

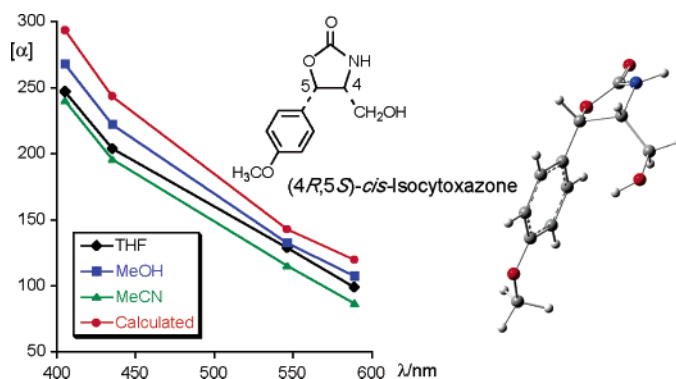
Determination of the Absolute Configuration of Flexible Molecules by *ab Initio* ORD Calculations: A Case Study with Cytoxazones and Isocytoxazones

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Ab initio calculations of the optical rotatory power of the natural cytokine modulator cytoxazone **1** and its *trans*-diastereomer **2**, as well as the structural isomers *cis*-**3** and *trans*-**4** isocytoxazones, have been performed at four different wavelengths (589, 546, 435, and 405 nm) by Density Functional Theory. The calculation of ORD curves provides a reliable method for the assignment of absolute configuration of these conformationally flexible molecules. The absolute configurations of isocytoxazones has been established as (+)-(4*R*,5*S*)-*cis*-**3** and (+)-(4*S*,5*S*)-*trans*-**4**.

Introduction

The cytoxazone (–)-**1**, (–)-(4*R*,5*R*)-*cis*-5-hydroxymethyl-4-(4-methoxyphenyl)-1,3-oxazolidin-2-one, an immunosuppressant agent produced by *Streptomyces* sp., is a novel cytokine modulator, which interferes with the cytokine IL-4, IL-10 and IgG production by selective inhibition of the signaling pathway of T_H2 cells.¹ The imbalance of cytokine production gives rise to a wide variety of

immunological disorders, i.e., allergy, progressive lymphoproliferation, and severe immunodeficiency.

The *cis* configuration of (–)-**1** was assigned by Osada et al.¹ on the basis of X-ray and NMR analysis, while the absolute configuration was first tentatively determined by comparison of the CD spectrum of (–)-**1** with that of a similar analog, (*S*)- and (*R*)-4-phenyl-2-oxazolidinone, which lacks hydroxymethyl and methoxy moieties of **1**.^{1b}

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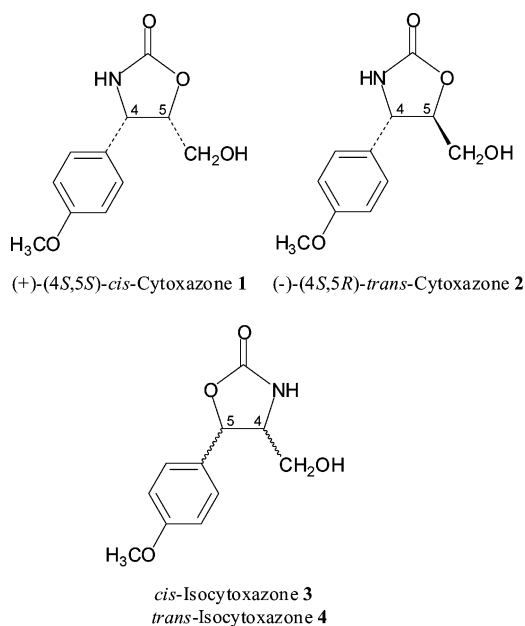
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Cytoxazone (–)-**1** was subsequently synthesized by Nakata and co-workers employing the Sharpless asymmetric dihydroxylation of *p*-methoxy cinnamic derivative as a key step, thus establishing its absolute configuration.^{2a} Recently, some of us reported the chemoenzymatic synthesis of optically active stereoisomers of cytoxazone **1** and *trans*-cytoxazone **2**,^{2b} further synthetic methods have also successfully yielded **1** and **2**.^{2c–k}

Positive biological results obtained with (–)-cytoxazone **1** led to the syntheses of two additional structural isomers of **1**, *cis*-isocytoxazone **3** and *trans*-isocytoxazone **4**. They were synthesized as a mixture of (±)-*cis*-**3** and (±)-*trans*-**4** stereoisomers, which were then separated by chiral HPLC.³ The *cis* and *trans* configurations of **3** and **4**, respectively, were determined on the basis of proton NMR spectra. Namely, while the ³J_{H4H5} of the *cis* isomer was 8.5 Hz, that of the *trans* isomer was a smaller value of 5.7 Hz.³ However, a careful and detailed analysis of the CD spectra of **3** and **4** to assign their absolute configuration was not carried out,³ so this problem remained open.



The aim of the present paper is to assign the absolute configuration of **3** and **4**. We will employ a new and interesting method, the *ab initio* calculation of optical rotation.^{4–14} In particular we will predict the Optical Rotatory Dispersion (ORD) curve, since it has been

clearly shown that the configurational assignment based on a comparison of predicted/experimental OR values at several wavelength is certainly more reliable than a comparison made at the single wavelength.¹⁵ Because nowadays spectropolarimeters are not so common in chemical laboratories, preventing the routine measure-

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(8) A possible cause of this solvent effect is most likely related to the presence of the carbamate NH and/or OH groups which in *trans*-**2** are participating in hydrogen bonding with THF solvent. The ¹H NMR of **2** in CD₃CN showed the amide proton at 6.0 ppm and the hydroxy proton at 3.2 ppm (see Supporting Information). When measured in THF-*d*₅, these protons shifted to the down shielded field, namely, the amide proton at 6.9 ppm and two distinct hydroxyl protons at 4.4 and 4.7 ppm (ratio of 1.7:1), respectively, while the chemical shifts of other protons of *trans*-**2** remained more or less unchanged. The downfield shifts of carbamate NH and observed two values for OH protons in THF clearly indicate a solvation of **2** by THF, giving rise to at least two different solution species.

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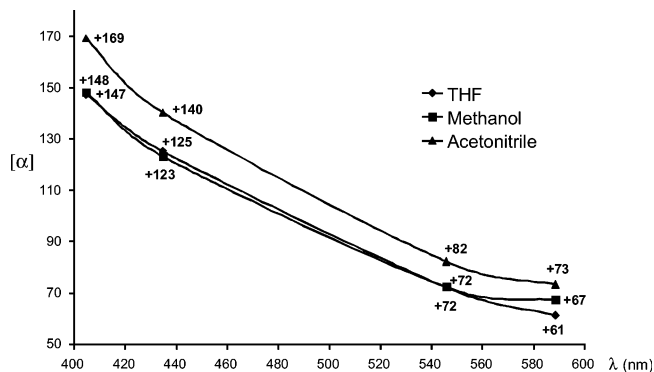


FIGURE 1. Experimental ORD of (+)-**1** in three different solvents ($c \approx 0.1$ g/100 mL).

ments of continuous ORD curves, we shall use $[\alpha]$ values obtained at some different wavelengths measured by a simple digital polarimeter equipped with both Na and Hg lamps. In this way it will be possible to measure the optical rotatory power at four different wavelengths: 589.3 (sodium D line), 546, 435, and 405 nm, so we can compare these values with those predicted by *ab initio* calculations. Our work is organized as follows: (i) Our original approach¹⁶ will be further tested using the known compounds **1** and **2**. This test is required because in the present investigation we are dealing with flexible molecules, while in the previous paper only rigid systems have been treated. Therefore we have to check if our ORD method can cope well with the problems related to the conformational mobility. We have to introduce an extra step, namely, a systematic conformational search of all the possible conformers. (ii) This modified approach will be then applied to assign the absolute configuration of the flexible and unknown **3** and **4**.

Results and Discussion

Only the $[\alpha]_D$ value in methanol has been reported for *cis*-cytoxazone **1**.² As shown in Figure 1, the optical rotation (OR) at four different wavelengths (589, 546, 435, and 405 nm) were measured in methanol, as well in THF and acetonitrile. Since *cis*-cytoxazone **1** is a flexible molecule existing as a mixture of conformers, the solvent effect is expected to play an important role. Generally, hydrocarbons such as hexane are considered the best solvents for comparison between experimental and calculated properties of isolated molecules.^{7c} Unfortunately, **1** is soluble neither in hydrocarbons nor in chlorinated solvents such as CH_2Cl_2 or CHCl_3 . Therefore,

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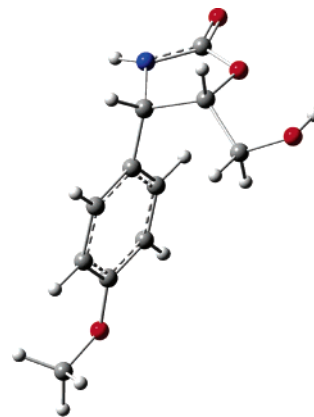


FIGURE 2. The most stable conformation of (4*S*,5*S*)-**1** optimized at the DFT/B3LYP/6-31G* level.

in addition to methanol, a protic and polar ($\epsilon = 32.63$) solvent, the ORD data were measured in other solvents of different characteristics; i.e., acetonitrile as an aprotic polar ($\epsilon = 36.64$) solvent and THF as an aprotic apolar ($\epsilon = 7.58$) solvent.

Figure 1 shows that the nature of the solvent has almost no effect on the OR values at all four wavelengths: the ORD values in THF and methanol are almost the same, while they are slightly higher in CH_3CN , the largest difference being ca. 20%. Importantly, the similarity in the sign, magnitude and trend of the ORD curves in all three solvents justified the comparison of experimental data with the theoretically predicted values in vacuo.

The conformational flexibility of (+)-(4*S*,5*S*)-cytoxazone **1** resulting in a large number of different conformers stems from the phenyl and hydroxymethyl moieties on the oxazolidinone ring that can adopt axial and/or equatorial orientations and also from the rotation around the single bond of hydroxymethyl, phenyl, and methoxy groups. The conformational distribution search was performed by molecular mechanics calculations using the MMFF94s force field by Spartan02 package.¹⁷ Details of the conformational analysis are reported in the Computational Methods section. This resulted in a total of 23 different conformers for cytoxazone **1**. All structures were fully optimized at DFT/B3LYP/6-31G* level leading to 10 different conformations that differ from the most stable one less than 2 kcal/mol. The most stable conformation is shown in Figure 2.

The optical rotatory power at four different wavelengths (589.3, 546, 435, and 405 nm) for all found conformers of (4*S*,5*S*)-cytoxazone **1** within the 2 kcal/mol energy range has been calculated at the TDDFT/B3LYP/6-31G* level using Gaussian03,¹⁸ and the results are shown in Table 1.

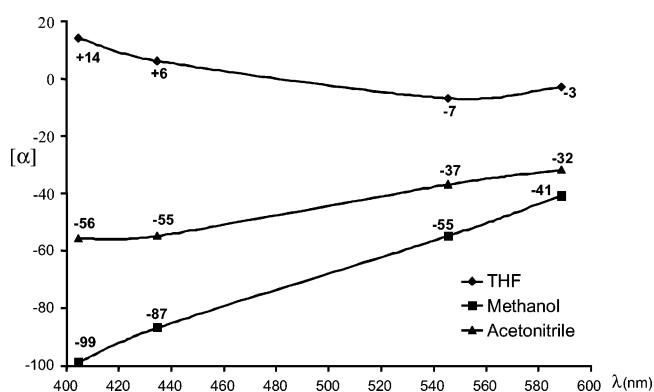
The sign, the order of magnitude, and the uniform trend in the experimental ORD curves (in all solvents), which showed increased OR values at shorter wavelength, have been correctly reproduced by the calculations, despite the fact that the predicted Boltzmann average ORD values are twice higher than the experimental ones: such difference may result from errors in

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TABLE 1. Calculated OR Values for the Optimized Conformations of (4*S*,5*S*)-1

(+)-(4 <i>S</i> ,5 <i>S</i>)-cytoxazone 1	ΔG	Boltzmann population ^a %	$[\alpha]_D$	$[\alpha]_{546}$	$[\alpha]_{435}$	$[\alpha]_{405}$
conformer 1	0.00	22.5	+89	+104	+171	+201
conformer 2 ^b	0.05	20.6	+9	+8	-6	-18
conformer 3	0.15	17.3	+195	+232	+395	+472
conformer 4	0.28	14.1	+287	+341	+591	+712
conformer 5	0.61	8.0	+166	+198	+346	+418
conformer 6	0.87	5.1	+212	+253	+448	+544
conformer 7	0.90	4.9	+171	+204	+353	+425
conformer 8	1.12	3.4	+234	+280	+492	+596
conformer 9	1.36	2.3	+18	+20	+21	+17
conformer 10	1.49	1.8	+122	+144	+246	+295
Boltzmann average values ^c			+139	+165	+279	+332
experimental OR in THF			+61	+72	+125	+147
experimental OR in MeOH			+67	+72	+123	+148
experimental OR in CH ₃ CN			+73	+82	+140	+169

^a Obtained from ΔG values using Boltzmann statistics. ^b The large difference is due only to the position of the methoxyphenyl group. ^c Conformational average obtained from individual conformer OR values and Boltzmann populations.

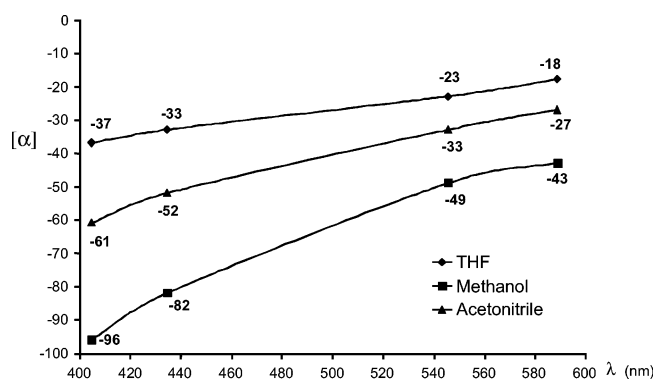
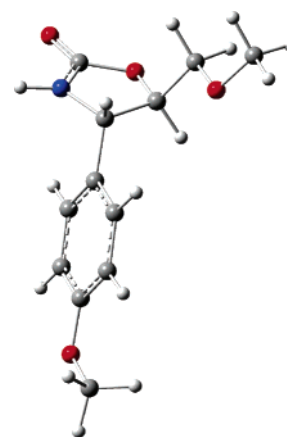
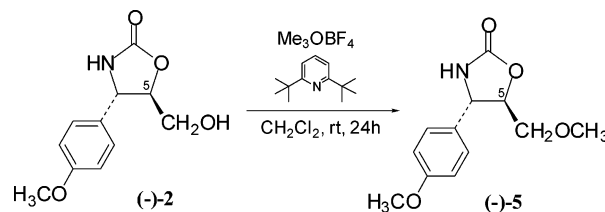
**FIGURE 3.** Experimental OR of (-)-**2** in three different solvents ($c \approx 0.1$ g/100 mL).

the theoretical OR predictions of respective conformers and/or from errors in the conformational distribution, which could become more significant as in the present case where several conformers are involved. However, it is noteworthy that all conformers at the four wavelengths shows positive OR except conformer **2**, which shows a small OR value of different sign. Thus, even large errors in the conformer distributions cannot change the sign and the trend of the calculated ORD curves, and therefore they actually follow well that of experimentally found dates for the known (+)-**1** as (4*S*,5*S*).

We followed the same approach with *trans*-cytoxazone **2** as well. The $[\alpha]$ values measured in three different solvents are shown in Figure 3.

As shown in Figure 3, a significant solvent effect passing from longer to shorter wavelengths was noted:

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**FIGURE 4.** Experimental OR of (-)-**5** in three different solvents ($c \approx 0.1$ g/100 mL).**FIGURE 5.** The most stable conformation of (4*S*,5*R*)-**5** at the DFT/B3LYP/6-31G* level.**SCHEME 1.** *O*-Methylation of (-)-**2**

$[\alpha]$ increases in THF while decreases in methanol and acetonitrile.⁸ Moreover, the absolute values of optical rotation are relatively small and this leads to further difficulties.^{7c} To avoid this solvent effect, (-)-*trans*-**2** was transformed into its methyl ether (-)-**5** by treatment with trimethyloxonium tetrafluoroborate in the presence of 2,6-di-*tert*-butylpyridine (Scheme 1).

The experimental OR values of **5** measured in THF, methanol, and acetonitrile are shown in Figure 4. Different from **2**, the methylated derivative **5** does not show any significant solvent effect in going from longer to shorter wavelengths; OR values decreased similarly in all three solvents. We then performed the conformational and ORD calculations of *O*-methyl derivative (-)-**5** following a similar conformational search protocol applied to cytoxazone **1**, upon which the MMFF94s force field provided 25 different conformers for the (4*S*,5*R*)-**5**. A full optimization of these conformations at DFT/B3LYP/6-31G* level led to 17 conformers within 2 kcal/mol. The most stable conformation is shown in Figure 5.

TABLE 2. Calculated OR Values for the Optimized Conformers of (4*S*,5*R*)-**5**

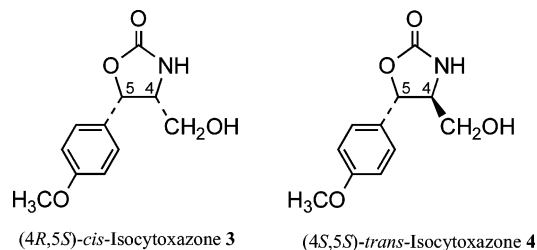
(-)-(4 <i>S</i> ,5 <i>S</i>)- 5	ΔG	Boltzmann population ^a %	$[\alpha]_D$	$[\alpha]_{546}$	$[\alpha]_{435}$	$[\alpha]_{405}$
conformer 1	0.00	17.3	-52	-62	-109	-132
conformer 2	0.07	15.4	-102	-122	-218	-267
conformer 3	0.17	13.1	-100	-120	-214	-262
conformer 4	0.29	10.6	+7	+9	+17	+22
conformer 5	0.39	9.0	+59	+71	+130	+161
conformer 6	0.41	8.7	-47	-55	-87	-100
conformer 7	0.43	8.4	-7	-6	+3	+11
conformer 8	1.10	2.7	+198	+235	+408	+491
conformer 9	1.13	2.6	+117	+138	+233	+277
conformer 10	1.17	2.4	-48	-57	-98	-118
conformer 11	1.23	2.2	-7	-10	-27	-37
conformer 12	1.24	2.1	+34	+42	+79	+99
conformer 13	1.40	1.6	+219	+259	+437	+520
conformer 14	1.46	1.5	+210	+248	+413	+490
conformer 15	1.69	1.0	+20	+24	+36	+41
conformer 16	1.75	0.9	-198	-236	-416	-504
conformer 17	1.98	0.6	-185	-221	-387	-469
Boltzmann average values ^b			-25	-29	-51	-62
experimental OR in THF			-18	-23	-33	-37
experimental OR in MeOH			-43	-49	-82	-96
experimental OR in CH ₃ CN			-27	-33	-52	-61

^a Obtained from ΔG values using Boltzmann statistics. ^b Conformational average obtained from individual conformer OR values and Boltzmann populations.

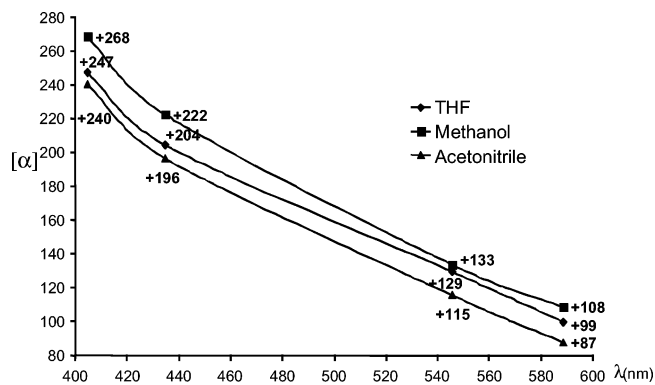
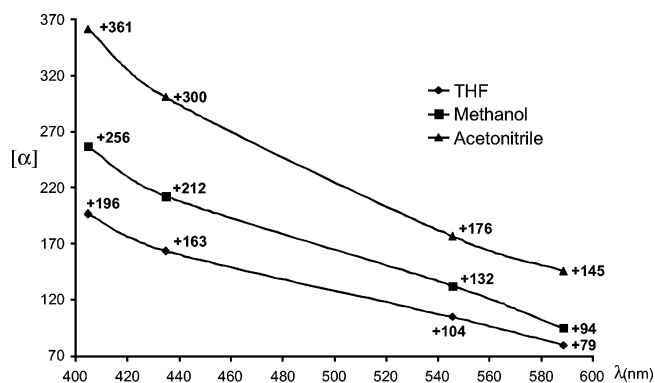
The optical rotatory power at four different wavelengths (589.3, 546, 435, and 405 nm) for all optimized conformations of (-)-(4*S*,5*R*)-**5** within 2 kcal/mol were calculated at the TDDFT/B3LYP/6-31G* level.

The results presented in Table 2 show that the predicted Boltzmann average ORD values for (4*S*,5*R*) absolute configuration of (-)-**5** are in excellent agreement with experimental data with respect to the sign, order of magnitude, and trend of ORD curve in all three solvents. The largest numerical difference of ca. 30% between the calculated and experimental values is observed only in the polar and protic solvent methanol. Noteworthy, in going from 589 to 405 nm, the absolute values of OR increase twice for both calculated and experimental values. These findings clearly make the current configurational assignment more reliable.^{7c,16}

Altogether, the success in theoretical prediction of OR of model compounds **1** and **2** with known absolute configurations has justified undertaking a similar approach to (+)-*cis*-isocytosaxone **3** and (+)-*trans*-isocytosaxone **4** for which no absolute configurational data exist. The (4*R*,5*S*) absolute configuration for *cis*-isocytosaxone **3** and (4*S*,5*S*) for *trans*-isocytosaxone **4** have been arbitrarily chosen for the calculation as shown below. The experimental $[\alpha]$ values of **3** are shown in Figure 6.



Similar to the case of (+)-**1**, the OR values of *cis*-**3** recorded at all four wavelengths are independent of the nature of the solvent, the sign, magnitude and trend of

**FIGURE 6.** Experimental OR of (+)-**3** in three different solvents ($c \approx 0.1$ g/100 mL).**FIGURE 7.** Experimental OR of (+)-**4** in three different solvents ($c \approx 0.1$ g/100 mL).

the ORD curves being almost the same in all solvents; the largest difference is only ca. 20%.

The observed $[\alpha]$ values of (+)-*trans*-**4** are shown in Figure 7. Here the OR values are more sensitive to the nature of the solvent, even though the optical rotations are always positive and quite large. By MMFF94s force field calculations, we found 12 different conformations for (4*R*,5*S*)-*cis*-isocytosaxone **3** and 20 for (4*S*,5*S*) for *trans*-isocytosaxone **4**. All conformations have been fully optimized at the DFT/B3LYP/6-31G* level; only 10 different conformations were found for both **3** and **4** within 2 kcal/mol. The most stable conformations for (4*R*,5*S*)-*cis*-isocytosaxone **3** and for (4*S*,5*S*)-*trans*-isocytosaxone **4** are shown in Figure 8.

Similar to **1** and **5**, the optical rotatory power of **3** and **4** were calculated for each of 10 conformers at four different wavelengths at TDDFT/B3LYP/6-31G* level.

The experimental and calculated data of **3** and **4** can be analyzed as follows. The order of magnitude and the trend of experimental ORD curves have been well reproduced in the calculations by taking into account the Boltzmann average values. In fact, the absolute value of the specific rotation increases going from long to short wavelengths for both experimental and calculated values. Moreover, it should be noted that the sum of the highly populated conformations (over 10% population for each conformer) constitutes more than 75% of overall population, i.e., conformers 1–4 for **3** and conformers 1–2 for **4**, show the same sign and the same trend in the calculated OR values. This indicates that the sign and the trend of ORD calculated curves will not be affected

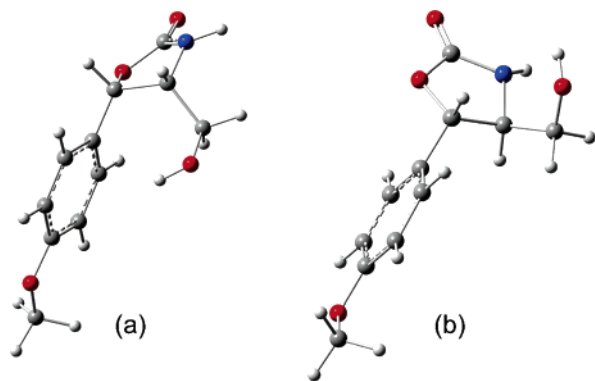


FIGURE 8. The most stable conformations of (a) (4*R*,5*S*)-*cis*-isocytosaxazone **3** and (b) (4*S*,5*S*)-*trans*-isocytosaxazone **4** optimized at the DFT/B3LYP/6-31G* level.

TABLE 3. Calculated OR Values for the Optimized Conformers of *cis*-(4*R*,5*S*)-**3**

(4 <i>R</i> ,5 <i>S</i>)- <i>cis</i> -isocytosaxazone 3	ΔG	Boltzmann population ^a %	$[\alpha]_D$	$[\alpha]_{546}$	$[\alpha]_{435}$	$[\alpha]_{405}$
conformer 1	0.00	22.0	+76	+91	+157	+189
conformer 2	0.06	20.1	+135	+159	+266	+315
conformer 3	0.13	17.7	+255	+303	+522	+627
conformer 4	0.20	15.7	+194	+232	+405	+498
conformer 5	0.55	8.7	+3	+3	+1	-1
conformer 6	0.84	5.3	+138	+165	+290	+352
conformer 7	0.88	5.0	-114	-138	-252	-310
conformer 8	1.10	3.5	-19	-23	-43	-53
conformer 9	1.75	1.2	-95	-115	-208	-256
conformer 10	1.93	0.9	+47	+56	+95	+113
Boltzmann average values ^b			+120	+143	+244	+294
experimental OR in THF			+99	+129	+204	+247
experimental OR in MeOH			+108	+133	+222	+268
experimental OR in CH ₃ CN			+87	+115	+196	+240

^a Obtained from ΔG values using Boltzmann statistics. ^b Conformational average obtained from individual conformer OR values and Boltzmann populations.

TABLE 4. Calculated OR Values for the Optimized Conformers of *trans*-(4*S*,5*S*)-**4**

(4 <i>S</i> ,5 <i>S</i>)- <i>trans</i> -isocytosaxazone 4	ΔG	Boltzmann population ^a %	$[\alpha]_D$	$[\alpha]_{546}$	$[\alpha]_{435}$	$[\alpha]_{405}$
conformer 1	0.00	43.8	+58	+69	+121	+146
conformer 2	0.10	36.9	+16	+19	+26	+27
conformer 3	1.44	3.8	-25	-30	-56	-69
conformer 4	1.58	3.0	+32	+37	+71	+87
conformer 5	1.69	2.5	+11	+12	+20	+22
conformer 6	1.76	2.2	+51	+60	+96	+111
conformer 7	1.78	2.2	+32	+39	+71	+87
conformer 8	1.83	2.0	+103	+122	+211	+255
conformer 9	1.89	1.8	+251	+298	+514	+618
conformer 10	1.90	1.8	-43	-52	-93	-114
Boltzmann average values ^b			+39	+47	+79	+93
experimental OR in THF			+79	+104	+163	+196
experimental OR in MeOH			+94	+132	+212	+256
experimental OR in CH ₃ CN			+145	+176	+300	+361

^a Obtained from ΔG values using Boltzmann statistics. ^b Conformational average obtained from individual conformer OR values and Boltzmann populations.

by the less populated conformers. Some unavoidable errors that occur in conformational energy calculation therefore will not affect the final results, at least in regard to the configurational assignment of (+)-*cis*-isocytosaxazone **3** and (+)-*trans*-isocytosaxazone **4** as (4*R*,5*S*) and (4*S*,5*S*), respectively.

As previously discussed,^{15,16} one of the main advantages of the *ab initio* prediction of the ORD curve for

TABLE 5. Calculated $[\alpha]_D$ Values for *cis*-(4*R*,5*S*)-**3** at TDDFT/B3LYP Level Using Two Different Basis Sets

(4 <i>R</i> ,5 <i>S</i>)- <i>cis</i> -isocytosaxazone 3	Boltzmann population %	$[\alpha]_D$ 6-31G*	$[\alpha]_D$ aug-cc-pVDZ
conformer 1	22.0	+76	+69
conformer 2	20.1	+135	+116
conformer 3	17.7	+255	+235
conformer 4	15.7	+194	+192
conformer 5	8.7	+3	-17
conformer 6	5.3	+138	+121
conformer 7	5.0	-114	-89
conformer 8	3.5	-19	+4
conformer 9	1.2	-95	-100
conformer 10	0.9	+47	+50
Boltzmann average values		+120	+111
experimental $[\alpha]_D$ in THF			+99
experimental $[\alpha]_D$ in MeOH			+108
experimental $[\alpha]_D$ in CH ₃ CN			+87

configurational assignments is the possibility to carry out calculations at the TDDFT/B3LYP/6-31G* level, i.e., at a small basis set level which ensures a small computational effort (vide infra). However, it was interesting to compare such calculations with those employing more extended basis sets. Thus, the OR calculations for (4*R*,5*S*)-*cis*-**3** were also performed at the TDDFT/B3LYP/aug-cc-pVDZ level, i.e., employment of an extended basis set, the use of which is strongly supported by Stephens and co-workers.^{7c} The results are shown in Table 5. It immediately appears that the values provided by the two different methods are quite similar except for conformers 5 and 8. In particular, similar OR values have been obtained for the four lowest-energy conformers 1–4, which provide 75% of the overall population. Conformers 5 and 8, which are predicted an opposite sign (12% of the overall population), have also weak OR values. Thus in the studied case, Boltzmann weighted OR values calculated with a larger basis set are practically the same as these predicted with a smaller basis set. Even though such comparison has been exemplified by only one chiral compound, the similarity in the results obtained by using different basis sets is noteworthy: an ORD calculation of a single conformer (4 wavelengths) requires 5 h at the 6-31G* level but the OR calculation at the single frequency requires 2 days at the aug-cc-pVDZ level, using the common desktop PC (see Computational Methods).

A final comment about the present ORD calculations concerns the lack of clear evidence that the quality of *ab initio* calculations at the sodium D line either improves or remains the same when moving from longer to shorter wavelengths. Therefore, in principle, the statistically averaged discrepancies that have been evaluated of the order of 30–40° dm⁻¹ (g/mL)⁻¹ at 589 nm by Stephens and co-workers^{7c} could become even larger. However, it is important to note that a comparison between prediction and experiment, carried out at several frequencies, leads to a reliable configurational assignment even if the numerical agreement at each frequency is not excellent, because the prediction reproduces the experimental trend of OR values as a frequency function.

Conclusions

In summary, the configurational correlation for *cis*-isocytosaxazone **3** and its *trans* isomer **4** has been established for the first time as (+)-(4*R*,5*S*)-**3** and (+)-(4*S*,5*S*)-

4, by using a new and practical approach based on the *ab initio* calculation of the ORD spectra. Although this result will be helpful in view of the potential therapeutic use of these compounds, it also deserves interest from a methodological point of view. In fact, these configurational assignments are based on both experimental data obtained quickly and simply with an inexpensive instrument (a polarimeter working at a few different wavelengths) and on theoretical predictions that have been made by a commercial quantum-chemical package, available at a moderate price for the chemical laboratories. Measurements at different and relatively short wavelengths up to 405 nm allows one to obtain OR values larger than that at 589 nm, thus making theoretical predictions more reliable¹⁶ even with relatively low level calculations.¹⁹ Specifically, a small computational effort with a common desktop PC suffices. Furthermore, the present results demonstrate that this theoretical approach could also be extended to flexible molecules, provided that a careful conformational analysis is carried out. If necessary, a simple derivatization, e.g., methylation of the hydroxyl group, can prevent undesirable solvent effects on ORD, thus resulting in a reliable absolute configurational assignment by OR calculations.

All of these features strongly indicate that the present polarimetric method represents an attractive alternative to Vibrational Circular Dichroism spectroscopy, which recently is becoming a versatile tool for configurational assignments²⁰ but still suffers from some limitations. The VCD measurements rely on an expensive instrument as compared to the present method that uses the inexpensive polarimeter found in all organic chemistry laboratories; VCD also requires a larger amount of samples, i.e., several milligrams, and quite often both enantiomers are necessary. This prevents VCD application when only small or even minuscule amounts of sample is available, e.g., natural product, whereas the ORD method needs much less material. The work described in this paper was performed on 1 mg of each compound. To conclude, polarimetric measurements at several wavelengths below the sodium D line, when coupled with an *ab initio* calculation package, can allow for a straightforward assignment of absolute configurations even in the hands of the experimental organic chemists.

(19) Actually, it could also be noticed that the difference between the results of a 6-31G* and an aug-cc-pVDZ calculation, which are small at the sodium D line, could be more significant at shorter wavelengths, as has been previously observed.^{7,11,14} In addition, TDDFT/B3LYP calculations tend to overestimate OR values at shorter wavelengths;^{11c,d,14} it is possible that the individual conformer OR values are too large, but the Boltzmann averaging effectively reduces this effect. In other words, the above noticed small difference could also result from some error cancellation. However, to assign the right absolute configuration by *ab initio* calculation of the ORD curve the use of the 6-31G* basis set is sufficient.¹⁶ All three referees of this paper commented on the use of the DFT/B3LYP/6-31G* level of theory to calculate the input geometries for the OR calculations. This choice has been made considering that a large part (probably the largest part) of the OR computations described in the literature employ geometries obtained at this level of theory as a good compromise between accuracy and computational efforts.

(20) (a) Stephens, P. J. In *Encyclopedia of Spectroscopy and Spectrometry*; Academic: London, 2000; pp 2415–2421. (b) Stephens, P. J.; Devlin, F. J. *Chirality*, **2000**, *12*, 172–179. (c) Stephens, P. J. In *Computational Medicinal Chemistry for Drug Discovery*; Bultinck, P., de Winter, H., Langenaeker, W., Tollenaere, J., Eds.; Dekker: New York, 2003; Vol. 26, pp 699–725. (d) Naife, L. A.; Freedman, T. B. In *Circular Dichroism, Theory and Practice*; Nakanishi, K., Berova, N., Woody, W., Eds.; Wiley: New York, 2000; p 97.

Experimental Section

Optical Rotation Measurement. The optical rotation measurements have been performed using a polarimeter endowed with a Na and Hg lamp and interference filter at 589 nm (using the Na lamp) and at 546, 435 and 405 nm (using the Hg lamp). In all solvents OR measurements have been carried out at room temperature, the concentration of solutions for **1–5** being about 0.1 gr/100 mL, which corresponds at about 0.0004 M, i.e., a value for which solute/solute interactions are certainly not significant.^{5h,j}

O-Methylated-(–)-(4S,5R)-cytoxazone (–)-5. A solution of (–)-**2** (3.2 mg, 14.3 mmol) and 2,6-di-*tert*-butylpyridine (41.1 mg, 4.8 mL, 215 mmol) in 2 mL of anhydrous CH₂Cl₂ was added onto trymethylxonium tetrafluoroborate (21.2 mg, 143.3 mmol). After 24 h of stirring at room temperature, brine was added and the product was extracted with CH₂Cl₂, washed with brine and dried over Na₂SO₄. Solvent removal furnished an oil that was purified by column chromatography (CH₂Cl₂/MeOH 9:1) to give (–)-**5** (3.2 mg, 94%) as a viscous colorless oil. ¹H NMR (300 MHz, CDCl₃) δ_H: 3.45 (s, 3H), 3.60 (dd, *J* = 3.5, 11.0 Hz, 1H), 3.65 (dd, *J* = 4.5, 11.0 Hz, 1H), 3.82 (s, 3H), 4.43 (ddd, *J* = 3.5, 4.5, 8.5 Hz, 1H), 4.75 (d, *J* = 6.5 Hz, 1H), 5.59 (s, 1H), 6.93 (d, *J* = 9 Hz, 2H), 7.28 (d, *J* = 9 Hz, 2H). HRMS: calcd for C₁₂H₁₆NO₄ 238.1079, found 238.1063.

Computational Methods. All calculations have been carried out on a simple PC endowed with a single PentiumIV 3.16 GHz processor. The preliminary conformational distribution search has been performed by Spartan02 package¹⁷ using the MMFF94s molecular mechanics force field.

The systematic search of all possible conformers has been performed using molecular mechanics method considering the degrees of freedom of system (i.e., different positions, axial or equatorial, of both the phenyl and the hydroxymethyl moieties on the rotation around the single bond of hydroxymethyl, phenyl and methoxy groups). In particular, we took into account different conformations in the five-membered ring, because using default parameters it is not possible to consider the flexibility in the five-membered ring. In other words, this means that a lot (3888) of different starting structures differ in the position of the flexible moieties. Each of them has been optimized using the above quoted molecular mechanics method, obtaining a limited number of the different real minima. The real minimum energy conformers found by molecular mechanics have been further fully optimized at the DFT/B3LYP/6-31G* level as implemented in Gaussian03 package.¹⁸ Only the conformers that differ from the most stable one by less than 2 kcal/mol have been taken into account, following a generally accepted protocol.^{7h,i} All conformers are real minima, no imaginary frequencies have been found, and the free energy values have been calculated and used for to get the Boltzmann population of conformers at 298.15 K.

The optical rotation calculations at different wavelengths have been carried out by means of time-dependent DFT methods using the hybrid B3LYP functional and the 6-31G* and aug-cc-pVDZ basis sets as available within Gaussian03. London orbitals (which ensure the origin independency of the results) have been used.

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Supporting Information Available: Copies of ¹H NMR spectra of *trans*-cytoxazone **2** in CD₃CN and THF-*d*₈ and its *O*-methyl derivative **5**; DFT/B3LYP/6-31G* full optimized geometries including Cartesian coordinates and computed total energies of molecules **1**, **3**, **4**, and **5** using Gaussian03 software package. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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