Induced fit phenomena in clathrate structures of cephalosporins

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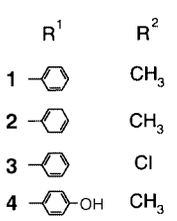
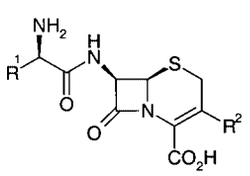
The antibiotics cephalexin, cephradine, cefaclor and cefadroxil form clathrate type inclusion compounds with naphthalene derivatives that readily crystallize from an aqueous solution. In these clathrates the antibiotic molecules form the hosting lattice and the naphthalene derivatives are the guest molecules, whereby water serves as "cement". A list of potential guest molecules was drawn up using the concept of molecular similarity. This list was extended by a series of compounds which are not supposed to fit. It was shown that a large variety of naphthalene derivatives can be hosted in clathrates with cephalexin, cephradine and cefaclor. Cefadroxil, however, is much more selective in accommodating guest molecules. Although cephalexin, cephradine and cefaclor form the principal hosting lattice and govern the overall crystal structure of the clathrates, the guest molecules are capable of inducing deviations in the framework of the host molecules, *i.e.* induced fit. Cefadroxil, however, lacks this adaptability due to the rigid three-dimensional hydrogen bonded structure of its hosting framework, and an exact fit of a guest molecule in the hosting framework of cefadroxil is thus required, *i.e.* lock and key concept. All four antibiotics have a limited adaptability by varying the number of water molecules in the clathrates. Certain guest molecules replace water in order to obtain the required space for inclusion, whereas other guest molecules cause incorporation of extra water, which is apparently beneficial for the crystal packing. However, the adaptability due to varying the water content cannot account for the remarkable flexibility in accommodating guest molecules exhibited by cephalexin, cephradine and cefaclor. The concept of induced fit is relevant for the understanding and design of clathrate type structures.

Introduction

Cephalexin **1**, cephradine **2**, cefaclor **3** and cefadroxil **4** are





widely used life-saving antibiotics belonging to the class of cephalosporins. One interesting feature they possess is their ability to form inclusion compounds with β-naphthol in the presence of water. These complexes are industrially of great importance as they can be used in the selective isolation of these cephalosporins from aqueous solution.[1] Although this phenomenon has been known for several years, the structural features of these clathrates have received almost no attention. Recently, we reported the crystal structures of complexes of β-naphthol with the four aforementioned cephalosporins.[2] In all four cases the antibiotic molecules appeared to be the host, while β-naphthol functions as the guest, and water fulfils the role of "cement" or "gluing" agent.[2,3] The crystal structures of the complexes of **1**, **2** and **3** with β-naphthol are isomorphous, all having channel-type frameworks based on a two-dimensional network of hydrogen bonds. These networks show some analogy with those present in the crystal structure of uncomplexed cefaclor dihydrate.[4] The crystal structure of the clathrate derived from cefadroxil **4** and β-naphthol, on the other hand, has a quite different morphology, *viz*. a three-dimensional network in which β-naphthol is hosted with a higher water content than found in the clathrates derived from **1**–**3**. Interestingly, the overall molecular geometry of cefadroxil in these complexes with β-naphthol is practically the same as in its uncomplexed monohydrate form.[5]

Having elucidated the crystal structures of the four clathrates of cephalosporins with β-naphthol, the interesting question arises as to whether guest molecules other than β-naphthol can be accommodated in these hosting antibiotic frameworks. Identifying other guest molecules may potentially open avenues for a more effective removal of cephalosporin from aqueous solutions. Moreover, complexing agents may be found with more environmentally acceptable properties than β-naphthol. For such a study, a series of compounds was selected, which, on a molecular level, show structural similarity with β-naphthol. This approach, based on the concept of molecular similarity, resembles that often used for the design of substrates for biological targets, such as receptors and enzymes. In this paper, the scope of clathrate formation of cephalosporins **1**–**4** with various selected guest molecules is described, whereby the role of these guests in the crystallization and crystal morphology is analysed. During this investigation some highly remarkable induced-fit phenomena were encountered in the clathrates.

Results and discussion

Applying the concept of molecular similarity, a series of potential guest molecules for the clathrates derived from cephalosporins **1**–**4**, having similar structural features to β-naphthol, was selected. These molecules were subjected

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Table 1 The isolated complexes of cephradine, cephalexin, cefaclor and cefadroxil with varying complexing agents

Entry	Complexing agent	Cephradine	Cephalexin	Cefaclor	Cefadroxil
1	β-Naphthol	A[a]	A	A	B[b]
2	α-Naphthol	A	A	A	B
3	Quinoline	A	A	A	[d]
4	Naphthalene	A	A	—[c]	[d]
5	1,2-Dihydroxynaphthalene	A	A	—	[d]
6	1,3-Dihydroxynaphthalene	A	A	—	[d]
7	1,4-Dihydroxynaphthalene	A	A	—	[d]
8	1,5-Dihydroxynaphthalene	A	A	—	[d]
9	1,6-Dihydroxynaphthalene	A	A	A	B
10	2,3-Dihydroxynaphthalene	A	A	—	[d]
11	2,6-Dihydroxynaphthalene	A	A	—	B
12	2,7-Dihydroxynaphthalene	A	A	—	B
13	Coumarin	A	A	—	[d]
14	8-Hydroxyquinoline	A	A	A	[d]
15	Indole	A	A	—	[d]
16	Indene	A	—	—	[d]
17	1-Acetonaphthone	A	—	—	[d]
18	2-Acetonaphthone	A	A	—	[d]
19	1-Chloronaphthalene	A	—	—	[d]
20	1,2,3,4-Tetrahydro-1-naphthol	A	—	—	[d]
21	1,5-Dihydroxy-1,2,3,4-tetrahydronaphthalene	A	—	—	[d]
22	2,2′-Bipyridyl	A	A	A	[d]

[a] A, Complex formed has type A structure. [b] B, Complex formed has type B structure. [c] — Not tried. [d] No complex formation.

to a molecular modelling study using docking, implying that the β-naphthol molecule was taken out of the lattice and the new molecules were fitted into the remaining cavity.[6] By docking it was found that only rather flat molecules can be accommodated in the hosting cavities formed by **1**–**4**. Whereas tetralin derivatives can adopt a conformation that is sufficiently flat to be hosted in the cavities formed by **1**–**4**, decalins are too thick, due to their puckered ring systems. The crystal structure of the clathrates formed by **1**–**3** with β-naphthol is referred to as type A, that of **4** with β-naphthol as type B. The procedure of selecting potential guests as outlined above, leads to a series of substituted naphthalenes and two other ring aromatics, listed in Table 1 (entries 1–16). This list was extended by a series of potential guests on more intuitive grounds, *viz.* entries 17–22 in Table 1. The compounds listed in Table 1 were all tested in clathrate formation experiments. Cephradine, cephalexin and cefadroxil were examined with all potential guests, while for cefaclor a limited number of experiments were performed because of the scarcity of this material. The cephalosporins **1**–**3** gave complexes with all compounds tested, whereas with cefadroxil **4** clathrate formation was only observed in a limited number of cases. These results are compiled in Table 1. The X-ray powder patterns of the clathrates derived from cephalexin **1**, cephradine **2** and cefaclor **3** reveal that they are all isomorphous with type A, while the few complexes obtained from cefadroxil **4** are all isomorphous with type B. These observations indicate that the cephalosporin host molecules strongly dictate the basic lattice in which the guest molecules are being accommodated. It is highly relevant to notice that for several potential guests, which were selected on intuitive grounds and for which docking experiments suggested that fitting into the β-naphthol cavity could not be achieved, the crystallization experiments show the opposite. In particular, type A complexes show a remarkable tolerance for guest molecules, much more so than type B complexes. This observation for type A clathrates clearly suggests that there must be a considerable flexibility in the accommodation of guests, much more than can be envisaged by a straightforward replacement of β-naphthol.

Several crystal structures of both type A and type B clathrates have been solved by single-crystal X-ray diffraction. These structures revealed an interesting, unpredicted role of water molecules in these clathrates. Initially, it was thought that the water molecules in the β-naphthol clathrates are essential for the stability of the basic host framework and were therefore regarded as fixed parts in these complexes.[2] However, the X-ray structures clearly showed that some guest molecules are able to remove water molecules from the cavity, whereas in other cases extra water molecules are incorporated. Such a possibility of having a variable amount of structural water was not taken into account during the docking studies, thus explaining the disagreement between prediction and experiments. The phenomena just mentioned are nicely illustrated in Fig. 1a–c for three type A clathrates derived from cephradine. Similar observations involving a varying number of water molecules were made for type B complexes as can be deduced from Fig. 2a–c. The hydrogen atoms of the water molecules could not be determined, hence only the oxygen atoms are shown in Fig. 1a–c and Fig. 2a–c. The phenomenon of varying amounts of structural water in the clathrates has some analogy with biological systems, in which often the scope of suitable substrates for a given receptor can be enlarged by removal of water molecules from the binding site.

The role of water as a "cementing" agent in these clathrates derived from cephalosporins allows certain flexibility in accepting guest molecules in these complexes. However, the higher guest tolerance in type A clathrates cannot be reconciled by solely varying the amount of water. More information became available by a detailed analysis of the structures of a series of type A clathrates, as determined by single-crystal X-ray diffraction. The dimensions and shapes of the hosting cavities in these complexes were compared. The distances between three sulfur atoms, which form three corners of a parallelogram (see Fig. 3) were measured. The S^1–S^2 distance is a measure of the length of the type A cavity as can be deduced from Fig. 1a–c and Fig. 3, because the line between S^1 and S^2 parallels the longest dimension of the guest molecule. For the type A structure, the distance (*d*) between two two-dimensional hydrogen bonded layers of cephalosporin molecules can be defined. In addition, the relative slip of two layers with respect to each other can be determined, as is indicated in Fig. 3. The sulfur–sulfur distances, the slip and the distance *d* are given in Table 2. These data reveal that the dimensions of the cavity vary with the guest accommodated in the complex. The S^1–S^2 distance, which is in the first approximation proportional to the size of the cavity, decreases from the large guest 2,2′-bipyridyl to the smaller guests naphthalene, quinoline and β-naphthol. Apparently, the hosting framework is able to adjust the dimensions of the cavity to match the size of the guest, in order to achieve the

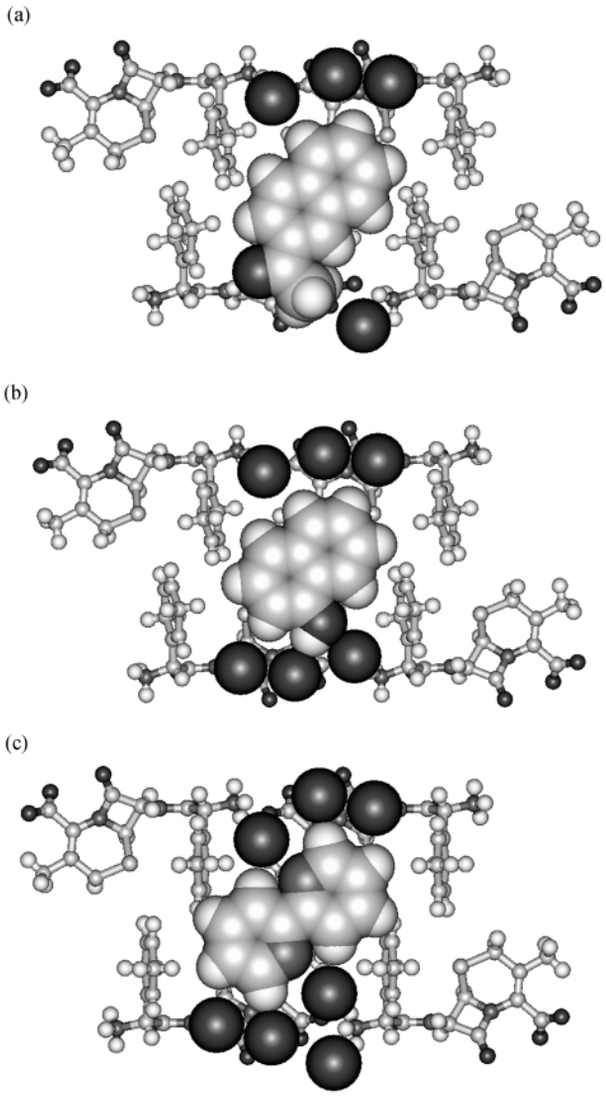

Fig. 1 Three type A complexes of cephradine with a varying amount of water. (a) Cephradine–2-acetonaphthone, host–guest–water is 2 : 1 : 4. (b) Cephradine–α-naphthol, host–guest–water is 2 : 1 : 6. (c) Cephradine–2,2′-bipyridyl, host–guest–water is 2 : 1 : 7.

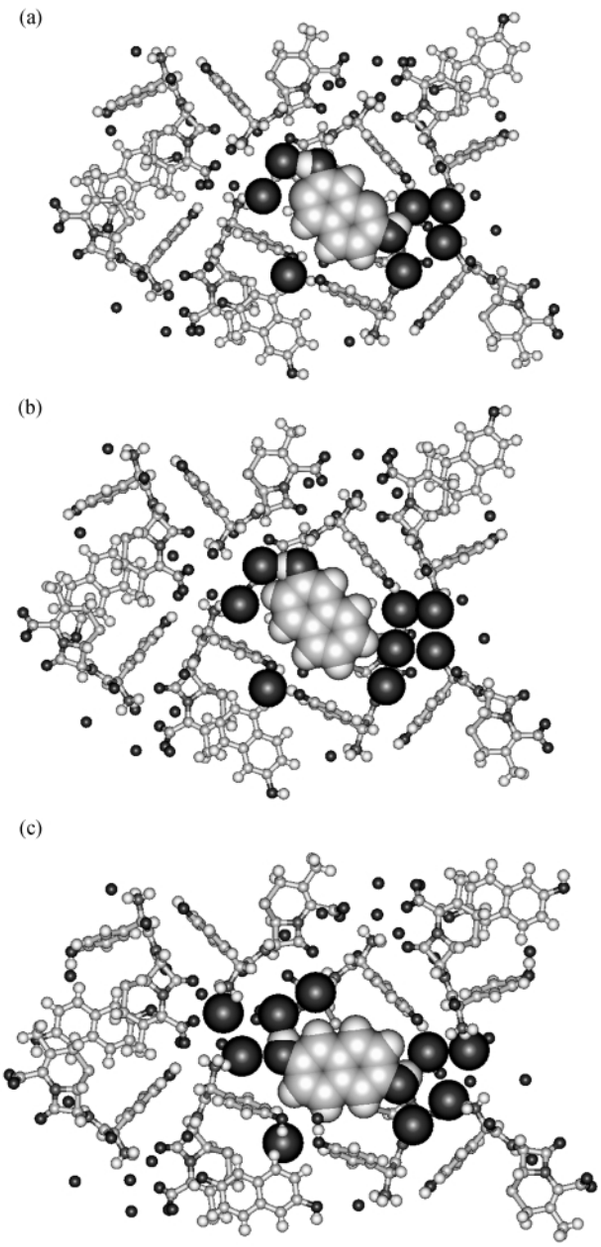

Fig. 2 Three type B complexes of cefadroxil with a varying amount of water. (a) Cefadroxil–2,7-dihydroxynaphthalene, host–guest–water is 2 : 1 : 7. (b) Cefadroxil–β-naphthol, host–guest–water is 2 : 1 : 8. (c) Cefadroxil–2,6-dihydroxynaphthalene, host–guest–water is 2 : 1 : 9.

most favourable crystal packing. The slip and the distance *d* are measures for the extent that the hosting framework is using its flexibility to adjust the size and shape of the cavity. For the cephradine complexes the distance *d* between the two-dimensional layers varies only marginally, *viz*. 0.10 Å. On the other hand, the slip shows a considerable decrease (1.15 Å) going from the largest to the smallest guest. These observations lead to the conclusion that the adjustment of the size and shape of the hosting cavity mainly takes place by varying the slip rather than the interlayer distance. The consequence of this adjustment of the cavity to the nature of the guest molecule is that a wider range of guest molecules than predicted can be accommodated in the cephradine framework, including those guest molecules that would not fit in the cavity arising from the removal of β-naphthol from the clathrate structures. The adaptability of the type A clathrate framework towards different guest molecules finds its origin in the rather weak interactions based on non-directional van der Waals forces between the two-dimensional layers of cephradine, allowing the slipping process to occur. This fitting of the guest into the hosting cavity has some analogy with the induced fit of substrates in enzyme cavities.

The situation with clathrates derived from cefadroxil is entirely different. This cephalosporin only forms complexes with a few guest molecules (Table 1), which is in strong contrast with the other three antibiotics. The four cefadroxil clathrates are isomorphous with the type B structure of its β-naphthol complex, as was established by powder diffraction analysis. This type B structure is three-dimensional in nature and is therefore lacking the adaptability arising from the slipping process as was observed for the type A clathrates. The type B framework is very rigid due to highly directional and strong hydrogen bonds, and as a consequence the flexibility in accommodating guest molecules is rather limited. Precise fitting is actually a prerequisite. In fact, only the adjustment of the number of water molecules accounts for the minimal adaptability. In other words cefadroxil conforms more to the lock and key model.

Concluding remarks

In conclusion, clathrate formation of cephalexin, cephradine and cefaclor can be achieved with a variety of complexing agents, designed by the concept of molecular similarity using the clathrate with β-naphthol as a basic model. The list of

Table 2 The dimensions of the cavities for different cephradine complexes[a]

Complex	S^1–S^2/Å	S^1–S^3/Å	S^2–S^3/Å	Slip/Å	*d*/Å
Cephradine–2,2′-bipyridyl	8.69	11.51	6.62	7.13	4.97
Cephradine–2-acetonaphthone	8.18	11.69	7.22	6.48	5.00
Cephradine–α-naphthol	8.12	11.74	7.33	6.39	5.01
Cephradine–naphthalene	8.06	11.73	7.33	6.34	4.97
Cephradine–quinoline	8.04	11.71	7.30	6.34	4.94
Cephradine–β-naphthol	7.73	11.71	7.54	5.98	4.90

[a] Distances were calculated after projection of S^1, S^2 and S^3 onto the *ac*-plane.

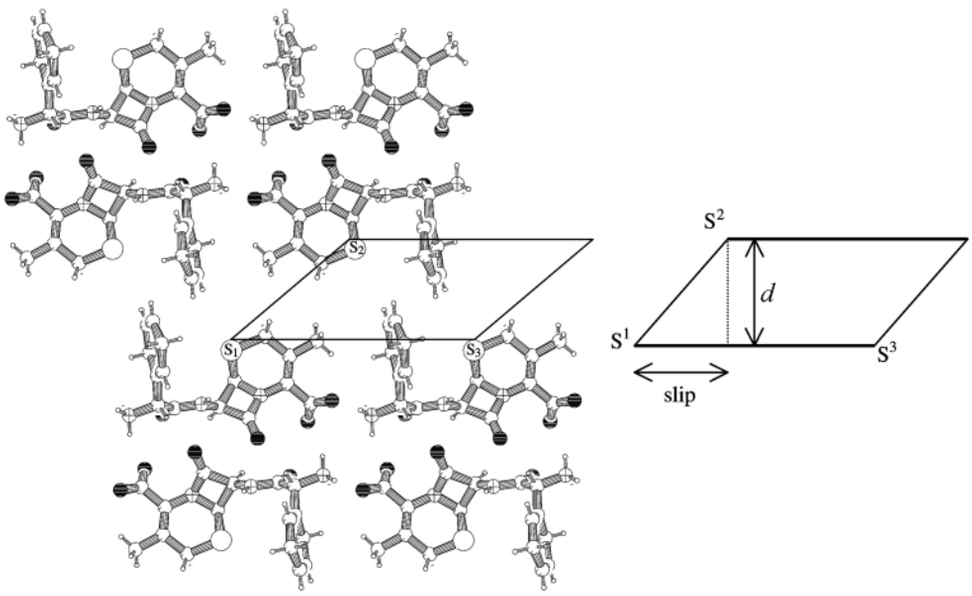

Fig. 3 A Pluton[7] drawing of the host-framework formed by cephradine viewed in the direction of the *b*-axis. Three sulfur-atoms have been numbered to illustrate the distances listed in Table 2.

effective clathrate-forming agents can be extended to a series of molecules that on the basis of molecular similarity and docking would not fit. This is due to the highly remarkable adaptability of the cephalosporin framework through an induced fit process. However, the clathrates derived from cefadroxil, containing an extra phenolic hydroxy function in comparison with the abovementioned cephalosporins, are very rigid and only a limited number of precisely fitting guest molecules can be accommodated in this antibiotic framework. For all four antibiotics a limited flexibility in accommodating guest molecules exists, due to variation of the number of cementing water molecules, but this effect is small compared with the induced fit process mentioned above. The research described in this paper is an example of crystal engineering, which is of great practical importance for identifying novel effective complexing agents for an efficient isolation of this important class of antibiotics.

Experimental

Cephalexin, cephradine, cefaclor and cefadroxil were obtained from DSM (Geleen). α-Naphthol, β-naphthol, 1,4-dihydroxynaphthalene, 2,7-dihydroxynaphthalene, 1,2,3,4-tetrahydro-1-naphthol and 1,5-dihydroxy-1,2,3,4-tetrahydronaphthalene were purchased from ACROS. 1,2-Dihydroxynaphthalene, 1,3-dihydroxynaphthalene, 1,5-dihydroxynaphthalene, 1,6-dihydroxynaphthalene, 2,3-dihydroxynaphthalene and 2,6-dihydroxynaphthalene were purchased from Aldrich.

Crystallization experiments

To a solution of cephalosporin (1.5 mmol) in 20% methanol (50 ml), complexing agent (0.75 mmol) dissolved in acetone (2 ml) was added. After one night at 4 °C the crystals were collected. The crystals were dried under a nitrogen flow and subjected to powder diffraction or, if appropriate, to single-crystal X-ray diffraction.

X-Ray structure determinations

Crystals were mounted on glass fibres and intensity data were collected on a Nonius CAD4 diffractometer. Intensity data were corrected for Lorentz and polarization effects. Semi-empirical absorption corrections (ψ-scan) were applied.[8] Details of all structure determinations are given in Table 3. The structures of **2a**, **2c**, **4a** and **4b** were solved using the ORIENT option of the DIRDIF program system.[9] The structures of **2b**, **2d** and **2e** were solved using the PATTY option of the DIRDIF program system.[10] Refinements were carried out with the SHELXL program.[11] All non-hydrogen atoms were refined with anisotropic temperature factors. The hydrogens were placed at calculated positions and refined isotropically in riding mode. Hydrogens attached to methyl groups and to the amine nitrogens were refined as rigid rotors with idealized sp^3 hybridisation to match maximum electron density in a difference Fourier map. For **2a** and **2e** the hydrogens attached to the cephradine molecule were freely refined subsequently. For **2c** all hydrogens, except the hydrogens attached to the amine group, were freely refined subsequently. All refinements were full-matrix least squares on F^2. In the case of **2a**, **2b**, **2c**, **2d** and **2e** a crystallographic twofold axis is passing through the centres of the cavities in which the guest molecules are situated. As a consequence, in the case of **2a**, **2b** and **2e** the guest molecules are disordered along this twofold axis. In all cases the two possible orientations of the guest molecules, which are related by twofold symmetry, could be refined using a disorder model. The naphthalene skeletons

Table 3 The crystal data of the complexes described in this paper[a]

	2a	2b	2c	2d	2e	4a	4b
Formula	$C_{41}H_{59}N_7O_{15}S_2$	$C_{42}H_{58}N_6O_{15}S_2$	$C_{42}H_{60}N_8O_{15}S_2$	$C_{42}H_{58}N_6O_{14.5}S_2$	$C_{44}H_{57}N_6O_{13.5}S_2$	$C_{42}H_{56}N_6O_{19}S_2$	$C_{42}H_{60}N_6O_{21}S_2$
M_w	954.07	951.06	981.10	943.06	950.08	1013.05	1049.08
T/K	293(2)	293(2)	173(2)	293(2)	293(2)	208(2)	293(2)
Crystal system	Monoclinic	Monoclinic	Monoclinic	Monoclinic	Monoclinic	Orthorhombic	Orthorhombic
Space group	$C2$	$C2$	$C2$	$C2$	$C2$	$P2_12_12_1$	$P2_12_12_1$
a/Å	23.4127(9)	23.471(2)	23.0227(8)	23.4584(6)	23.3855(5)	7.0902(7)	7.1079(18)
b/Å	7.1091(2)	7.1215(10)	7.1467(4)	7.1179(2)	7.1965(3)	21.273(3)	21.863(5)
c/Å	14.8060(6)	14.9304(19)	14.5544(4)	14.8922(5)	14.7588(4)	31.004(4)	32.306(4)
α/°	90	90	90	90	90	90	90
β/°	108.146(3)	108.268(14)	104.644(3)	108.571(2)	108.580(3)	90	90
γ/°	90	90	90	90	90	90	90
U/Å^3	2341.79(15)	2369.8(5)	2316.94(16)	2357.12(12)	2354.36(11)	4676.4(9)	5020.4(18)
Z	2	2	2	2	2	4	4
D_c/Mg m^{-3}	1.353	1.333	1.406	1.329	1.340	1.439	1.388
μ (Mo-Kα)/mm^{-1}	1.660	1.633	1.700	1.629	1.620	1.759	1.687
Refl. col./uniq.	2468/2402	4988/4411	2453/2387	2522/2425	2507/2410	5017/5017	5384/5384
R_{int}	0.0096	0.0347	0.0102	0.0149	0.0167		
Final R indices	$R1 = 0.0329$	$R1 = 0.0756$	$R1 = 0.0370$	$R1 = 0.0435$	$R1 = 0.0329$	$R1 = 0.1085$	$R1 = 0.0771$
$[I > 2\sigma(I)]$	$wR2 = 0.0915$	$wR2 = 0.1792$	$wR2 = 0.1005$	$wR2 = 0.1229$	$wR2 = 0.0899$	$wR2 = 0.2737$	$wR2 = 0.1898$

[a] **2a** Cephradine–quinoline. **2b** Cephradine–α-naphthol. **2c** Cephradine–2,2′-bipyridyl. **2d** Cephradine–naphthalene. **2e** Cephradine–2-acetonaphthone. **4a** Cefadroxil–2,7-dihydroxynaphthalene. **4b** Cefadroxil–2,6-dihydroxynaphthalene.

belonging to the two possible orientations of the 2-acetonaphthone molecule do not overlap but are shifted away from the twofold axis.

Crystal data

The β-naphthol complexes of cephalexin, cephradine, cefaclor and cefadroxil have been previously published.[2] The crystal data of the complexes described in this paper are summarized in Table 3. CCDC reference number 188/243. See http://www.rsc.org/suppdata/p2/b0/b001692f/ for crystallographic files in .cif format.

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