Amines That Transport Protons across Bilayer Membranes: Synthesis, Lysosomal Neutralization, and Two-Phase pK_a Values by NMR

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Received February 21, 1996[⊗]

It is desirable to be able to control the pH of lysosomes. A collection of lipophilic, nitrogenous bases, designed to act as membrane-active, catalytic proton transfer agents, were prepared and their effective pK_as measured in a vigorously stirred, two-phase system. One phase was a phosphate buffer whose pH was varied over the range ca. 1−11. The other was an immiscible, deuterated organic solvent in which the compounds preferentially resided even when protonated. When chemical shift changes versus the pH of the buffer were plotted, clear pK_a curves were generated that are relevant to transmembrane proton transfer behavior. The two-phase pK_as increased with increasing counterion lipophilicity and with increasing organic solvent polarity. The compounds were also tested for their ability to neutralize the acidity of lysosomes, a model for other acidic vesicles involved in drug sorting. The most successful of these, imidazole **6a**, has >100 times the neutralizing power of ammonia, a standard lysosomotropic amine, causing a 1.7 unit rise in lysosomal pH of RAW cells at 0.1 mM, compared to a 0.2 and 1.4 unit rise for ammonium chloride at 0.1 and 10 mM, respectively.

Introduction

The ability of eukaryotic cells to internally segregate aqueous compartments differing in pH using lipid bilayer membranes and ATP-driven proton pumps has allowed the evolutionary development of sophisticated mechanisms for cell nutrition and defense (lysosomes and endosomes)[1] and protein synthesis, modification, and sorting (Golgi and endoplasmic reticulum).[2] However, harmful organisms[3] and disease states[4] have also evolved that depend upon this compartmentalization. There is increasing evidence, for example, that the phenomenon of multiple drug resistance (MDR), one of the greatest problems in cancer chemotherapy, is linked to intracellular pH and may involve the sorting of anticancer drugs in low pH vesicles before expulsion from the cancer cell.[5] In a wholly different therapeutic area, many viruses such as influenza use the low pH of endosomes in an elaborate mechanism that results in membrane fusion and ultimately infection of the host cell.[6]

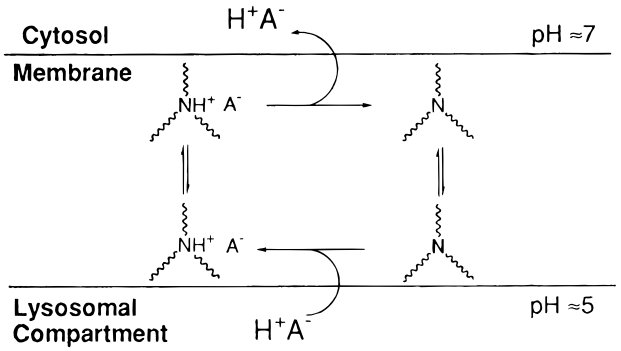

Figure 1. Schematic model of lysosomal neutralization by a membrane-active proton transporter.

As part of a program in which we seek to modulate these pH differences in order to treat disease, we have prepared membrane-active agents capable of transporting protons across lipid bilayers resulting in the dissipation of pH gradients. Unlike standard lysosomotropic amines such as ammonia, which transport protons from aqueous to lipid to aqueous phases, these compounds are designed to carry protons across bilayer membranes much more efficiently by picking them up on one side and depositing them on the other while remaining all the while within the bilayer (Figure 1). They consist of a basic nitrogen, aliphatic or heterocyclic, to which are attached three lipophilic groups, usually alkyl chains of varying length. Having only one alkyl chain would make the molecule a detergent, which would disrupt the membrane. Two alkyl chains would make it a bilayer former and thus very slow to move from one face of the membrane to the other. An exception is the adamantyl group which is probably too bulky to contribute to bilayer formation. Our experience with lysosomotropic detergents suggested that the basic portion of the molecule should have a pK_a between 5 and 7 so that it would

⊗ Abstract published in *Advance ACS Abstracts,* June 15, 1996.

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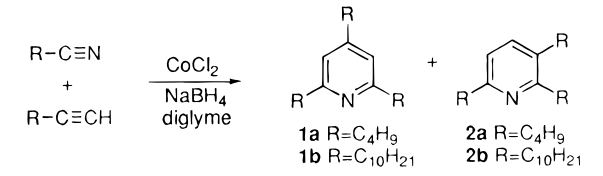
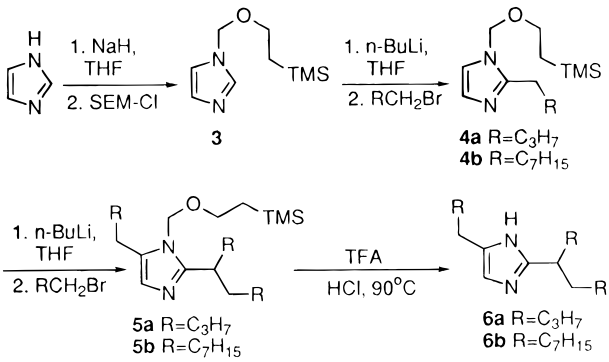
S0022-3263(96)00367-2 CCC: $12.00

Scheme 1



Scheme 2



selectively localize in lysosomal membranes.[7] However, since these new agents are intended to protonate and deprotonate while inside the membrane, and not in water, we decided to investigate their acidity constants in various organic solvents, as models for the environment of the lipid bilayer. For this we designed a series of NMR experiments in which the compounds, in water-immiscible, deuterated solvents, were stirred with an aqueous phase consisting of a buffer whose pH could be varied. Clear pK_a curves were produced by monitoring the change in chemical shift of various protons in the molecules.[8] As might be expected, these pK_as were dependent both on organic solvent and on counterion. Members of each class of compounds were then tested to determine whether they caused lysosomal neutralization *in vitro* at low concentrations.

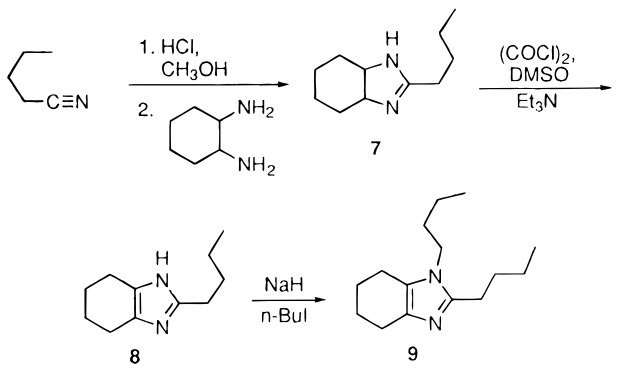
Results and Discussion

Synthesis. The trialkylated pyridines **1** were prepared in one step by the method of Bönneman,[9] using cobalt-mediated reductive cyclization of 2 equiv of alkynes with 1 equiv of nitrile (Scheme 1). Trialkylated benzenes and the unsymmetrical pyridines **2** are also formed but are easily separated from the desired products.

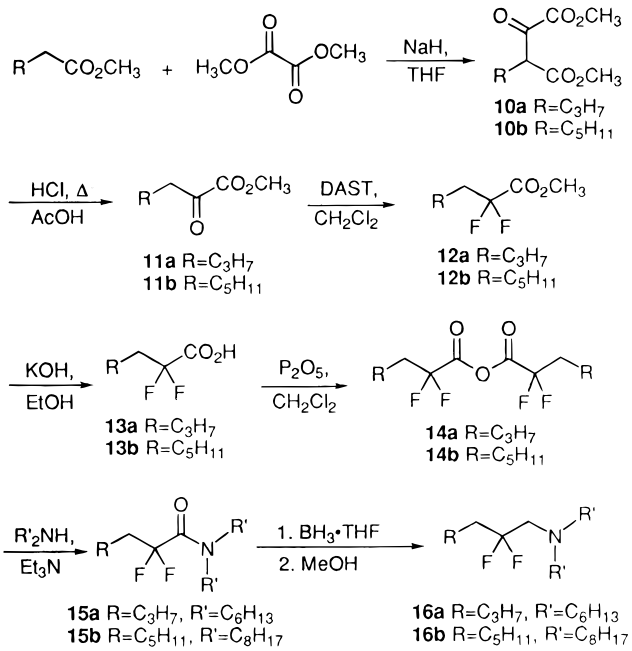
Branched imidazoles **6** were obtained from repetitive lithiation and alkylation of SEM-protected imidazole **3** (Scheme 2).[10] The first alkylation occurs exclusively at C-2, while subsequent reactions occur at both C-5 and at the new α-position with little apparent discrimination. Removal of the SEM group from the crowded nitrogen in **5** requires vigorous conditions (HCl/TFA, 90 °C, 40 h) which, nevertheless, proceeds cleanly.

Fused bicyclo imidazole **9** was synthesized as shown in Scheme 3. The methyl imidate from valeronitrile was

Scheme 3



Scheme 4



prepared *in situ* and then reacted with 1,2-diaminocyclohexane. The resulting imidazoline **7** was oxidized to the imidazole **8** using Swern conditions. N-Alkylation was effected by treatment with sodium hydride followed by iodobutane to give **9**.

Scheme 4 shows the preparation of the 2,2-difluorotrialkylamines **16**. Methyl valerate and methyl heptanoate were acylated with dimethyl oxalate, and the intermediates **10** were decarboxylated.[11] Treatment with DAST converted the 2-oxo esters **11** to 2,2-difluoro esters **12** in high yield,[12] and basic hydrolysis afforded the free acids **13**. Formation of the tertiary amides **15** was found to proceed most cleanly through addition of the appropriate secondary amines to the symmetric anhydrides **14**. The extra equivalent of **13** was easily recovered by basic extraction of the reaction mixture. Reduction of the amides **15** with diborane, followed by methanolysis, gave the 2,2-difluoro amines **16** in good yield.

A series of (adamantylmethyl)amines (**18** and **20**) was synthesized as shown in Scheme 5 by straightforward acylation with adamantanecarbonyl chloride and reduction of the resulting amides (**17** and **19**). (Adamantylmethyl)imidazole **22** was prepared by the very slow

(7) Miller, D. K.; Griffiths, E.; Lenard, J.; Firestone, R. A. *J. Cell Biol.* **1983**, *97*, 1841−1851. (b) Firestone, R. A.; Pisano, J. M.; Bonney, R. J. *J. Med. Chem.* **1979**, *22*, 1130−1133.

(8) A recent paper describes the determination of pK_a curves of pyrrole carboxylic acids by plotting ^{13}C NMR chemical shifts versus pH in a single phase (H_2O−DMSO-d_6): Holmes, D. L.; Lightner, D. A. *Tetrahedron* **1995**, *51*, 1607−1622.

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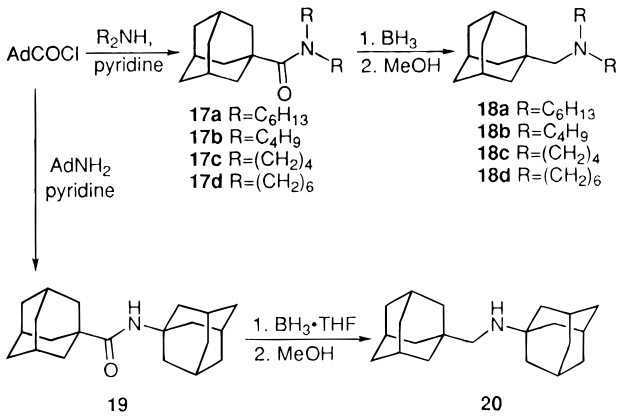
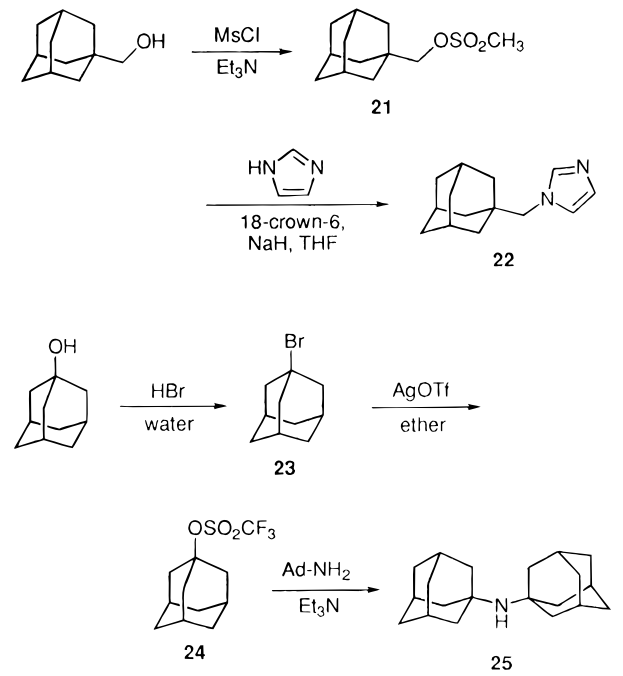
Scheme 5



Scheme 6



reaction of imidazole with mesylate **21** under phase transfer conditions (Scheme 6). Formation of the very crowded 1,1-diadamantylamine (**25**), even in low yield, required generation of adamantyl 1-triflate **24** *in situ* by treatment of the bromide **23** with silver triflate, followed by quenching with adamantylamine. Neither **23** itself, nor the corresponding nitrate (from treatment with silver nitrate), was reactive enough to couple with adamantylamine.

Hydrocarbon Mass. In order for our compounds to localize in the hydrocarbon region of the phospholipid bilayer and remain there in the protonated state, they must carry sufficient hydrocarbon mass. On the other hand, for effective delivery of these agents through aqueous media, lipophilicity must be minimized. To determine the minimum effective hydrocarbon bulk, we used chloroform as a surrogate for the interior of the membrane and tried to extract two borderline compounds (tributylamine and tripentylamine) with pH 4 buffer (somewhat more acidic than lysosomes) or 10% HCl. In the case of tributylamine (C_{12}), 21% was extracted into pH 4 buffer, while none of the tripentylamine (C_{15}) was detected there. In 10% HCl the acid-extractable fractions were 31% and 12%, respectively. This demonstrated to us that a hydrocarbon mass of C_{12} was too low since a

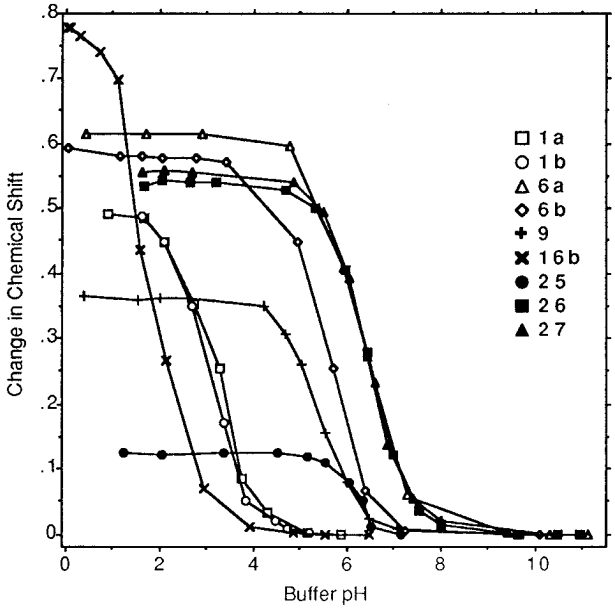


Figure 2. Two-phase pK_as measured in $CDCl_3$ stirred with phosphate buffer containing sodium chloride (1 M).

significant amount of the compound might dissolve inside the acidic vesicle where it would have only a bulk effect and not the desired catalytic one.

Two-Phase pK_a Measurements. Our aim was to determine the effective pK_as of these agents in an environment that would be as close as possible to the one they would experience if they were acting as we designed them, i.e., undergoing protonation and deprotonation while residing within the lipid bilayer. In any case, aqueous pK_as measured in the usual ways would be gross approximations because all of these compounds, in their unprotonated form, are practically insoluble in water. We also wished to avoid using standard pH electrodes in mixed aqueous/organic media because of the uncertain correction factors involved.

The solution that we found was a two-phase system: one an aqueous buffer whose pH could be varied and then measured using a standard glass electrode and the other an immiscible, deuterated NMR solvent containing the compound under study. Indeed, a two-phase system is more realistic than the standard single-phase titration because it mimics more closely the actual situation in living cells, where the drug will be inside the bilayer, influencing the pH in an adjacent aqueous compartment. By vigorously stirring the two phases over 10 min, we found that the protonation state of the compound reached an equilibrium that depended on three factors: the inherent basicity of the compound, the nature of the organic solvent (our substitute for the interior of the lipid bilayer), and the lipophilicity of the counterion. We assumed that the most lipophilic anion in the aqueous phase, as long as it was present in excess, would be carried into the organic phase as the counterion. Portions of the organic phase were removed and the proton NMR spectra measured. The extent of protonation of each compound was clearly mirrored by the downfield shifts of proton signals close to the basic site. When these shifts were plotted versus the pH of the buffer, clear titration curves were generated (Figure 2). In order to ensure that all of the protonated compound remained in the deuterated solvent, the aqueous phase at the acidic limit of the experiment (pH $\leq$ 2) was removed, basified

Table 1. Two-Phase pK_as Determined by Proton NMR in $CDCl_3$ After Stirring with Chloride-Containing Phosphate Buffer at Various pH Values

compound	two-phase pK_a	parent pK_a[a]	deviation
trihexylamine (**26**)	6.43	10.65[b]	4.2
trioctylamine (**27**)	6.42	10.65[b]	4.2
1a	3.32	7.48[c]	4.2
1b	3.11	7.48[c]	4.4
6a	6.23	8.3[d]	2.1
6b	5.51	8.3[d]	2.8
9	5.42	8.3[d]	2.9
16b	1.77	7.5[e]	5.7
18a	5.3	10.65[b]	5.3
18b	5.5	10.65[b]	5.1
18c	6.1	10.46[f]	4.4
18d	5.5	10.30[g]	4.6
25	6.25	10.91[h]	4.7

[a] See ref 14. [b] Triethylamine. [c] 2,4,6-Trimethylpyridine. [d] 2,4-Dimethylimidazole. [e] (2,2-Difluoroethyl)diethylamine. [f] *N*-Methylpyrrolidine. [g] *N*-Methylazacycloheptane. [h] Bis-*sec*-butylamine.

to >pH 12, and extracted with chloroform. Proton NMR showed that only (adamantylmethyl)imidazole **22**, the least lipophilic compound in our study, was extracted into the acid to any detectable extent.[13]

For most of our compounds a steady downfield shift, with no change in the signal patterns, was observed with increasing protonation. The exceptions were the adamantylmethyl series of compounds (**18a**−**d**), which displayed broadened signals for two distinct species in the pH range near the apparent pK_a. Fully protonated **18a**−**d** showed two very complex multiplets for each of the alkyl chain (or cyclic) methylene groups next to the nitrogen. These were separated by as much as 1.1 ppm (**18c**) or as little as 0.2 ppm (**18a** and **b**). The unprotonated species, on the other hand, showed the expected single triplet signal for these protons. Conversion from one state to the other in either direction did not result in a significant shift in any of the signals for **18a**−**d**, only a broadening and a gradual replacement by the signals for the new specie, suggesting hindered rotation around the C−C and C−N bonds between the bulky adamantyl group and the nitrogen. Two factors are most likely to account for this increased barrier to rotation: the decreased rate of nitrogen lone pair inversion on protonation and the fact that the proton on nitrogen is an added steric element that might encounter bridgehead adamantyl methylene groups. The two-phase pK_as of these compounds could be estimated by integration of the interconverting species.

The derived pK_a values of our compounds in deuterated chloroform with chloride ion are collected in Table 1. Also included are aqueous pK_a values, from the literature,[14] of similar compounds that contain significantly less hydrocarbon mass. Most of these comparison compounds substitute a methyl or ethyl group in place of the hydrocarbon chain or the adamantylmethyl group in the compound of interest. The two-phase pK_as are all significantly lower than those of the parent compounds. In addition, the range of deviation is quite large, extending from 2 to 3 pK_a units for the imidazole compounds (**6** and **9**) to almost 6 units for the β-difluoro amine **16b**. It is not surprising that the pK_as measured in this way

Table 2. Two-Phase pK_as Determined by Proton NMR in $CDCl_3$ After Stirring with a Phosphate Buffer Containing Different Counterions at Various pH Values

compound	PO_4^{2-}	Asp^{2-}	Cl^-	Br^-	I^-	TsO^-
27	3.29	3.88	6.42	7.32	8.21	8.42
18c			6.11	7.02	8.10	
16b			1.77	2.64	4.02	

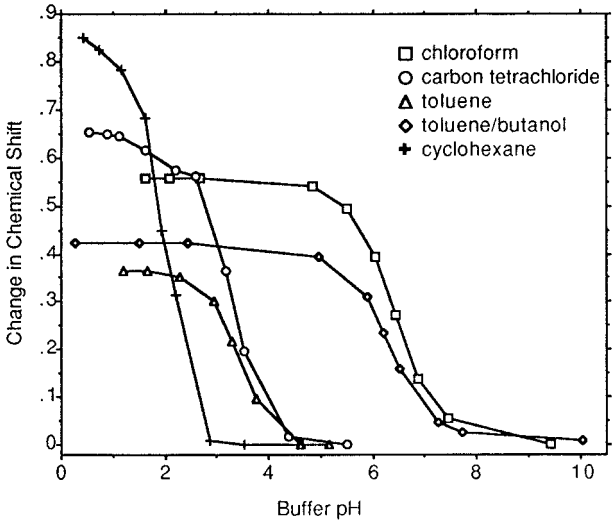


Figure 3. Two-phase pK_as measured in various solvents stirred with phosphate buffer containing sodium chloride (1 M).

should be lower than would be expected in water because the solvation energy of the ion pair is much greater in water.[15,16] This is shown dramatically in Table 2 which represents experiments in which the anion available in the buffer is varied to include a range of hydrophobicities. The relative solvation energy that is sacrificed in going from water to the organic solvent is reflected in the two-phase pK_as, i.e., the less lipophilic the anion (e.g., PO_4^{2-}), the lower the pH required to carry it into the organic phase. In the case of trioctylamine **27**, phosphate depresses the two-phase pK_a in comparison with that measured with chloride by more than 3 pK_a units while tosylate increases it by 2 units. The magnitude of this effect appears to be consistent as well since the two-phase pK_as of all three compounds in Table 2 increase by nearly the same amounts on going from chloride to bromide and then to iodide. The ordering of these anion-dependent, two-phase pK_as are also mirrored in the order of lysosomal membrane anion permeability.[17]

Significant variations in two-phase pK_as of **27** (with chloride ion) were also seen in experiments in which the organic phase was varied (Figure 3). A bulk organic solvent can only be a rough approximation of the hydrophobic environment inside a lipid bilayer, especially since it cannot model aspects of molecular packing and ease of motion of "solute" molecules.[18] Chloroform is most widely used to mimic the lipid bilayer in bulk liquid membrane transport experiments.[19] Early studies were carried out using toluene, though a more useful model

(13) Another compound whose pK_a could not be measured using our method was *N*-(adamantylmethyl)adamantylamine (**20**), the protonated form of which was insoluble in both the aqueous and organic phases.

(14) Perrin, D. D. *Dissociation Constants of Organic Bases in Aqueous Solution: Supplement 1972*; Butterworths: London, 1972.

(15) Reichardt, C *Solvents and Solvent Effects in Organic Chemistry*; VCH: New York, 1988.

(16) For a discussion of pK_a measurement in nonaqueous solvents, see: Bordwell, F. G. *Acc. Chem. Res.* **1988**, *21*, 456−463 and refernces therein.

(17) Casey, R. P.; Hollemans, M.; Tager, J. M. *Biochim. Biophys. Acta* **1978**, *508*, 15−26.

(18) Datta, D. B. *A Comprehensive Introduction to Membrane Biochemistry*; Floral Publishing: Madison, 1987.

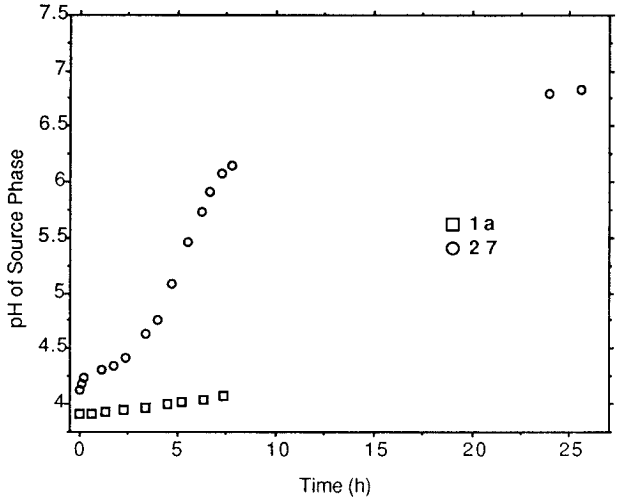


Figure 4. Change in pH of an unbuffered HCl source phase starting at ca. pH 4 during U-tube experiment in which trioctylamine (**27**) and tetradecylpyridine (**1b**) in chloroform (0.002 mM) transported protons to a phosphate buffer receiving phase at pH 7.4.

was later found by adding 30% 1-butanol.[20] Perhaps not coincidentally chloroform and 7:3 toluene/1-butanol yield virtually the same two-phase pK_a values (6.2−6.4). When chloroform is replaced by carbon tetrachloride, the pK_a falls to 3.3. This may represent a significant stabilization of the chloride ion by hydrogen bonding in chloroform. The pK_as shown in Figure 3 correlate roughly with the dielectric constants of the organic solvents. Note the especially low pK_a in cyclohexane, demonstrating extreme difficulty in creating ions in this highly nonpolar medium. The case of the butanol/toluene mixture is of course more complex since the dielectric constants of mixtures of solvents whose properties are different are not additive. In a case like this it is conceivable that an ion pair could assemble around itself an environment of higher polarity if the loss in entropy is compensated by the gain in charge stabilization.[15]

We also carried out several U-tube experiments in which trioctylamine (**27**) and tridecylpyridine **1b** were dissolved in chloroform (0.002 mM) between an unbuffered HCl/NaCl source phase at ca. pH 4 and a phosphate buffer (0.1 M) receiving phase at pH 7.4. All three phases were gently stirred, and the pH of the unbuffered side was monitored. These experiments were clumsy to run so we did not carry them out for each compound. However, the effort that we put into them gave us some confirmation of our two-phase pK_a data. As shown in Figure 4 proton transport by **27** is relatively fast until the pH of the unbuffered side approaches the two-phase pK_a of the transporting agent (ca. 6.4). From there, progress to the buffered side pH of 7.4 is very slow. Proton transport by **1b** is slow from the outset since the pH of the acid side (3.9) is already above the two-phase pK_a (3.1).

***In Vitro* Lysosomal Neutralization.** The best models for intracellular acidic vesicles are lysosomes. Nitrogenous bases such as ammonium chloride and methylamine, which raise lysosomal pH, are well-known through the work of Ohkuma and Poole.[21] Most of these agents cause vacuolization of lysosomes as a result of a buildup of osmotic stress. This is because the unprotonated bases pass easily into lysosomes through their membranes, but cannot readily depart because, after protonation, they are trapped in the aqueous phase, so that the phospholipid membrane now becomes a barrier. An exception is tributylamine which, Poole and Ohkuma noted,[21,22] may be lipophilic enough to cross freely even when protonated.

Table 3. RAW Cell Lysosomal Neutralization Induced by Lysosomotropic Amines Measured by Flow Cytometry

compound	pK_a	concn (mM)	vehicle	ΔpH[c]
NH_4Cl	9.21[a,b]	10	water	1.4
NH_4Cl	9.21[a,b]	1	water	0.7
NH_4Cl	9.21[a,b]	0.1	water	0.2
CH_3NH_3Cl	10.62[a,b]	10	water	1.4
tributylamine	10.89[a,b]	10	water	0
6a	6.23	0.1	lipid emulsion	0.5
6a·HCl	6.23	0.1	water	1.0
6a·HCl	6.23	0.1	Molecusol complex[d]	1.7
8·HCl		1	water	0.4
9·HCl	5.42	1	water	0.6
18b·HCl	5.3	0.1	water	0.2
18b	5.5	1	lipid emulsion	0.3
18d	5.5	1	lipid emulsion	<0.1
18c	6.1	1	lipid emulsion	<0.1
22	*e*	1	lipid emulsion	<0.1
1a	3.32	0.1	lipid emulsion	0.2
16a	1.77	1	lipid emulsion	<0.1

[a] Measured in a single-phase aqueous system. [b] Hall, H. K. *J. Am. Chem. Soc.* **1957**, *79*, 5441−5444. [c] Where pH 4.8 is taken for the starting value (see ref 20). [d] Molecusol alone had no effect on the pH of the cells and was nontoxic. [e] Extracted into aqueous acid.

A selected number of our compounds were tested for their ability to cause lysosomal neutralization of RAW cells over time by measurement of the fluorescence emission of fluorescein isothiocyanate (FITC)−dextran using a flow cytometer (Table 3). RAW cells are a murine monocytic macrophage line that are rich in lysosomes. Upon incubation, the cells selectively localize FITC−dextran in lysosomes by endocytosis. The degree of neutralization over time differed greatly depending on the compound. As observed by Ohkuma and Poole,[21] ammonium chloride caused an almost instantaneous rise in pH. We have used their standard of pH 6.2 at 10 mM ammonium chloride as the yardstick by which we measured the pH differences induced by our new compounds. Methylamine hydrochloride, also at 10 mM, raised the pH to the same degree but over several minutes. We were unable, however, to duplicate their results with tributylamine (using no dispersing agent). Tributylamine was not very soluble in the medium, and little may have reached the cells. Our compounds caused a steady rise in lysosomal pH over ca. 15 min, at which time it leveled off.

All of the compounds exhibited some degree of activity and were nontoxic to the cells at the concentrations tested. Being very lipophilic, they would not simply dissolve unaided in medium. At first we used a milky emulsion formed by sonicating the compounds with egg yolk phosphatidylcholine in medium. This was sufficient

(19) Recent examples: (a) Lambert, E; Breinlinger, E. C.; Rotello, V. M. *J. Org. Chem.* **1995**, *60*, 2646−2647. (b) Miyake, H.; Yamashita, T.; Kojima, Y.; Tsukube, H. *Tetrahedron Lett.* **1995**, *36*, 7669−7672. (c) Colton, I. J.; Kazlauska, R. J. *J. Org. Chem.* **1994**, *59*, 3626−3635.

(20) (a) Behr, J.-P.; Lehn, J.-M. *J. Am. Chem. Soc.* **1973**, *95*, 6108−6110. (b) Lehn, J.-M. In *Physical Chemistry of Transmembrane Ion Motions*; Elsevier: Amsterdam, 1983.

(21) Ohkuma, S; Poole, B. *Proc. Natl. Acad. Sci. U.S.A.* **1978**, *75*, 3327−3331.

(22) Poole, B; Ohkuma, S. *J. Cell Biol.* **1981**, *90*, 665−669.

to determine which compounds looked promising but was hardly satisfactory. Imidazole **6a** was clearly superior in these initial tests even when it was obvious that much of the compound was not reaching the cells. At 1 mM most of the other compounds showed detectable but very slight neutralization. Exceptions were the tributylpyridine **1a** and (adamantylmethyl)dibutylamine **18b**. Neither retained enough activity in subsequent tests to warrant continued interest.

It became clear to us in the course of these studies that these compounds displayed much less activity when assays were done using plastic, presumably because of binding of the lipophilic compounds to the walls of the vessel. Therefore, whenever possible, biological testing was done in glass vessels.

We found that delivery of **6a** to the cells could be improved greatly, first by forming the hydrochloride salt and adding it to the cells as a water solution, thereby doubling the lysosomal pH increase, and then by forming the hydroxypropylated β-cyclodextrin complex of the hydrochloride. This was completely water soluble to a concentration of **6a** of at least 10 mM. At 0.1 mM, **6a** raises lysosomal pH to ca. 6.5 within 15 min as its cyclodextrin complex. This is superior to ammonium chloride at 100 times the concentration, and taken together with the fact that the hydrochloride salt of **6a** is not extracted out of chloroform into water, argues strongly for its acting by the catalytic mechanism proposed above. The fused cyclohexyl imidazoles **8** and **9** also exhibited activity that was significant, but still greatly inferior to that of **6a**.

Imidazole **6a** exhibited potent reversal of doxorubicin (DOX) resistance in the HCT116-VM46 cell line by a factor of 14 over the DOX-sensitive strain and superior to that of widely used verapamil by a factor of 1.75 at 0.02 mM. In addition, replication of influenza virus was inhibited by **6a** (ED_{50} = 0.048 mM (13.3 μg/mL)).[5a]

Experimental Section

Molecusol (hydroxypropylated β-cyclodextrin) was purchased from Pharmatec Inc. (Alachua, FL). Chloroquine and FITC−dextran were purchased from Sigma Chemical Co. (St. Louis, MO). All other regents were purchased from Aldrich Chemical Co. (Milwaukee, WI) and were used as received. THF was distilled from sodium benzophenone ketyl, and chloroform was stored over basic alumina. All other dry solvents were Aldrich anhydrous grade and were used as received. Culture medium was purchased from GIBCO Laboratories (Grand Island, NY). Cell lines (RAW and HCT116-VM46) were obtained from the American Type Culture Collection. NMR spectra were measured on a Varian Gemini 300 spectrometer in $CDCl_3$, unless indicated. Microanalyses were carried out at Oneida Research Services.

Preparation of Hydrochloride Salts. The compound in dry ether at 0 °C was treated with 2 equiv of 1.0 M HCl in ether. After 1 h of stirring, excess HCl and solvent were removed on the rotary evaporator. The residue was flushed several times with CH_2Cl_2 and then dried *in vacuo* (ca. 0.05 mmHg).

2,4,6-Tributylpyridine (1a). Valeronitrile (2.39 g, 28.7 mmol) was added to cobalt(II) chloride hexahydrate (68 mg, 0.01 equiv) under argon at rt. The reaction was cooled to −40 °C and treated with 1-hexyne (1.18, 0.5 equiv), followed by $NaBH_4$ (22 mg, 0.04 equiv). The mixture was stirred at rt for 24 h and then diluted with hexane. The solution was washed with water, dried over Na_2SO_4, and concentrated *in vacuo*. The residue was purified by flash chromatography, eluting with 5% ethyl acetate/hexane, to give 713 mg (40%) of **1a** as an oil: ^{1}H NMR δ 0.89 (m, 9H), 1.42 (m, 6H), 1.59 (m, 6H), 2.49 (t, 2H), 2.68 (t, 4H), 6.82 (s, 2H); MS (DCI) 248 (MH^+): HRMS calcd for $C_{17}H_{30}N$ 248.2378, found 248.2369. **Hydrochloride**: ^{1}H NMR δ 0.88 (m, 9H), 1.36 (m, 6H), 1.59 (m, 2H), 1.73 (m, 4H), 2.69 (t, 2H), 3.17 (t, 4H), 7.12 (s, 2H); MS (FAB) 248 (MH^+). Anal. Calcd for $C_{17}H_{29}NCl$: C, 71.93; H, 10.65; N, 4.93. Found: C, 72.13; H, 10.51; N, 4.66.

2,4,6-Tridecylpyridine (1b) was prepared as described above for **1a** (38%): ^{1}H NMR δ 0.83 (m, 9H), 1.31 (m, 42H), 1.58 (m, 2H), 1.64 (m, 4H), 2.48 (t, 2H), 2.68 (t, 4H), 6.73 (s, 2H); MS (FAB) 500 (MH^+); HRMS calcd for $C_{35}H_{65}N$ 500.5195, found 500.5185. Anal. Calcd for $C_{35}H_{65}N$: C, 84.09, H, 13.11, N, 2.80. Found: C, 83.78, H, 12.73, N, 2.45. **Hydrochloride**: ^{1}H NMR δ 0.81 (t, 9H), 1.23 (m, 42H), 1.60 (m, 2H), 1.72 (t, 4H), 2.68 (t, 2H), 3.08 (t, 4H), 7.02 (s, 2H).

1-[[2-(Trimethylsilyl)ethoxy]methyl]imidazole (SEM-imidazole) (3). A stirred suspension of sodium hydride (60% dispersion in mineral oil, 0.68 g, 1.1 equiv) in THF (14 mL) under argon was cooled to 0 °C and then treated with a solution of imidazole (1.05 g, 15.4 mmol) in THF (6 mL). After 5 min the reaction was warmed to rt for 30 min, recooled to 0 °C, and treated with [2-(trimethylsilyl)ethoxy]methyl chloride (2.86 mL, 1.05 equiv). After the solution was warmed gradually to rt over 4 h, the reaction was quenched with excess saturated NH_4Cl and the solution was concentrated *in vacuo*. The aqueous residue was extracted with CH_2Cl_2 (4×), and the combined extracts were dried over $MgSO_4$. The crude product was purified by short path distillation to give 2.56 g of **3** as a colorless oil (83%) (bp 112−113 °C/0.1 mm Hg, lit.[10] bp 115−118 °C/0.15 mmHg): ^{1}H NMR δ 0.02 (t, 9H), 0.93 (t, 2H), 3.48 (t, 2H), 5.29 (s, 2H), 7.07 (s, 1H), 7.14 (s, 1H), 7.63 (s, 1H); MS (FAB) 199 (MH^+); HRMS calcd for $C_9H_{19}N_2OSi$ 199.1267, found 199.1262.

1-[[2-(Trimethylsilyl)ethoxy]methyl]-2-(1-propyl-1-pentyl)-4-butylimidazole (5a). A stirred solution of SEM-imidazole (**3**) (0.59 g, 3 mmol) in THF (6 mL) under argon at −78 °C was treated with *n*-BuLi (1.15 M in hexanes, 3.1 mL, 1.2 equiv). After 15 min at −78 °C, 1-bromobutane (0.34 mL, 1.05 equiv) was added. After 10 min the solution was warmed to rt for 1 h. The reaction was recooled to −78 °C, and the procedure was repeated three times, each with *n*-BuLi (3.39 mL, 1.3 equiv) and bromobutane (0.35 mL, 1.1 equiv), afterward allowing the reaction to stir at rt for 16 h. The mixture was then concentrated, diluted with CH_2Cl_2, washed with saturated NH_4Cl (2×) and water, and dried over $MgSO_4$. Flash chromatography on silica gel eluting with 15% ethyl acetate/hexane gave 440 mg of **5a** as an oil (40%). Mono- and dialkylated imidazoles were obtained and recycled. **5a:** ^{1}H NMR δ 0.04 (s, 9H), 0.84 (m, 11H), 1.07−1.31 (m, 6H), 1.31−1.44 (m, 2H), 1.52−1.78 (m, 6H), 2.51 (t, 2H), 2.74 (m, 1H), 3.41 (t, 2H), 5.10 (s, 2H), 6.68 (s, 1H); MS (DCI) 367 (MH^+); HRMS calcd for $C_{21}H_{43}N_2OSi$ 367.3145, found 367.3151. Anal. Calcd for $C_{21}H_{42}N_2OSi$: C, 68.79; H, 11.54; N, 7.64. Found: C, 68.73; H, 11.38; N, 7.58.

1-[[2-(Trimethylsilyl)ethoxy]methyl]-2-(1-heptyl-1-nonyl)-4-octylimidazole (5b) was prepared as described for **5a** (19%): ^{1}H NMR δ 0.02 (s, 9H), 0.91 (m, 11H), 1.05−1.49 (m, 30H), 1.71 (m, 6H), 2.58 (t, 2H), 2.78 (m, 1H), 3.49 (t, 2H), 5.18 (s, 2H), 6.74 (s, 1H); ^{13}C NMR δ (selected) 0.00, 15.62, 19.50, 24.22, 25.99, 29.33, 30.88, 33.47, 36.90, 38.99, 67.07, 73.32, 126.24, 133.12, 154.48; MS (ESI) 535.4 (MH^+); HRMS calcd for $C_{33}H_{67}N_2OSi$ 535.5023, found 535.5021. Anal. Calcd for $C_{33}H_{66}N_2OSi$: C, 74.09; H, 12.43; N, 5.24. Found: C, 73.87; H, 12.25; N, 5.19.

2-(1-Propyl-1-pentyl)-4-butylimidazole (6a). The SEM-protected imidazole **5a** (2.16 g, 5.9 mmol) under argon at rt was treated with a mixture of TFA (20 mL) and concd HCl (15 mL). The reaction was heated at 91 °C for 40 h. After concentration on the rotary evaporator, CH_2Cl_2 was added and the aqueous layer was basified with 1 M NaOH, extracted with CH_2Cl_2 (4×), and dried over Na_2SO_4. The crude product was purified by chromatography on alumina, eluting with 10% ethyl acetate/hexane, giving 1.09 g of **6a** as an oil (79%): ^{1}H NMR δ 0.76−0.89 (m, 9H), 1.08−1.40 (m, 8H), 1.47−1.71 (m, 6H), 2.50 (t, 2H), 2.72 (m, 1H), 6.60 (s, 1H), 8.8−9.2 (broad, 1H); MS (DCI) 237 (MH^+). **Hydrochloride**: mp 82−84 °C; ^{1}H NMR (D_2O) δ 0.72, 0.76, and 0.82 (3 × t, 9H), 0.98 (m, 2H), 1.16−1.37 (m, 6H), 1.59−1.80 (m, 2H), 1.80−1.99 (m, 2H), 2.58

(t, 2H), 2.97 (m, 1H), 6.98 (s, 1H). Anal. Calcd for $C_{15}H_{29}N_2$-Cl: C, 66.03; H, 10.71; N, 10.27. Found: C, 65.66; H, 10.43; N, 10.00.

2-(1-Heptyl-1-nonyl)-4-octylimidazole (6b) was prepared as described for **6a**: ^{1}H NMR δ 0.83 (m, 9H), 1.17 (brs, 14H), 1.26 (m, 18H), 1.59 (m, 6H), 2.51 (t, 2H), 2.75 (m, 1H), 6.59 (s, 1H); MS (DCI) 406 (MH^+). Anal. Calcd for $C_{27}H_{52}N_2$: C, 80.13; H, 12.95; N, 6.92. Found: C, 79.87; H, 12.88; N, 6.86.

Cyclodextrin Complex of 6a. A solution of the hydrochloride salt of **6a** (19.6 mg, 71.8 mmol) in absolute ethanol (0.2 mL) was treated with 0.40 mL (ca. 1.25 equiv) of a 37% w/v solution of Molecusol (hydroxypropylated β-cyclodextrin) in water. The solvents were evaporated by passing a stream of N_2 over the stirred mixture at rt. When only a glassy solid remained, residual solvent was removed *in vacuo* (ca. 0.05 mmHg) over 24 h. The solid was dissolved in water (7.2 mL), giving a final concentration of 10 mM of **6a**. The proton NMR of **6a** in D_2O showed the expected changes in chemical shift upon complexation: δ 2.58 and 2.97 (free) to 2.62 and 3.02 (complexed).[23]

2-Butyl-3a,4,5,6,7,7a-hexahydro-1*H*-benzimidazole (7). A stirred solution of valeronitrile (10.0 mL, 95.6 mmol) in ether (50 mL) and methanol (5.8 mL, 1.5 equiv) and cooled to 0 °C. HCl gas was bubbled through the solution at 0 °C over which was passed a continuous stream of N_2. After 3 h the solvents were removed on the rotary evaporator and the residue was flushed with methanol several times, giving a white foam. Methanol (30 mL) was added, and the mixture was cooled to 0 °C and treated with 1,2-diaminocyclohexane (11.5 mL, 1 equiv) in methanol (13 mL), dropwise over 45 min. The reaction was allowed to warm to rt overnight. The solvent was then evaporated and the residue basified with 1 M NaOH (30 mL) followed by extraction with CH_2Cl_2. The organic phase was washed with brine (1×), dried over Na_2SO_4, filtered, and concentrated. The crude product was purified by flash chromatography on silica gel, eluting with 13:1 CH_2Cl_2/methanol, to yield 13.79 g (80%) of a white solid: ^{1}H NMR δ 0.89 (t, 3H), 1.33 (m, 6H), 1.55 (m, 2H), 1.74 (d, 2H), 2.15 (d, 2H), 2.11 (t, 2H), 2.88 (m, 2H); MS (DCI) 181 (MH^+); HRMS calcd for $C_{11}H_{21}N_2$ 181.1705, found 181.1700. Anal. Calcd for $C_{11}H_{20}N_2 \cdot 0.5H_2O$: C, 69.79; H, 11.18; N, 14.80. Found: C, 69.86; H, 10.97; N, 14.69.

2-Butyl-4,5,6,7-tetrahydro-1*H*-benzimidazole (8). A stirred solution of oxalyl chloride (3.82 mL, 1.1 equiv) in CH_2Cl_2 (45 mL) under argon at −78 °C was treated with DMSO (6.22 mL, 2,2 equiv) in CH_2Cl_2 (10 mL), dropwise over 5 min. After 10 min, imidazoline **7** (7.18 g, 39.8 mmol) in CH_2Cl_2 (11 mL) was added over 10 min, followed by triethylamine (27.8 mL, 5.0 equiv), dropwise over 10 min. The mixture was stirred with gradual warming overnight, and then the reaction was quenched with water. The organic phase was washed with brine, dried over $MgSO_4$, filtered, and concentrated to give a yellow solid. The crude product was purified by flash chromatography on silica gel using 20:1 CH_2Cl_2/methanol to give 5.82 g (82%) of a waxy solid: ^{1}H NMR δ 0.88 (t, 3H), 1.46 (m, 2H), 1.67 (m, 2H), 1.78 (s, 4H), 2.53 (s, 4H), 2.67 (t, 2H). **Hydrochloride**: ^{1}H NMR δ 0.84 (t, 3H), 1.31 (m, 2H), 1.78 (m, 6H), 2.60 (s, 4H), 2.98 (t, 2H); MS (DCI) 179 (MH^+); HRMS calcd for $C_{11}H_{19}N_2$ 179.1548, found 179.1544. Anal. Calcd for $C_{11}H_{20}N_2Cl$: C, 61.53; H, 8.92; N, 13.05. Found: C, 61.24; H, 8.84; N, 12.85.

1,2-Dibutyl-4,5,6,7-tetrahydro-1*H*-benzimidazole (9). A stirred suspension of NaH (60% dispersion in mineral oil, 0.61 g, 1.1 equiv) in THF (50 mL) under argon at 0 °C was treated with 2-butyl bicyclo imidazole **8** (2.46 g, 13.8 mmol) in THF (20 mL). The stirred mixture was warmed to rt for 30 min and then heated to 56 °C. After 1 h iodobutane (1.65 mL, 1.05 equiv) was added and heating was continued overnight. The reaction was quenched with saturated NH_4Cl, and the solution was concentrated *in vacuo*. The aqueous phase was extracted with CH_2Cl_2 (4×). The combined organic phases were dried over Na_2SO_4 and concentrated. The crude product was purified by flash chromatography on silica gel, eluting with 27:1 CH_2Cl_2/methanol, to give the product as an oil (1.94 g, 60%): ^{1}H NMR δ 0.92 (q, 6H), 1.38 (m, 4H), 1.60 (m, 2H), 1.75 (m, 6H), 2.44 (m, 2H), 2.52 (m, 2H), 2.59 (t, 2H), 3.66 (t, 2H); MS (DCI) 235 (MH^+); HRMS calcd for $C_{15}H_{27}N_2$ 235.2174, found 235.2166. **Hydrochloride**: ^{1}H NMR δ 0.94 (q, 6H), 1.40 (m, 4H), 1.68 (m, 2H), 1.84 (m, 6H), 2.51 (brt, 2H), 2.74 (brt, 2H), 2.95 (t, 2H), 3.85 (t, 2H). Anal. Calcd for $C_{15}H_{27}N_2Cl$: C, 66.52; H, 10.05; N, 10.34. Found: C, 66.84; H, 10.08; N, 10.19.

Methyl 2-Oxooctanoate (11a). A stirred suspension of sodium hydride (278.7 mg, 11.61 mmol) in THF (5 mL) under argon at 0 °C was treated with methanol (0.11 mL, 0.25 equiv) and gradually allowed to warm to rt. To this was added dimethyl oxalate (1.247 g, 0.9 equiv) followed by methyl heptanoate (1.523 g, 0.9 equiv). The mixture was heated at reflux for 3 h and cooled to rt, and the reaction was carefully quenched with water (5 mL). THF was removed *in vacuo*, and the residue was dissolved in acetic acid (15 mL) and treated with concd HCl (5 mL). The solution was heated at reflux for 6 h and then poured into ice water. The oily residue was extracted with ethyl acetate (3×), and the organic phase was dried over $MgSO_4$ and concentrated *in vacuo*. The residue in methanol (20 mL) was treated with a catalytic amount of *p*-toluenesulfonic acid (15 mg) and the solution heated at reflux for 2 h. The mixture was concentrated *in vacuo*, diluted with ethyl acetate, washed with 20% K_2CO_3 (3×), and dried over Na_2SO_4. Evaporation of the solvent and chromatography on silica gel, eluting with 5% ethyl acetate/hexane, gave 0.60 g (33%) of an oil: ^{1}H NMR δ 0.87 (t, 3H), 1.27 (m, 6H), 1.58 (m, 2H), 2.79 (t, 2H), 3.85 (s, 3H); MS (DCI) 173 (MH^+); HRMS calcd for $C_9H_{17}O_3$ 173.1178, found 173.1176. Anal. Calcd for $C_9H_{16}O_3$: C, 62.77; H, 9.36. Found: C, 62.52; H, 9.31.

Methyl 2-oxohexanoate (11b) was prepared as described for **11a**: ^{1}H NMR δ 0.89 (t, 3H), 1.33 (m, 2H), 1.58 (m, 2H), 2.81 (t, 2H), 3.83 (s, 3H); MS (DCI) 145 (MH^+). Anal. Calcd for $C_7H_{12}O_3$: C, 58.32; H, 8.39. Found: C, 58.28; H, 8.32.

Methyl 2,2-Difluorooctanoate (12a). To a stirred solution of methyl 2-oxooctanoate (**11a**) (107.7 mg, 0.63 mmol) in CH_2Cl_2 (1.5 mL) under nitrogen at 0 °C was added (diethylamino)sulfur trifluoride (DAST) (0.091 mL, 1.1 equiv), dropwise over 30 s. The reaction mixture was allowed to warm to rt, and was stirred overnight. Water (5 mL) was then added, and the pH was adjusted to ca. 7 by the addition of small amounts of solid $NaHCO_3$. The mixture was extracted with CH_2Cl_2 and the organic phase dried over Na_2SO_4 and concentrated *in vacuo*. The residue was chromatographed on silica gel, eluting with 10% ethyl acetate/hexane to give the product as a thick oil (83.7 mg, 69%): ^{1}H NMR δ 0.85 (t, 3H), 1.25 (m, 6H), 1.42 (m, 2H), 2.03 (m, 2H), 3.84 (s, 3H); MS (DCI) 195 (MH^+); HRMS calcd for $C_9H_{17}O_2F_2$ 195.1197, found 195.1194.

Methyl 2,2-difluorohexanoate (12b) was prepared as described for **12a**: ^{1}H NMR δ 0.89 (t, 3H), 1.39 (m, 6H), 2.04 (m, 2H), 3.85 (s, 3H); MS (DCI) 167 (MH^+); HRMS calcd for $C_7H_{13}O_2F_2$ 167.0884, found 167.0883.

2,2-Difluorooctanoic Acid (13a). A stirred solution of methyl 2,2-difluorooctanoate (**12a**) (352.6 mg, 1.82 mmol) in ethanol (5 mL) at 0 °C was treated with KOH (87%, 1.17 g, 10 equiv). The reaction mixture was stirred at rt for 2 days, concentrated, and diluted with water. After acidification to pH 2 with 10% HCl, the mixture was extracted with hexane. The organic phase was dried over $MgSO_4$ and concentrated under reduced pressure to give 272 mg (83%) of crude product which was used without further purification: ^{1}H NMR δ 0.86 (t, 3H), 1.27 (m, 6H), 1.47 (m, 2H), 2.06 (m, 2H); MS (DCI) 181 (MH^+); HRMS calcd for $C_8H_{15}O_2F_2$ 181.1040, found 181.1037.

2,2-Difluorohexanoic acid (13b) was prepared as described for **13a**: ^{1}H NMR δ 0.90 (t, 3H), 1.39 (m, 6H), 2.07 (m, 2H); MS (DCI) 153 (MH^+); HRMS calcd for $C_6H_{11}O_2F_2$ 153.0727, found 153.0725.

***N*,*N*-Dioctyl-2,2-difluorooctanamide (15a).** A stirred solution of 2,2-difluorooctanoic acid (**13a**) (118 mg, 0.66 mmol) in CH_2Cl_2 (2 mL) at 0 °C was treated with P_2O_5 (140 mg, 1.5 equiv). The reaction mixture was stored at 5 °C for 2 days, then recooled to 0 °C, and treated with dioctylamine (0.1 mL, 0.5 equiv) and triethylamine (0.05 mL, 0.55 equiv). After 2 days at rt, the mixture was washed with 10% KOH to remove

(23) Cabral Marques, H. M.; Hadgraft, J.; Kellaway, I. W.; Pugh, W. J. *Int. J. Pharm.* **1990**, *63*, 267−274.

starting material (recycled) and then with 10% HCl. The organic phase was dried over Na_2SO_4 and then concentrated to give 69 mg (quant.) of crude product which was used without further purification: ^{1}H NMR δ 0.85 (m, 9H), 1.25 (br, 26H), 1.51 (m, 6H), 2.08 (m, 2H), 3.26 (t, 2H), 3.37 (t, 2H); MS (DCI) 404 (MH^+); HRMS calcd for $C_{24}H_{48}NOF_2$ 404.3704, found 404.3703.

***N,N*-Dihexyl-2,2-difluorohexanamide (15b)** was prepared as described for **15a**: ^{1}H NMR δ 0.90 (m, 9H), 1.29 (m, 16H), 1.57 (m, 4H), 2.06 (m, 2H), 3.28 (t, 2H), 3.39 (t, 2H); MS (DCI) 320 (MH^+); HRMS calcd for $C_{18}H_{36}NOF_2$ 320.2765, found 320.2758.

2,2-Difluorotrioctylamine (16a). A stirred solution of *N,N*-dioctyl-2,2-difluorooctanamide (**15a**) (112 mg, 0.28 mmol) in THF (3.5 mL) under argon at 0 °C was treated with diborane (1.0 M in THF, 2.8 mL, 10 equiv). The reaction mixture was allowed to warm to rt and was stirred overnight. The mixture was then cooled in an ice bath, the reaction was carefully quenched with methanol (15 mL), and the mixture was concentrated *in vacuo*, diluted with more methanol (30 mL), and heated at reflux overnight. After evaporation of the solvent, the residue was purified by flash chromatography on silica gel, eluting with 2% ethyl acetate/hexane, giving the product (105 mg, 97%) as an oil: ^{1}H NMR δ 0.85 (t, 9H), 1.24 (br, 26H), 1.36 (m, 6H), 1.85 (m, 2H), 2.46 (t, 4H), 2.67 (t, 2H); MS (DCI) 390 (MH^+); HRMS calcd for $C_{24}H_{50}NF_2$ 390.3911, found 390.3910. Anal. Calcd for $C_{24}H_{49}NF_2$: C, 73.98; H, 12.67; N, 3.59; F, 9.75. Found: C, 74.02; H, 12.67; N, 3.58; F, 9.81.

2,2-Difluorotrihexylamine (16b) was prepared as described for **16a**: ^{1}H NMR δ 0.85 (m, 9H), 1.28 (brs, 14H), 1.39 (m, 6H), 1.88 (m, 2H), 2.47 (t, 4H), 2.69 (t, 2H); MS (DCI) 306 (MH^+); HRMS calcd for $C_{18}H_{38}NF_2$ 306.2972, found 306.2960. Anal. Calcd for $C_{18}H_{37}NF_2$: C, 70.77; H, 12.21; N, 4.58; F, 12.44. Found: C, 70.63; H, 12.35; N, 4.46; F, 12.54.

***N*-(Adamantylcarbonyl)dihexylamine (17a).** To a solution of 1-adamantanecarbonyl chloride (5.15 g, 25.9 mmol) in CH_2Cl_2 (25 mL) at 0 °C were added dihexylamine (6.1 mL, 1 equiv) and pyridine (6.3 mL, 3 equiv), dropwise over 5 min. The reaction mixture was allowed to warm to rt. After 2 h the mixture was washed with 10% HCl and brine. The organic phase was dried over Na_2SO_4 and concentrated to give an oil which was purified by flash chromatography on silica gel, eluting with 4% ethyl acetate/hexane, to give 6.96 g of **17a** as a thick oil (77%): ^{1}H NMR δ 0.83 (t, 6H), 1.22 (m, 12H), 1.48 (m, 4H), 1.67 (brs, 6H), 1.94 (s, 6H), 1.99 (s, 3H), 3.28 (brt, 4H); MS (ESI) 348.2 (MH^+). Anal. Calcd for $C_{23}H_{41}NO$: C, 79.48; H, 11.89; N, 4.03. Found: C, 79.26; H, 11.71; N, 4.01.

***N*-(Adamantylcarbonyl)dibutylamine (17b)** was prepared as described above for **17a** (88%): ^{1}H NMR δ 0.87 (t, 6H), 1.30 (m, 4H), 1.48 (m, 4H), 1.65 (brs, 6H), 1.92 (s, 6H), 1.98 (s, 3H), 3.31 (brt, 4H); MS (ESI) 292.2 (MH^+). Anal. Calcd for $C_{19}H_{33}NO$: C, 78.29; H, 11.41; N, 4.81. Found: C, 77.98; H, 11.29; N, 4.79.

***N*-(Adamantylcarbonyl)pyrrolidine (17c)** was prepared as described above for **17a** (93%): ^{1}H NMR δ 1.67 (s, 6H), 1.76 (m, 4H), 1.93 (s, 6H), 1.99 (brs, 3H), 3.52 (m, 4H); ^{13}C NMR δ 27.84, 28.60, 36.52, 36.93, 38.42, 41.95, 176.77; MS (ESI) 234.1 (MH^+). Anal. Calcd for $C_{15}H_{23}NO$: C, 77.21; H, 9.93; N, 6.00. Found: C, 77.07; H, 9.95; N, 6.01.

***N*-(Adamantylcarbonyl)azacycloheptane (17d)** was prepared as described above for **17a** (89%): ^{1}H NMR δ 1.50 (m, 4H), 1.68 (m, 10H), 1.99 (brs, 9H), 3.51 (brt, 4H); MS (ESI) 262.1 (MH^+). Anal. Calcd for $C_{17}H_{27}NO$: C, 78.11; H, 10.41; N, 5.36. Found: C, 77.95; H, 10.24; N, 5.29.

[(Adamantylcarbonyl)amino]adamantane (19) was prepared as described for **17a** and purified by column chromatography on silica, eluting with 30% ethyl acetate/hexane (1.46 g, 89%): ^{1}H NMR δ 1.62 (m, 3H), 1.70 (m, 9H), 1.77 (s, 3H), 1.90 (s, 6H), 1.94 (m, 3H), 2.03 (brs, 6H), 5.20 (brs, 1H); ^{13}C NMR δ 29.15, 29.66, 35.59, 36.64, 36.76, 39.61, 40.72, 41.86, 53.22, 194.45; MS (ESI) 314.1 (MH^+). Anal. Calcd for $C_{21}H_{31}NO$: C, 80.46; H, 9.97; N, 4.47. Found: C, 80.30; H, 9.86; N, 4.49.

***N*-(Adamantylmethyl)dihexylamine (18a).** A stirred solution of *N*-(adamantylcarbonyl)dihexylamine (**17a**) (5.51 g, 15.84 mmol) in THF (10 mL) under argon at 0 °C was treated with diborane (1.0 M in THF, 32 mL, 2.0 equiv). Over 2 h the reaction mixture was allowed to warm to rt. After 16 h the mixture was cooled to 0 °C and the reaction was quenched with methanol (50 mL). The solvent was evaporated and the residue dissolved in methanol (150 mL) and heated at 70 °C for 45 min. The solvent was removed *in vacuo* and the crude product purified by flash chromatography on silica gel, eluting with 2% ethyl acetate/hexane, to give 4.00 g of **18a** as a thick oil (76%): ^{1}H NMR δ 0.86 (t, 6H), 1.22 (m, 12H), 1.31 (m, 4H), 1.43 (s, 6H), 1.63 (ABq, 6H), 1.89 (brs, 3H), 1.98 (s, 2H), 2.32 (ABq, 4H); MS (ESI) 334.3 (MH^+). Anal. Calcd for $C_{23}H_{43}N$: C, 82.81; H, 12.99; N, 4.20. Found: C, 83.01; H, 12.77; N, 4.03.

***N*-(Adamantylmethyl)dibutylamine (18b)** was prepared as described above for **18a** (82%): ^{1}H NMR δ 0.88 (t, 6H), 1.30 (m, 8H), 1.44 (s, 6H), 1.62 (ABq, 6H), 1.90 (brs, 3H), 1.97 (s, 2H), 2.33 (ABq, 4H); ^{13}C NMR δ 14.39, 20.89, 28.90, 30.19, 35.06, 37.62, 41.64, 57.21, 69.25; MS (ESI) 278.2 (MH^+); HRMS calcd for $C_{19}H_{35}N$ 277.2769, found. Anal. Calcd for $C_{19}H_{35}N$: C, 82.24; H, 12.71; N, 5.05. Found: C, 82.40; H, 12.94; N, 5.09. **Hydrochloride**. Anal. Calcd for $C_{19}H_{36}NCl$: C, 72.69; H, 11.56; N, 4.46; Cl, 11.29. Found: C, 72.68; H, 11.60; N, 4.40; Cl, 11.34.

***N*-(Adamantylmethyl)pyrrolidine (18c)** was prepared as described above for **18a** (92%): ^{1}H NMR δ 1.48 (d, 6H), 1.61 (m, 6H), 1.67 (m, 4H), 1.89 (brs, 3H), 2.08 (s, 2H), 2.50 (m, 4H); MS (ESI) 220.2 (MH^+). Anal. Calcd for $C_{15}H_{25}N$: C, 82.13; H, 11.49; N, 6.38. Found: C, 82.40; H, 11.63; N, 6.40. **Hydrochloride**. Anal. Calcd for $C_{15}H_{26}NCl$: C, 70.42; H, 10.24; N, 5.48; Cl, 13.86. Found: C, 70.38; H, 10.17; N, 5.21; Cl, 13.62.

***N*-(Adamantylmethyl)azacycloheptane (18d)** was prepared as described above for **18a** (94%): ^{1}H NMR δ 1.30 (m, 2H), 1.45 (m, 10H), 1.62 (m, 6H), 1.83 (s, 2H), 1.90 (brs, 3H), 2.34 (m, 4H); ^{13}C NMR δ 27.45, 28.83, 29.10, 35.83, 37.63, 41.19, 59.23, 71.85; MS (ESI) 248.2 (MH^+). Anal. Calcd for $C_{17}H_{29}N$: C, 82.53; H, 11.81; N, 5.66. Found: C, 82.58; H, 11.79; N, 5.70. **Hydrochloride**. Anal. Calcd for $C_{17}H_{30}NCl$: C, 71.93; H, 10.65; N, 4.93; Cl, 12.49. Found: C, 72.02; H, 10.85; N, 4.90; Cl, 12.49.

[(Adamantylmethyl)amino]adamantane (20) was prepared as described for **18a** and purified by chromatography on silica, eluting with 20% ethyl acetate/hexane (519.2 mg, 91%): ^{1}H NMR δ 1.43 (d, 6H), 1.54 (d, 6H), 1.60 (m, 12H), 1.91 (brs, 3H), 2.00 (brs, 3H), 2.17 (s, 2H); ^{13}C NMR δ 28.39, 28.77, 29.88, 37.12, 37.58, 39.30, 41.17, 43.05, 53.20, 74.21; MS (ESI) 300.2 (MH^+). Anal. Calcd for $C_{21}H_{33}N$: C, 84.22; H, 11.11; N, 4.68. Found: C, 84.04; H, 11.08; N, 4.65. **Hydrochloride**. Anal. Calcd for $C_{21}H_{34}NCl$: C, 75.08; H, 10.20; N, 4.17; Cl, 10.55. Found: C, 75.11; H, 10.38; N, 4.14; Cl, 10.85.

Adamantylmethyl Methanesulfonate (21). A stirred solution of adamantylmethanol (784.0 mg, 4.72 mmol) in CH_2Cl_2 (15 mL) at 0 °C was treated with methanesulfonyl chloride (0.55 mL, 1.5 equiv) and triethylamine (1 mL, 1.5 equiv). After being warmed to rt, the mixture was stirred for 16 h and then diluted with ethyl acetate (80 mL). The solution was washed with 10% citric acid, water, and brine, dried over Na_2SO_4, and concentrated *in vacuo*. The crude product was carried on without further purification (1.073 g, 93%): ^{1}H NMR δ 1.41 (brs, 6H), 1.55 (m, 6H), 1.88 (brs, 3H), 2.88 (s, 3H), 3.66 (s, 2H); MS (DCI) 243 (MH^+). Anal. Calcd for $C_{12}H_{20}O_3S$: C, 58.99; H, 8.25; S, 13.12. Found: C, 59.22; H, 8.40; S, 12.92.

***N*-(Adamantylmethyl)imidazole (22).** A two-phase system consisting of adamantylmethyl methanesulfonate (**21**) (532.9 mg, 2.18 mmol), imidazole (297.0 mg, 2 equiv), and Aliquat 336 (25 mg) in toluene (5 mL) and 1 M NaOH (5 mL) was heated at reflux for 1 week. The organic phase was separated, washed with water and brine, dried over Na_2SO_4, and concentrated. The residue was chromatographed on alumina, eluting with 25% ethyl acetate/hexane, to give 141.5 mg of **22** (30%) as a waxy solid: ^{1}H NMR δ 1.38 (s, 6H), 1.53 (ABq, 6H), 1.89 (brs, 3H), 3.48 (s, 2H), 6.70 (s, 1H), 6.91 (s, 1H), 7.27 (s, 1H); ^{13}C NMR δ 28.25, 34.26, 36.79, 40.49, 59.87, 121.40, 128.96, 138.85; MS (ESI) 217.1 (MH^+). **Hydrochlo-**

ride. Anal. Calcd for $C_{14}H_{21}NCl$: C, 66.52; H, 8.37; N, 11.08; Cl, 14.02. Found: C, 66.50; H, 8.59; N, 10.93; Cl, 14.07.

1-Bromoadamantane (23). 1-Adamantanol (5.05 g, 33.2 mmol) was weighed into a 500 mL separatory funnel. HBr (48% aqueous, 170 mL) was added, and the mixture was shaken vigorously for 10 min. The solid was collected by filtration and washed several times with water. The crude material (6.42 g, 90%) was dried *in vacuo* and used without further purification: ^{1}H NMR δ 1.70 (t, 6H), 2.07 (s, 3H), 2.35 (d, 6H); MS (DCI) 215 (MH^+). Anal. Calcd for $C_{10}H_{15}Br$: C, 55.83; H, 7.03; Br, 37.14. Found: C, 55.71; H, 7.05; Br, 36.93.

1,1-Diadamantylamine (25). A solution of 1-bromoadamantane (**23**) (1.39 g, 1.05 equiv) in ether (16 mL), under argon at rt, was added to silver triflate (1.66 g, 1.05 equiv) in ether (10 mL). The mixture was protected from light. After being stirred at rt for 1 h, the reaction mixture was filtered through Celite in the dark and rinsed with ether. The filtrate was immediately added to a solution of 1-adamantanamine (0.93 g, 6.14 mmol) and triethylamine (1.5 mL, 1.16 equiv) in CH_2Cl_2 (10 mL). The mixture was stirred at rt for 2 days. The solution was washed with 10% aqueous KOH (2×) and brine (2×). The organic phase was dried over $MgSO_4$. After evaporation of the solvent the crude product was purified by flash chromatography on silica gel, eluting with 10:1 CH_2Cl_2/methanol, to yield 39.9 mg (2.3%) of a solid foam: ^{1}H NMR δ 1.61 (b, 6H), 2.00 (b, 9H); MS (DCI) 286 (MH^+); HRMS calcd for $C_{20}H_{32}N$ 286.2535, found 286.2534. Anal. Calcd for $C_{20}H_{31}N$: C, 84.15; H, 10.95; N, 4.91. Found: C, 84.04; H, 11.04; N, 4.80.

Measurement of pK_as by Proton NMR. In a 100 mL pear-shaped flask equipped with a magnetic stirrer, the compound under study (ca. 50 mg) in deuterated solvent (3 mL) was vigorously stirred with a solution of KH_2PO_4 (1 M) in 1 M HCl (pH 0.4−1) for 10 min. A portion of the organic phase was removed with a pipet, and the proton NMR was measured. The sample was returned to the flask, and the aqueous phase was basified with portions of a solution of NaCl (1 M) in 1 M NaOH. The procedure was repeated at least eight times along the pH scale until the aqueous phase had reached ca. pH 10. The aqueous pH was monitored before and after stirring so that the difference was never greater than 0.05 unit. To ensure that the fully protonated substrates did not extract into the aqueous solution, at the end of the experiment the mixture was acidified to pH <1 and the aqueous layer was separated, basified, and extracted with $CHCl_3$. Only in the case of **22** was any aqueous solubility detected by NMR. Depending on the nature of the compound, the change in chemical shift of α, β, and ring protons was plotted against the buffer pH values. In each case clear, overlapping pK_a curves were generated. The data included in the graphs and tables represent the signals that showed the largest shifts. When the influence of counterions other than chloride was investigated, NaCl and HCl were replaced by the corresponding sodium salt and acid of the counterion of interest.

U-Tube Experiments. In a 150 mL U-shaped glass tube, equipped with a magnetic stirrer, the compound under study (0.002 mM) in $CHCl_3$ (65 mL) was continuously stirred between two aqueous solutions: a pH 7.4 phosphate buffer (0.1 M, 35 mL) receiving phase and an unbuffered solution of NaCl (1 M) in HCl at ca. pH 4 (the source phase). The two aqueous solutions were continuously stirred using microstirrers (VWR Scientific). The pH of the unbuffered source phase was monitored continuously. When the organic phase contained no proton transport agent, the pH of the source phase was stable to within ±0.05 pH unit over the period of study.

Aqueous Acid Extractions. Tributylamine. Tributylamine (366.4 mg) in $CHCl_3$ (30 mL) was shaken vigorously with 10% HCl (2 × 50 mL). The aqueous layer was separated, washed with $CHCl_3$, basified with 15% NaOH to pH 11, and then extracted with $CHCl_3$ (3 × 30 mL). The combined organic phases were dried over $MgSO_4$ and concentrated *in vacuo* to give 113.3 mg (31%) of tributylamine. When this procedure was repeated using a pH 4 buffer (0.05 M biphthalate) in place of 10% HCl, 21% of the tributylamine was recovered from the acid washings.

Tripentylamine. Tripentylamine (315.8 mg) in $CHCl_3$ (30 mL) was shaken vigorously with 10% HCl (2 × 50 mL). The aqueous layer was separated, washed with $CHCl_3$, basified with 15% NaOH to pH 11, and extracted with $CHCl_3$ (3 × 30 mL). The combined organic phases were dried over $MgSO_4$ and concentrated *in vacuo* to give 38.0 mg (12%) of tripentylamine. When this procedure was repeated using a pH 4 buffer (0.05 M biphthalate) in place of 10% HCl, no detectable tripentylamine was recovered from the acid washings.

***In Vitro* Lysosomal Neutralization.** RAW cells were incubated with fluorescein isothiocyanate−dextran (FITC−dextran) (final concentration 5 mg/mL), which concentrates selectively in lysosomes, for 24 h at 37 °C. They were then spun down, washed four times with RPMI medium (without serum or glutamine), and diluted to 10^7 cells/mL in RPMI; 10^6 cells were removed, diluted to 1 mL with RPMI, and loaded on the flow cytometer (Epics V, Coulter Electronics). The sample cell was excited at 488 nm and emission measured at 525 nm with a band pass of ±5 nm. The amplitude of emission of fluorescein increases proportionally with pH over the range of interest. A base line pH (ca. 4.8) was measured, and the compound of interest was added. The change in lysosomal pH was measured over time. Ammonium chloride at 10 mM, which has been shown to raise lysosomal pH to ca. 6.2, was used as a standard. The viability of treated cells was evaluated by monitoring the forward angle light scatter and the 90° angle light scatter in the flow cytometer.

Supporting Information Available: Copies of NMR spectra of compounds **12a** and **12b** (2 pages). This material is contained in libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

JO960367F