Circular Dichroism Spectra of the Achiral Guest *N*-Aryl-*N*-nitrosamines Included in the Crystal Host Matrices of Cholic Acid

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The crystalline inclusion complexes of cholic acid with three symmetric *N*-aryl-*N*-nitrosamines were prepared, and their X-ray structures were solved. As a result of chiral conformations adopted by the enclathrated guest nitrosamines, the solid-state CD spectra were measured in KBr disks. The observed Cotton effect sign, corresponding to the n−π^* transition, was correlated with the helicity of the twisted nitrosamine chromophore conjugated with the *N*-aryl substituent. In addition, the absolute configuration of the enantiomorphous crystals of *N*-benzyl-*N*-nitroso-4-chloroaniline was established on the basis of the solid-state CD and X-ray crystallographic results.

Introduction

The chemistry of *N*-nitrosamines is a subject of considerable interest with regard to their strong carcinogenic and mutagenic properties.[1] Since their biological activity is critically influenced by the molecular geometry, the stereochemistry of these compounds has been investigated by various experimental and theoretical methods.[2] Particularly circular dichroism (CD) spectroscopy, owing to its sensitivity to changes in the molecular geometry, is a useful tool for conformational and configurational assignments.[3,4] Recently, we have shown that the CD spectra can be measured also for formally achiral molecules that gain chirality by inclusion complexation with optically active hosts.[5] This is apparently due to chiral conformations assumed by the guest molecules enclosed in the chiral host matrix.[5,6]

Easily accessible, naturally occurring cholic (**CA**) and deoxycholic acid (**DCA**) have proved to be particularly useful and versatile host compounds.[5,7] The crystal lattices of these bile acids contain void helicoidal channels that can be filled with guest molecules of suitable shape and size.[7] For example, a chiral discrimination occurring during the complexation of simple *N*-nitrosopiperidines allowed us to obtain these compounds in the optically active form, as manifested by their CD spectra.[5b] Since the configuration of the bile acids is well established, the X-ray crystallographic analysis of single crystals of the corresponding inclusion complexes allows unequivocal assignment of the absolute configuration of the guest molecules. Generally, determination of the crystal structures of the **CA** complexes is much more straightforward than that of the corresponding **DCA** clathrates because of a severe guest disorder in the last.[8] Continuing our interest in this subject, we turned attention toward *N*-aryl-*N*-nitrosamines as potential guest molecules. Among various model nitrosamines studied by the chiroptical methods, there are only very few examples of this class of substances.[4] Therefore we obtained several inclusion complexes of **CA** with the nitrosamines **1**−**3** and measured their solid-state CD spectra. We were also able to detect the CD of the chiral crystal of **4**. Combining the X-ray crystallographic results for the inclusion complexes with the solid-state CD spectra we were able to correlate the observed Cotton effect signs with the molecular geometry of the guest nitrosamine molecules.

Results and Discussion

The pale yellow needlelike crystals of the 1:1 complex **1·CA** were prepared by a crystallization of **CA** from the guest nitrosamine **1**. The 1:2 complexes **2·CA** and **3·CA** were obtained by cocrystallization of **CA** with an excess of the corresponding guest compounds from a small amount of 2-butanol, which is one of very few solvents

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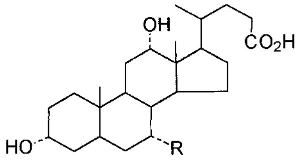
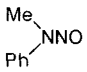


CA, R = OH
DCA, R = H



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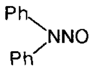

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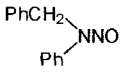

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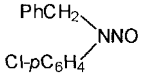

4

that do not form inclusion complexes with **CA**.[9] The crystal structures of **1·CA**, **2·CA**, and **3·CA** resemble those found in many of the **CA** tubulate inclusion compounds.[7] The **CA** molecules self-assemble through intermolecular hydrogen bonds into corrugated amphiphilic bilayers. The channels formed between the stacked hydrophobic layers are filled with the guest nitrosamine molecules. In the case of **1·CA** the guest molecules are organized in infinite helicoidal columns propagating along the *y* axis (Figure 1). The phenyl ring in the enclathrated **1** (Figure 2) is significantly twisted from the NNO plane in the *M* sense [C═C−N−N torsion angle is 10.2(9)°], whereas the nitrosamino group is nearly planar [$C_{Me}-N-N{=}O$ and $C_{Ph}-N-N{=}O$ are 3.5(1)° and 176.5(6)°, respectively]. The guest nitrosamine in **2·CA** shows a static disorder similar to that observed in many **DCA** inclusion complexes (see Experimental Section), and an additional disorder is observed for the *N*-nitroso group (Figure 2). The guest molecule **2** assumes a helicoidal geometry with both phenyl rings strongly twisted from the plane of the NNO group [the torsional angles C═C−N−N are 30.8(7)° and 65.4(6)°] in the *P* sense. The complex **3·CA** is isostructural with the recently reported benzophenone·**CA** clathrate;[5c] two crystallographically independent guest molecules assume similar conformations and pack separately in two independent and topologically similar channels (Figure 1b). Their *N*-nitrosoaniline fragment adopts a skewed conformations of the *M* helicity [C═C−N−N are −28.3(11)° and −24.3(9)°]. The nitrosamino group in one of these molecules is nearly planar [$C_{CH_2}-N-N{=}O$ and $C_{Ph}-N-N{=}O$ are −2.5(10)° and 177.6(6)°, respectively], and in the second one the amino nitrogen is slightly pyramidalized [$C_{CH_2}-N-N{=}O$ and $C_{Ph}-N-N{=}O$ are −0.1(13)° and 173.6(7)°, respectively]. In the complexes **1·CA** and **3·CA** the nitroso oxygen of the guest nitrosamines adopts the *anti* orientation to the phenyl group.

The CD spectra of the complexes **1·CA**-**3·CA** presented in Figures 3 and 4 show relatively strong Cotton effects near 400 nm with a pronounced vibronic fine structure. They unequivocally can be assigned to the forbidden n−π^* electronic transition of the nitrosamino chromophore.[4] Their position is shifted by ca. 20 nm to the red in comparison to the n−π^* band in the aliphatic nitrosamines that is apparently due to a conjugation between the NNO group and the phenyl substituent. An intriguing behavior is shown by **3·CA**, since in the region of the n−π^* transition it exhibits a bisignate Cotton effect composed of a strong positive band at 402 nm and a weaker negative one centered at 373 nm (Figure 4).

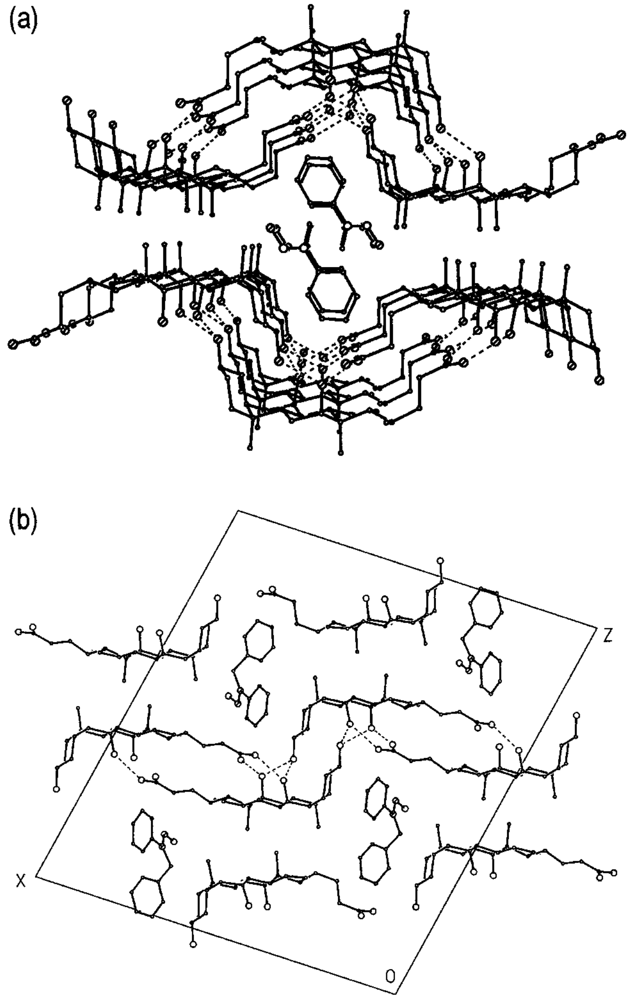

Figure 1. The crystal packing of the inclusion complexes (a) **1·CA** and (b) **3·CA** viewed along the *y* axis. The hydrogen atoms are omitted for clarity. The broken lines represent the hydrogen bonds.

Usually, the equilibria between two or more conformers contributing with opposite CD signs are responsible for the bisignate Cotton effects observed in the solution CD spectra. Obviously, this is not possible for the crystalline clathrate **3·CA**. This also cannot be due to the host−guest interaction within the complex because a spectrum very similar to that of **3·CA** is displayed by the uncomplexed *p*-chloro analogue **4** (Figure 4). The CD measurement for the last compound was possible since it fortunately crystallizes in the enantiomorphic space group $P2_1$ as a conglomerate,[10] which means that the solid nitrosamine **4** is a mechanical mixture of homochiral crystals.[11] Therefore by picking a suitable single crystal we were able to measure its solid-state CD spectrum and perform X-ray crystallographic analysis of a carefully cut piece of the same crystal. It revealed that the helicity of the molecule **4** showing a positive Cotton effect at 405 nm is *M*. We believe that the bisignate Cotton effects displayed by **3·CA** and **4** can be explained by assuming that two types of the nitrosamine chromophores contribute to the

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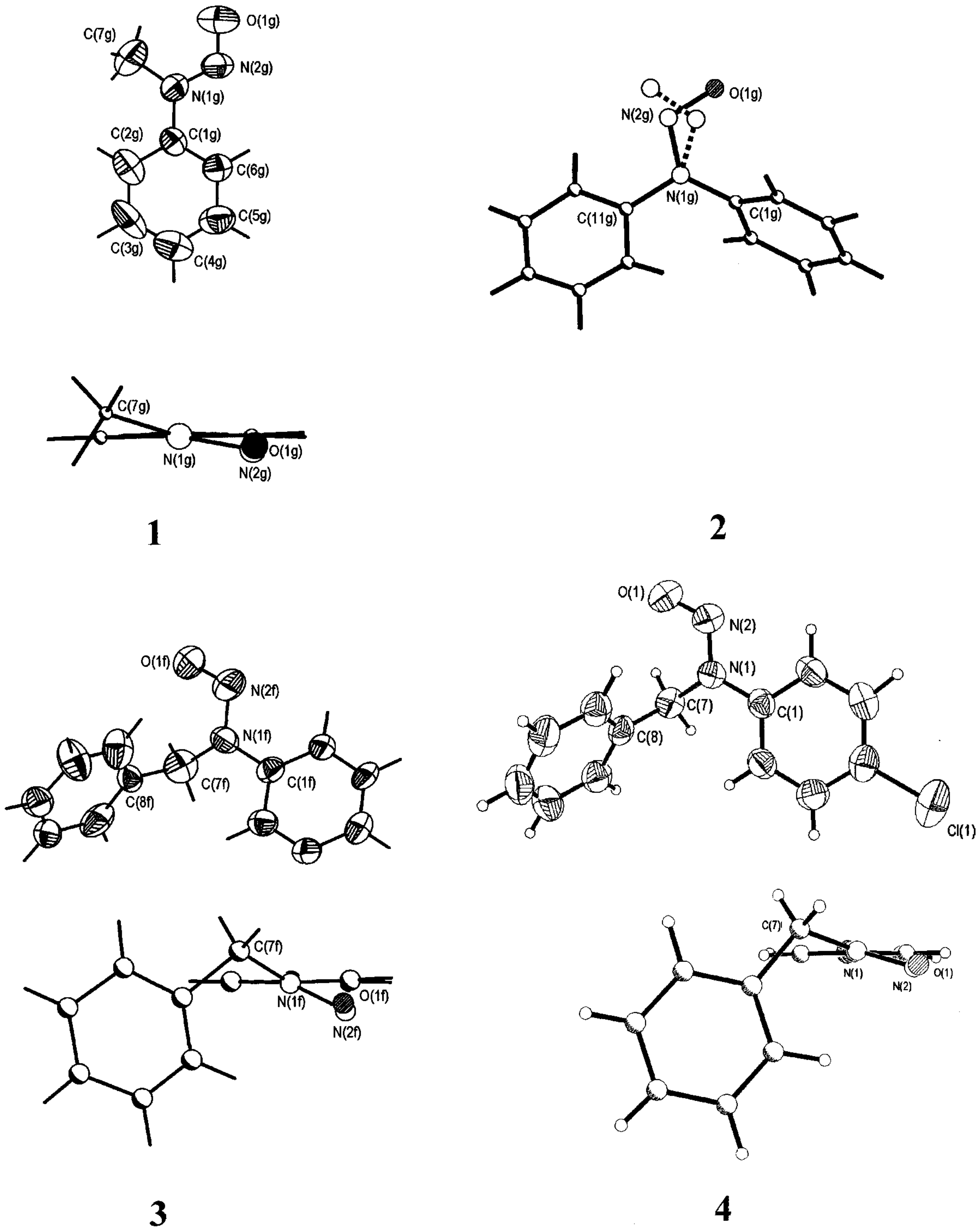
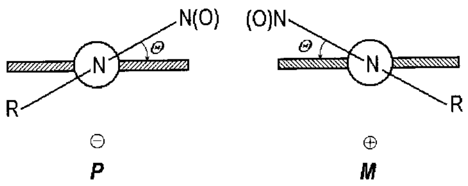


Figure 2. Drawings of the guest molecules **1**−**3** showing their absolute configurations in the inclusion complexes with **CA** and the nitrosamine **4** in the chiral crystal.

CD of these molecules: one conjugated and the second homoconjugated with the phenyl ring being a part of the aniline and benzylamine unit, respectively. The first one is responsible for the long-wavelength Cotton effect and the second one for that at the slightly higher energy.



By analogy with other conjugated systems, such as α,*β*-unsaturated ketones,[12] any distortion of the *N*-arylnitrosamine moiety from planarity results in formation of an inherently chiral chromophore, the helicity of which should determine the Cotton effect sign. Indeed, the observed positive Cotton effect of a moderate intensity for **1·CA** and the strong negative one for **2·CA** can be correlated with the *M* and *P* helicity of the **1** and **2** guest molecules, respectively, revealed by the crystal struc-

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[12] Lightner, D. A.; Gurst, J. E. *Organic Conformational Analysis and Stereochemistry from Circular Dichroism Spectroscopy*; Wiley-VCH: New York, 2000; Chapter 11.

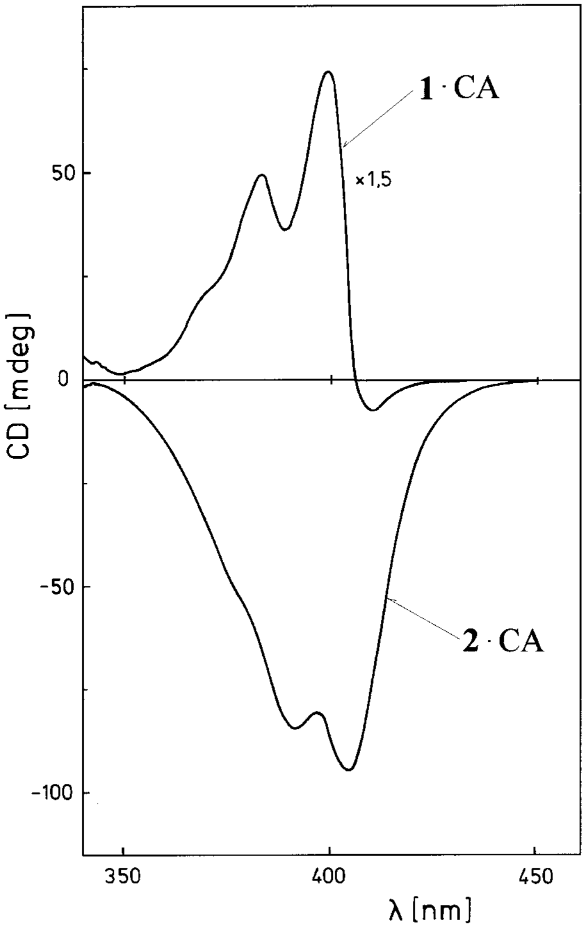

Figure 3. Solid-state CD spectra of the complexes **1·CA** and **2·CA** taken in KBr disks.

tures.[13] Similarly, the long-wavelength positive CD is due to the *M* chirality of the *N*-nitrosoaniline unit in **3·CA** and **4**, whereas the sign of the second Cotton effect is determined by the helicity of the homoconjugated *N*-benzylnitrosamine system. The last system resembles β,γ-unsaturated ketones, where a homoconjugation between the carbonyl and olefin chromophores leads to the strong n−π^* Cotton effects, the sign of which is governed by the mutual orientation of these chromophores.[12] The above correlations of the long-wavelength Cotton effect sign were also confirmed by the INDO/S-CI calculations[14,15] of the rotatory strength performed for the molecular geometries observed in the solid state (Table 1). Generally, the INDO/S method with standard parametrization[16] provides a reasonable description of the electronic spectra.[17] Though, as usual, the calculated n−π^* transition energies are too low, the oscillator strengths are adequately accounted for, and first of all, the calculations predict correct sign of the long-wavelength CD band for the chiral conformations of **1**−**4**.

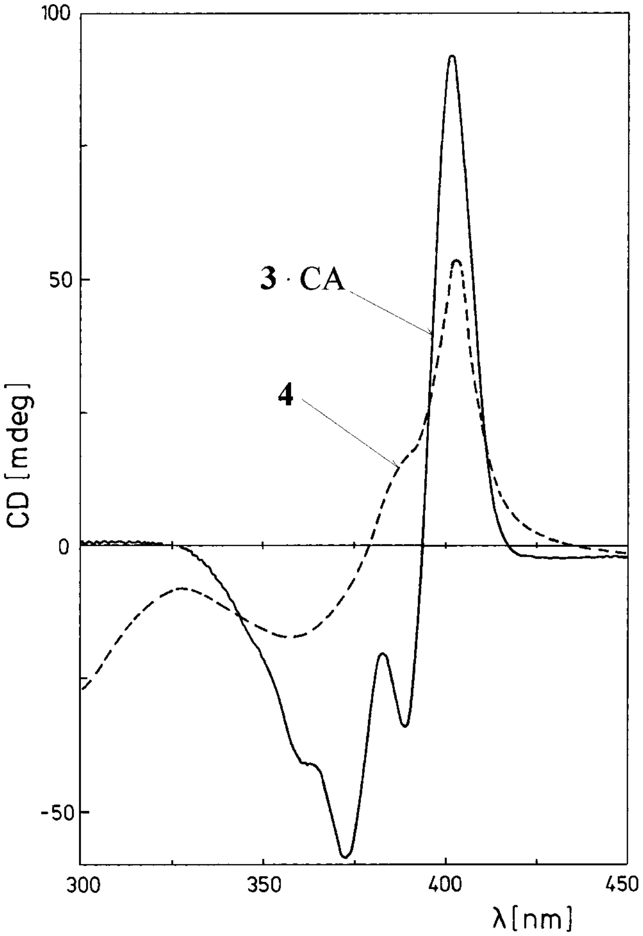

Figure 4. Solid-state CD spectra of the complex **3·CA** and the chiral crystal of **4** taken in KBr disks.

Table 1. Calculated Parameters for the First (n−π^*) Electronic Transition of *N*-Nitrosamines 1−4 in Chiral Conformations

compd	ΔE^a (eV)	f[b]	[*R*] (Dμ_B)[c]	CD λ, nm ([Θ])[d,e]
1	2.29	0.0020	0.1135	398 (1630)
2	1.97	0.0014	−0.0959	406 (−4600)
3	1.96	0.0014	0.1158	402 (5000), 373 (−3160)
4	2.23	0.0016	0.1834	406 (890), 360 (−380)

[a] Transition energy. [b] Oscillator strength in cgs. [c] Rotatory strength in Debye-Bohr-Magneton (DBM) units. [d] Molecular ellipticity in deg cm^2 dmol^{-1}. [e] Approximate experimental value determined by considering the weight concentration (KBr density 2.75 g cm^{-3}).

In conclusion, the chirality of *N*-aryl-*N*-nitrosamines can be generated by inclusion complexation with cholic acid. The guest molecules trapped in the host crystal matrices are frozen in chiral conformations that can be detected by the solid-state CD measurements. The helicity of the enclathrated guest molecules can be determined either by X-ray crystallography or deduced from the Cotton effect sign corresponding to the lowest energy n−π^* transition of the nitrosamino chromophore.

Experimental Section

The *N*-nitrosamines studied were all known and were prepared by standard methods.[18] The solid-state CD spectra were taken in freshly prepared KBr disks and recorded with

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[13] If the turn from the nitroso group to the *cisoidal* side of the benzene ring is clockwise, the helicity is *P*, and if counterclockwise, it is *M*.

[14] (a) Thompson, M. A.; Zerner, M. C. *J. Am. Chem. Soc.* **1990**, *112*, 7828. (b) Thompson, M. A.; Zerner, M. C. *J. Am. Chem. Soc.* **1991**, *113*, 8210.

[15] The standard INDO parameters with the interaction scaling factors $f_\sigma = 1.267$ and $f_\pi = 0.585$ were used. The CI calculations were restricted to 16 (**1**) and 33 (**2**−**4**) singly excited configurations. A quantum electronic structure program ARGUS 1.1 was kindly provided by Dr. Mark A. Thompson (Pacific Northwest Lab., Richland, WA).

[16] Zerner, M. C. *Theor. Chim. Acta* **1973**, *32*, 111.

[17] Clark, L. B. *J. Am. Chem. Soc.* **1995**, *117*, 7974.

[18] Looney, C. E.; Phillips, W. D.; Reilly, E. L. *J. Am. Chem. Soc.* **1957**, *79*, 6136.

a JASCO J-715 dichrograph. A mixture of 2−5 mg of the sample and 250 mg of dried KBr was ground and formed into a disk 0.5 mm thick and with radius of 15 mm. The disk was rotated around the optical axis and the CD recordings were made for several positions in order to check the reproducibility of the spectra.

X-ray Structure Analysis. Diffraction data for **1·CA** were obtained at 293 K on a Kuma KM-4 diffractometer, and those for **2·CA** and **3·CA** were determined at 130 K on a KUMACCD diffractometer with graphite monochromated Cu Kα radiation ($\lambda = 1.54178$ Å). The structures were solved by direct methods with the program SHELXS-86.[18] Full matrix least-squares refinement was carried out with SHELXL-97.[20]

Crystal data for 1·CA: $C_{24}H_{40}O_5 \cdot C_7H_8N_2O$, monoclinic, $P2_1$, $a = 13.562(4)$ Å, $b = 8.083(2)$ Å, $c = 14.301(4)$ Å, $\beta = 113.07(3)°$, $V = 1442.3(7)$ Å^3, $Z = 2$, $D_x = 1.254$ g cm^{-3}, $\mu = 0.086$ mm^{-1}. The structure was refined on 4204 reflections; 353 refined parameters; $R_1 = 0.0585$, $wR_2 = 0.1571$, GOF = 0.992 for 2369 reflections with $F > 4\sigma(F)$ [$R_1 = 0.1661$, $wR_2 = 0.1817$ for all 4204 independent reflections].

Crystal data for 2·CA: $2C_{24}H_{40}O_5 \cdot C_{12}H_{10}N_2O$, monoclinic, $P2_1$, $a = 13.096(3)$ Å, $b = 7.891(2)$ Å, $c = 13.954(3)$ Å, $\beta = 105.63(3)°$, $V = 1388.7(6)$ Å^3, $Z = 1$, $D_x = 1.190$ g cm^{-3}, $\mu = 0.081$ mm^{-1}. The structure was refined on 3028 reflections; 555 refined parameters; $R_1 = 0.0484$, $wR_2 = 0.1142$, GOF = 1.049 for 2619 reflections with $F > 4\sigma(F)$ [$R_1 = 0.0584$, $wR_2 = 0.1240$ for all independent reflections]. The nearest guest molecules in the channel are not related by the crystallographic 2_1 axis directed along the channel but are transformed by the unit translation along y. In consequence, a superposition of the two guests images (each with occupancy 0.5) related by the 2_1 axis is observed on electron density maps. The guest molecule was refined with constraints imposed on planarity of the phenyl rings and 1−2 and 1−3 distances of the N−NO fragment. The *N*-nitroso group shows an additional disorder, as shown in Figure 2, adopting two different orientations with respect to the phenyl rings with occupancies 0.35 and 0.15. The guest molecule was refined anisotropically, except for the NO group atoms, which was refined with isotropic displacement parameters.

Crystal data for 3·CA: $2C_{24}H_{40}O_5 \cdot C_{13}H_{12}N_2O$, $P2_1$, $a = 25.444(6)$ Å, $b = 7.930(2)$ Å, $c = 28.171(6)$ Å, $\beta = 100.47(2)°$, $V = 5589(2)$ Å^3, $Z = 4$, $D_x = 1.223$ g cm^{-3}, $\mu = 0.083$ mm^{-1}. The structure was refined on 10599 reflections; 1333 refined parameters; $R_1 = 0.0758$, $wR_2 = 0.1790$, GOF = 1.057 for 8363 reflections with $F > 4\sigma(F)$ [$R_1 = 0.0961$, $wR_2 = 0.1964$ for all independent reflections].

Crystal data for 4: $C_{13}H_{11}N_2OCl$, $P2_1$, $a = 6.0626(8)$ Å, $b = 7.3696(7)$ Å, $c = 13.5502(12)$ Å, $\beta = 96.578(9)°$, $V = 601.42(11)$ Å^3, $Z = 2$, $D_x = 1.362$ g cm^{-3}, $\mu = 0.301$ mm^{-1}. The structure was refined on 2333 reflections; 199 refined parameters; $R_1 = 0.0382$, $wR_2 = 0.0989$, GOF = 1.051 for 2078 reflections with $F > 4\sigma(F)$ [$R_1 = 0.0427$, $wR_2 = 0.1025$ for all independent reflections]; the absolute structure Flack parameter[21] $x = 0.05(8)$.

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Supporting Information Available: X-ray crystallographic data for **1·CA**, **2·CA**, **3·CA**, and **4** including atomic positions and atomic displacement parameters, as well as complete list of bond lengths and angles. This material is available free of charge via the Internet at http://pubs.acs.org.

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(21) Flack, H. D. *Acta Crystallogr.* **1990**, *A39*, 876.