Novel Generation of pH Indicators for Proton Magnetic Resonance Spectroscopic Imaging

Jordi Soler-Padrós,[†] Elena Pérez-Mayoral,[†] Laura Domínguez,[‡] Pilar López-Larrubia,[§] Elena Soriano,[§] José Luis Marco-Contelles,[‡] Sebastián Cerdán,[§] and Paloma Ballesteros*,[†]

Laboratorio de Síntesis Orgánica e Imagen Molecular por Resonancia Magnética, Instituto Universitario de Investigación, UNED, Paseo Senda del Rey 9, 28040 Madrid, Spain, Laboratorio de Radicales Libres, Instituto de Química Orgánica General (CSIC), c/Juan de la Cierva 3, 28006 Madrid, Spain, and Laboratorio de Imagen y Espectroscopía por Resonancia Magnética (LIERM), Instituto de Investigaciones Biomédicas "Alberto Sols" (CSIC), c/Arturo Duperier 4, 28029 Madrid, Spain

Received January 12, 2007

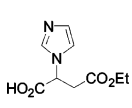
We describe the synthesis of 1,*ω*-di-1*H*-imidazoles **2** and **3**, derived from L-threitol and D-mannitol, respectively, showing suitable magnetic and toxicological properties, as novel extracellular pH indicators for ^{1}H spectroscopic imaging by magnetic resonance methods.

Introduction

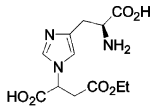
The imidazole ring[1] is a major biochemical contributor found in many natural products such as histidine, histamine, and guanine, as well as a crucial component of nucleic acids DNA and RNA. Additionally, a number of pyrrole−imidazole compounds, such as those derived from oroidin,[2] and indole−imidazole alkaloids[3] with interesting biological properties have been isolated from marine sponges and terrestial plants. At the same time, synthetic fluoro-2-nitroimidazoles have been extensively investigated and considered as ideal markers for the measurement of tumor hypoxia by noninvasive methods.[4] These ^{18}F (or ^{19}F) labeled bioreductive drugs bind to hypoxic cells in tumors, a process that can be detected by positron emission tomography (PET) or ^{19}F magnetic resonance spectroscopy (^{19}F MRS). As part of our current work on molecular imaging and diagnosis, we reported the synthesis[5] and in vivo evaluation of 3-(ethoxycarbonyl)-2-imidazol-1-ylpropionic acid (IEPA)[6] (Chart 1), the first probe made available for the noninvasive measurement of extracellular pH (pH_e) in tumors by ^{1}H NMR spectroscopic imaging (^{1}H MRSI[a]). The pH_e in tumors is usually acid compared to the surrounding normal tissue. This feature has been confirmed with numerous microelectrode measurements[7] and confirmed by ^{31}P magnetic resonance spectroscopy (MRS)[8] as a less invasive method. Moreover, pH_e in tumors is known to provide valuable information concerning the most appropriate chemotherapic agent, as well as in the characterization of the tumoral microenvironment, a crucial variable determining tumoral invasion and metastatic behavior.[9] On these grounds, the development of optimal probes for the measurement of extracellular pH in tumors is of considerable clinical interest.

The selection of an imidazole ring in the design of IEPA is based on two reasons: (i) imidazole pK_a (∼7.0) is very close to physiological pH, and (ii) its H-2, H-4 and H-5 proton resonances are easily resolved and observed in the aromatic region of ^{1}H NMR spectra, far away enough from the water resonance and the more crowded aliphatic regions of the spectrum. In vivo pH_e measurements using this probe were obtained from chemical shifts of its H-2 proton, as the indicator is distributed within the different pH_e environments of the tumors.[6] These results provided the first pH_e map in vivo from a model of C6 glioblastoma multiforme implanted in the brain of adult rats. Although IEPA was an excellent reporter molecule for the in vitro measurements, the presence of only one proton (H-2 of imidazole ring) demanded the use of relatively high concentrations of the probe, often resulting in unfavorable signal-to-noise ratios in the corresponding in vivo ^{1}H MRSI spectra. Moreover, IEPA became rapidly removed from the circulation by renal clearance, a circumstance that imposed very high delivery doses to compensate its elimination. To overcome some of these drawbacks, we recently described a new pH probe derived from L-histidine **1**[10] to increase the polar nature of the molecule and increase its retention time within the circulation (Chart 1). However, its apparent p$K_a' = 5.58$ remained far from the physiological range, a circumstance that precluded further developments as a pH_e indicator.

Chart 1. ^{1}H MRSI pH Indicators Containing an Imidazole Ring

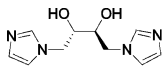


IEPA

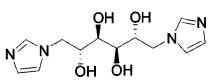


1

Chart 2. Polyhydroxylated 1,*ω*-Diimidazoles **2** and **3**



2



3

To further improve the performance of these pH_e probes, we herein report the design and synthesis of **2** and **3** containing two imidazole rings linked to a polyhydroxylated skeleton (Chart 2). The hydroxy groups of the polyalcohol moiety should provide higher polarity to the molecule, while the presence of two magnetically equivalent imidazole rings would increase the intensity of the H2 MRSI resonance, improving both the unfavorable dose and elimination rates of the previous probes. Diimidazoles **2** and **3** can easily be prepared from readily available carbohydrate templates, being very useful in the preparation of novel families of pH_e probes with optimized magnetic and pharmacological properties.

Results and Discussion

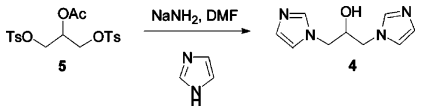
In order to setup the experimental conditions for the synthesis of **2** and **3**, we first pursued the preparation of **4**[11] from the previously known acetate **5**[12] as a model compound (Scheme

* To whom correspondence should be addressed. Phone: 34 913987320. Fax: 34 913986697. E-mail: pballesteros @ccia.uned.es.
† Instituto Universitario de Investigación.
‡ Instituto de Química Orgánica General (CSIC).
§ Instituto de Investigaciones Biomédicas "Alberto Sols" (CSIC).

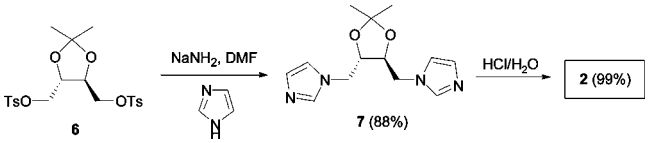
[a] Abbreviations: ^{1}H MRSI, proton magnetic resonance spectroscopic imaging.

10.1021/jm070044j CCC: $37.00
Published on Web 08/11/2007

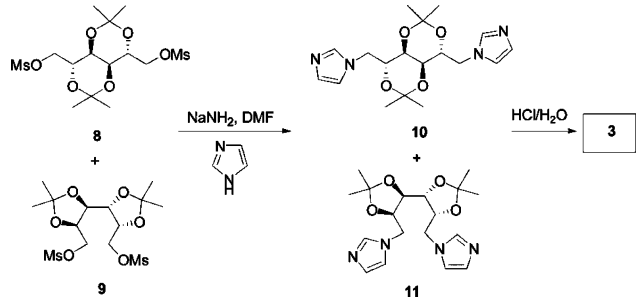
Scheme 1. Synthesis of 1,3-Diimidazol-1-yldeoxyglycerol (**4**)



Scheme 2. Synthesis of Compound **2** from Commercial Tosylate **6**



Scheme 3. Synthesis of Diimidazole **3** from the Known Mesylates **8** and **9**



1). Reaction of **5** with imidazole in the presence of sodium amide in dry DMF afforded product **4** in 17% yield (not optimized) (Scheme 1). Considering this, we tried the synthesis of **2** from commercial 1,4-di-*O*-*p*-toluenesulfonyl-2,3-*O*-isopropylidene-L-threitol (**6**) under the same experimental conditions. In this case, **7** was obtained in high chemical yield (88%) and was submitted to acid hydrolysis to give **2** (Scheme 2), characterized as its dihydrochloride salt, in almost quantitative yield. In the same way, **3** was synthesized from a mixture of mesylates **8** and **9**,[13,14] leading to diimidazoles **10** and **11** in 77% chemical yield that afforded the dihydrochloride salt of **3** after acid hydrolysis without separation (Scheme 3).

With these compounds in hand, we pursued the determination of the corresponding apparent pK_a' of *N*-3 of diimidazoles **2**, **3**, and **4**. Values of pK_a were determined by ^{1}H NMR (500.13 MHz, 37 °C) from pH titrations of the solutions (25 mM) of the corresponding compounds in deuterated water solutions and by simulating biological conditions through the use of fetal bovine serum (FBS). Table 1 shows the calculated and experimental pK_a' values for **2**−**4**. The experimental values were determined from the titration behavior of H2 resonance of the imidazole ring in the range $3 < pH < 11$. As indicated in Table 1, the pK_a' of these indicators remained in the range 6.45−7.00, in good agreement with the theoretical values determined by computational calculations.[15] Noteworthy is the fact that pK_a' values determined in D_2O become higher for longer chain lengths, from three to six carbons. The pK_a' values of **2** and **3** in FBS are 6.67 and 6.63, respectively. Under these conditions the pK_a' of **3** is slightly lower than the calculated and experimental pK_a' in D_2O, although it remains in the range of physiological pH.

As mentioned above, the presence of only one H2 proton per molecule in IEPA limited the sensitivity of the in vivo determination because of signal-to-noise considerations.[6] The compounds reported herein are ideally functionalized to overcome this inconvenience because they contain two magnetically equivalent imidazole rings per molecule, improving by 2 the resonance intensity and by 1.43 the signal-to-noise ratio for the same number of scans and acquisition conditions.

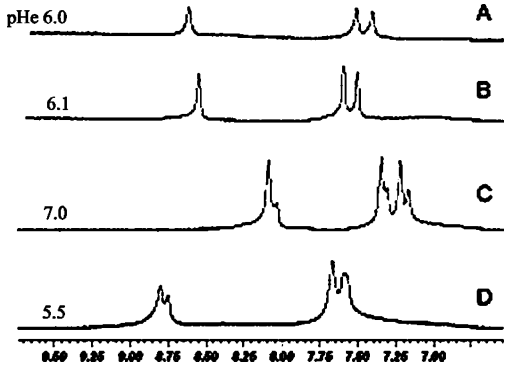


Figure 1. Proton NMR of (A) 25 mM IEPA in cultures of C6 glioma cells, (B) 25 mM **3** in cultures of C6 glioma cells, (C) 12.5 mM IEPA and 12.5 mM **3** erythrocytes, and (D) 12.5 mM IEPA and 12.5 mM **3** in cultures of C6 glioma cells, at 500.13 MHz. Numbers in the left indicate the final pH_e value of the suspension.

Table 1. Apparent pK_a (pK_a') Values of Compounds **2**−**4** Determined by ^{1}H NMR (500.13 MHz, 37 °C)

compd	pK_a' (D_2O)[a]	pK_a' (FBS)[b]	pK_a' (calcd)[c]
2	6.56	6.67	6.65
3	7.00	6.63	7.12
4	6.45		6.38

[a] Model solution of 25 mM **2**, **3** or **4** in D_2O. [b] Model solution of 25 mM **2**, **3**, or **4** in FBS (20% D_2O). [c] Calculated values are those described by Soriano et al.[15]

We then performed in vitro NMR experiments using erythrocyte preparations (from healthy rats) and cultures of C6 glioma cells (a well-characterized tumor astrocytoma cell line) in order to compare the chemical shifts of the H2 protons from the probes in different environments (parts C and D of Figure 1). Although the imidazole ring resonances of **3** and IEPA appeared partially overlapping, the double intensity of the H2 resonance from the diimidazolic probe was well observed. The assessments of chemical shift of H2 proton of imidazole rings in **3** were achieved in the presence and absence of IEPA as a reference compound. It is important to remark here that the pK_a' value of **3** was not affected by the presence of IEPA, a circumstance confirmed by the combined titration of **3** and IEPA (see Figure 1 in Supporting Information).

Figure 1 shows only the aromatic resonances of the tested compounds, confirming the uncrowded environment of the aromatic region of ^{1}H NMR spectra from cells and tissues, and the absence of the overlap of the H2 resonance from our probes with other endogenous resonances from the cells. As shown in parts A and B of Figure 1, the in vitro spectra (cultures of C6 cells) depict only the resonances from added IEPA and **3**, but very similar results were obtained with **2**. These resonances correspond to the extracellular space, since they reveal a pH very similar to that measured by potentiometric methods with a pH electrode in the same suspension. It is important to remember that methylimidazol-1-yl acetate, considered as an indicator of intra- and extracellular pH (pH_i), has two distinct H2 resonances, derived from the intra- and extracellular spaces, respectively. This is due to the fact that the uncharged form of this probe freely crosses the cellular membranes[5] distributing in the intracellular and extracellular spaces and consequently originating two resolved resonances as previously reported.[5] This was not observed in the present study. In contrast, our results reveal the presence of only one resonance, corresponding to a pH virtually identical to that measured with a potentiometric

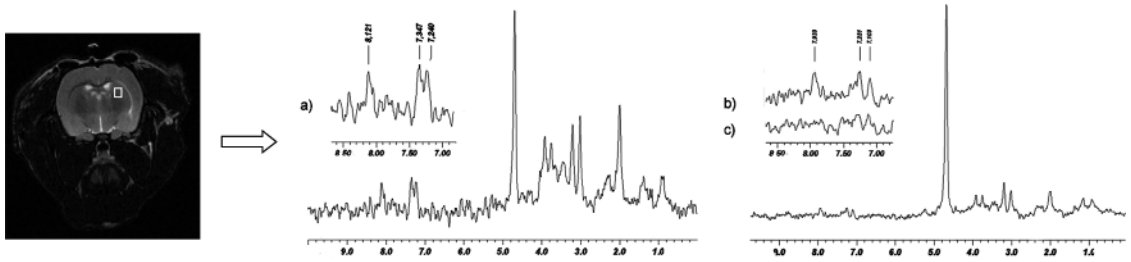


Figure 2. T_2-weighted spin−echo anatomical image acquired with a RARE sequence in axial orientation (TR = 3300 ms, TE = 60 ms, RARE factor = 8, av = 3, FOV = 38 × 38 mm, acquisition matrix = 256 × 256, in-plane resolution of 148 × 148 μm^2, slice thickness = 1.00 mm, and number of slices = 24) of a representative rat. In vivo spectroscopy protocol using a point-resolved spectroscopy sequence (PRESS) combined with VAPOR water suppression, located in the injection zone of the white matter in the right ventricle (TR = 3000 ms, TE = 35 ms, av = 128, dummy scans = 8, volume = 3 mm^3): (a) 60 min after injection of **3** (125 mM, 25 μL); (b) 25 min after injection of IEPA (125 mM, 25 μL); (c) 35 min after injection of IEPA (125 mM, 25 μL). Note the faster elimination of IEPA compared to **3**.

glass electrode. This finding allows us to conclude that the new probes monitor primarily the extracellular pH compartment.

Furthermore, we initiated the in vivo spectroscopic imaging of **3** by intravenous injection of this probe in rats bearing an implanted C6 glioma in their brains. Earlier studies using IEPA required the iv infusion of a 0.6 M solution of this probe (4−5 mL total infusion volume). However, the maximal concentration of **3** was limited by its solubility at physiological pH to 0.175 M, making it very difficult to detect the corresponding resonances by in vivo ^{1}H NMR. To overcome this limitation, **3** was administered by direct injection into the brains of healthy Wistar rats, and in vivo PRESS spectra were acquired at 7 T as indicated in the Experimental Section (Figure 2). In this case, the resonances of the imidazole ring could easily be detected and adequately resolved even 60 min after the intracranial injection of **3** (Figure 2a). However, the resonances from IEPA injected intracraneally under the same conditions became undetectable 5 min after the injection, revealing a much faster elimination rate (Figure 2b and Figure 2c, respectively). These experiments confirmed that the retention time of **3** in cerebral tissue was higher than that of IEPA, overcoming one of the main limitations of the parental compound.

Finally, in vitro toxicity of **2** and **3** was separately assayed in cultures of C6 glioma cells by monitoring the intracellular release of lactic dehydrogenase (LDH) to the incubation medium. The amount of LDH released was measured after 1−6 h of incubation of the C6 cells with the concentration of the probes ranging between 20 and 150 mM. No apparent signs of toxicity were detected, with LDH release values being smaller than 10% for **2** or **3**, even at the highest concentrations.

In summary, the polyhydroxylated diimidazoles **2** and **3** were easily obtained from available L-threitol and D-mannitol derivatives, exhibited low toxicity, have pK_a' values in the range of physiological extracellular pH (6.45−7), double the intensity of H-2 imidazole resonance, and have higher retention times in cerebral tissue than earlier monoimidazolic pH$_e$ probes. Taken together, these favorable circumstances indicate that the new probes are good and promising pH$_e$ indicators for ^{1}H MRSI.

Experimental Section

General Methods. Melting points were determined on a digital melting-point apparatus (Electrothermal) and are uncorrected. ^{1}H and ^{13}C NMR spectra were recorded in $CDCl_3$ at 200 and at 50 MHz. TLC was performed on silica F254 (Merck), and detection was by UV light at 254 nm or by charring with phosphomolybdic H_2SO_4 reagent. Column chromatography was carried out on silica gel 60 (Merck, 230 mesh).

General Method for the Synthesis of the Diimidazole Derivatives. To a solution of imidazole (4 equiv) and $NaNH_2$ (4 equiv) in dry DMF (0.8 M), the corresponding ditosylate (1 equiv) was added. The mixture was left to react at the temperature and time stated in each case. The solvent was evaporated, and the residue was purified by column chromatography using mixtures of CH_2Cl_2/MeOH as eluent.

{3-[(2*S*,3*S*)-(2,3-Dihydroxybutane-1,4-diyl)]}-di-1*H*-imidazolium Dihydrocholoride (2). Following the general method for the synthesis of diimidazole derivatives above, imidazole (0.441 g, 6.48 mmol), $NaNH_2$ (0.253 g, 6.48 mmol) in dry DMF (9 mL), and 1,4-di-*O*-*p*-toluenesulfonyl-2,3-*O*-isopropylidene-L-threitol (**6**) (0.764 g, 1.62 mmol) were mixed and stirred at room temperature for 24 h. Purification by column chromatography (CH_2Cl_2/MeOH, 98:2) gave (−)-1,4-deoxy-1,4-diimidazol-1-yl-2,3-*O*-isopropylidene-L-threitol (**7**) (0.373 g, 88%). Compound **7** (0.180 g, 0.69 mmol) was dissolved in 1.2 M HCl (3 mL) at 50 °C for 3 h. Then the solvent was evaporated, and the product was purified by recrystallization from methanol to give **2** (0.149 g, 97%) as a colorless solid: mp 213−5 °C; $[\alpha]_D^{25}$ −25 (*c* 0.67, MeOH); IR (KBr) ν 3232, 1576, 1544, 1436, 1283, 1087 cm^{-1}; ^{1}H NMR (D_2O, 200 MHz) δ 8.61 (s, 2 H, H-2 Im), 7.38 (s, 2 H, H-5 Im), 7.32 (s, 2 H, H-4 Im), 4.32−4.16 (m, 4 H, 2 × CH_2), 3.98−3.91 (m, 2 H, 2 × CH); ^{13}C NMR (D_2O, 50 MHz) δ 135.2 (2 × C-2 Im), 122.3 (2 × C-5 Im), 119.7 (2 × C-4 Im), 69.7 (2 × CH), 52.0 (2 × CH_2); MS (ES) *m*/*z* 223 [M + 1]$^+$, 445 [2M + 1]$^+$. Anal. ($C_{10}H_{14}N_4O_2$·2HCl·H_2O) C, H. N calcd, 38.35, 5.79, 17.89; found, 38.41, 5.80, 18.01.

{3-[(2*R*,3*R*,4*R*,5*R*)-(2,3,4,5-Tetrahydroxyhexane-1,6-diyl)]}di-1*H*-imidazolium Dihydrocholoride (3). Following the general method for the synthesis of diimidazole derivatives, imidazole (0.462 g, 6.80 mmol), $NaNH_2$ (0.265 g, 6.80 mmol), dry DMF (10 mL), and 1,6-dimethanesulfonyl-2,4;3,5-di-*O*-isopropylidene-D-mannitol (**8**)[14]/1,6-dimethanesulfonyl-2,3;4,5-di-*O*-isopropylidene-D-mannitol (**9**)[14] (0.710 g, 1.70 mmol) were mixed, stirred at 50 °C for 24 h, and purified and separated by chromatography (CH_2Cl_2/MeOH, 97:3), affording (−)-1,6-deoxy-1,6-diimidazol-1-yl-2,4:3,5-di-*O*-isopropylidene-D-mannitol (**10**) (0.312 g, 51%) and (−)-1,6-deoxy-1,6-diimidazol-1-yl-2,3:4,5-di-*O*-isopropylidene-D-mannitol (**11**) (0.159 g, 26%). Total yield was 77%. A mixture of the isomers **10** and **11** (0.470 g, 1.29 mmol) was dissolved in 1.2 M HCl (10 mL) at 50 °C for 2 h. Then the solvent was evaporated, and the residue was washed several times with absolute ethanol. The product was recrystallized from methanol to give **3** (0.215 g, 51%) as a colorless solid: mp 210−2 °C; $[\alpha]_D^{25}$ +10 (*c* 0.43, MeOH); ^{1}H NMR (D_2O, 200 MHz) δ 8.57 (s, 2 H, H-2), 7.36 (s, 2 H, H-5), 7.31 (s, 2 H, H-4), 4.46−4.13 (m, 4 H, 2 × CH_2), 3.86 (m, 2H, 2 × CH), 3.47 (d, 2H, 2 × CH); ^{13}C NMR (D_2O, 50 MHz) δ 135.4 (2 × C-2), 122.7 (2 × C-5), 119.7 (2 × C-4), 69.6 (2 × CH), 68.8 (2 × CH), 52.6 (2 × CH_2); MS (ES) *m*/*z* 283 [M + 1]$^+$, 565 [2M + 1]$^+$. Anal. ($C_{12}H_{18}N_4O_4$·2HCl·$2H_2O$) C, H. N calcd, 36.84, 6.18, 14.32; found, 37.01, 6.21, 14.45.

Acknowledgment. This work was supported in part by MEC (Grants SAF2003-01810 and CTQ2006-06505/BQU to P.B.), (Grants SAF 2004-02145, NAN2004-09125-C07-03, FIS C03/10, FIS C03/08, and FIS G03/155 to S.C.), European Community (MEDITRANS 2006, Integrated EU Project FP6-2004-

NMP-NI-4/IP 026668-2), Grant S-BIO/0179/2006 from the Community of Madrid to P.B. and S.C., and Grant BQU2003-00813 to J.L.M.-C.

Supporting Information Available: Experimental procedures and characterization of **2**−**4** and figure of combined titration of IEPA and **3**. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Grimmett, M. R. *Imidazole and Benzimidazole Synthesis*; Academic Press: London, 1997.

(2) Al Mourabit, A; Poitier, P. Sponge's Molecular Diversity Through the Ambivalent Reactivity of 2-Aminoimidazole: A Universal Chemical Pathway to the Oroidin-Based Pyrrole−Imidazole Alkaloids and Their Palau'amine Congeners. *Eur. J. Org. Chem.* **2001**, 237−243.

(3) Pindur, U.; Lemster, T. Advances in Marine Natural Products of the Indole and Annelated Indole Series: Chemical and Biological Aspects. *Curr. Med. Chem.* **2001**, *8*, 1681−1698.

(4) Aboagye, E. O.; Kelson, A. B.; Tracy, M.; Workman, P. Preclinical Development and Current Status of the Fluorinated 2-Nitroimidazole Hypoxia Probe *N*-(2-Hydroxy-3,3,3-trifluoropropyl)-2-(2-nitro-1-imidazolyl) Acetamide (SR 4554, CRC 94/17): A Non Invasive Diagnostic Probe for the Measurement of Tumor Hypoxia by Magnetic Resonance Spectroscopy and Imaging, and by Positron Emission Tomography. *Anti-Cancer Drug Des.* **1998**, *13*, 703−730. Seddon, B. M.; Payne, G. S.; Simmons, L.; Ruddle, R.; Grimshaw, R.; Tan, S.; Turner, A.; Raynaud, F.; Halbert, G.; Leach, M. O.; Judson, I.; Workman, P. A Phase I Study of SR-4554 via Intravenous Administration of Tumor Hypoxia by Magnetic Resonance Spectroscopy in Patients with Malignancy. *Clin. Cancer Res.* **2003**, *9*, 5101−5112.

(5) Gil, M. S.; Zaderenko, P.; Cruz, F.; Cerdán, S.; Ballesteros, P. Imidazol-l-ylalkanoic Esters and Their Corresponding Acids. A Novel Series of Extrinsic ^{1}H NMR Probes for Intracellular pH. *Bioorg. Med. Chem. Lett.* **1992**, *2*, 1717−1722. Gil, M. S.; Zaderenko, P.; Cruz, F.; Cerdán, S.; Ballesteros, P. Imidazol-l-ylalkanoic Acids as Extrinsic ^{1}H NMR Probes for the Determination of Intracellular pH, Extracellular pH and Cell Volume. *Bioorg. Med. Chem*. **1994**, *2*, 305−314. Zaderenko, P.; Gil, M. S.; Ballesteros, P.; Cerdán, S. Synthesis and Regioselective Hydrolysis of 2-Imidazol-l-ylsuccinic Esters. *J. Org. Chem*. **1994**, *59*, 6268−6273. Zaderenko, P.; López, M. C.; Ballesteros, P. Addition of Azoles and Amines to Unsymmetrical Fumaric Esters. *J. Org. Chem*. **1996**, *61*, 6825−6828.

(6) van Sluis, R.; Bhujwalla, Z. M.; Raghunand, N.; Ballesteros, P.; Alvarez, J.; Cerdán, S.; Galons, J.-Ph.; Gillies, R. In vivo Imaging of Extracellular pH Using ^{1}H MRSI. *J. Magn. Reson. Med.* **1999**, *41*, 743−750. García-Martín, M. L.; Herigault, G.; Remy, C.; Flarion, R.; Ballesteros, P.; Coles, J.; Cerdán, S.; Ziegler, A. Mapping Extracellular pH in Rat Brain Gliomas in Vivo by ^{1}H Magnetic Resonance Spectroscopy: Comparison with Maps of Other Metabolites. *Cancer Res.* **2001**, *61*, 6524−6531. Bhujwala, Z.; Artemov, D.; Ballesteros, P.; Cerdán, S.; Gillies, R.; Solaiyappan, M. Combined Vascular and Extracellular pH Imaging of Solid Tumors. *NMR Biomed.* **2002**, *15*, 114−119.

(7) Wike-Hooley, J. L.; Haveman, J.; Reinhold, H. S. The Relevance of Tumor pH to the Treatment of Malignant Disease. *Radiother. Oncol*. **1984**, *2*, 343−366.

(8) Gillies, R. J.; Liu, Z.; Bhujwalla, Z. ^{31}P MRS Measurement of Extracellular pH of Tumor Using 3-Aminopropylphosphonate. *Am. J. Physiol.* **1994**, *267*, C195−C203.

(9) Cairns, R.; Papandreou, I.; Denko, N. Overcoming Physiologic Barriers to Cancer Treatment by Molecularly Targeting the Tumor Microenvironment. *Mol. Cancer Res.* **2006**, *4*, 61−70. Wong, P.; Lee, C.; Tannock, I. F. Reduction of Intracellular pH as a Strategy to Enhance the pH-Dependent Cytotoxic Effects of Melphalan for Human Breast Cancer Cells. *Clin. Cancer Res.* **2005**, *11*, 3553−3557. Raghunand, N.; He, X.; van Sluis, R.; Mahoney, B.; Baggett, B.; Taylor, C. W.; Paine-Murrieta, G.; Roe, D.; Bhujwalla, Z. M.; Gillies, R. J. Enhancement of Chemotherapy by Manipulation of Tumour pH, *Br. J. Cancer* **1999**, *80*, 1005−1011. Engin, K.; Leepers, D. B.; Thistlethwaite, A. J.; Tupchon, L.; McFarlane, J. D. Tumor Extracellular pH as a Prognostic Factor in Thermoradiotherapy. *Int. J. Radiat. Oncol., Biol., Phys.* **1994**, *29*, 125−132.

(10) López-Larrubia, P.; García-Amo, M.; Mayoral, E. P.; Gillies, R. J.; Cerdán, S.; Ballesteros, P. τ-Regioselective Addition of (−)-*N*(α)-*tert*-Butoxycarbonyl-L-histidine Methyl Ester to Diethyl Fumarate. *ARKIVOC* **2004**, 26−31.

(11) Scheithauer, S.; Otto, R.; Reitenbach, U.; Friese, J. DD 284225, 1990. The *O*-stearoyl derivative of compound **5** has been described in a study directed to the synthesis of dicephalic (two-headed) surfactants: Sommerdijk, N. A. J. M.; Hoeks, T. L.; Booy, K. J.; Feiters, M. C.; Nolte, R. J. M.; Zwanenburg, B. Dicephalic Surfactants. *Chem. Commun*. **1998**, 743−744.

(12) Wallace, S.; Yang, D. J.; Cherif, A. U.S. Patent 5,728,843, 1998. Cherif, A.; Yang, D. J.; Tansey, W.; Kim, E. E.; Wallace, S. Rapid Synthesis of 3-[^{18}F]fluoro-1-(2′-nitro-1′-imidazolyl)-2-propanol ([^{18}F]-fluoromisonidazole). *Pharm. Res*. **1994**, *11*, 466−469.

(13) Brown, J. M.; Dayrit, F. M.; Sinou, D. Chiral Ligands for Organometallic Catalysis Containing Two Dioxolane Rings. *J. Chem. Soc., Perkin Trans. 2* **1987**, 91−95.

(14) Acton, E. M.; Keyanpour-Rad, M.; Christensen, J. E.; Tong, H. H.; Kwok, R. P.; Goodman, L. Potential Antitumor Agents. Some 1,6-Disubstituted Galactitol and Mannitol Derivatives. *Carbohydr. Res.* **1972**, *22*, 477−486. Toldy, L.; Vargha, L. Benzylidene Derivatives of L-iditol. *Chem. Ber*. **1959**, *92*, 2694−2700.

(15) Soriano, E.; Ballesteros, P.; Cerdán, S. Computational Determination of pKa values. A Comparison of Different Theoretical Approaches and a Novel Procedure. *THEOCHEM* **2004**, *684*, 121−128.

JM070044J