Molecular Selectivity and Cooperativity in the Clathrate-Type Complexation of Cephradine

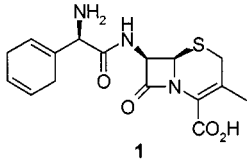
Gerardus J. Kemperman,[a] René de Gelder,[b] F. J. Dommerholt,[a] Antonius J. H. Klunder,[a] and Binne Zwanenburg*[a]

Keywords: Antibiotics / Clathrates / Inclusion compounds / Selective inclusion

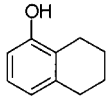
The cephalosporin antibiotic cephradine can form clathrate-type complexes with appropriate guest molecules. When a mixture of *o*- and *p*-disubstituted benzene derivatives is subjected to complexation with cephradine, a high preference and in some cases complete selectivity for one isomer is shown by the host molecule. It is demonstrated that clathration with cephradine is an effective method for the separation of *o* and *p* isomers. Another interesting feature is observed when a cocktail of appropriate guest molecules is used in the complexation with cephradine. The observed preference for inclusion of more than one type of complexing agent strongly points to a cooperative effect of guest molecules in the resulting complex. This cooperative effect, however, does not result in more efficient complexation – that is, a lower residual concentration of the antibiotic – which is desirable for industrial applications.

Introduction

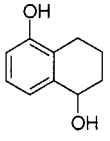
The cephalosporin antibiotic cephradine forms clathrate-type complexes with a variety of naphthalene- and benzene-derived guest molecules.[1] Clathration of cephalosporins is an industrially relevant method to remove these antibiotics from aqueous reaction mixtures obtained by enzymatic synthesis from a β-lactam nucleus and a D-amino acid side chain.[2] The accommodation of guest molecules in the clathrate-type complexation of the cephalosporin antibiotic cephradine (**1**) has considerable adaptability.[1] Nevertheless, this induced-fit phenomenon has its limitations, as is demonstrated by, for instance, the acceptance of tetrahydronaphthols **2** and **3** as guest molecules while decalinol **4** does not form a complex.[1] It may further be hypothesized that the difference in complexation behavior between molecules of related structures may be sufficient to give selective complexation. Separation of isomeric molecules then becomes an interesting option. When effective, separation by selective complexation is attractive, as it is a fast and inexpensive methodology. Toda et al. used inclusion with 1,1,6,6,-tetraphenylhexa-2,4-diyne-1,6-diol (**5**) as the host molecule for the separation of *o*- and *p*-disubstituted benzene derivatives and mono- and disubstituted naphthalenes.[3] In this paper the separation of *o*- and *p*-disubstituted benzene derivatives through selective complexation with cephradine (**1**) is described.



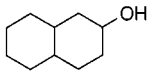
1



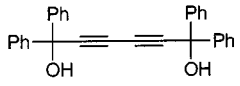
2



3



4



5

In the process of identifying suitable complexing agents for cephalosporin-type antibiotics, individual candidates were subjected to complexation conditions. These experiments were conducted most efficiently on a series of candidates in a parallel fashion, as described in a preceding paper.[1] Sada et al., used the parallel methodology to develop inclusion compounds derived from ammonium carboxylates as the hosting system.[4] An alternative approach would be to expose a hosting compound to a mixture of

[a] Department of Organic Chemistry, NSR Institute for Molecular Structure, Design and Synthesis, University of Nijmegen,
Toernooiveld, 6525 ED, Nijmegen, The Netherlands
Fax: (internat.) + 31-24/3653393
E-mail: zwanenb@sci.kun.nl
[b] Department of Inorganic Chemistry, NSR Institute for Molecular Structure, Design and Synthesis, University of Nijmegen,
Toernooiveld, 6525 ED, Nijmegen, The Netherlands

  1434–193X/02/0102–0350 $ 17.50+.50/0

potential complexants, assuming that complexation would preferentially occur with the best guest molecule. In fact, this cocktail approach would be very attractive for identifying the optimal complexing agent. An additional feature of this cocktail approach might be that two or more compounds would display a cooperative effect in filling up the hosting cavities. The combined properties of two or more complexants would in such a case be more appropriate for complexation than those of the individual compounds. Complexes with more than one guest molecule are often referred to as solid solutions. An example of such a process in which two or more molecules are involved is the resolution of racemates by the *Dutch resolution* methodology.[5] Here, potential resolving agents are exposed to a racemate in a cocktail fashion. Solid solutions may have a higher density and a lower solubility than pure components.[6] From this point of view, complexation of cephradine with more than one guest molecule could result in a lower residual concentration of the antibiotic than could be attained by using a single complexant. For industrial applications, the residual concentration of antibiotic must be minimized, as this is the part that is lost during the workup of reaction mixtures. This paper describes cooperativity of complexants in complexation with cephradine as well as the effect of this on the residual concentration of the antibiotic.

Results and Discussion

Separation of *o*- and *p*-Disubstituted Benzene Derivatives by Preferential Complexation with Cephradine

A series of *o*- and *p*-disubstituted benzene derivatives was subjected to complexation with cephradine. The underlying assumption is that the hosting framework of this antibiotic may show preferential or even selective complexation with one of the isomers. The *o*/*p* mixtures were exposed to the antibiotic, with either water or ethyl acetate containing 5% of water as the medium. In the latter case, the cephalosporin formed a suspension. These heterogeneous conditions resemble those used by Toda et al. for experiments with tartrate-derived bis(carbinol) host molecules.[7] The complexant can readily be separated from cephradine by acidic or basic hydrolysis of the complex followed by extraction with an organic solvent. This separation process makes use of the zwitterionic nature of the antibiotic.

There is a considerable solvent effect on the composition of the precipitated molecular complexes. In some cases, the selectivity of the complexation is entirely opposite for the two media (Table 1), as is the case for hydroxyacetophenone and methylbenzoic acid. This difference in behavior may be attributed to different Gibbs energies of solvation of the guest molecule in the two media. The effect of the Gibbs energy of solvation during the complexation has been noted previously.[8]

Cooperative Effect of Complexants in the Clathration with Cephradine

The exposure of a solution of cephradine to a mixture of complexing agents may result either in preferential (or selective) complexation or in the simultaneous inclusion of more than one guest molecule. In the latter case, a solid solution will be obtained. In order to investigate the possible formation of such a solid solution, cephradine was treated with a cocktail of mono-, di-, and trihydroxybenzenes. Prior to this investigation, these compounds had all been subjected individually to complexation conditions with cephradine. In this way it was ensured that all compounds could form complexes with cephradine. The complexes were characterized by X-ray powder diffraction (Figure 1a,b) and the efficiency of complexation for each complexant was determined by HPLC measurement. From the X-ray powder patterns it was concluded that the structures of the cephradine complexes with phenol and 1,3-dihydroxybenzene (Figure 1a) were isomorphous. It was also found that the cephradine complexes with 1,2-dihydroxybenzene, 1,2,3-trihydroxybenzene, and 1,3,5-trihydroxybenzene (Figure 1b) were isomorphous, but with a structure different from that observed for the first two guest molecules mentioned. The residual concentrations of cephradine in the complexation with the mono-, di-, and trihydroxybenzenes employed in this study are collected in Table 2. The lower the residual concentration of antibiotic, the amount of antibiotic that is lost during workup, the more economically viable the complexation process will be.

Table 1. Cephradine complexation with *o*/*p* mixtures of disubstituted benzene derivatives

ortho/*para* mixture	Purity[a]	
	Ethyl acetate	Water
Hydroxybenzoic acid	84% *ortho*	52% *ortho*
Aminophenol	81% *ortho*	100% *ortho*
Dihydroxybenzene	90% *para*	57% *ortho*
Nitrophenol	67% *para*	[b]
Aminobenzoic acid	[b]	100% *ortho*
Nitrotoluene	72% *para*	[b]
Hydroxyacetophenone	100% *para*	100% *ortho*
Methoxyacetophenone	62% *ortho*	100% *ortho*
Methylbenzoic acid	100% *para*	100% *ortho*
Methyl hydroxybenzoate	73% *para*	100% *para*

[a] The purity of the major product after decomplexation. [b] No complex formation.

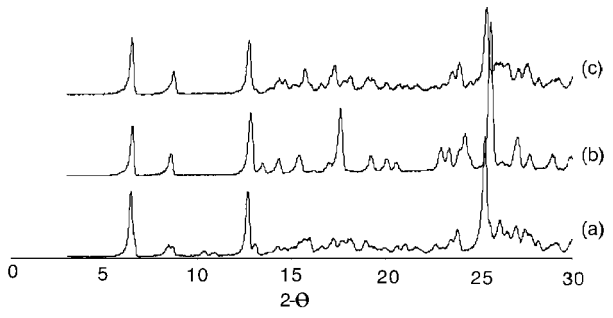


Figure 1. The powder pattern of the cephradine complexes with 1,3-dihydroxybenzene (**a**), 1,3,5-trihydroxybenzene (**b**), 1,3-dihydroxybenzene and 1,3,5-trihydroxybenzene (**c**)

Table 2. The residual concentration of cephradine in the complexation with mono-, di-, and trihydroxybenzenes

Complexant (1 equiv.)	*c*(cephradine) [mM]
Phenol	18.1
1,2-Dihydroxybenzene	21.5
1,3-Dihydroxybenzene	10.9
1,2,3-Trihydroxybenzene	> 29
1,3,5-Trihydroxybenzene	> 29

The components in the cocktail of mono-, di-, and trihydroxybenzenes are all reasonably soluble in water. This has the advantage that complexation can start from a completely homogeneous solution, with equal concentrations of each component. On treatment with cephradine, this mixture of hydroxybenzenes gave needle-shaped crystals within a couple of hours. HPLC analysis of the needles revealed that all five compounds were incorporated in the obtained crystalline material. The ratio of the individual components in the crystals was not the same in successive experiments and turned out to be dependent on the precise conditions of the complexation experiments. In all cases, however, the needles appeared unstable and spontaneously turned into powders upon drying. Single-crystal X-ray diffraction was therefore not possible. According to the powder diffraction pattern of the resulting powder, the structure did not correspond to any of those found for the complexes of cephradine with any of the individual guest molecules. Another interesting observation was that, on standing in the aqueous mother liquor, the initially formed needles recrystallized to form cubes. Unlike the needles, the cubes were fully stable and could be analyzed by single-crystal X-ray diffraction. The crystal structure, depicted in Figure 2, proved that two different guest molecules − 1,3-dihydroxybenzene and 1,3,5-trihydroxybenzene − were included in this cube-shaped cephradine complex. In addition, the complex contained water molecules, which formed hydrogen bonds with polar groups of both host and guest molecules, and thus fulfilled the role of cement. The guest molecules displayed only nonpolar van der Waals interactions with the hosting framework, and were included in the cavities merely by steric barriers, which is typical for a clathrate-type structure. Two types of cavities could be recognized in this structure, one of which was entirely occupied by 1,3-dihydroxybenzene, the other 50% by 1,3-dihydroxybenzene and 50% by 1,3,5-trihydroxybenzene. This resulted in an overall ratio of the respective constituents of 3:1. Powder diffraction showed that this crystal structure was isomorphous with that of the powder originating from the initially obtained needles (Figure 1c). Apparently, the formation of these needles is kinetically preferred, but when the needles are kept in the mother liquor they recrystallize to the thermodynamically favored cubes containing only 1,3-dihydroxybenzene and 1,3,5-trihydroxybenzene. It was found that this three-component complex of cephradine, 1,3-dihydroxybenzene, and 1,3,5-trihydroxybenzene could also be prepared directly by treating a cephradine solution with a mixture of the two complexants.

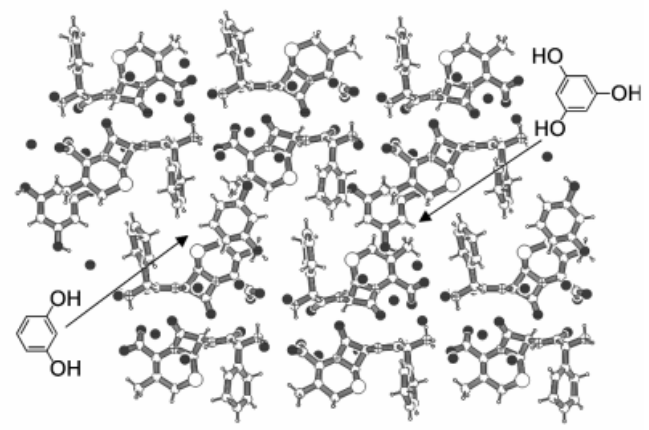


Figure 2. The complex of cephradine and 1,3-dihydroxybenzene and 1,3,5-trihydroxybenzene as guest molecules

The formation of a molecular complex of cephradine with two complexants clearly points to a cooperative effect of the two complexants; otherwise there would have been preferential formation of a complex with only the most efficient component, 1,3-dihydroxybenzene. The thermodynamic preference for complexation with two guest molecules in the hosting framework of cephradine raised the question of whether this complex would afford a lower residual concentration of the antibiotic than that obtained when the complex with only one of the complexing agents is used. The residual concentrations of cephradine obtained when 1,3-dihydroxybenzene, the more efficient complexant of the two, was used as a single complexant and that obtained when a complex with a 3:1 or 2:1 ratio of 1,3-dihydroxybenzene and 1,3,5-trihydroxybenzene was applied were therefore compared. The resulting precipitates were subjected to HPLC analysis to ensure that both guest molecules were enclathrated by cephradine. In addition, X-ray powder diffraction confirmed that the precipitates were clathrates isomorphous to that shown in Figure 2. It was found that at best similar efficiencies were achieved in the two cases, as is evident from Table 3. There is therefore no clear advantage in the use of the complex with two complexants in this case.

Table 3. The efficiency of cephradine complexation with 1,3-dihydroxybenzene and 1,3,5-trihydroxybenzene

Equiv. of complexant	1,3-Di-/1,3,5-trihydroxybenzene	*c*(cephradine) [mM]
1	100:0	10.9
1	75:25	12.6
1	67:33	12.9
0.5	100:0	13.8
0.5	75:25	13.8
0.5	67:33	14.9

Another case of cooperativity of two complexants was encountered when cephradine was treated with α-naphthol and methyl 4-hydroxybenzoate (paraben). Similarly, β-naphthol and paraben were jointly accommodated in a complex with cephradine. Both crystalline complexes were analyzed by X-ray powder diffraction; the resulting powder patterns are compared in Figure 3. The powder diagrams clearly revealed that these complexes with two components had a *C*2 cavity structure, which is also obtained with α-naphthol or β-naphthol as the single complexant.[1,9] Figure 4 shows this *C*2 cavity structure of cephradine and α-naphthol, with a host/guest ratio of 2:1, as was apparent from single-crystal X-ray diffraction analysis.[9] Remarkably, the complex with paraben as the single complexant had the *C*2 channel-type structure with a host/guest ratio of 1:1.[9] For the complex of cephradine and methyl 3-hydroxybenzoate, an isomorphous *C*2 cavity structure was observed, as was shown by single-crystal X-ray diffraction (Figure 5). Apparently, the inclusion of paraben into the complex together with naphthol results in a thermodynamic preference for the *C*2 cavity structure. A striking demonstration of this thermodynamic preference for the *C*2 cavity structure is the fact that the complex of cephradine and paraben adopts the *C*2 cavity structure when cephradine is treated with paraben containing a small amount of α- or β-naphthol as a seed molecule (5% suffices). The inclusion of even a minor amount of naphthol has a profound effect on the structure of the complex.

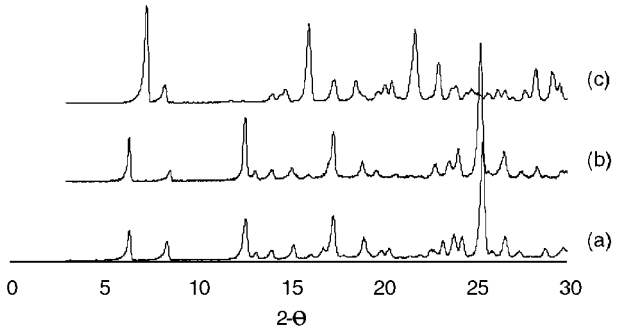


Figure 3. The X-ray powder patterns of the cephradine complexes with α-naphthol **(a)**, mixture of α-naphthol and paraben **(b)**, and paraben **(c)**

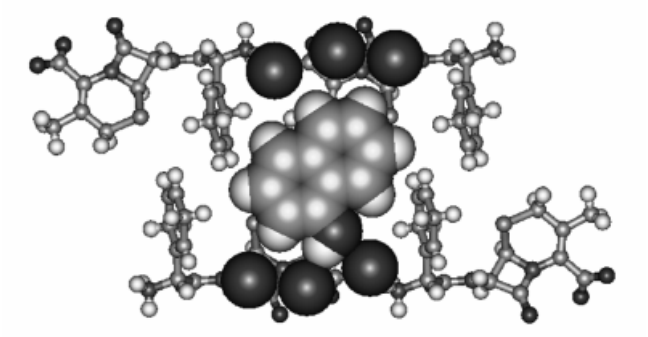


Figure 4. The complex of cephradine and α-naphthol

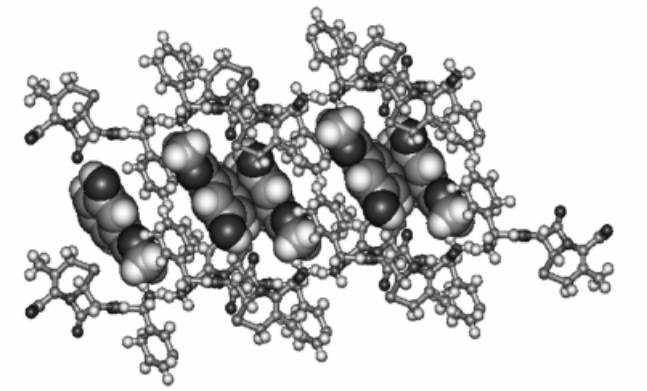


Figure 5. The complex of cephradine and methyl 3-hydroxybenzoate

In this case as well, the residual concentrations of antibiotic were compared for the case with only α-naphthol or paraben as the complexant and that in which a two-complexant complex with paraben was used. However, cephradine complexation with the combination of the two complexants was less efficient than complexation using either α-naphthol or paraben, as can be concluded from the data in Table 4. The cooperative effect of two complexants does not produce more efficient complexation.

Table 4. Complexation of cephradine with α-naphthol and paraben

Equiv. of complexant	Paraben/ α-naphthol	Structure type	*c*(cephradine) [mM]
2	0:100	*C*2 cavity	1.3
2	100:0	*C*2 channel	3.7
1	90:10	*C*2 cavity	6.0
2	90:10	*C*2 cavity	4.6

Conclusion

The separation of *o*/*p* mixtures by selective complexation with cephradine has been demonstrated. There is a notable effect from the medium used in the complexation experiments. In some cases complete selectivity was achieved, thus allowing the separation of those *o* and *p* isomers.

Cooperativity of two complexants results in complexes in which two guest molecules are incorporated in the hosting framework of cephradine. Hence, the accommodation of two components must be thermodynamically preferred over selective complexation with one of the complexing agents. This positive outcome of the cooperative effect must be attributed to a strain-relieving effect and a subtle pattern of molecular interactions throughout the overall structure of the complex, which results in an energetic advantage. The two-guest complexes studied do not give lower residual concentrations of the antibiotic and so the use of two-compon-

ent complexation has no clear advantage in the enzymatic synthesis of cephalosporin-type antibiotics in comparison with the single-component complexation discussed in preceding papers.[8] Nevertheless, the cooperative effect of two guest molecules in molecular complexation is a remarkable phenomenon that is worth further study.

Experimental Section

General Remarks: Cephradine monohydrate was a generous gift of DSM Life Sciences Group (Geleen, The Netherlands). All complexing agents used are commercially available and were purchased either from Acros or from Aldrich. X-ray powder patterns were recorded with a Philips PW1820 Automatic Powder Diffractometer equipped with a Philips PW1830 High-Voltage Generator. For HPLC analysis, a Pharmacia LKB.LCC 2252 HPLC with a reversed phase column (Merck 50983 LiChrospher 100RP18, 5 μm, 250 × 4 mm) was used. For detection, a Farmacia LKB.UV-MII UV detector (λ = 254 nm) was used. An appropriate eluent for analysis was a mixture of acetonitrile (HPLC grade) and a 50 mM phosphoric acid buffer with pH = 2.7.

Cephradine with a Mixture of *o*- and *p*-Disubstituted Benzene Derivatives: Cephradine (525 mg, 1.5 mmol) was dissolved in water (40 mL), and a mixture of the *o*- and *p*-disubstituted benzene derivatives (0.75 mmol of each) dissolved in methanol (2 mL) was added to the stirred solution. After one night, the resulting crystals were collected by filtration. The complex was hydrolyzed in 2 M HCl, followed by extraction of the included compound with ethyl acetate. The ethyl acetate was evaporated and the resulting product was analyzed by HPLC. The experiments were also performed in ethyl acetate. To this end, a mixture of *o*- and *p*-disubstituted benzene derivative (0.75 mmol of each) was dissolved in ethyl acetate (10 mL) containing 0.5 mL of water, and cephradine (525 mg, 1.5 mmol) was added to this stirred solution. After the resulting suspension had been stirred overnight, the complex was isolated by filtration and analyzed as described above.

Cephradine Complexation with Mono-, Di-, and Trihydroxybenzenes: Cephradine (525 mg, 1.5 mmol) was dissolved in water (50 mL). A mixture of phenol, catechol, resorcinol, pyrogallol, and phloroglucinol (0.75 mmol of each), dissolved in methanol (4 mL), was added to the cephradine solution. The crystalline complexes that formed on standing were collected and analyzed by HPLC and X-ray powder diffraction. The cube-shaped single crystals that resulted from these experiments were analyzed by single-crystal X-ray diffraction, as described further on in this section. Efficiency measurements were performed as follows. A solution of 1,3-dihydroxybenzene and 1,3,5-trihydroxybenzene (in a total amount of either 0.75 mmol or 1.5 mmol, comprising the components in a ratio of 100:0, 75:25, or 67:33) in methanol (2 mL) was added to a stirred solution of cephradine (525 mg, 1.5 mmol) in water (50 mL). Filtrate samples, taken 90 min and 24 h after the addition of the complexing agents, were analyzed by HPLC to determine the residual concentration of cephradine. After 24 h, the precipitated complex was filtered, dried under a flow of nitrogen, and analyzed by X-ray powder diffraction. The complex was hydrolyzed in 2 M HCl, which was extracted with ethyl acetate. The ethyl acetate was evaporated and the residue was analyzed by HPLC to determine the ratio of the included guest molecules.

Cephradine with α- or β-Naphthol and Methyl 4-Hydroxybenzoate (Paraben): Cephradine (525 mg, 1.5 mmol) was dissolved in water (50 mL), and a mixture of α-(or β-)naphthol (0.75 mmol) and paraben (0.75 mmol), dissolved in methanol (2 mL), was added to the cephradine solution. After one night, crystals were collected by filtration and analyzed by HPLC and X-ray powder diffraction. Efficiency measurements were performed as follows. A solution of α-naphthol and paraben (total amount either 0.75 mmol or 1.5 mmol in a ratio of 100:0, 0:100, 10:90, respectively), dissolved in methanol (2 mL), was added to a stirred solution of cephradine (525 mg, 1.5 mmol) in water (50 mL). Filtrate samples, which were taken 90 min and 24 h after the addition of the complexing agents, were analyzed by HPLC to determine the residual concentration of cephradine.

Crystal Structure Determination of the Cephradine/1,3-Dihydroxybenzene/1,3,5-Trihydroxybenzene Complex: A transparent, colorless crystal (0.31 × 0.31 × 0.29 mm) was mounted on a glass fiber and intensity data were collected with a Nonius CAD4 diffractometer. The radiation used was Cu-K_α (graphite-monochromated) with λ = 1.54184Å. Intensity data were corrected for Lorentz and polarization effects. Semiempirical absorption corrections (ψ-scan) were applied.[10] The structures were solved by using the DIRDIF program system.[11] Structure refinement was performed by full-matrix, least-squares on F^2 (SHELXL program).[12] $C_{76}H_{116}N_{12}O_{34}S_4$, M_w = 1870.05, T = 293(2) K, triclinic, $P\bar{1}$, a = 7.0822(4) Å, b = 15.5835(11) Å, c = 21.6513(14) Å, α = 105.877(14)°, β = 94.636(11)°, γ = 91.243(10)°, V = 2288.5(3) Å^3, Z = 1, $D_{calcd.}$ = 1.357 Mgm^{-3}, refl. collected/unique 8928/8928, GOF on F^2 = 1.062, SHELXL-97 weight parameters 0.100300 and 0.309400, R (all data): $R1$ = 0.0686, $wR2$ = 0.1570, largest diff. peak and hole 0.612 and −0.332 e·A^{-3}. The crystal structure data for the complex of cephradine and α-naphthol and the complex of cephradine and methyl 3-hydroxybenzoate were reported in a previous paper.[9] Crystallographic data (excluding structure factors) for the structure reported in this paper have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication no. CCDC-163018. Copies of the data can be obtained free of charge on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK [Fax: (internat.) + 44-1223/336-033; E-mail: deposit@ccdc.cam.ac.uk].

Acknowledgments

We are indebted to DSM Life Sciences Group (Geleen, The Netherlands) and the Dutch Ministry of Economical Affairs for their financial support.

[1] G. J. Kemperman, R. de Gelder, F. J. Dommerholt, P. C. Raemakers-Franken, A. J. H. Klunder, B. Zwanenburg, *J. Chem. Soc., Perkin Trans. 2* **2000**, 1425−1429.
[2] A. Bruggink, *Chimia* **1996**, *50*, 431−432; A. Bruggink, E. C. Roos, E. de Vroom, *Org. Proc. Res. Dev.* **1998**, *2*, 128−133; WO 93/12250, EP 618979, US 5470717 [*Chem. Abstr.* **1993**, *119*, 137533w].
[3] F. Toda, *Adv. Supramol. Chem.* **1992**, *2*, 141−191; F. Toda, *Top. Curr. Chem.* **1987**, *140*, 43−69.
[4] K. Sada, K. Yoshikawa, M. Miyata, *Chem. Commun.* **1998**, 1763−1764.
[5] T. Vries, H. Wynberg, E. van Echten, J. Koek, W. Ten Hoeve, R. M. Kellog, Q. B. Broxterman, A. Minnaard, B. Kaptein, S. van der Sluis, L. Hulshof, J. Kooistra, *Angew. Chem. Int. Ed.* **1998**, *37*, 2349−2354.

[6] A. Collet, *Angew. Chem. Int. Ed.* **1998**, *37*, 3239−3241.
[7] F. Toda, Y. Tohi, *J. Chem. Soc., Chem. Commun.* **1993**, 1238−1240.
[8] G. J. Kemperman, R. de Gelder, F. J. Dommerholt, P. C. Raemakers-Franken, A. J. H. Klunder, B. Zwanenburg, *J. Chem. Soc., Perkin Trans. 2* **2001**, 633−638.
[9] G. J. Kemperman, R. de Gelder, F. J. Dommerholt, P. C. Raemakers-Franken, A. J. H. Klunder, B. Zwanenburg, *Eur. J. Org. Chem.* **2001**, 3641−3650.
[10] A. C. T. North, D. C. Philips, F. S. Mathews, *Acta Crystallogr., Sect. A* **1968**, *24*, 351.
[11] P. T. Beurskens, G. Beurskens, W. P. Bosman, R. de Gelder, S. Garcia-Granda, R. O. Gould, R. Israel, J. M. M. Smits, *DIRDIF-96.* A computer program system for crystal structure determination by Patterson methods and direct methods applied to difference structure factors, Crystallography Laboratory, University of Nijmegen, The Netherlands, **1996**.
[12] G. M. Sheldrick, *SHELXL-97. Program for the refinement of crystal structures*, University of Göttingen, Germany, **1997**.

Received May 9, 2001
[O01226]