Absolute Configuration and Polymorphism of 2-Phenylbutyramide and α-Methyl-α-phenylsuccinimide

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Supporting Information

ABSTRACT: Crystal structures of racemic and homochiral forms of 2-phenylbutyramide (1) and 3-methyl-3-phenylpyrrolidine-2,5-dione (2) were investigated in detail by a single crystal X-ray diffraction study. Absolute configurations of the homochiral forms of 1 and 2, obtained by chromatographic separation of racemates, were determined. It was revealed that racemate and homochiral forms of 1 are very similar in terms of supramolecular organization (H-bonded ribbons) in crystal, infrared (IR) spectral characteristics, and melting points. The presence of two different molecular conformations in homochiral forms of 1 allowed mimicking of crystal packing of the H-bonded ribbons in racemate 1. Two polymorph modifications (monoclinic and orthorhombic) comprising very similar H-bonded zigzag-like chains were found for the homochiral forms of compound 2 that were significantly different in terms of crystal structure, IR spectra, and melting points from the racemic form of 2. Unlike compound 1, homochiral forms of compound 2 have a higher density than the corresponding racemate which contradicts the Wallach rule and indicates that, in this case, homochiral forms are more stable than racemate forms.

INTRODUCTION

It is universally recognized that shape of a molecule, or a molecule’s spatial structure, plays a key role in determining its physiological and pharmacological properties. The chiral nature of all living organisms suggests that the most suitable forms of drugs for organisms might be not the racemic form of an active compound (a mixture of equal quantities of two molecules that are mirror images of one another) but rather one of its chiral forms. Significant attention to this problem was raised by the thalidomide tragedy in late 50s to early 60s of the 20th century, when the racemic form of thalidomide was marketed as a sedative and antinausea treatment for pregnant women. Soon after the beginning of thalidomide usage, it was found that this drug produced embryotoxic and teratogenic effects.1−3 At that time, these effects were believed to be related to only one form of thalidomide, its (S)-(−)-enantiomer.4 However, since thalidomide undergoes fast racemization under physiological conditions,5 administration of only the (R)-(+)−enantiomer most probably would not help to avoid those tragic consequences. Nevertheless, these events attracted significant attention to the separation of enantiomers, testing of their bioactivity, and estimation of their absolute configuration using X-ray diffraction method.6,7

Thalidomide, (RS)-2-(2,6-dioxopiperidin-3-yl)-1H-isooindole-1,3(2H)-dione

Recently, the attention of our research groups was aroused toward crystallographic studies of compounds with anticonvulsant activity. The thalidomide story and examples of stereoselective pharmacological activity clearly demonstrate that enantiomeric composition and relation of molecular absolute configuration to its bioactivity need to be taken into account for all established or prospective chiral pharmaceuticals.

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We have recently discovered that α-substituted lactams, succinimides, and carboxamides inhibit the function of the neuronal acetylcholine receptor (nAChR) in vitro with a potency that correlates with their ability to prevent maximal electroshock (MES)-induced seizures in vivo. One of the simplest compounds that inhibit the receptor is 2-phenylbutyramide (1, Scheme 1). It shows promising antiepileptic activity in several rodent models of epilepsy. Another compound that inhibits the receptor is α-methyl-α-phenylsuccinimide (2, Scheme 1), which is the pharmacologically active, N-demethylated metabolite of a well-known antiepileptic drug methsuximide. Methsuximide marketed under the trade name of Celontin by Pfizer and is considered to be a safe and effective antiepileptic drug. Both 1 and 2 are chiral, and we recently reported chromatographic resolution and pharmacological testing of (+) and (−) enantiomers of these drugs. To the best of our knowledge, no polymorphic modifications have been reported for either 1 or 2.

The main goal of the presented project was a detailed characterization of all racemic and enantiomeric forms of mentioned compounds with the single crystal X-ray diffraction method and discussion of the relationship of their molecular and crystal structure to the physicochemical properties of different forms of compounds 1 and 2. The following notations are used for the studied compounds: 2-phenylbutyramide 1, α-methyl-α-phenylsuccinimide 2, their racemic forms are called rac-1 and rac-2; after racemic forms have been separated we call them in accordance with an order of eluted fractions 1a and 2a, and 1b and 2b, respectively. The enantiomeric forms characterized with X-ray analysis in accordance with their stereochemistry are called R or S, and in accordance with their optical activity are called + (plus) or − (minus). In the case of a polymorphic compound a name of singony was added to the corresponding notation.

### MATERIALS AND METHODS

#### Chemicals

2-Phenylbutyramide and α-methyl-α-phenylsuccinimide (3-methyl-3-phenylpyrrolidine-2,5-dione) were purchased from Alfa Aesar and Sigma-Aldrich, respectively. Acetonitrile and methanol (Omnisolv, gradient grade), hexanes, and acetone were obtained from Alfa Aesar and Sigma-Aldrich, respectively.

### Table 1. Crystallographic Data for Compound 1

| compound | rac-1 | (R)-1 | (S)-1 |
|----------|-------|-------|-------|
| empirical formula | C_{12}H_{19}NO | C_{12}H_{19}NO | C_{12}H_{19}NO |
| fw | 163.21 | 163.21 | 163.21 |
| radiation | Mo Kα | Cu Kα | Cu Kα |
| temperature (K) | 100 | 100 | 100 |
| cryt size (mm) | 0.30 × 0.18 × 0.15 | 0.44 × 0.10 × 0.08 | 0.28 × 0.24 × 0.04 |
| space group | C2/c | triclinic | triclinic |
| a (Å) | 24.197(2) | 5.1916(1) | 5.1941(1) |
| b (Å) | 5.1143(3) | 9.4525(2) | 9.4531(1) |
| c (Å) | 17.669(1) | 10.0367(2) | 10.0232(1) |
| α (deg) | 90 | 96.326(1) | 96.343(1) |
| β (deg) | 121.267(1) | 102.938(1) | 102.945(1) |
| γ (deg) | 90 | 104.938(1) | 104.938(1) |
| V (Å³) | 1869.02(2) | 455.23(2) | 455.97(1) |
| Z | 2 | 2 | 2 |
| d_{s} (g cm⁻³) | 1.160 | 1.188 | 1.189 |
| F(000) | 704 | 176 | 176 |
| μ (mm⁻¹) | 0.075 | 0.607 | 0.607 |
| θ range (deg) | 1.97−30.00 | 4.59−69.99 | 4.60−70.00 |
| index range | −33 ≤ h ≤ 34 | −6 ≤ h ≤ 6 | −6 ≤ h ≤ 6 |
| reflections collected | 11537 | 11121 | 10827 |
| reflections unique/Rint | 2717/0.026 | 3010/0.038 | 2980/0.022 |
| reflections with I > 2σ(I) | 2181 | 2991 | 2955 |
| R_{I,wR_{2}} (I > 2σ(I)) | 0.0412/0.1123 | 0.0321/0.0815 | 0.0288/0.0750 |
| R_{I,wR_{2}} (all data) | 0.0530/0.1185 | 0.0323/0.0818 | 0.0290/0.0755 |
| data/parameters | 2717/116 | 3010/231 | 2980/231 |
| GOF on F² | 1.001 | 1.000 | 1.001 |
| Flack/Hooft parameters | not appl | 0.24(19)/— | 0.09(18)/— |
| no. of Bipolet pairs | — | 1315 (76%) | 1286 (74%) |
| largest diff peak/hole (e·Å⁻³) | 0.356/−0.185 | 0.121/−0.171 | 0.172/−0.175 |
| abs cor T_{max} T_{min} | 0.989; 0.978 | 0.955; 0.776 | 0.976; 0.848 |
EM Science. Ultrapure (18.2 MΩcm) water was produced in-house using Barnstead NANOpure Diamond system (Thermo Scientific).

**Chiral Liquid Chromatography.** Resolution of enantiomers of 1 and 2 was performed by chiral high-performance liquid chromatography (HPLC) in reversed phase mode. Briefly, enantiomers of 1 were separated on Chiralcel OD stationary phase, and enantiomers of 2 on Chiralpak OJ stationary phase (both from Daicel Chemical Industries, Ltd.) using gradients of MeOH in H2O. Collected fractions were dried in vacuo to give powder materials used in the experiments described in this paper. On the basis of analytical chiral HPLC, the enantiomeric purity of enantiomers of 1 thus prepared was 93–95%, and enantiomeric purity of enantiomers of 2, due to the better resolution achieved in preparative chromatography, was 96–98%.

**X-ray Single Crystal Structure Analysis.** Data were collected on a Bruker APEX-II CCD diffractometer (graphite monochromator, ω and φ scan mode) and corrected for absorption.13 For details of experiments and structure solution, see Tables 1 and 2. The structures were determined by direct methods and refined by a full-matrix least-squares technique on F² with the anisotropic displacement parameters for non-hydrogen atoms. For the enantiomers of 2, the absolute configurations were reliably determined by the refinement of Flack parameters14 and confirmed by calculations of Hoof parameters15 (Tables 1 and 2). For (R)-1 and (S)-1, it was impossible to calculate the Hoof parameters because of an insufficient number of the measured Friedel pairs, and thus only the Flack parameters were used. The hydrogen atoms of the amino groups were localized in the difference Fourier maps and included into refinement with fixed isotropic displacement parameters (U(eq)(H) = 1.2U(eq)(N)), except for compound rac-2, in which the positional parameters of these atoms were also fixed. The other hydrogen atoms were placed in calculated positions and refined in riding model with fixed isotropic displacement parameters (U(eq)(H) = 1.5U(eq)(C) for the CH₂-groups and U(eq)(H) = 1.2U(eq)(C) for the other groups). All calculations were carried out by use of the SHELXTL program package.16 Crystallographic data have been deposited with the Cambridge Crystallographic Data Center, CCDC 938765–938772. Copies of this information may be obtained free of charge from the Director, CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (Fax: +44 1223 336033; e-mail: deposit@ccdc.cam.ac.uk or www.ccdc.cam.ac.uk).

**IR Spectroscopy, CD Spectroscopy, and Melting Point Determination.** Powder infrared (IR) spectra of finely grounded crystals were recorded in attenuated total reflection (ATR) mode at room temperature (22 °C) on Magna-IR 550 FT-IR spectrometer (Thermo Nicolet) with the Linear Baseline Correction. Far-UV (200–280 nm) circular dichroism (CD) spectra of 1-mM solutions of 1 and 2 in AcCN/H2O (1:1) were recorded on a model 420 CD spectrometer (Aviv Biomedical, Lakewood, NJ) in a 0.1 cm path length quartz optical cell. A 2 nm spectral bandwidth was used, and three or five scans were collected and averaged for each sample. The optical bench was purged with dry N₂.

Melting points were measured on OptiMelt automated melting point system (Stanford Research Systems Ltd.) controlled by MeltView software version V.1.107. Heating rate of 1 °C/min was used, and the detection thresholds were set as follows: 10% for clear point, 50% for single (meniscus) point, and 70% for onset point.

### Table 2. Crystallographic Data for Compound 2

| compound | rac-2 | (R)-2m | (S)-2m | (R)-2o | (S)-2o |
|----------|-------|--------|--------|--------|--------|
| empirical formula | C₁₁H₃NO₂ | C₁₁H₃NO₂ | C₁₁H₃NO₂ | C₁₁H₃NO₂ | C₁₁H₃NO₂ |
| fw | 189.21 | 189.21 | 189.21 | 189.21 | 189.21 |
| radiation | Mo Kα | Cu Kα | Cu Kα | Cu Kα | Cu Kα |
| temperature (K) | 296 | 100 | 100 | 100 | 100 |
| cryst size (mm) | 0.30 × 0.20 × 0.20 | 0.30 × 0.25 × 0.20 | 0.30 × 0.20 × 0.20 | 0.30 × 0.24 × 0.21 | 0.35 × 0.30 × 0.25 |
| cryst syst | monoclinic | monoclinic | monoclinic | orthorhombic | orthorhombic |
| space group | P2₁/c | P2₁ | P2₁ | P2₁, P2₁, P2₁, P2₁, P2₁ |
| a (Å) | 7.3699(9) | 6.7072(1) | 6.7076(5) | 6.7078(1) | 6.7089(1) |
| b (Å) | 22.392(3) | 7.0142(7) | 7.0112(5) | 7.1693(1) | 7.1714(1) |
| c (Å) | 11.7988(15) | 10.0182(1) | 10.0210(7) | 19.0492(3) | 19.0480(2) |
| α (deg) | 90 | 90 | 90 | 90 | 90 |
| β (deg) | 90.308(2) | 102.6398(3) | 102.635(2) | 90 | 90 |
| γ (deg) | 90 | 90 | 90 | 90 | 90 |
| V (Å³) | 1964.5(4) | 459.895(9) | 459.86(6) | 916.08(3) | 916.44(2) |
| Z | 8 | 2 | 2 | 4 | 4 |
| dₒ (g cm⁻³) | 1.280 | 1.366 | 1.366 | 1.372 | 1.371 |
| f(000) | 800 | 200 | 200 | 400 | 400 |
| μ (mm⁻¹) | 0.089 | 0.773 | 0.776 | 0.776 | 0.776 |
| θ range (deg) | 0.90–30.00 | 4.52–72.12 | 4.52–69.94 | 4.52–71.98 | 4.52–71.95 |
| index range | −10 ≤ h ≤ 10 | −8 ≤ h ≤ 8 | −8 ≤ h ≤ 8 | −8 ≤ h ≤ 8 | −8 ≤ h ≤ 8 |
| reflections collected | 25061 | 8828 | 13074 | 16767 | 22269 |
| reflections unique/R_re | 5725/0.022 | 1631/0.024 | 1696/0.035 | 1793/0.035 | 1797/0.027 |
| reflections with I > 2σ(I) | 4559 | 1631 | 1696 | 1792 | 1795 |
| R₁/τ₀R₁ (I > 2σ(I)) | 0.0490/0.1314 | 0.0266/0.0668 | 0.0257/0.0688 | 0.0258/0.0647 | 0.0250/0.0624 |
| R₁/τ₀R₁ (all data) | 0.0637/0.1406 | 0.0266/0.0668 | 0.0257/0.0688 | 0.0258/0.0647 | 0.0250/0.0624 |
| data parameters | 5725/256 | 1631/132 | 1696/132 | 1793/132 | 1797/132 |
| GOF on F² | 1.002 | 1.006 | 1.002 | 1.002 | 1.002 |
| Flack/Hoof parameters | not appl | 0.00(19)/0.07(4) | 0.17(18)/0.11(4) | 0.06(20)/0.01(4) | 0.05(19)/0.05(2) |
| No. of Bijvoet pairs | 663 (80%) | 683 (80%) | 754 (95%) | 718 (99%) | 719 (99%) |
| largest dif peak/hole (e Å⁻³) | 0.251/–0.210 | 0.195/–0.137 | 0.187/–0.130 | 0.188/–0.141 | 0.186/–0.134 |
| abs corr | Trmax/Tr | 0.982/0.974 | 0.861/0.801 | 0.861/0.801 | 0.854/0.801 | 0.830/0.773 |
RESULTS AND DISCUSSION

Chromatographic Resolution, Relative Configuration, and Crystallization of 1 and 2. Enantiomers of compounds 1 and 2 were resolved by chiral reversed phase HPLC (see Materials and Methods). Far-UV CD spectra of enantiomers of 1 (Figure 1a) show a positive Cotton effect around 225 nm for the enantiomer that elutes first, and a negative Cotton effect for the enantiomer that elutes second. Far-UV CD spectra of enantiomers of 2 (Figure 1b) are more complex: the enantiomer that elutes first has a positive Cotton effect around 220 nm and a negative one around 250 nm, while the enantiomer that elutes second has opposite signs of the Cotton effects. Thus, for both compounds (+) enantiomers elute first, and (−) enantiomers second. This is in accordance with our polarimetry data. Far-UV (200–300 nm) CD spectrum of (−)-2 in AcCN/H₂O (1:1) (Figure 1b) closely resembles the far-UV (210–280 nm) spectrum of this enantiomer in MeOH previously reported by Knabe and Koch.17

After trying several different crystallization conditions, we found that crystals of racemic and enantiomerically pure compounds 1 and 2 obtained by chiral chromatography separations have been investigated by X-ray diffraction analysis. The absolute configurations of enantiomers have been established based on the anomalous dispersion effects observed with Cu Kα radiation. Because the presence of only one or two oxygen atoms in compounds 1 and 2, we have carried out the experimental X-ray diffraction studies for both enantiomers of each compound to get more indicative values of Flack parameters. Evidently, the structures of (R)- and (S)-enantiomers of the same compound are mirror images of each other. Therefore, only (R)-enantiomers of 1 and 2 are described below (for full X-ray data of the (S)-enantiomers of 1 and 2, see Supporting Information).

The molecular structures of (R)-1 and rac-1 are shown in Figure 2 along with the atomic numbering schemes. Enantiomer (R)-1 crystallizes in the triclinic space group $P\textbar1$ with the two crystallographically independent molecules are of a quality well suitable for single-crystal X-ray diffraction crystallography.

MOLECULAR AND CRYSTAL STRUCTURES

The structures of both racemic and enantiomerically pure compounds 1 and 2 obtained by chiral chromatography separations have been investigated by X-ray diffraction analysis. The absolute configurations of enantiomers have been established based on the anomalous dispersion effects observed with Cu Kα radiation. Because the presence of only one or two oxygen atoms in compounds 1 and 2, we have carried out the experimental X-ray diffraction studies for both enantiomers of each compound to get more indicative values of Flack parameters. Evidently, the structures of (R)- and (S)-enantiomers of the same compound are mirror images of each other. Therefore, only (R)-enantiomers of 1 and 2 are described below (for full X-ray data of the (S)-enantiomers of 1 and 2, see Supporting Information).

The molecular structures of (R)-1 and rac-1 are shown in Figure 2 along with the atomic numbering schemes. Enantiomer (R)-1 crystallizes in the triclinic space group $P\textbar1$ with the two crystallographically independent molecules

Figure 1. Far-UV CD spectra of enantiomers of 1 (a) and 2 (b) in AcCN/H₂O (1:1).

Figure 2. Molecular structures of (a) rac-1 (conformer A) and (b) (R)-1 (the two crystallographically independent molecules—conformers A and B distinguishing by rotation of the 1-phenylpropyl substituent about the C1—C2 bond).
representing different conformers A and B (Figure 1b). The conformers A and B are distinguished by rotation of the amide group around the C1–C2 bond (the N1–C1–C2–C3 torsion angles are 32.26(18) and −144.08(13)°, in conformers A and B respectively). A superposition of two conformers shown in Figure 3 demonstrates significant difference between them attributed to rotation of amide group by ~180° around the C1–C2 bond. Unlike (R)-I, rac-1 crystallizes in the monoclinic space group C2/c and consists of the only A conformers (the N1–C1–C2–C3 torsion angle is −130.06(9)°). The geometrical parameters of molecules (R)-I and rac-1 are very similar and are in good agreement with those found in the literature.

The molecule of 1 contains one hydrogen-bond acceptor and two donor sites. In order to satisfy condition of H-bonds formation by all potential hydrogen-bonding acceptors and donors, each carbonyl group should act as a bifurcated hydrogen-bond acceptor, and both hydrogen atoms of each amide groups should act as hydrogen-bond donors. As a result, the molecules (R)-I and rac-1 produce very similar crystal structures despite the differences in molecular structures. The presence of two conformers in (R)-I also helps to satisfy this condition. Thus, in the crystals of both (R)-I and rac-1, there are the infinite ribbons built via the N–H...O hydrogen bonds (Figure 4, Table 3). The ribbons in (R)-I are chiral and are composed of both conformers A and B (Figure 4b), whereas the ribbons in rac-1 are centrosymmetric (Figure 4a). The amide groups within the chains are practically coplanar, and the bulk phenyl substituents are arranged in anti-position relative to the acetamide fragment plane. The infinite ribbons are further packed in similar parquet pattern in both (R)-I and rac-1 structures (Figure 5). It is interesting to note that the analogous H-bonded ribbons are also formed in the crystals of other related amides.18,19 Similarity of hydrogen bonds characteristics and molecular packings for (R)-I and rac-1 suggests that their physicochemical properties might be similar as well. This is supported by the close similarity of IR spectral characteristics of racemic and enantiomeric forms and by very close values of melting points for all forms of 1.

Crystallographic studies revealed that enantiomers of 2 form two different polymorphs when crystallized from different solvents (Table 2). Crystallization of 2 from AcCN/H2O (1:1) gives monoclinic form (2m, space group P21/c), while its crystallization from hexanes/acetic acid (2:1) gives orthorhombic form (2o, space group P212121). The crystal shape of two polymorphs is presented in Figure 6. rac-2 crystallizes from hexane/acetic acid and acetone/water solution in the monoclinic space group P21/c with the two crystallographically independent molecules.

The molecular structures of (R)-2 and rac-2 are shown in Figure 7 along with the atomic numbering schemes. The geometrical parameters of molecules (R)-2m, (R)-2o, and rac-2 are very close to each other and comparable to those found for the related compounds.20–26 Superposition of molecules 2 from monoclinic and orthorhombic polymorphs (Figure 8) shows close molecular similarity in these forms.

In contrast to 1, the molecule 2 contains two hydrogen-bond acceptor and one donor sites. As a result, in the crystal, the succinimides usually form either dimers20,26 or infinite chains21–25 by hydrogen bonding between the imide hydrogen atom and carbonyl oxygen atom of adjacent molecules. In the case of 2, the molecules both in the enantiomerically pure 2m and 2o and in the racemate rac-2 are linked by the intermolecular N1–H1...O2 hydrogen bonds (Table 3) into one-dimensional zigzag-like chains running in the b and a directions, respectively (Figure 9, left). The fact that only O2 oxygen atom acts as a protonoacceptor is apparently explained by the steric reasons. The succinimide rings within the chains are almost coplanar. Nevertheless, despite the obvious similarity of these chains, there is a striking distinction between them, namely, anti (in the enantiomerically pure 2m and 2o), similar to 1, and syn (in the racemic rac-2), unlike 1, mutual disposition of the phenyl substituents relative to the succinimide ring plane (Figure 9, right). It should be noted that, to the best our knowledge, the less sterically favorable syn configuration of the phenyl rings within the H-bonded chains is observed for the first time among compounds of this type.27 The main structural motifs the H-bonded zigzag-like chains in the two polymorphs 2m and 2o are quasi-identical. The crystal structures of these polymorphs are characterized by the different packing of the H-bonded chains. In the crystal structure of 2o, the H-bonded chains are packed congruently, i.e., without changing of the conformations relative to each other (Figure 9a, right), while, in the crystal structure of 2m, these chains are packed incongruently, with rotation by 180 deg in turn along the c axis (Figure 9b, right). Due to the syn-configuration of the phenyl rings, the crystal packing of the H-bonded chains in rac-2 is zipper-like (Figure 9c).

Solid-State IR Spectroscopy of Racemic and Homochiral Forms of 1 and 2. No substantial differences were observed in powder IR spectra between racemic and homochiral forms of 1. However, a powder IR spectrum of racemic form of 2 is different from spectra of homochiral forms of this compound in the N–H stretch region (Figure 10).

It appears that spectral data are closely related to distinctions of supramolecular organization in racemate–enantiomer and polymorphs pairs. For compound 1, the presence of two conformers in enantiomeric crystal allows the racemic H-bonded chain to be closely mimicked, which leads to very close similarity of solid state IR spectra for rac-1, R-1 and S-1. On the contrary, dissimilarity of supramolecular organization for rac-2 and both polymorphs of 2 (H-bonded molecular chains with transoid orientation for enantiomeric polymorphs and with cisoid orientation for racemate) leads to differences in
Figure 4. Infinite H-bonded ribbons in (a) rac-1 (centrosymmetric) and (b) (R)-1 (chiral).

Table 3. Intermolecular N–H···O Hydrogen Bonds (Å and deg) in 1 and 2

|          | d(D–H) | d(H–A) | d(D–A)     | θ(D–H–A) |
|----------|--------|--------|------------|-----------|
| rac-1    |        |        |            |           |
| N1–H1A–O1 [x, −1+y, z] | 0.888(16) | 2.067(15) | 2.8797(11) | 151.8(14) |
| N1–H1B–O1 [1/2–x, 3/2–y, 1−z] | 0.896(19) | 2.042(19) | 2.9335(14) | 173.2(13) |
| (R)-1    |        |        |            |           |
| N1–H1A–O1A | 0.84(2)   | 2.05(2)   | 2.8882(16) | 175(2)    |
| N1–H1B–O1 [−1+x, y, z] | 0.84(2)   | 2.19(2)   | 2.9591(16) | 152(2)    |
| N1A–H1C–O1A [1+x, y, z] | 0.88(2)   | 2.16(2)   | 2.9497(15) | 150(2)    |
| N1A–H1D–O1 | 0.89(2)   | 2.07(2)   | 2.9581(17) | 178(2)    |
| rac-2    |        |        |            |           |
| N1A–H1A–O2B [x, y, −1+z] | 0.86      | 1.97      | 2.823(2)   | 170       |
| N1B–H1B–O2A [1+x, y, 1+z] | 0.86      | 2.04      | 2.862(2)   | 160       |
| (R)-2m   |        |        |            |           |
| N1–H1A–O2 [−x, −1/2+y, 2−z] | 0.864(19) | 1.961(16) | 2.8241(15) | 176(2)    |
| (R)-2o   |        |        |            |           |
| N1–H1–O2 [−x, 1/2+y, 3/2−z] | 0.916(14) | 1.933(14) | 2.8374(14) | 168.99(14) |

*D* – proton donor, A – proton acceptor.
geometric characteristics of hydrogen bonding and crystal packing and, as a consequence, to different spectral characteristics (Figure 10, Table 4). Due to similarity of molecular packing in polymorphs of 2 (2m and 2o), their spectral characteristics are also similar to small deviations related to slight differences in hydrogen bonding.

Crystal Density and Melting Points of Racemic and Homochiral Forms of 1 and 2. Usually denser compounds with tightly packed molecules are considered to be more...
thermodynamically stable.\textsuperscript{7} Comparison of eight pairs of homochiral and racemic compounds conducted by Wallach in 1895 allowed him to formulate a rule that racemic compounds have a trend to be denser than enantiomers.\textsuperscript{28} Statistical analysis carried out by Brock at al.\textsuperscript{29} based on CSD data in 1991 revealed that such trend in general exists for racemic crystals composed of enantiomers that can be resolved chemically. However, exceptions to this rule are described in the literature.\textsuperscript{30,31} In contrast to the Wallach rule, compound 2, unlike compound 1, demonstrates a higher density of chiral crystals in comparison to its racemate (difference is ca. 0.1 g cm\textsuperscript{-3}, Table 2). That might be an indication that compound 2 in chiral forms is at least as stable as its racemate.

Interesting observations were obtained for melting points of studied materials. If for racemic and homochiral forms of 1 melting points are very close, for 2 melting points differ

Figure 9. Infinite H-bonded chains (left) and their crystal packing (right) in (a) rac-2, (b) (R)-2m, and (c) (R)-2o. The dashed lines indicate the intermolecular N–H···O hydrogen bonds.
significantly (Table 4). These results can be explained by the close similarity of molecular packings in all forms of 1 and by dissimilarities in supramolecular aggregates and molecular packings in crystals 2 (chains with transoid orientation for enantiomeric polymorphs and with uncommon cisoid orientation for racemate). For racemate 2, lower crystal density and lower melting temperature (difference ~33°C) suggest its lower stability.

CONCLUSIONS

Enantiomers of compounds 1 and 2 were separated by chiral chromatography, and their absolute configurations were reliably established by analysis of anomalous X-ray scattering using refinement of Flack and Hooft parameters. A combination of X-ray and CD data allowed the following designations for the enantiomers: R-(−)-1, S-(+)-1, R-(+)-2, S-(−)-2. The assignments for 2 are in accordance with the study by Knabe and Koch,17 who determined relative and absolute configurations of enantiomers of this compound by establishing a correlation between their optical activity and optical activity of their synthetic precursors of known absolute configuration. In crystals of racemate and enantiomers of 1, the robust molecular synthons with very similar structure and hydrogen bonding pattern were found. The existence of such synthons in the enantiomers was due to the presence of two molecular conformations that allowed mimicking of molecular hydrogen bonded chains found in the racemate. Structural similarity of racemate and homochiral forms for 1 and polymorphs for 2 explains the close similarity of their IR spectra and melting points. On the basis of presented structural characteristics, it is possible to speculate that bioactivity for all forms of 1 will be similar, while for 2 it might be different.

ASSOCIATED CONTENT

Supporting Information

Crystallographic information file. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

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