Electronic Supplementary Information

Distinguishing between Polymorphic Forms of Linezolid by Solid-Phase Electronic and Vibrational Circular Dichroism

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List of Supplementary Information

1. General experimental information

IR spectra

The IR spectra of the obtained polymorphic forms of linezolid were recorded in nujol and KBr pellets on FTIR Jasco 6200 apparatus.

DSC

Thermal analysis were carried out by means of the DSC 822 cell with IntraCooler (Mettler Toledo) in the nitrogen atmosphere. Accurately weighed samples (5-7 mg) were packed in aluminium pans with pierced lids. Samples were heated from 30 to 200 °C, with the scanning rate of 10 °C/min. To stabilize a sample under starting measurement conditions the two-minute isothermal step at the temperature of 30 °C preceded the dynamic temperature regime for DSC measurements. The DSC instrument was calibrated using indium and zinc as standards.

Powder X-Ray Diffraction

XRPD studies were carried out by means of the MiniFlex diffractometer (Rigaku Coporation, Tokyo Japan) using CuK_{α} radiation. Samples were gently pressed on a glass plate and the instrument was operated in the continuous scan mode with the step size of 0.02 ° and the scan speed of 0.5 °/min in the range from 3 to 40 °. The generator was operated at 30 kV and 15 mA and diffraction data were recorded by a scintillation detector.

Solid-phase ECD measurements

The diffused transmission circular dichroism (DTCD) spectra were recorded between 400 - 200 nm at room temperature using a JASCO J-815 CD Spectropolarimeter equipped with the Powder CD Attachment (*i.e.* the integrating sphere compartment coating with barium sulphate). All spectra were obtained using 100 nm/min scanning speed, a step size of 0.2 nm, a bandwidth of 5 nm, a response time of 0.5 sec, and an accumulation of 5 scans. The spectra were background corrected.

Solid-phase samples were prepared with the KCl pellet technique (0.132 mg of form II per 100 mg KCl and 0.214 mg of form III per 100 mg KCl). The crystalline compound was mixed with dried KCl, finely ground in Specac Mill for 10 min and pressed at 10 ton under vacuum for 5 min to make a 13 mm diameter transparent and spotless disk. Several samples (at least 5) were prepared for each compound to guarantee the data. The pellet was mounted on a rotatable holder in spectropolarimeter. On each sample, several ECD spectra were measured upon rotation of the disk around the incident axis direction at various rotation angles. These spectra were almost identical, demonstrating the absence of detectable spectral artifacts. Linear dichroism (LD) was also measured, the order was *ca.* $3x10^{-3}$, which was negligibly small.[1] Each measurement was additionally repeated on JASCO J-715 Spectropolarimeter under the same conditions of measurement. During the measurements on JASCO J-715 the sample was rotated around the optical axis during entire measurement using an original JASCO equipment for this purpose.

Solid-phase IR and VCD measurements

Both the IR and VCD spectra were recorded on a Chiral*IR-2X* dual PEM VCD spectrometer[2] (BioTools, Inc) at a resolution of 4 cm^{-1} using Synchrocell (2.75 sec/cycle). The ZnSe photoelastic modulator of the instrument was set to 1400 cm^{-1}. To improve the S/N ratio, the spectra were measured for 5 h. Solid-phase samples were prepared with the KBr pellet technique (using 0.5 mg of linezolid per 130 mg of KBr) in the same way like ECD measurements. Baseline correction was achieved by subtracting the spectrum of a reference KBr pellet obtained under the same conditions.

The IR and VCD spectra of both polymorphic forms of linezolid were also measured with a Bruker PMA 37 VCD/PM-IRRAS module connected to an Equinox 55 FTIR spectrometer. The ZnSe photoelastic modulator of the instrument was set to 1600 cm^{-1} and an optical filter with a transmission range of 1960–1250 cm^{-1} was used in order to increase the sensitivity in the carbonyl region. Solid-phase samples were prepared with the KBr pellet technique using 0.5 mg of linezolid per 130 mg of KBr. On each sample, VCD spectra were measured upon rotation of the disk around the incident axis direction at 0 ° and 90 °. These spectra were almost identical, demonstrating the absence of detectable spectral artifacts. To improve the S/N ratio, the spectra were averaged for 6 h (corresponding to ~21000 accumulated interferograms). Baseline correction was achieved by subtracting the spectrum of a reference KBr pellet obtained under the same conditions.

For each polymorphic form at least 5 pellets were prepared and measured with each instrument.

2. Computational details

ECD

In order to correlate the observed chiroptical phenomena with the structures of investigated molecule, the ECD spectra were calculated in the framework of TD-DFT in Gaussian 09 package.[3] Computation was done by using as an input structure the X-ray crystal geometry after optimization of hydrogen atoms at B3LYP/6-31G(d) level. In order to predict ECD spectra calculations were performed in vacuum using the B3LYP hybrid functional in conjunction with the 6-31G(d), 6-311G++(d,p), and TZVP basis sets. The best agreement was obtained at the B3LYP/6-311++G(d,p) level of theory, hence, we focus here only on these data. Rotatory strengths were calculated using both length and velocity representations. In the present study, the differences between the length and velocity of calculated values of rotatory strengths were quite small, and for this reason, only the velocity representations were taken into account. The ECD spectra were simulated by overlapping Gaussian functions for each transition. The calculated UV spectra were red-shifted by *ca.* 5-15 nm in relation to the experimental, so ECD spectra were also wavelength corrected.

VCD

For quantum mechanical calculations the reoptimized X-ray crystal geometry was used as an input structure. Computation were performed in the framework of TD-DFT in Gaussian 09 package.[3] In order to predict VCD spectra calculations were performed in vacuum using the B3LYP, CAM-B3LYP and B3PW91 hybrid functional in conjunction with the 6-31G(d), 6-311G(d,p), 6-311G++(d,p), TZVP, and aug-cc-pVDZ basis sets. Results were similar, but best agreement was obtained at the at B3LYP/aug-cc-pVDZ level of theory, hence, we focus here only on these data. Calculated IR and VCD spectra were simulated from calculated rotatory strength and wavenumbers (scaled by a factor between 0.961-0.968) with Lorentzian curves using half width at half-height values of 4 cm^{-1}.

3. Preparation of polymorphic forms of linezolid and their characterization

Linezolid form II. Linezolid (7.42 g) was dissolved in hot dichloromethane (60 mL) and to the stirred solution ethyl acetate (100 mL) was added slowly. The resulting mixture was concentrated under normal pressure by distilling off 47 mL of solvents. Then the mixture was cooled to r.t. The crystals were filtered off and dried at r.t. under reduced pressure to give 6.163 g of polymorph II. IR (Nujol): ν=3362, 1749, 1675, 1535, 1445, 1410, 1328, 1236, 1220 cm^{-1}; IR (KBr): ν=3362, 1749, 1675, 1537, 1445, 1410, 1328, 1236, 1220 cm^{-1}. On DSC trace of sample of form II two endotherms of an irreversible solid-solid transition from form II to III are visible. Melting point of form II is about 155°C (see Figure S1). The diagnostic XRPD peaks of our form II at 2Θ 14.3, 16.8, 21.5, 23.5, 27.1 agree well with those presented in patent application of form II (14.2, 16.8, 21.6, 23.5, 27.0).[4]

Linezolid form III. A suspension of linezolid form II (4.4 g) in toluene (40 mL) was stirred and heated under reflux for 2 h. Then it was stirred at r.t. overnight. The crystals were filtered off, washed with toluene (5 mL) and dried at r.t. under reduced pressure to give 4.232 g of polymorph III. IR (Nujol): ν=3333, 1740, 1661, 1335, 1228, 1197, 1175 cm^{-1}; IR (KBr): ν=3337, 1741, 1662, 1547, 1424, 1335, 1228, 1199, 1176 cm^{-1}. Sample of form III is characterized in DSC by a single endotherm at about 178°C (see Figure S1). The diagnostic XRPD peaks of our form III at 2Θ 7.4, 13.5, 14.7, 18.7, 29.6 agree with those in patent application of form III (7.6, 13.6, 14.9, 18.9, 29.9)[5] as well as with those in patent application of form "IV" (7.5, 13.5, 18.7).[6]

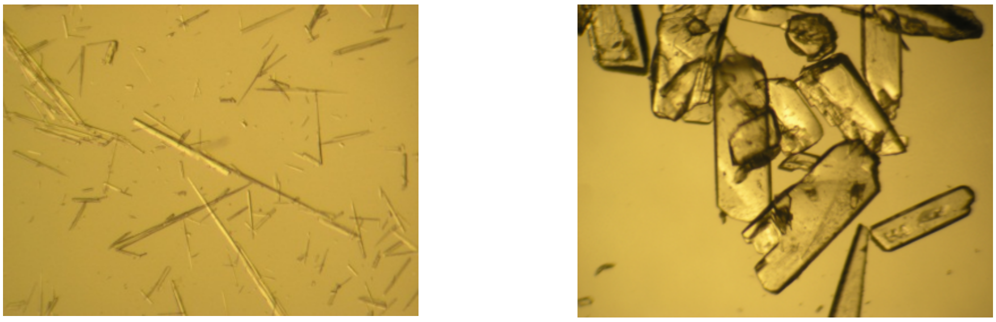

Figure S1. Optical micrographs of needle of form II (left) and of prismatic crystals of form III (right).

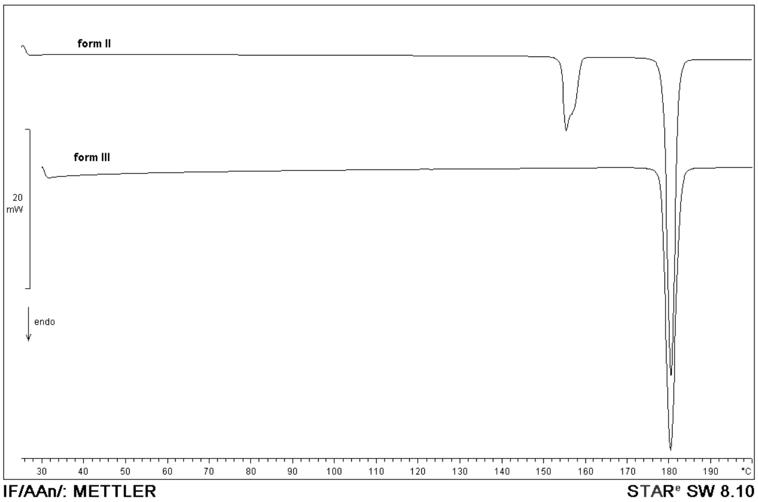

Figure S2. Comparison of the DSC curves for our samples of form II (top) and form III (bottom).

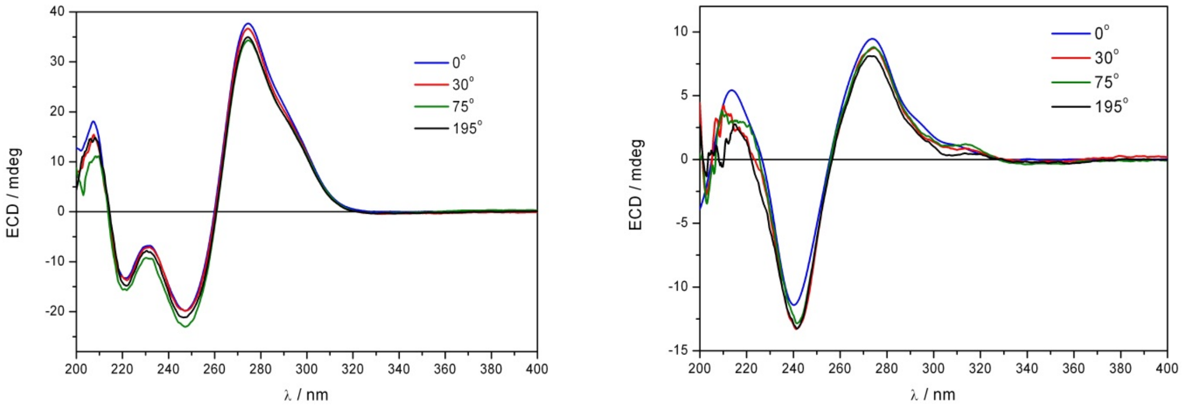

Figure S3. ECD spectra at various rotation angles with respect to the cell surface normal (form II – left, form III – right) taken on JASCO J-815.

225.4 nm 88→91 41%
222.8 nm 89→101 76%

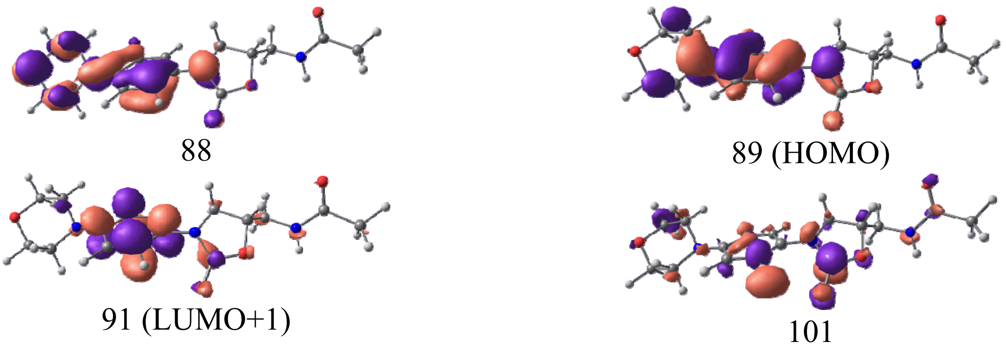

Figure S4. The main transitions for ECD diagnostic band of form II at 221 nm and plots of involving orbitals.

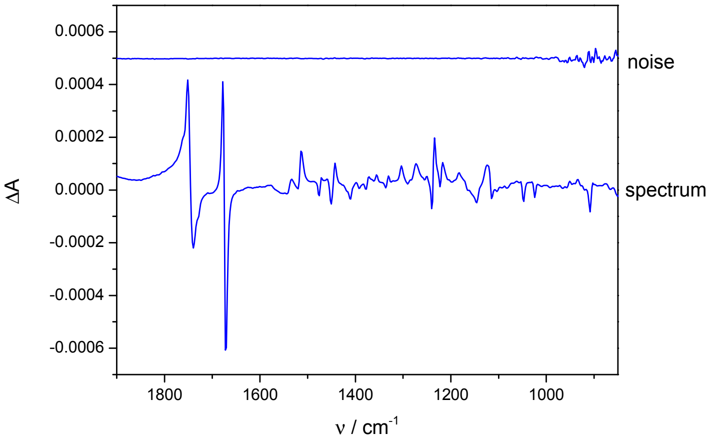

Figure S5. VCD spectrum of linezolid form II (bottom) with the noise level (top) recorded in the KBr pellet on the Chiral*IR-2X* dual PEM (BioTools) instrument.

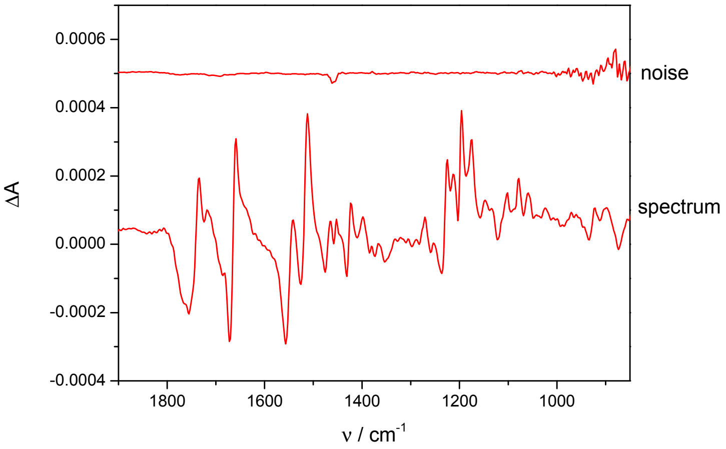

Figure S6. VCD spectrum of linezolid form III (bottom) with the noise level (top) recorded in the KBr pellet on the Chiral*IR-2X* dual PEM (BioTools) instrument.

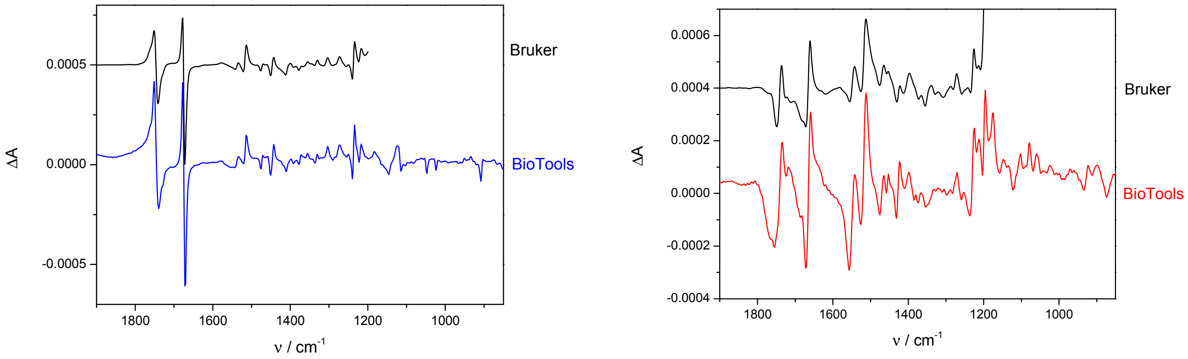

Figure S7. Comparison of VCD spectra of linezolid form II (left) and form III (right) recorded with Bruker (top) and BioTools (bottom) instruments.

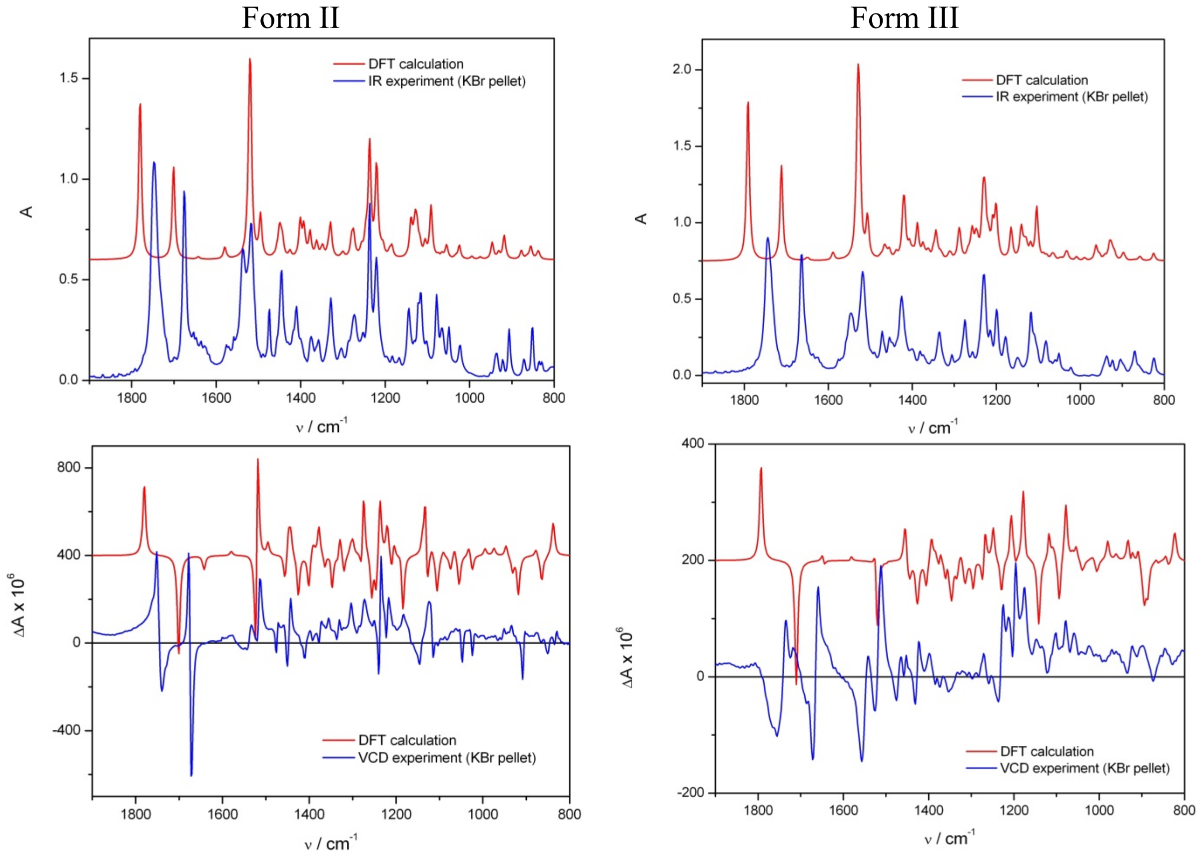

Figure S8. Experimental (blue) and calculated (red) IR (top) and VCD (bottom) spectra of linezolid form II and form III in KBr pellet.

References for Electronic Supplementary Information:

[1] R. Kuroda, T. Honma, *Chirality*, 2000, **12**, 269.

[2] L. A. Nafie, *Vibrational Optical Activity: Principles and Applications,* WILEY-VCH, 2011.

[3] Gaussian 09, Revision A.02, M. J. Frisch, G. W. Trucks, H. B. Schlegel, G. E. Scuseria, M. A. Robb, J. R. Cheeseman, G. Scalmani, V. Barone, B. Mennucci, G. A. Petersson, H. Nakatsuji, M. Caricato, X. Li, H. P. Hratchian, A. F. Izmaylov, J. Bloino, G. Zheng, J. L. Sonnenberg, M. Hada, M. Ehara, K. Toyota, R. Fukuda, J. Hasegawa, M. Ishida, T. Nakajima, Y. Honda, O. Kitao, H. Nakai, T. Vreven, J. A. Montgomery, Jr., J. E. Peralta, F. Ogliaro, M. Bearpark, J. J. Heyd, E. Brothers, K. N. Kudin, V. N. Staroverov, R. Kobayashi, J. Normand, K. Raghavachari, A. Rendell, J. C. Burant, S. S. Iyengar, J. Tomasi, M. Cossi, N. Rega, J. M. Millam, M. Klene, J. E. Knox, J. B. Cross, V. Bakken, C. Adamo, J. Jaramillo, R. Gomperts, R. E. Stratmann, O. Yazyev, A. J. Austin, R. Cammi, C. Pomelli, J. W. Ochterski, R. L. Martin, K. Morokuma, V. G. Zakrzewski, G. A. Voth, P. Salvador, J. J. Dannenberg, S. Dapprich, A. D. Daniels, O. Farkas, J. B. Foresman, J. V. Ortiz, J. Cioslowski, and D. J. Fox, Gaussian, Inc., Wallingford CT 2009.

[4] *WO 2001/57 035 A1 Pat*. 2001.

[5] *WO 2005/035 530 A1 Pat.* 2005.

[6] *WO 2006/004 922 A1 Pat.* 2006.