an efficient method of synthesis of symmetric diaroyl phosphates where Ar = Ar′.

The previously described **5**, **6**, **8**, and **10** react with serine β-lactamases, as inhibitory substrates, as described (*3*, *4*). Rapid acylation of the enzyme active site is followed by slow deacylation (Scheme 1). The new asymmetric compounds **7**, **9**, and **11** appear to behave qualitatively in the same manner with typical examples of class A (TEM-2), class C (*Enterobacter cloacae* P99), and class D (OXA-1) enzymes. Apparent values of k_2 and K_i ($=k_2/k_i$) were determined as described in Materials and Methods and the preceding article (*4*). Figure 1A, for example, shows data for the determination of k_2 for the P99 β-lactamase on reaction with **7**. The essentially linear initial rates, independent of the concentration of **7**, indicate $K_i^{app} \ll 10$ μM and directly yield the k_2^{app} value. These are apparent values because of the uncertainty at that stage as to the mode of inhibition (Scheme 2). Figure 1B shows data for the experimental determination of K_i^{app} for the OXA-1 β-lactamase on reaction with **7**. A final example, Figure 1C, shows the data employed for the direct determination of k_2^{app} and K_i^{app} for reaction of the TEM-2 β-lactamase with **9**, by nonlinear least-squares fitting of the Henri−Michaelis−Menten equation.

Steady-state kinetics data from the experiments described above, for slow turnover of **7**, **9**, and **11** by the various enzymes, is shown in Table 1. Also shown, for comparison, are previously determined values of these parameters for **5**, **6**, **8**, and **10** (*3*, *4*). Values for **8** and **10** for the OXA-1 enzyme were also determined. The apparent values for **7**, **9**, and **11** should be composites of the two possible modes of reaction (Scheme 2). This proposition was supported by mass spectrometry. For example, incubation of the P99 β-lactamase with **7** yielded a protein with mass peaks at 39,189, 39,295, and 39,376 amu. These correspond to the free enzyme (M), benzoyl-E (expected, M + 104), and 4-phenylbenzoyl-E (expected, M +180), respectively. Similarly, with the OXA-1 enzyme, peaks at 28,130, 28,234, and 28,311 amu were observed. It thus seems likely that the two reaction modes of Scheme 2 will, in general, be observed in the reactions of **7**, **9**, and **11** with β-lactamases.

The quantitative kinetic parameters of the two modes of reaction were deconvoluted by means of Scheme 6 and eqs 1 and 2, which are derived from it. This is possible when it is realized that k_2 for **7** will be the same as that for **5** (already determined; Table 1) and k_2' will have the value of k_2 for **6** (Table 1), if it is assumed, reasonably, that the asymmetric compound **7** will produce the same acyl-enzymes as those from the symmetrical compounds **5** and **6**. Simultaneous solution of eqs 1 and 2 with the data of Table 1 yielded the values of Table 2.

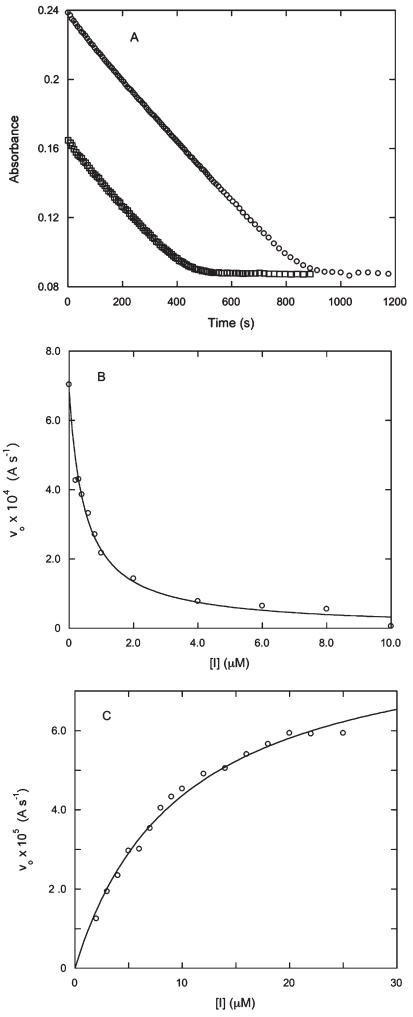
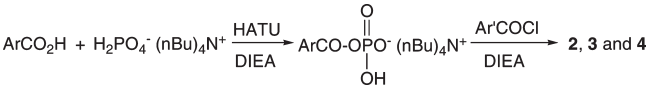


FIGURE 1: (A)Total progress curves for the hydrolysis of **7** (□, 10 μM; ○, 20 μM) by the P99 β-lactamase (0.98 μM). Absorbance changes at 300 nm are shown. (B) Inhibition of cephalothin (200 μM) hydrolysis in the presence of the OXA-1 β-lactamase (0.11 μM) by the diaroyl phosphate **7**. The circles represent experimental points, and the solid line is a least-squares fit to a competitive inhibition equation. (C) Initial rates of hydrolysis of **9** by the TEM-2 β-lactamase (0.14 μM) as a function of the concentration of **9**. The circles represent experimental points, and the solid line is a least-squares fit to the Henri−Michaelis−Menten equation.

Scheme 5



$$k_2^{app} = (k_2K_i' + k_2'K_i)/(K_i + K_i') \quad (1)$$

$$K_1^{app} = K_iK_i'/(K_i + K_i') \quad (2)$$

where $K_i = k_2/k_i$, and $K_i' = k_2'/k_i'$.

Immediately noticeable from Table 1 for **7** is the fact that, within experimental uncertainty, the k_2 values for **6** and **7** are equal for all three enzymes while that for **5** is different. Thus, in terms of Scheme 6, $k_2^{app} \approx k_2'$ and $k_2 < k_2'$. This will be possible (eq 1) if $K_i \gg K_i'$ and, therefore, $k_i' \gg k_i$. It also follows from Scheme 6 that the steady state ratio of acyl enzymes [EI′]/[EI] is equal to K_i/K_i', which is thus much greater than unity. Thus, the dominant acyl-enzyme generated by the reaction of all three enzymes with **7** must be the 4-phenylbenzoyl species rather than the benzoyl, i.e., for all three enzymes, the 4-phenylbenzoyl group prefers the acylation site and benzoylphosphate the leaving group site, rather than vice versa. Within experimental limits, therefore, k_2 and k_i for **7** are indeterminable, and k_2' and k_i' (Table 2) are equal to the apparent values (Table 1).

Scheme 6

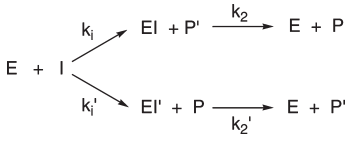


Table 1: Rate and Equilibrium Constants for Inhibition and Turnover of Diaroyl Phosphates by β-Lactamases[a]

enzyme	diaroyl phosphate	$k_i \times 10^{-6}$ ($s^{-1}M^{-1}$)	$k_2 \times 10^3$ (s^{-1})	K_i (nM)
OXA-1	**5**	0.028	5.5	200
	6	10.8	1.2	0.11
	7[b,c]	0.04	1.3	32
	8[b]	0.63	37	58
	9[b,c]	0.069	6.8	99
	10[b]	0.029	407	14,200
	11[b,c]	0.030	15	510
P99	**5**	0.007	9.3	1330
	6	0.87	27	32
	7[b,c]	0.55	27	49
	8	70	210	3.0
	9[b,c]	5.1	110	22
	10	0.27	350	1300
	11[b,c]	0.14	240	1700
TEM-2	**5**	0.0005	38	76,000
	6	0.30	24	80
	7[b,c]	0.039	23	590
	8	0.054	79	1500
	9[b,c]	0.006	65	10,000
	10	0.0018	74	41,000
	11[b,c]	0.002	63	27,000

[a]Uncertainties in k_2 and K_i values were ± 8%, on average. [b]k_i values determined from k_2/K_i. [c]Apparent values (see text).

For the other asymmetric compounds **9** and **11**, the k_2^{app} values lie between those of k_2 and k_2' (the relevant values for the two cognate symmetrical compounds, **5** and **8** for **9**, and **5** and **10** for **11**) (Table 1). In these cases, individual k_2, k_i, k_2', and k_i' values could be calculated from eqs 1 and 2 (see Table 2). It can be seen from Table 2 that, in all cases but one, that of the OXA-1 enzyme with **9**, the asymmetric compounds react more rapidly where the aroyl group acylates the enzyme rather than benzoyl ($k_i' > k_i$). In most cases, the asymmetric compound, in one orientation, reacts more slowly than the dibenzoyl compound **5**, and in one case, that of the OXA-1 enzyme with **11**, both do. It is noticeable also that in no case, taking into account experimental uncertainties (notice the case of the TEM β-lactamase with **11**) is an asymmetric compound superior to *both* of the related symmetric compounds, both with respect to k_i and to k_2. Although, in principle, there is no reason why this should necessarily be so, it does seem to be true with these particular enzymes and diacyl phosphates. There is, however, quite noticeable nonadditivity in most cases between the aroyl groups as one is changed in the presence of another, i.e., certainly some degree of cooperativity, positive or negative, between the groups is evident. This is best seen from the changes in free energy of activation of enzyme acylation, $\delta\Delta G^{\ddagger}$, on replacement of one aroyl group with another (Scheme 3), calculated from eq 3, where k_y/k_x would contain the relevant k_i and k_i' values for the various compounds and reactions. These calculations lead to the free energy diagrams of Figure 2.

$$\delta\Delta G^{\ddagger} = -RT \ln(k_y/k_x) \qquad \text{(eq3)}$$

It is clear from the diagrams of Figure 2 that the energetic effect of changing a benzoyl group to another aroyl group in the acylation site depends on the nature of the leaving group and vice versa, i.e., the contributions of each type of substitution to the activation free energy are not additive, and therefore, in general, there must be some degree of cooperativity between the two aroyl groups in the acylation transition state. In most cases, the change in activation energy from replacement of benzoyl by another aroyl group in the acylation site leads to faster acylation when the leaving group is aroyl phosphate (A, B, C, F, H, and I) rather than benzoyl phosphate, but there is one counter-example (E); two cases are uncertain (D, G). The converse is also, of course,

Table 2: Rate and Equilibrium Constants for the Two Modes of Reaction of Asymmetric Diaroyl Phosphates[a]

enzyme	diaroyl phosphate	$k_i \times 10^{-6}$ (s^{-1} M^{-1})	$k_i' \times 10^{-6}$ (s^{-1} M^{-1})	K_i (nM)	K_i' (nM)
OXA-1	**7**	n[b]	0.04	n[b]	32
	9	0.053	0.016	100	2300
	11	0.010	0.020	520	20,000
P99	**7**	n[b]	0.55	n[b]	49
	9	0.21	4.9	44	43
	11	0.0017	0.14	5500	2600
TEM-2	**7**	n[b]	0.039	n[b]	590
	9	0.0013	0.0053	30,000	15,000
	11	0.00045	0.0019	84,000	40,000

[a]k_i and K_i refer to formation and dissociation of the benzoyl-enzyme (Ar = benzoyl, Scheme 2) and k_i' and K_i' to those of the aroyl-enzyme. [b]n: not determined (see text).

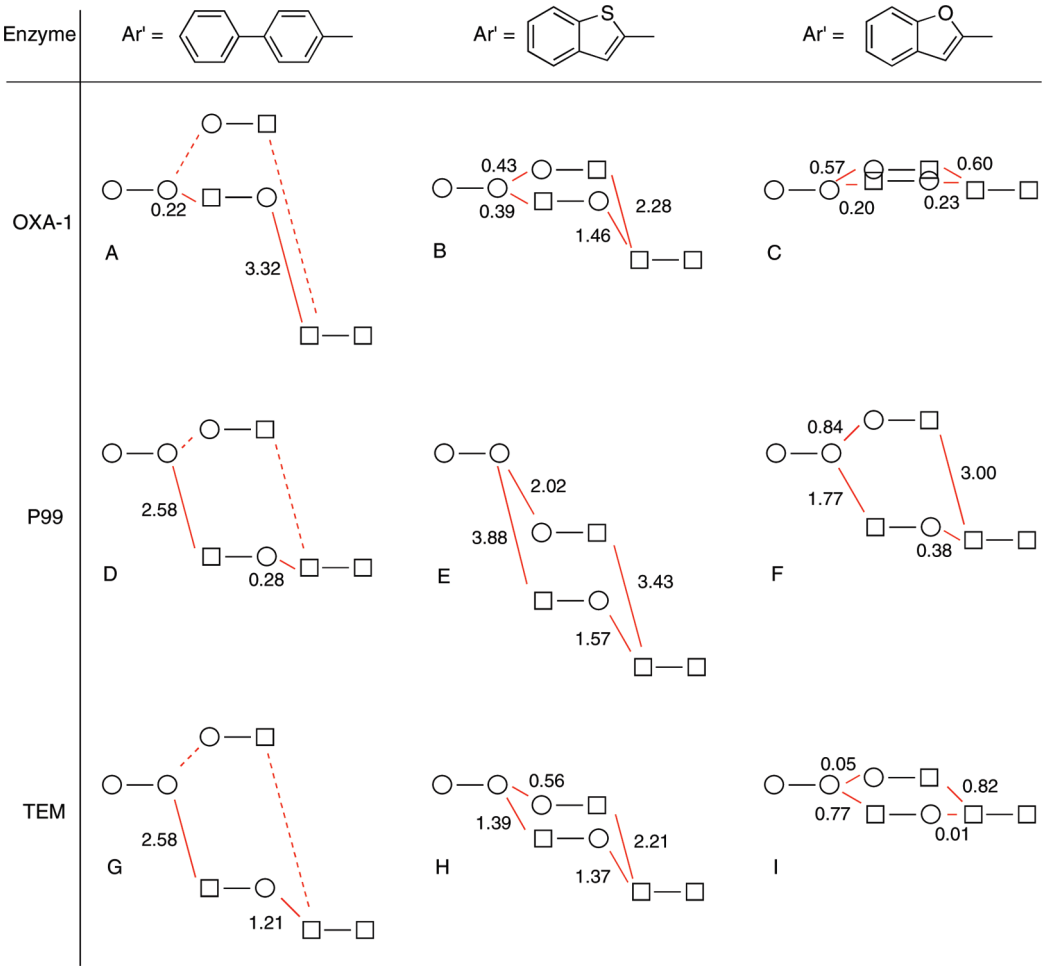


FIGURE 2: Relative free energies of activation ($\delta\Delta G^{\ddagger}$, kcal/mol) of acylation of β-lactamases by diaroyl phosphates. The symbol ○ corresponds to the benzoyl moiety, □ to ArCO, and the line connecting them to phosphate. The left-hand character of each structure refers to the acylating moiety and the right-hand character to the leaving group, i.e., ○—□ represents a benzoyl aroyl phosphate which reacts with an enzyme by benzoylation, with aroyl phosphate as a leaving group.

true, i.e., the change from benzoyl phosphate to aroyl phosphate in the leaving group site usually leads to faster acylation when the acylating group is aroyl rather than benzoyl (with the same counter-example, E). In most cases, therefore, there is a greater positive cooperativity between the larger aroyl groups than between a benzoyl group and another aroyl group, irrespective of whether the single aroyl group is in the acylating or leaving group site. The differences are most dramatic, perhaps, in A and F. In F, for example, replacement of benzoyl by benzofurancarbonyl leads to a decrease in activation energy of 1.77 kcal/mol if the leaving group is benzoyl phosphate but a decrease of 3.0 kcal/mol if the leaving group is benzofurancarbonyl phosphate. However, a change in leaving group from benzoyl phosphate to benzofurancarbonyl phosphate leads to an *increase* in activation energy of 0.84 kcal/mol when the acyl group is benzoyl but a *decrease* of 0.38 kcal/mol when the acyl group is benzofurancarbonyl.

There is a general qualitative similarity between the various diagrams of Figure 2, dictated by the observation, noted above, that in most cases, the most reactive species is the diaroyl phosphate, although, even in the comparison of benzothiophenecarbonyl (B,E,H) with benzofurancarbonyl (C,F,I), which have very similar molecular shapes, there are considerable differences in the enzymes' quantitative responses. These must arise from differences in very specific interactions, dictated, for example, by the differences in dipole and quadrupole moments between these heterocycles (*16*). There are clear differences, also, in how the different enzymes interact with a particular aroyl group.

The molecular mechanism(s) of the cooperativity effects are not known at present. They could arise from direct interaction between the aroyl groups when at the active site of the OXA-1 enzyme, for example, where molecular modeling suggests that the two aroyl groups may be in close proximity to each other in the acylation transition state (*3*). In other cases, where the aroyl groups may be further apart in the acylation complex, such as with the P99 and TEM-2 enzymes (*4*), the cooperative effects may arise from modulation of protein structure by the substituents. A combination of the two is the likely general scenario.

We have showed that it is possible to prepare and employ asymmetric diaroyl phosphates as β-lactamase inhibitors. These have reactivities with typical β-lactamases not linearly predictable from their symmetric parents. A combination of this fact and the likely variability in physical properties of those asymmetric compounds may allow diaroyl phosphates to be even more versatile β-lactamase inhibitors than previously indicated.

SUPPORTING INFORMATION AVAILABLE

Details of the inhibition kinetics experiments. This material is available free of charge via the Internet at http://pubs.acs.org.

REFERENCES

1. Li, N., and Pratt, R. F. (1998) Inhibition of serine β-lactamases by acyl phosph(on)ates: a new source of inert acyl (and phosphyl) enzymes. *J. Am. Chem. Soc. 120*, 4264–4268.
2. Kaur, K., and Pratt, R. F. (2001) Mechanism of reaction of acyl-phosph(on)ates with the β-lactamase of *Enterobacter cloacae* P99. *Biochemistry 40*, 4610–4621.
3. Majumdar, S., Adediran, S. A., Nukaga, M., and Pratt, R. F. (2005) Inhibition of class D β-lactamases by diaroyl phosphates. *Biochemistry 44*, 16121–16129.
4. Majumdar, S. Pratt, R. F. (2009) Inhibition of class A and class C β-lactamases by diaroyl phosphates. Biochemistry , in press.
5. Morrison, M. J., Li, N., and Pratt, R. F. (2001) Inverse acyl phosphates: substrates or inhibitors of β-lactam-recognizing enzymes? *Bioorg. Chem. 29*, 271–281.
6. Ahn, Y.-M., and Pratt, R. F. (2004) Kinetics and structural consequences of the leaving group in substrates of a class C β-lactamase. *Bioorg. Med. Chem. 12*, 1537–1542.
7. Buynak, J. D. (2006) Understanding the longevity of the β-lactam antibiotics and of antibiotic/β-lactamase inhibitor combinations. *Biochem. Pharmacol. 71*, 930–940.
8. Schellenberger, V., Schellenberger, U., Mitin, Y. V., and Jakubke, H.-D. (1990) Characterization of the S′-subsite specificity of bovine pancreatic α-chymotrypsin via acyl transfer to added nucleophiles. *Eur. J. Biochem. 187*, 163–167.
9. Schellenberger, U., Braune, K., Hofmann, H.-J., and Jakubke, H.-D. (1991) The specificity of chymotrypsin: a statistical analysis of hydrolysis data. *Eur. J. Biochem. 199*, 623–636.
10. Berti, P. G., Faerman, C. H., and Storer, A. C. (1991) Cooperativity of papain-substrate interaction energies in the S2 to S2′ subsites. *Biochemistry 30*, 1394–1402.
11. Schiller, O., and Overall, C. M. (2008) Proteome-derived, database-searchable peptide libraries for identifying protease cleavage sites. *Nat. Biotechnol. 26*, 685–694.
12. Tilbeurgh, H., Loontiens, F. G., Engelborgs, Y., and Claeyssens, M. (1989) Studies of the cellulolytic system of *Trichoderma reesei* QM 9414: Binding of small ligands to the $1,4^{\beta}$-glucan cellobiohydrolase II and influence of glucose on their affinity. *Eur. J. Biochem. 184*, 533–539.
13. Jen-Jacobson, L., Engler, L. E., Lesser, D. R., Kurpiewski, M. R., Yee, C., and McVerry, B. (1996) Structural adaptations in the ineraction of EcoRI endonuclease with methylated GAATTC sites. *EMBO J. 15*, 2870–2882.
14. Perumal, S. K., Adediran, S. A., and Pratt, R. F. (2008) β-Ketophosphates as β-lactamase inhibitors: intramolecular cooperativity between the hydrophobic subsites of a class D β-lactamase. *Bioorg. Med. Chem. 16*, 6987–6994.
15. Chantrenne, H. (1948) Mixed anhydrides of benzoic and phosphoric acids. *Compt. Rend. Trav. Lab. Carlsberg, Ser. Chim. 26*, 297–314.
16. Weston, G. S., Blázquez, J., Baquero, F., and Shoichet, B. K. (1998) Structure-based enhancement of boronic acid-based inhibitors of AmpC β-lactamase. *J. Med. Chem. 41*, 4577–4586.