Polymorphism of racemic imidazol-1-ylsuccinic acid derivatives. Suitable probes for extracellular pH by magnetic resonance spectroscopic imaging

Carmen Ubide-Barreda,[a] Mª Luisa Rojas,[b] Pedro José Martínez de Paz,[c] Valeria Righi,[d] and Paloma Ballesteros [a]*

[a]Laboratorio de Síntesis Orgánica e Imagen Molecular por Resonancia Magnética, Facultad de Ciencias, UNED, Pº Senda del Rey 9,28040 Madrid, Spain
[b]Departamento de Química Inorgánica y Química Técnica, Facultad de Ciencias, UNED, Pº Senda del Rey 9, 28040 Madrid, Spain
[c]Grupo de Biología y Toxicología Ambiental, Departamento de Física Matemática y Fluidos, Facultad de Ciencias,UNED, Pº Senda del Rey 9, 28040 Madrid, Spain
[d]Dipartimento di Chimica, Università di Modena e Reggio Emilia, via G.Campi 183,41125 Modena, Italy
E-mail: pballesteros@ccia.uned.es

Dedicated to Professor Julio Álvarez Builla on the occasion of his 65th birthday

DOI: http://dx.doi.org/10.3998/ark.5550190.0012.314

Abstract

The polymorphism of racemic imidazol-1ylsuccinic acid derivatives, a series of compounds previously used as extracellular pH probes for NMR spectroscopy, is investigated by crystallization in different solvents. The mono-ester, (±)-3-(ethoxycarbonyl)-2-(imidazol-1-yl) propionic acid, shows two different polymorphs when recrystallized in absolute ethanol (Form I) or water (Form II), respectively. These two forms have been characterized by optical microscopy, powder X-ray diffraction analysis, and thermogravimetry. Polymorphs I and II can also be distinguished by solid state CP-MAS ^{13}C NMR spectroscopy.

Keywords: Polymorphism; racemic imidazol-1-ylsuccinic acid derivatives; crystallization; optical microscopy; powder X-ray diffractometry; thermogravimetry

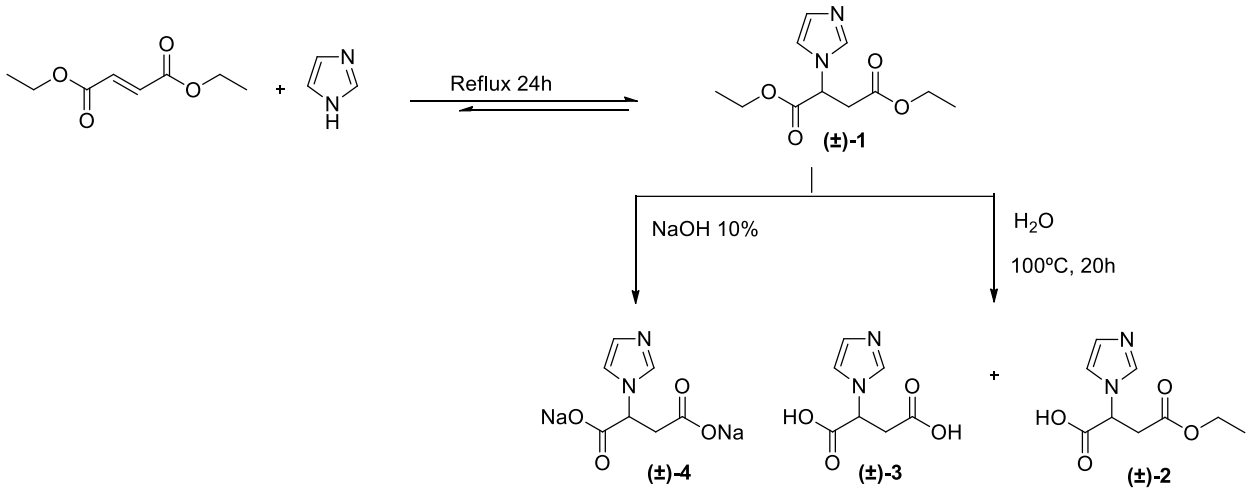
Introduction

The ability of a substance to exist in different forms is known as polymorphism. Polymorphs of a specific compound are chemically identical, but differ with respect to their physical properties,

 ©ARKAT USA, Inc.

such as density, crystal habit, spectra, melting point, solubility, etc. In a classic paper by Haleblian and McCrone, polymorphism was defined as the ability of any compound to crystallize as more than one distinct crystal species.[1] However, in physical pharmacy the word 'polymorphism' is often used nowadays to cover a variety of solid forms of active pharmaceutical ingredients (APIs) and excipients including crystalline, amorphous, and also solvate/hydrate forms. Polymorphism of drug substances has been the subject of intensive research for many years. The knowledge of the physical properties and polymorphism of the drug is essential for the successful development of a new medicine.[2] Presently, solid form screening is a standard procedure in drug development, but it was not until the last decades of the 20th century that the whole pharmaceutical industry became aware of the importance of polymorphism, even though the phenomenon had been known since the early 19th century.[3]

We have previously described that imidazol-1yl succinic acid derivatives are excellent probes for the measurement of extracellular pH.[4] Racemic compounds **1-4** are easily prepared by imidazole addition to diethyl fumarate and subsequent hydrolysis (Scheme 1). (±)-Di-ester **1** was previously resolved by enantioselective inclusion methodology.[5] Racemization of the resolved di-ester was easy in biological media.



Scheme 1. Syntheses of (±)-imidazol-1-ylsuccinic acid derivatives (±)-**1-4**.

Results and Discussion

Neutral hydrolysis of di-ester (±)-**1** yields a mixture of mono-ester (±)-**2** and di-acid (±)-**3** easily separated by recrystallization in absolute ethanol. Mono-ester (±)-**2** melts to 144-145 ºC. Crystal packing of racemic compound (±)-**2** was previously determined[6] by X-ray diffraction analysis revealing that it exists in zwitterion form in the solid state. Proton from carboxylic oxygen migrates to the N3 imidazole ring forming the zwitterion. Besides, there are some interactions

 ©ARKAT USA, Inc.

between neighbouring chains. Moreover, compound (±)-**2** was further linked into antiparallel pairs through ring interactions (distance between centroids of 3.52 Å) and by a short contact between the centroid and the aliphatic chain. These features clearly indicate that compound (±)-**2** is a pure racemic species. We consider this crystalline compound as Form I.

In absence of theoretical methods to predict how atoms are arranged in a molecule and how molecules pack into a crystal,[7] we started a systematic study of crystallization[8] of compound (±)-**2**. Thus, Form I was dissolved in hot absolute ethanol and crystallized in different solvent mixtures giving different types of crystalline polymorphs with different melting points (Table 1). Furthermore, recrystallization in plain water produces crystalline compound (±)-**2** with a molecule of water included. The presence of the water molecule in the crystal was confirmed by elemental analysis (see experimental) and thermometric analysis. We consider this polymorph as Form II.

Optical microscopic analysis

Crystal shapes and melting points of the different polymorphs of compounds (±)-**2** and (±)-**4** were determined on a hot stage microscope and the data are depicted in Tables 1 and 2. Melting point values are compared with those obtained by calorimetric (DSC) determination.

Table 1. Solvents, crystal shape and melting points of compound (±)-**2** determined in hot stage microscope and DSC

Solvent	Crystal shape	Compound (±)-**2** (m.p. ºC)	DSC ºC
Abs. Ethanol	prisms	144-145	145
Abs. Ethanol /Methylene Chloride	prisms	129-130	149
Abs. Ethanol /Dioxane	prisms	141-143	139
Abs. Ethanol /Toluene	microcrystals	139-141	138
Abs. Ethanol /Cyclohexane	prisms	140-141	146
Abs. Ethanol /Benzene	prisms	139-140	146
Abs. Ethanol /Butanol	prisms	142-143	146
Abs. Ethanol /2-Propanol	prisms	142-143	145
Abs. Ethanol /Acetone	microcrystals	143-146	147
Abs. Ethanol/Ethyl acetate	prisms	141-143	148
Abs. Ethanol/Hexane	prisms	140-141	148
Abs. Ethanol/Tetrahydrofurane	prisms	142-143	147
Water	prisms	140-141	144*

*water lost in a 60-125 ºC range.

Di-sodium salt (±)-**4** crystallizes as microcrystals from water which decompose above 264 ºC (Table 2). Recrystallization from different mixtures of water and protic and aprotic solvents did

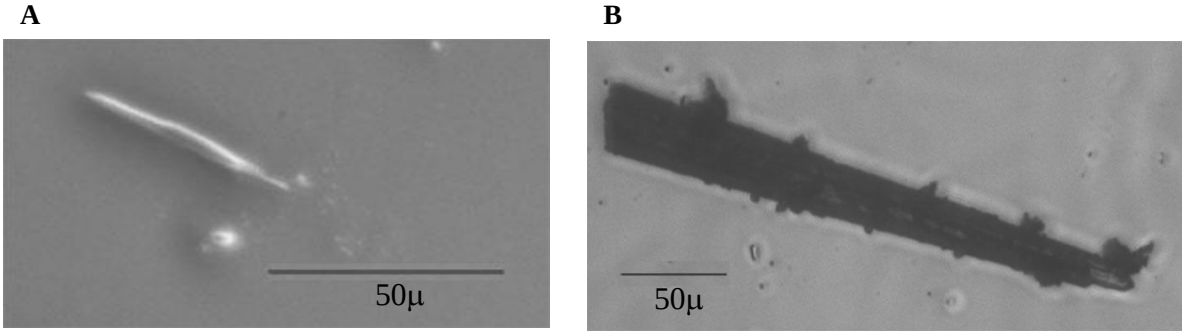
 ©ARKAT USA, Inc.

not yield different crystalline species. These results prompt us to study their decomposition in detail by DSC and TG showing four decomposition temperatures.

Table 2. Solvents, crystal shape and melting points of compound (±)-**4** determined in hot stage microscope and DSC and TG data

Solvent	Crystal shape	m.p.ºC (decomp.)	DSC-TG ºC
Water	microcrystals	269	(51-167) (203-293) (293-341) (341-500)
Water/Ethanol	microcrystals	264	(51-156) (192-302) (302-400) (400-500)
Methanol/Ethyl Acetate	microcrystals	275	(50-131) (293-330) (330-370) (370-500)
Water/ 2-Propanol	microcrystals	270	(52-108) (108-190) (286-333) (333-500)
Water/ Acetonitrile	microcrystals	271	(52-156) (203-310) (310-398) (398-500)
Methanol/1-Butanol	microcrystals	275	(51-144) (290-332) (332-384) (384-500)
Methanol/ Cyclohexane	microcrystals	278	(53-147) (246-303) (303-378) (378-500)
Methanol/ Ethanol	microcrystals	277	(50-164) (229-332) (332-401) (401-500)
Methanol/Acetonitrile	microcrystals	270	(50-164) (229-332) (332-401) (401-500)

Crystal forms and size of compound (±)-**2** recrystallized in ethanol (Form I) and water (Form II) were observed in more detail in an optical microscope 160x at room temperature. Optical microscopy showed clearly the presence of crystals of different sizes. The size of prisms from Form I (A: left panel) is approximately three times smaller than that from prisms Form II (B: right panel).



 ©ARKAT USA, Inc.

Figure 1. Prisms of compound (±)-**2** recrystallized in (A): absolute ethanol (Form I); (B) in water (Form II). Observed in a Microscope 160x.

Thermal analysis (TG and DSC)

Thermogravimetry (TG) and differential scanning calorimetry (DSC) curves of Form I are displayed in Figure 2. A very wide endothermic peak evolving a heat of 103 kJ/mol is observed in the 60-125 ºC range. This peak can be associated with the reorganization of the molecular structure, probably due to the breaking of both the zwitterions interactions between the molecules of mono-esters and the ring interactions between neighboring chains. The peak corresponding to the melting process is centered at 145 ºC. Finally, in the 160-260 ºC range the decomposition of compound (±)-**2** occurs, leading to a carbonous residue of around 13 wt%.

The TG and DSC curves of Form II are depicted in Figure 3. The TG curve is very similar to that of Form I, with the exception of the mass loss of ca. 3 % observed in the 60-100 ºC, which can be assigned to the removing of the molecules of water of crystallization (approximately 0.3 mols of water by mol of compound (±)-**2**, which is in agreement with the results obtained by elemental analysis. The process of reorganization of the molecular structure overlaps with that of water removing, therefore the heat evolved (497 kJ/mol) in the endothermic peak centered at 79 ºC being ca. 5 times higher than that observed for compound crystallized from ethanol.

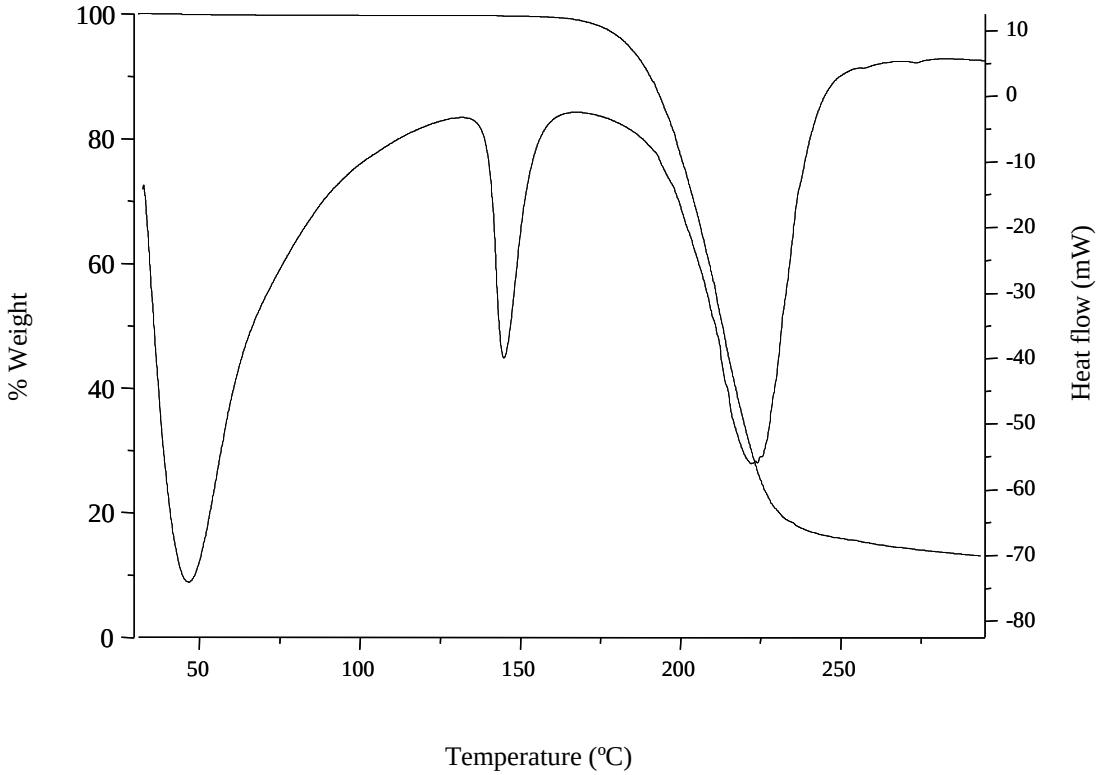


Figure 2. TG and DSC of compound (±)-**2** recrystallized from absolute ethanol (Form I).

 ©ARKAT USA, Inc.

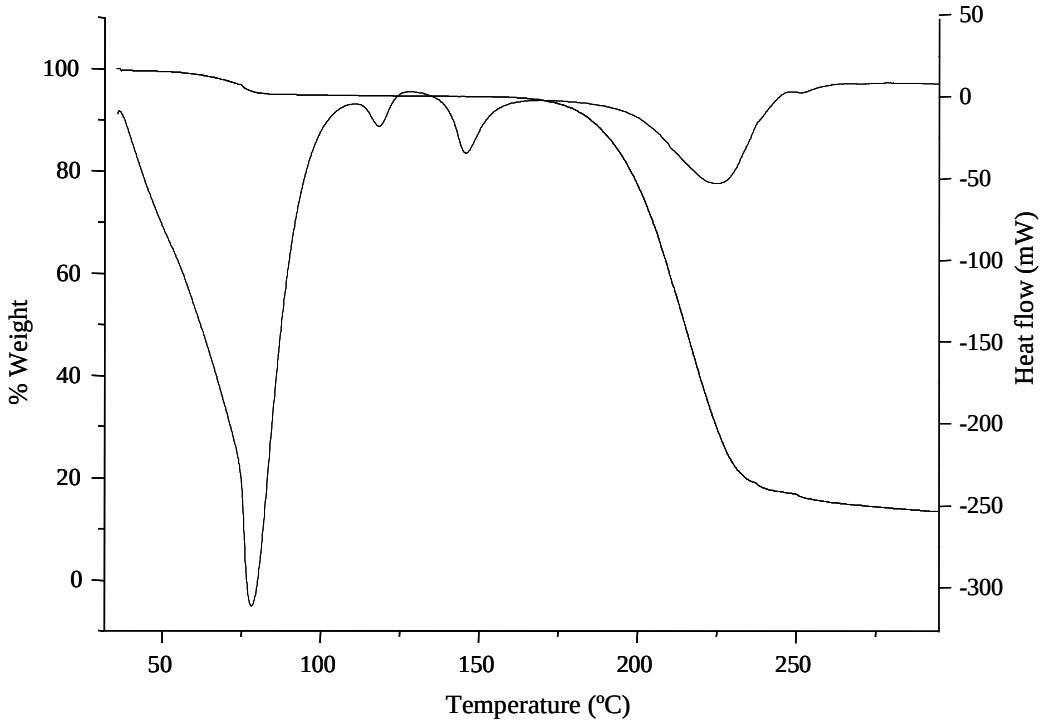


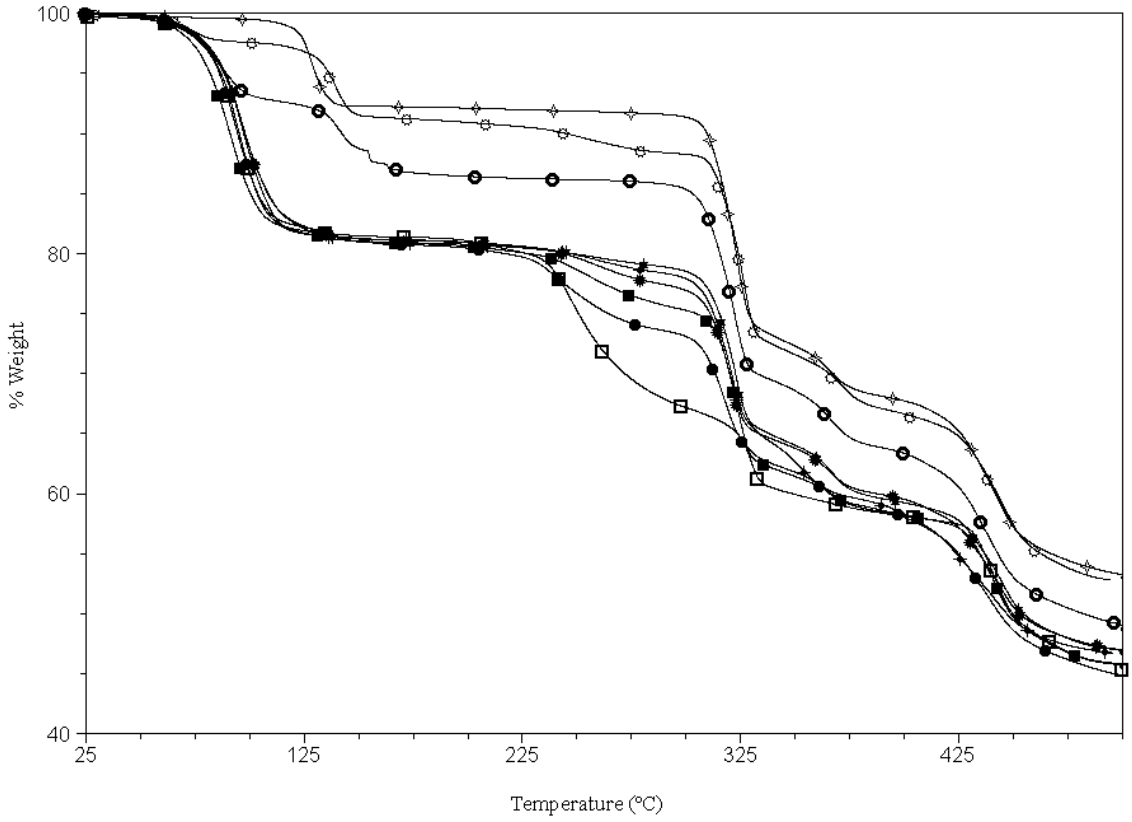
Figure 3. TG and DSC of compound (±)-**2** recrystallized from water (Form II).

Thermogravimetry (TG) curves of compound (±)-**4** recrystallized from different solvent mixtures depict four different decomposition intervals (Figure 4). Analyzing the curve from (±)-**4** recrystallized from water (●) we can observe that the decomposition start at the interval 51-167 ºC. The melting point described in Table 2 corresponds into the second decomposition interval 203-293 ºC. Further decomposition corresponds to the last two intervals at 293-341 and 341-500 ºC. Similar behaviors are observed in the case of the microcrystals obtained from the other solvent mixtures.

Powder X-Ray Diffraction (PXRD)

The X-ray diffraction patterns of Form I dissolved in ethanol and crystallized with different protic and aprotic solvents are depicted in Figure 5. The diffractograms show similar diffraction lines, differing only in their intensities, suggesting a different degree of crystallinity but also from the packing differences. Furthermore, it appears a peak with a strong intensity around 12 degrees in the first three compounds from the top might crystallize in one packing arrangement while the rest adopt a different system. Interestingly, in the case of propanol and benzene mixtures, clearly profile splitting is noticed in the 20 deg and 12 deg peaks, respectively. This should undoubtedly suggest the formation of another crystalline arrangement or a conformational polymorphism.[9]

 ©ARKAT USA, Inc.



(●)-Water; (🞏)-Water/Ethanol; (✦)-Methanol/Ethyl Acetate; (❍)-Water/2-Propanol; (■)-Water/Acetonitrile; (✧)-Methanol/ 1-Butanol; (✷)-Methanol/ Cyclohexane; (✶)-Methanol /Ethanol; (☼)-Methanol/Acetonitrile. DSC curves have been omitted for simplicity.

Figure 4. Comparison of thermal analysis (TG) of compound (±)-**4** recrystallized from different solvent mixtures.

Comparison of X-ray diffraction pattern of Form I and that from Form II is depicted in Figure 6. Clearly, the crystalline structures obtained in both cases are different, as deduced by the presence of new diffraction lines centered at 2θ = 9 and 11.5 º in the diffractogram of the compound recrystallized in water and the absence of the diffraction line at 2θ = 13 º. On this basis, it can be deduced that two different polymorphs of compound (±)-**2** are obtained, depending on the solvent, ethanol or water, used in the recrystallization.

 ©ARKAT USA, Inc.

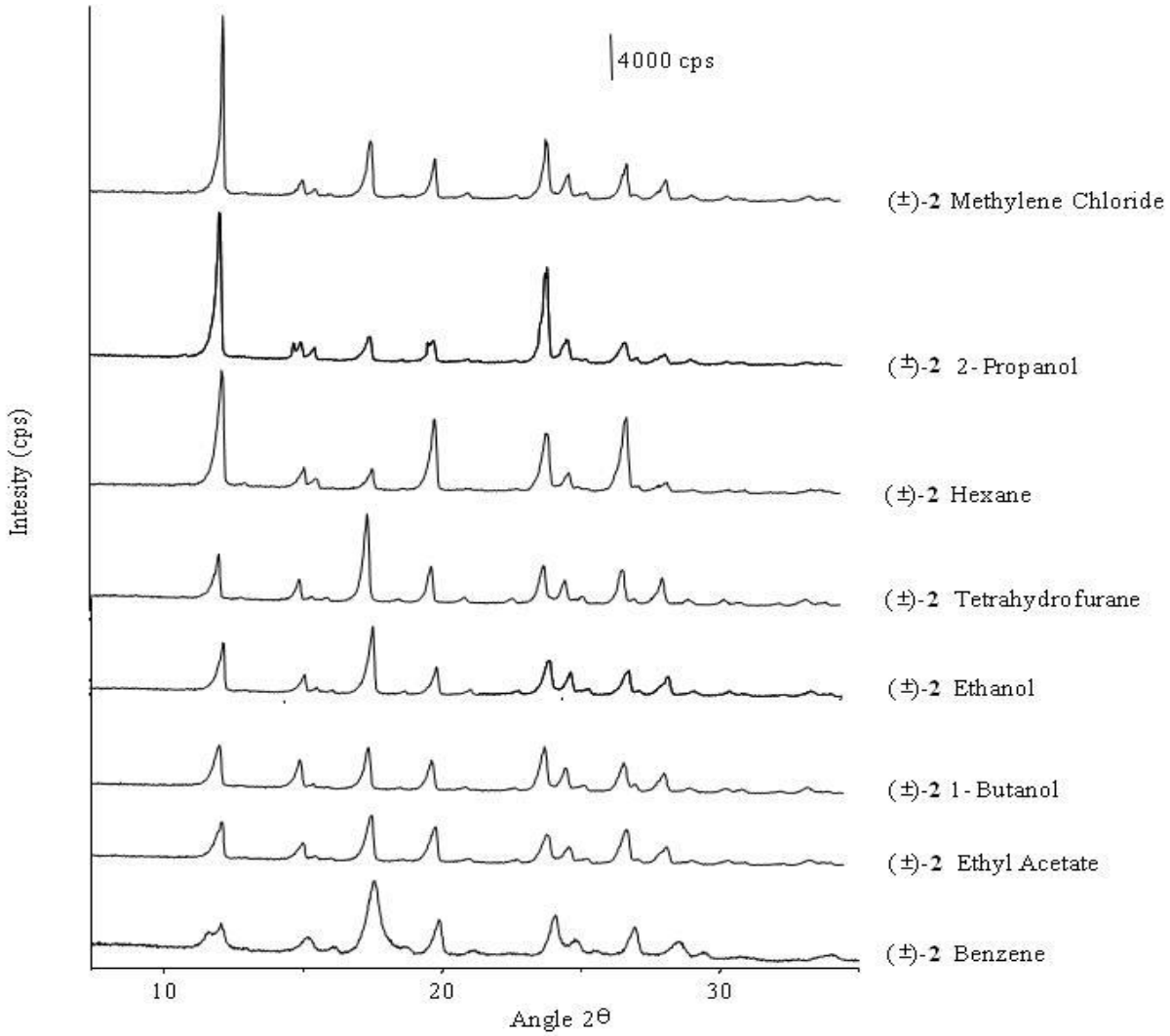


Figure 5. Powder X-ray diffraction pattern of crystals obtained from Form I dissolved in ethanol and crystallized with mixtures of different solvents.

^{13}C NMR study

Forms I and II were also analyzed by ^{13}C NMR spectroscopy in D_2O solution and in the solid state. As it can be observed in Table 3 both forms I and II show the same chemical shift in solution. However, when spectra were acquired by Cross Polarization Magic Angle Spinning (CPMAS) solid techniques different chemical shifts were observed for the C2, C4, and C5 imidazolic carbon atoms, indicating a different crystalline arrangement. (Table 3 and Figure 7).

 ©ARKAT USA, Inc.

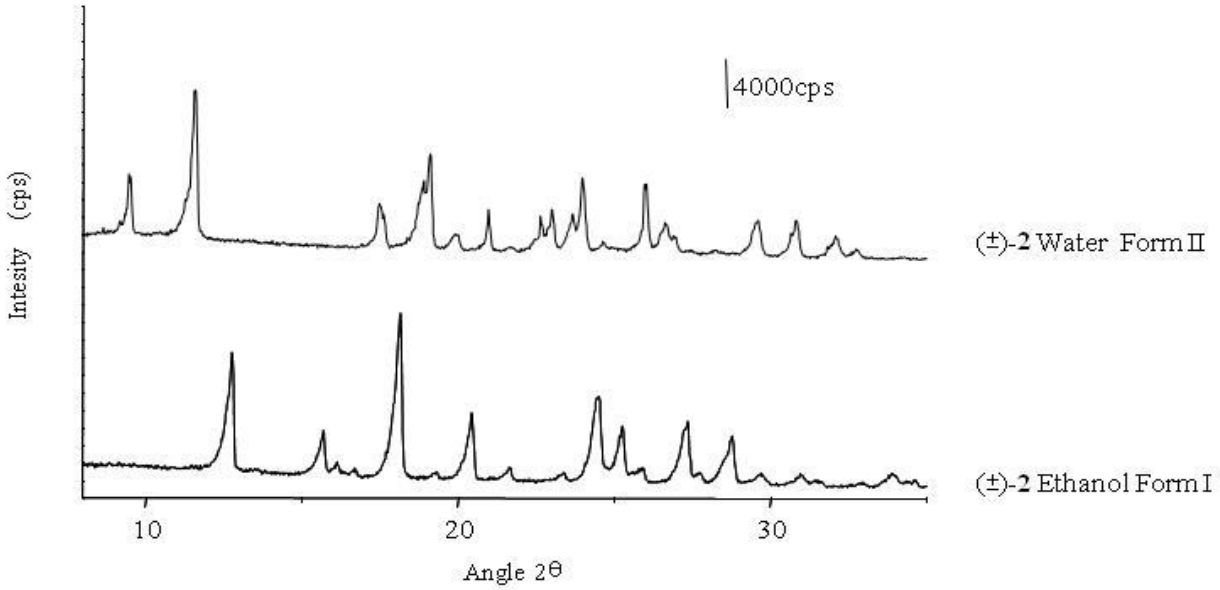


Figure 6. Comparison of Powder X-Ray Diffraction Pattern of Form I and Form II.

Table 3. ^{13}C Chemical shifts of forms I and II of compound (±)-**2** in D_2O and CP-MS at 100.61 MHz

Compounds	^{13}C 100.61 MHz (D_2O), δ (ppm)
Form I	13.1 (CH_3), 37.2 (CH_2), 60.3 (CH), 62.2 (OCH_2), 119.3 (C5), 121.5 (C4), 135.1
pD: 3.69	(C2), 171.9 (CO), 172.3 (CO)
	CP-MAS ^{13}C 100.61 MHz, δ (ppm)
	15.0 (CH_3), 43.0 (CH_2), 65.0 (CH), 65.0 (OCH_2),124.0 (C5), 126.0 (C4), 138.0 (C2), 177.0 (CO), 179.0 (CO)
	^{13}C 100.61 MHz (D_2O), δ (ppm)
Form II	13.1 (CH_3), 37.2 (CH_2), 60.3 (CH), 62.2(OCH_2), 119.3(C5), 121.5(C4), 135.1
pD: 3.21	(C2),171.9 (CO), 172.3 (CO)
	CP-MAS ^{13}C 100.61 MHz, δ (ppm)
	16.0 (CH_3), 43.0 (CH_2), 65.0 (CH), 65.0 (OCH_2),125.0 (C5), 127.0 (C4), 139.0 (C2),176.0 (CO), 178.0 (CO)

 ©ARKAT USA, Inc.

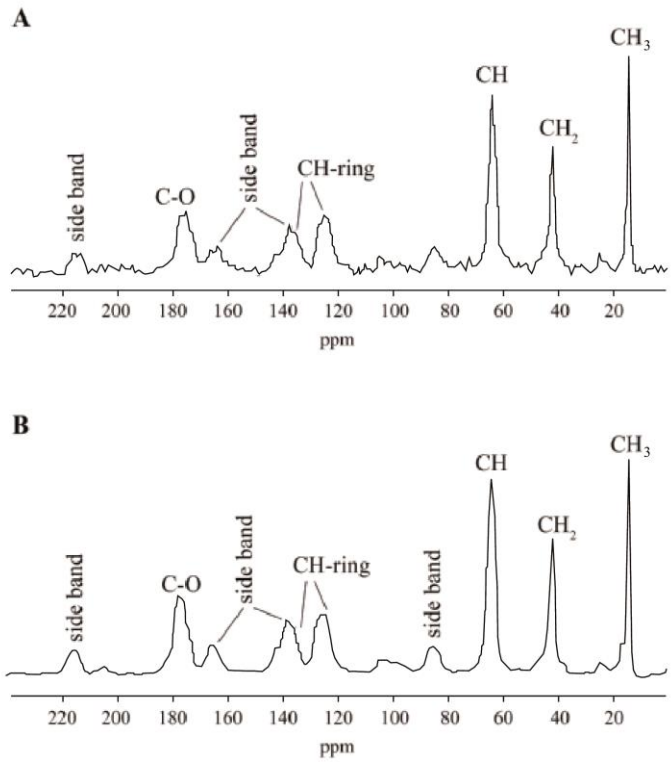


Figure 7. CP-MAS ^{13}C NMR of A) solid Form I and B) solid Form II.

Conclusions

Racemic mono-ester **(±)-2**, (±)-3-(ethoxycarbonyl)-2-(imidazol-1-yl)propionic acid, shows two different polymorphs when recrystallized in absolute ethanol (Form I) or water (Form II), respectively. Both forms have been unambiguously characterized by optical microscopy and powder X-ray diffractometry showing two different crystalline patterns. The presence of these two forms was also assessed by thermogravimetry (TG) and differential scanning calorimetry (DSC). Small differences can be observed in the solid state by CP-MAS ^{13}C NMR on the chemical shifts of the imidazolic carbons atoms. However, thermogravimetry (TG) curves of microcrystals from compound **(±)-4** depict four different decomposition intervals and no stable polymorphs were isolated. Taken together, we can conclude that, in the absence of single crystal X-ray diffraction, the best techniques to identify the polymorphs investigated here are the combination of optical microscopy and powder X-ray diffractometric analysis. The differences in bioavailability of the two polymorphs (Forms I and II) may be important for an agent used in NMR imaging will be investigated in the future.

 ©ARKAT USA, Inc.

Experimental Section

General. Elemental analyses were performed with a Perkin-Elmer 24000 apparatus NMR spectra were recorded with a Bruker Avance III Plus (400.13 MHz for ^{1}H, 100.61 MHz for ^{13}C). Products were purchased from commercial sources and were used without additional purification. Compounds (±)-**1**-(±)-**4** were prepared according literature procedures.[4a,b]

(±)-3-(ethoxycarbonyl)-2-(imidazol-1-yl)propionic acid (±)-2[4a,b] **(Form I).** Anal. Calcd. for $C_9H_{12}N_2O_4$: C, 50.94; H, 5.70; N, 13.20. Found: C, 50.68; H, 5.49; N,13.10
(±)-3-(ethoxycarbonyl)-2-(imidazol-1-yl)propionic acid (±)-2[4a,b] **(Form II).** Anal. Calcd. for $C_9H_{12}N_2O_4$: C, 50.94; H, 5.70; N, 13.20. Found: C, 49.61; H, 5.59; N, 13.10

Recrystallization method

Compounds **(±)-2** or **(±)-4** were dissolved in hot absolute ethanol or distilled water and subsequent crystallization with the corresponding solvent and cooling. The resulting crystals were filtered off under vacuum and dried in a desiccator at 60 ºC.

Optical microscopy

Optical microscopy studies were carried out on a Hot Stage on a Leica Galen III model microscope with crossed polarized light and a Zeiss Axiophot light microscope.

Thermal analysis (TG-DSC)

Thermal analysis was carried out using a Seiko SSC 5200 TG-TA 320 System. Samples of compounds (±)-**2** (about 20 mg) were heated in nitrogen from 30 up to 125 ºC (flow rate = 50 mL·min^{-1}) with a heating rate of 10 ºC·min^{-1}, soaked at this temperature for 3 min, then heated up to 160 ºC at a heating rate of 0.5 ºC·min^{-1} and finally from 160 up to 400 ºC at a heating rate of 10 ºC·min^{-1}.

Powder X-Ray diffraction data

The X-ray powder diffraction patterns between 5 and 50 º of 2θ were obtained using a Seifert C-3000 diffractometer with filtered Cu-Kα radiation operated at 40 kV and 30 mA, over non-oriented powder samples.

^{13}C NMR measurements

^{13}C in D_2O are given from external DMSO-d_6, with an accuracy of ± 0.1 ppm for ^{13}C. ^{13}C Cross-polarization magic angle spinning (CP-MAS) NMR spectra were obtained at 100.61 MHz.
Spectra were acquired using a ^{1}H 90 ° pulse with 5.40 µs, a Hartmann-Hahn contact time of 1.5 ms (tppm15, sequence used for decoupling), a data acquisition time 42 ms. The powder sample was packed in 4-mm zirconia rotor which was spun at 4kHz. ^{1}H-^{13}C cross-polarization pulse sequence (cp.av) is used. Acquisition Data: 128 scans, ds 0, TD 1676, sweep width in Hz 23980,

 ©ARKAT USA, Inc.

spectral width 240 ppm, d1 = 5s recycle delay, lb applied 50. Also in we performed the ^{1}H-^{13}C cross-polarization pulse sequence with sidebands suppression (cptoss.av sequence) in IEPA sample.

Acknowledgments

We acknowledge Prof. S. Cerdán for helpful suggestions. We also wish to thank partial support from projects S-BIO2006-170 from the Community of Madrid, MEDITRANS (FP VI 2006, EU IP NMP-02668) and CTQ2009-14146-C01 to PB.

References

1. Haleblian, J.; McCrone, W. *J. Pharm. Sci*. **1969**, *58*, 58911.
2. Hilfiker R. Ed. *Polymorphism in the Pharmaceutical Industry*. Wiley-VCH Weinheim, 2006.
3. (a) Thun, J.; Seyfarth, L.; Senker, J.; Dinnebier, R.E.; Breu, J. *Angew. Chem., Int. Ed*. **2007**, *46*, 6729. (b) Aaltonen, J.; Allesø, M.; Mirza, S.; Koradia, V. Gordon, K. C., Rantanen, J. *Europ. J. Pharma. Biopharma*, **2009**, *71*, 23.
4. (a) Zaderenko, P.; Gil, M. S.; Ballesteros, P.; Cerdán, S. *J. Org. Chem.* **1994**, *59*, 6268. (b) Gil, S.; Zaderenko, P.; Cruz, F.; Cerdán, S.; Ballesteros, P. *Bioorg. Med. Chem*. **1994**, *2*, 305. (c) Sluis, R. v.; Bhujwala, Z. M.; Raghunand, N.; Ballesteros, P.; Álvarez, J.; Cerdán, S.; Gallons, J. P.; Gillies, R. *J. Mag. Res. Med*. **1999**, *41*, 743. (d) García-Martín, M. L.; Herigault, G.; Remy, C.; Farion, R.; Ballesteros, P.; Coles, J.; Cerdán, S.; Ziegler, A. *Cancer Res*. **2001**, *61*, 6524. (e) Provent, P.; Benito, M.; Hiba, B.; Farion, R.; Lopez-Larrubia, P.; Ballesteros, P.; Remy, Ch.; Segebarth, Ch.; Cerdan, S.; Coles, J. A.; Garcia-Martin, M. L. *Cancer Res*. **2007**, *67*, 7638. (f) Penet, M. F.; Pathak, A. P.; Raman, V.; Ballesteros, P.; Artemov, D.; Bhujwalla, Z. M. *Cancer Res*. **2010**, *69*, 8822.
5. Zaderenko, P.; López, P.; Ballesteros, P.; Tamuki, H.; Toda, F. *Tetrahedron: Asymmetry* **1995**, *6*, 381-384.
6. López, P.; Zaderenko, P.; Balcazar, J. L.; Fonseca, I.; Hernández Cano, F.; Ballesteros, P. *J. Mol. Struct*. **1996**, *377*, 105.
7. Sanderson, K. *Nature* **2007**, *450*, 771.
8. (a) Wishkermann, S.; Bernstein, *J. Cryst. Eng. Comm*. **2006**, *8*, 245. (b) Guo, J. Ulrich, *J. Cryst. Res. Technol.* **2010**, *45*, 267.
9. Nangia A. *Acc. Chem. Res.* **2008**, *41*, 595.

 ©ARKAT USA, Inc.