J|A|C|S
A R T I C L E S

The Influence of Short Strong Hydrogen Bonding on the Structure and the Physicochemical Properties of Alkyl-*N*-iminodiacetic Acids in Solid State and Aqueous Systems

Leif Häggman,[†] Cecilia Lindblad,[†] Hans Oskarsson,[‡] Ann-Sofi Ullström,[†] and Ingmar Persson*,[†]

Contribution from the Department of Chemistry, Swedish University of Agricultural Sciences, Box 7015, SE-750 07 Uppsala, Sweden, and Akzo Nobel Surface Chemistry AB, SE-444 85 Stenungsund, Sweden

Received July 23, 2002; Revised Manuscript Received November 5, 2002; E-mail: Ingmar.Persson@kemi.slu.se

Abstract: Alkyl-*N*-iminodiacetic acids with varying alkyl chain lengths have been prepared and characterized with respect to structure, acidic properties, and ability to form aggregates in water. The alkyl-*N*-iminodiacetic acids are the group of ligands with the lowest molecule weight which can be characterized as chelating surfactants, compounds with surface chemical properties which at the same time have a high ability to bind metal ions. The solid alkyl-*N*-iminodiacetic acids have a unique structure with neutral zwitterionic units linked together to polymer chains through a short strong hydrogen bond, $d(O(-H)\cdots O) \approx 2.46$ Å, and where the $\nu(O-H)$ stretching vibration at ca. 720 cm^{-1} supports the presence of such a hydrogen bond. The polymer chains are cross-linked together to bilayers through relatively strong hydrogen bonds between ammonium and carboxylate groups, and where the parallel alkyl groups are interdigitating each other; the bilayer surface consists of hydrophilic iminodiacetic acid groups. The acidic properties of monomeric alkyl-*N*-iminodiacetic acids in water are in the expected ranges with pK_a values of about 1.7, 2.3, and 10.3. *n*-Octadecyl-*N*-iminodiacetic acid, present as aggregates in water, displays very acidic properties of the first proton, and a substantially weakened acidity of the second proton, pK_{a2} = 5.5−7.5, depending on ionic strength, and pK_{a3} = 9.5−10.5. This pattern of the acidic constants strongly indicates that the polymer structure with short strong hydrogen bonds is maintained in the aggregates and that such bonds can exist in aqueous systems if they are supported by a strong and rigid backbone structure, as the bilayers of well-organized long interdigitating alkyl chains in the studied systems. Hydrogenbis(methyl-*N*-iminodiacetic acid) perchlorate precipitates from perchloric acidic solutions of methyl-*N*-iminodiacetic acid. The structure is built up of dimers of zwitterionic methyl-*N*-iminodiacetic acid units linked together by an extra proton in a short strong hydrogen bond, $d(O(-H)\cdots O) \approx 2.456(6)$ Å, and $\nu(O-H)$ = 789 cm^{-1}.

Introduction

The interest for surface-active compounds, which at the same time have the ability to bind metal ions strongly and/or specifically, so-called chelating surfactants, is markedly increasing as many technical applications are in sight, such as, for example, soil remediation. The number of physical-chemical studies on chelating surfactants is scarce, as it in many instances is difficult to obtain reliable information due to the difficulty to prepare pure and well-defined compounds. In this study, we have chosen to study the group of ligands with the lowest molecule weight which can be defined as chelating surfactants, the alkyl-*N*-iminodiacetic acids, R−N(CH$_2$−COOH)$_2$. The alkyl-*N*-iminodiacetic acids will serve as a model for larger molecules with longer and/or more alkyl chains and with EDTA-like multidentate entities for efficient and/or specific complex formation of metal ions.

The aim of this paper is to understand the crucial chemical requirements for a chelating surfactant system with predictable properties and to set the basis for the following papers in this project describing the structural and physical-chemical properties of the alkyl-*N*-iminodiacetic acids in the presence of different metal ions in aqueous systems. This paper presents the preparation of three alkyl-*N*-iminodiacetic acids, R = *n*-hexyl, *n*-dodecyl, and *n*-octadecyl, the determination of the solid-state structures of three alkyl-*N*-iminodiacetic acids, R = methyl, *n*-hexyl, and *n*-dodecyl, and of one singly protonated dimer, hydrogenbis(methyl-*N*-iminodiacetic acid) perchlorate, the acidic properties of the methyl-, *n*-hexyl-, *n*-dodecyl-, and *n*-octadecyl-*N*-iminodiacetic acids including variation of total concentration and ionic strength, the aggregation properties of the *n*-hexyl- and *n*-dodecyl-*N*-iminodiacetic acids as a function of pH and total concentration as determined with steady-state fluorescence measurements, and infrared, mass, and ^{1}H NMR spectroscopic data and elemental analyses of the alkyl-*N*-iminodiacetic acids for characterization purposes.

[†] Swedish University of Agricultural Sciences.
[‡] Akzo Nobel Surface Chemistry AB.

This study shows clearly that the hydrogen bonding plays a very important role in the structure and the physical-chemical behavior of alkyl-*N*-iminodiacetic acids, as it also does in many other chemical systems. Hydrogen bonding is the key factor in explaining many chemical and biological structures and processes such as, for example, the unique properties of water,[1] enzyme conformation,[2] the behavior of lipid membranes,[3] catalysis,[4] and the double-helical structure of DNA.[2] It is therefore not surprising that the hydrogen bond energy can vary over a very wide range, ca. 5−150 kJ mol^{−1}.[5] In recent years, much attention has been put on the upper energy range, often referred as short strong hydrogen bonds (SSHBs) or low-barrier hydrogen bonds (LBHBs). The basic demands for being an SSHB/LBHB include (a) a short distance between hydrogen bond acceptor and donor, in the range 2.36−2.65 Å in oxygen-containing systems,[6] and (b) that the energy of the hydrogen bond is at least 40 kJ mol^{−1}.[7] It has also been proposed that high polarity and entropic disorder of the surroundings weaken a hydrogen bond,[8] while equal pK_a values of the hydrogen bond acceptor and donor should strengthen it.[9] There has been strong controversy over the existence of strong hydrogen bonds in aqueous solution. Cleland and Kreevoy have stated "formation of a short (less than 2.5 Å), very strong low barrier hydrogen bond in the transition state or in an enzyme-intermediate complex can be an important contribution to enzymatic catalysis."[10] Guthrie has argued against this view and pointed out that so far no short strong hydrogen bond has been proven to exist in aqueous solution.[7] Theoretical calculations support Guthries view that short and strong hydrogen bonds do not exist in media with permittivities larger than 10, such as, for example, in water.[11] This paper will discuss the possibility of forming SSHBs in surface-active systems in aqueous systems using results from acidic constant determinations of alkyl-*N*-iminodiacetic acid systems in water.

Experimental Section

Chemicals. Methyl-*N*-iminodiacetic acid (Aldrich, 99%), perchloric acid 70−72% (Merck, p.a.), sodium hydroxide solution (Merck, Titrisol), pyrene (Aldrich, twice recrystallized from ethanol), and ethanol (Kemetyl, 99.5%, spectroscopic grade) were used as purchased, and all water used in this study has been deionized and Milli-Q-filtered.

Analyses of Methyl-*N*-iminodiacetic Acid. Elemental analysis of methyl-*N*-iminodiacetic acid: C, calc. 40.82%, exp. 40.5, 40.6%; H, calc. 6.15%, exp. 6.1, 6.2%; N, calc. 9.52%, exp. 9.4, 9.4%. The elemental analyses were performed by Mikrokemi AB, Uppsala. The methods used are described at http://www.mikrokemi.se.

Mass spectroscopy: Calc. 147.131 g mol^{−1}; exp. 147.051 g mol^{−1}.

^{1}H NMR data in Me$_2$SO-d_6: δ 2.38 (3H), δ 3.34 (4H); see Table S1 for details.

(1) Silverstein, K. A. T.; Haymet, A. D. J.; Dill, K. A. *J. Am. Chem. Soc.* **2000**, *122*, 8037.
(2) Brändén, C.-I.; Tooze, J. *Introduction to Protein Structure*; Garland Publishing: New York and London, 1991.
(3) Evans, D. F.; Wennerström, H. *The Colloidal Domain*; Wiley-VCH: New York, 1999.
(4) Shan, S.; Loh, S.; Herschlag, D. *Science* **1996**, *272*, 97.
(5) Kumar, G. A.; McAllister, M. A. *J. Am. Chem. Soc.* **1998**, *120*, 3159.
(6) Bertolasi, V.; Gilli, P.; Ferretti, V.; Gilli, G. *Chem.-Eur. J.* **1996**, *2*, 925 and references therein.
(7) Guthrie, J. P. *Chem. Biol.* **1993**, *3*, 163.
(8) Pan, Y.; McAllister, M. A. *J. Am. Chem. Soc.* **1998**, *120*, 166. Perrin, C. L. *Science* **1994**, *266*, 1665. Perrin, C. L.; Thoburn, J. D. *J. Am. Chem. Soc.* **1992**, *114*, 8559.
(9) Gerlt, J. A.; Gassman, P. G. *Biochemistry* **1993**, *32*, 11943; *J. Am. Chem. Soc.* **1993**, *115*, 11532.
(10) Cleland, W. W.; Kreevoy, M. M. *Science* **1994**, *264*, 1887.
(11) Chen, J.; McAllister, M. A.; Lee, J. K.; Houk, K. N. *J. Org. Chem.* **1998**, *63*, 4611.

Syntheses of Alkyl-*N*-iminodiacetic Acids. Chemicals. 2-Propanol (IPA) (analytical grade, Merck), ethanol (Primalco, 99.7%), *n*-hexyl-amine (Fluka, 99%), *n*-dodecylamine (Fluka, >98%), *n*-octadecylamine (Aldrich, 99%), and sodium monochloroacetate (SMCA) (Skoghall, >97%) were used as purchased.

n-Hexyl-*N*-iminodiacetic Acid. *n*-Hexylamine (102.14 g, 1.009 mol) was dissolved in 2-propanol (250 cm[3]) and charged to a double jacket 1000 cm[3] glass-reactor with a cooler, a Teflon-covered mechanical stirrer (RW 20 DZM, Janke&Kunkel, IKA Labotechnique), a pH-stat (Titrino 719S, Metrohm), and a digital thermometer (Ebro TFX 392). Next 270.66 g (2.30 mol) of SMCA, dissolved in 300 cm[3] of deionized water, was added to the reaction mixture at 350−355 K during a period of 1.5 h at a constant pH of 8.5, adjusted by addition of 30% sodium hydroxide from a Dosimat (Titrino 719 from Metrohm Instruments). The postreaction continued for 7 h at 350 K and pH 8.5. Thereafter, the product was purified and concentrated. Excess SMCA was hydrolyzed at 350 K and pH 12 for 2.5 h. The product was poured in an E-flask and concentrated, and thereafter 4 mol dm^{−3} of hydrochloric acid was added until pH 1.80 was reached. The solvent was partly evaporated off, which caused precipitation of sodium chloride and the product; the filter-cake contained 14.5% sodium chloride. The product/salt filter-cake was dried and dissolved in ethanol (99.7%). In hot ethanol, sodium chloride precipitated and was filtered off. The filtrate was allowed to cool slowly overnight. By the following day, the product had precipitated to a hard solid. The solid was divided into small pieces, which were dried with diethyl ether. The compound was recrystallized from aqueous solution at pH 1.9 to get single crystals suitable for X-ray diffraction studies.

Analyses. Elemental analysis of *n*-hexyl-*N*-iminodiacetic acid: C, calc. 55.28%, exp. 54.0, 54.3%; H, calc. 8.81%, exp. 8.6, 8.7%; N, calc. 6.44%, exp. 6.3, 6.3%.

Mass spectroscopy: Calc. 217.265 g mol^{−1}; exp. 217.131 g mol^{−1}.

^{1}H NMR data in Me$_2$SO-d_6: δ 0.86 (3H), δ 1.22 (6H), δ 1.34 (2H), δ 2.60 (2H), δ 3.38 (4H); see Table S1 for details.

n-Dodecyl-*N*-iminodiacetic Acid. First 100.88 g (0.544 mol) of *n*-dodecylamine was dissolved in 250 cm[3] of IPA and charged to the reactor described above. Next 129.94 g (1.116 mol) of SMCA, dissolved in 195 cm[3] of deionized water, was added during a period of 1.5 h at 350−355 K and at a constant pH of 8.5, adjusted by the addition of 30% sodium hydroxide from a Dosimat. The length of the postreaction, the purification and concentration of the product, and the hydrolysis of excess SMCA were the same as those described for *n*-hexyl-*N*-iminodiacetic acid above. The product was poured in an E-flask, and ca. 150 mL of 4 mol dm^{−3} hydrochloric acid was added to the reaction mixture until a pH value of 4.5 was obtained. The solvent was evaporated off, which was complicated by formation of a lot of foam during the evaporation. The resulting product was dissolved and refluxed in ethanol (99.7%). The sodium chloride salt precipitated out from the warm ethanol and was filtered off. The filtrate was slowly cooled, and crystals were formed. The crystals were filtered off, and a second crop of crystals was collected. The third crop of crystals was dissolved in water/ethanol (75/25), and the pH was adjusted from 6.1 to 1.82. It was a clear solution after 1 h of reflux. The solution was slowly cooled. No crystals had been formed after 2 h, and the solution was left overnight. By the next day, nice flake-shaped crystals had formed. The crystals were filtered off and washed with cold ethanol under nitrogen atmosphere. The crystals were dried, first under vacuum and then in a desiccator.

Analyses. Elemental analysis of *n*-dodecyl-*N*-iminodiacetic acid: C, calc. 63.76%, exp. 63.1, 63.2%; H, calc. 10.37%, exp. 10.3, 10.3%; N, calc. 4.64%, exp. 4.6, 4.6%.

Mass spectroscopy: Calc. 301.426 g mol^{−1}; exp. 301.222 g mol^{−1}.

^{1}H NMR data in Me$_2$SO-d_6: δ 0.85 (3H), δ 1.23 (18H), δ 1.36 (2H), δ 2.60 (2H), δ 3.39 (4H); see Table S1 for details.

n-Octadecyl-*N*-iminodiacetic Acid. First 99.54 g (0.369 mol) of *n*-octadecylamine was dissolved in 295 cm[3] of IPA and charged to the

Table 1. Summary of the Acidic Constants of Methyl-, *n*-Hexyl-, and *n*-Octadecyl-*N*-iminodiacetic Acid (C_nH_{2n+1}—IDA, *n* Is Number of Carbon Atoms in the Aliphatic Chain) in Aqueous System (Temperature Was 295 ± 1 K)

	pK_{a1}	pK_{a2}	pK_{a3}	ionic medium
MIDA	1.82(5)	2.22(2)	9.59(1)	0.1 M NaClO$_4$
C$_6$—IDA	1.55(4)	2.37(2)	10.412(7)	0.1 M NaClO$_4$
C$_{18}$—IDA[a]		7.61(9)	9.45(12)	no supp. electrolyte
		6.97(8)	9.4(2)	0.01 M NaClO$_4$
		6.04(4)	10.65(14)	0.1 M NaClO$_4$
		5.72(4)	9.92(8)	0.5 M NaClO$_4$
		5.64(10)	9.48(16)	1.5 M NaClO$_4$

[a] Present as aggregates.

reactor described above. Next 88.19 g (0.757 mol) of SMCA, dissolved in 100 cm³ of deionized water, was added during a period of 1.5 h at a constant pH of 8.5 adjusted by the addition of 30% sodium hydroxide from a Dosimat. The length of the postreaction, the purification and concentration of the product, and the hydrolysis of excess SMCA were the same as those described for *n*-hexyl-*N*-iminodiacetic acid above. The product precipitated together with sodium chloride, and the bottom phase of sodium chloride and product was separated. The product/salt mixture was acidified in ethanol/water by adding 5 mol dm⁻³ of hydrochloric acid. The addition of acid was stopped at pH 3 as the product precipitated. The solvent was removed, and the product was recrystallized in dichloromethane and 2-propanol.

The product was finally dissolved in ethanol (99.7%). At 333—343 K, the product appeared as a cloudy upper phase, and sodium chloride precipitated in a bottom phase. The product was filtered warm, the sodium chloride was filtered off, and the solvent was finally evaporated off from the product.

Analyses. Elemental analysis of *n*-octadecyl-*N*-iminodiacetic acid: C, calc. 68.53%, exp. 67.6, 68.0, 68.3%; H, calc. 11.24%, exp. 11.2, 11.2, 11.3%; N, calc. 3.63%, exp. 3.6, 3.6, 3.7%.

Mass spectroscopy: Calc. 385.5877 g mol⁻¹; exp. 385.3204 g mol⁻¹.

¹H NMR data in Me₂SO-*d₆*: δ 0.86 (3H), δ 1.22 (30H), δ 1.35 (2H), δ 2.60 (2H), δ 3.41; see Table S1 for details.

Synthesis of Hydrogenbis(methyl-*N*-iminodiacetic Acid) Perchlorate, [(CH₃—N(CH₂—COOH)₂H]ClO₄. Methyl-*N*-iminodiacetic acid was dissolved in 1.0 mol dm⁻³ of perchloric acid in the stoichiometric ratio 1:1 giving a pH ≈ 0.50. Water was gently evaporated off, and crystals were formed.

Elemental analysis of hydrogenbis(methyl-*N*-iminodiacetic acid) perchlorate: C, calc. 30.43%, exp. 30.4, 30.5%; H, calc. 4.85%, exp. 4.8, 4.9%; N, calc. 7.10%, exp. 7.0, 7.1%.

The mass spectroscopy spectrum of hydrogenbis(methyl-*N*-iminodiacetic acid) perchlorate shows the exact same fragmentation pattern as methyl-*N*-iminodiacetic acid (Figure S7a). It therefore was not possible to determine the molecule weight of hydrogenbis(methyl-*N*-iminodiacetic acid) perchlorate by mass spectroscopy.

Preparation of Solutions. pH Titrations. All solutions prepared had the ionic strength 0.100 mol dm⁻³ using sodium perchlorate as supporting electrolyte, except for the *n*-octadecyl-*N*-iminodiacetic acid aqueous systems where the ionic strength was varied (Table 1). Solutions of neutral methyl- and *n*-hexyl-*N*-iminodiacetic acids were titrated with both 0.100 mol dm⁻³ of sodium hydroxide and 0.100 mol dm⁻³ of perchloric acid, and alkaline solutions of methyl- and *n*-hexyl-*N*-iminodiacetate were titrated with 0.100 mol dm⁻³ of perchloric acid. The solutions of methyl- and *n*-hexyl-*N*-iminodiacetic acid contained 20.0 mmol dm⁻³ of neutral iminodiacetic acid and 80.0 mmol dm⁻³ of supporting electrolyte, and the alkaline solutions contained 20 mmol dm⁻³ of iminodiacetic acid and 80 mmol dm⁻³ of sodium hydroxide. The acidic *n*-dodecyl-*N*-iminodiacetic acid aqueous systems contained 1.0 and 5.0 mmol dm⁻³ of iminodiacetic acid, 2 or 3 equiv of perchloric acid and supporting electrolyte, and the alkaline solutions contained 20, 5, 1, and 0.5 mmol dm⁻³ of iminodiacetic acid, 4 equiv of sodium

hydroxide and supporting electrolyte. The alkaline solutions of methyl-, *n*-hexyl-, and *n*-dodecyl-*N*-iminodiacetate were prepared from degassed water, and the solutions were kept under nitrogen atmosphere. A 0.4 mmol dm⁻³ suspension of *n*-octadecyl-*N*-iminodiacetic acid was prepared by ultrasonication during 1 h. The solution is turbid in the investigated pH range 2—13, and it seems obvious that the *n*-octadecyl-*N*-iminodiacetic acid is present as aggregates. These turbid solutions were titrated with both sodium hydroxide and perchloric acid solutions. The titrations showed that the stoichiometry of weighed product is correct and that all iminodiacetic acid groups in the aggregates are in contact with water, and in equilibrium with acids, bases, and other particles they may react with. Heavy stirring was necessary to maintain an aqueous phase of evenly dispersed aggregates; otherwise a slow accumulation of an amorphous phase took place at the bottom of the vessel.

Fluorescence Measurements. A stock solution of 5.0×10^{-7} mol dm⁻³ of aqueous pyrene was prepared; this is below the solubility of pyrene in water. *n*-Hexyl-*N*-iminodiacetic acid was dissolved in the pyrene stock solution by means of ultrasonication close to isoelectric pH. Aqueous solutions of *n*-dodecyl-*N*-iminodiacetate at neutral pH, 6.1—7.9, were prepared by dissolving monosodium *n*-dodecyl-*N*-iminodiacetate in the aqueous pyrene stock solution. Alkaline solutions of *n*-dodecyl-*N*-iminodiacetate, pH = 10.8—13, were prepared by dissolving *n*-dodecyl-*N*-iminodiacetic acid in a 4.5×10^{-7} mol dm⁻³ of pyrene and 0.10 mol dm⁻³ of sodium hydroxide stock solution by ultrasonication. Lower concentrations of *n*-hexyl-iminodiacetic acid, 1.5—150 mmol dm⁻³, and neutral and alkaline *n*-dodecyl-*N*-iminodiacetic acid, 0.1—200 and 0.01—200 mmol dm⁻³, respectively, were prepared by dilution of the pyrene-containing stock solutions. The samples where kept in darkness to prevent photodegradation.

Potentiometry. In the acid—base titrations of the alkyl-*N*-iminodiacetic acid systems in aqueous solution, the pH was recorded by an Orion Research EA 940 potentiometer with a Mettler Toledo InLab422 electrode or an Orion 720A potentiometer with an Orion 9102AP pH electrode. Calibrations were performed with Orion standards at pH 4.01, 7.00, and 10.01. All measurements were performed at ambient room temperature, 295 ± 1 K. The titrator solution was manually added from a digital buret (Brand Bürette Digital II or Metrohm 665 Dosimat). Titrations of the aqueous systems of *n*-octadecyl-*N*-iminodiacetic acid showed that the time to reach a stable pH reading was ca. 15 min. up to the second half-equivalence point and above the second equivalence point, while ca. 2 h was required for the range in between. The time to reach stable pH values was significantly faster for the other alkyl-*N*-iminodiacetic acid systems, but with the observation that the slowest reading up to 45 min was in the region between the second half-equivalence and equivalence points in *n*-dodecyl-*N*-iminodiacetic acid. The acidic constants were calculated from pH titration data by means of the program MAINPH.[12]

Single-Crystal X-ray Diffraction. Data were collected on a Bruker Smart Platform equipped with a 1k CCD area detector[13] and a graphite monochromator using a fine-focus sealed X-ray tube generating Mo Kα ($\lambda = 0.71073$ Å) radiation. The crystal to detector distance was 50 mm. The temperature was 295 ± 1 K. A hemisphere of data (1271 frames) was collected for each structure using the omega scan method (0.3° frame width). The first 50 frames were remeasured at the end of each data collection to monitor crystal and instrument stability. Intensity decay was negligible for all crystals in this study. The structures were solved by the direct methods in Bruker SHELXTL[14] and refined using full-matrix least squares on F^2. Non-hydrogen atoms were treated anisotropically. The ammonium and acetic acid hydrogen atoms in methyl-*N*-iminodiacetic acid were located in the difference map, while

(12) Sandell, A., personal communication; Physical Chemistry 1, Chemical Center, Lund University, Sweden. The program is available from the corresponding author of this paper.

(13) Bruker SMART and SAINT, area detector control and integration software, Bruker Analytical X-ray Systems, Madison, WI, 1995.

(14) Sheldrick, G. M. *Acta Crystallogr., Sect. A* **1990**, *46*, 467.

Table 2. Crystallographic Data of the Alkyl-*N*-iminodiacetic Acids Determined in This Study

chemical formula	$C_5H_9NO_4$	$C_{10}H_{19}NO_4$	$C_{16}H_{31}NO_4$	$C_{22}H_{43}NO_4$	$C_{10}H_{19}ClN_2O_{12}$
formula wt (g mol^{-1})	147.13	217.26	301.42	385.57	394.720
space group	*Pbca* (No. 61)	*Pbca* (No. 61)	*Pbca* (No. 61)	*Pbca* (No. 61)	$P\bar{1}$ (No. 2)
a (Å)	9.291(6)	8.412(2)	8.3774(17)	8.60(1)	5.5253(15)
b (Å)	10.469(7)	9.589(2)	9.7376(19)	10.07(2)	7.169(2)
c (Å)	13.489(8)	28.063(7)	41.525(8)	56.95(9)	10.823(3)
α (deg)	90	90	90	90	81.321(5)
β (deg)	90	90	90	90	88.518(5)
γ (deg)	90	90	90	90	76.042(5)
vol (Å^3)	1213.0(14)	2263.5(10)	3387.4(12)	4932(23)	411.28(19)
formula units Z	8	8	8	8	1
λ (Å)	0.71073	0.71073	0.71073	0.71073	0.71073
T (K)	295	295	295	295	295
ρ_{calc} (g cm^{-3})	1.490	1.275	1.182		1.594
μ (cm^{-1})	1.30	0.98	0.83		3.00
R_{int}	0.1947	0.1325	0.1493		0.0244
R indices R_1, wR_2 [$I > 2\sigma(I)$]^a	0.0614, 0.1286	0.0519, 0.1019	0.0601, 0.1130		0.0666, 0.1764
R indices R_1, wR_2 [all data]^b	0.0996, 0.1421	0.1045, 0.1161	0.1307, 0.1299		0.1079, 0.1907

^{a} Definition of R: SHELXTL, $R = \sum||F_o| - |F_c||/\sum|F_o|$; $R_w = [\sum w(|F_o| - |F_c|)^2/\sum(w|F_o|)^2]^{1/2}$; SHELXL, R1 $= \sum||F_o| - |F_c||/\sum|F_o|$. ^{b} w$R_2 = (\sum[w(F_o^2 - F_c^2)^2]/\sum[[wF_o^4]])^{1/2}$.

in the other structures the acetic acid protons were calculated in estimated idealized positions on the basis of the relationship between O−H bond distances and ν(O−H) stretching vibrations.[6] The hydrogen atoms in the alkyl chains were calculated in ideal positions riding on their respective carbon atom. Crystal and experimental data are summarized in Table 2.

Steady-State Fluorescence Measurements. The steady-state fluorescence measurements were performed on a SPEX Fluorolog 1680 combined with a SPEX Spectroscopy Laboratory Coordinator DM1B. The excitation wavelength was set to 337 nm, and the emission was collected at front face, typically in the range 360−560 nm. The background emission due to solvent and alkyl-*N*-iminodiacetic acid was subtracted by pyrene-free solution spectra.

The aggregational behavior of the *n*-hexyl- and *n*-dodecyl-*N*-iminodiacetic acids was examined using steady-state fluorescence spectroscopy of the emission from pyrene in aqueous solutions of alkyl-*N*-iminodiacetic acids. The dissolution of pyrene in micelles is well established, as well as its different spectrophotometric behavior in hydrophobic and hydrophilic environments.[15,16] From the changes in the total emission intensity in the region 360−560 nm,[17] and the pyrene monomer bands, first and third vibrational peaks, at ca. 383 and 372 nm, respectively, conclusions can be drawn of the critical micelle concentration.[18] The excited pyrene might also be quenched by a nonemissive deactivation process by another quenching molecule.[19] The Stern−Volmer equation for collisional quenching is given as $I_o/I = 1 + K_D[Q]$, where I_o and I are the fluorescence intensities of the unquenched and quenched fluorophore, respectively, K_D is the Stern−Volmer quenching constant, and [Q] is the quencher concentration.

Vibration Spectroscopy. The infrared spectra of the solid samples were recorded at room temperature for characterization. A Perkin-Elmer 1720 X spectrometer with DTGS detector and Mylar beam splitter was used to record the mid-IR spectra in the range 400−4000 cm^{-1}. The solid samples were measured as KBr pastilles containing 5−10 wt % sample. In each spectrum, 200 scans were collected and averaged at 4 cm^{-1} resolution.

Mass Spectroscopy. Mass spectra were obtained using MS-1 of a JEOL JMS-SX/SX102A (JEOL, Japan) tandem mass spectrometer operating at an accelerating voltage of 10 kV and equipped with JEOL's XMS software. Electron ionization (EI) mass spectra were recorded

using a direct probe with evaporation of the samples into the ion source kept at ca. 475 K and an electron beam energy of 70 eV. High-resolution (HREI) measurements were made at a resolution of 5000 and using perfluorokerosene as internal standard.

NMR Spectroscopy. ^{1}H NMR spectra were recorded on a DRX400 Bruker Avance spectrometer operating at 400.13 MHz and 303 K. A pulse angle of 30° was used in all experiments, the pulse repetition time was 5.0 s, and the number of scans was 32. Deuterated dimethyl sulfoxide (Me$_2$SO-d_6, 99.9%), ethanol (C$_2$D$_5$OD-d_6, 99.9%), and water (D$_2$O-d_2, 99.9%) were used as solvents.

Results

Vibration Spectroscopy. The IR spectra of the solid alkyl-*N*-iminodiacetic acids in the neutral zwitterionic form are given in Figures 1 and S1. The C−H stretching vibrations in the aliphatic chain fall in the region 3000−2850 cm^{-1}, and absorption bands in the region 3700−3100 cm^{-1} are ordinarily due to various O−H and N−H stretching vibrations.[20,21] However, strong hydrogen bonding tends to broaden the peaks and move them toward lower wavenumbers.[6] The crystallographic characterization of the alkyl-*N*-iminodiacetic acids shows the presence of a short strong intermolecular hydrogen bond, see below.

It has been shown that there is a semiquantitative relationship between the stretching frequency ν(O−H) and the O−H and O(−H)···O distances in a hydrogen bond.[6] According to this relationship, the observed oxygen−oxygen distances around 2.46 Å in the alkyl-*N*-iminodiacetic acids should be in conformity with a ν(O−H) stretching frequency down to 1200−750 cm^{-1}. The spectra of *n*-hexyl-, *n*-dodecyl-, and *n*-octadecyl-*N*-iminodiacetic acid show a broad unsymmetric peak at ca. 730 cm^{-1}, which consists of a fairly sharp band at ca. 730 cm^{-1} and broader band at ca. 720 cm^{-1}, as seen from the second derivatives of the spectra (Figure S2). The band at 730 cm^{-1} is assigned to unbranched alkyl chains longer than four methylene groups,[21] and the 720 cm^{-1} band is assigned to the ν(O−H) stretching vibration (Figures 1, S1, and S2). There is also a very broad

(15) Almgren, M.; Griezer, F.; Thomas, J. K. *J. Am. Chem. Soc.* **1979**, *101*, 279.
(16) Lianos, P.; Viriot, M.-L.; Zana, R. *J. Phys. Chem.* **1984**, *88*, 1098.
(17) (a) Zhao, C.-L.; Winnik, M. A.; Riess, G.; Croucher, M. D. *Langmuir* **1990**, *6*, 514. (b) Wang, Y.; Winnik, M. A. *Langmuir* **1990**, *6*, 1437.
(18) Kalyanasundaram, K.; Thomas, J. K. *J. Am. Chem. Soc.* **1977**, *99*, 2039.
(19) Lakowicz, J. R. *Principles of Fluorescence Spectroscopy*; Plenum Press: New York, 1983.

(20) Williams, D. H.; Fleming, I. *Spectroscopic Methods in Organic Chemistry*, 3rd ed.; McGraw-Hill Book Company (U.K.) Limited: London, 1980; Chapter 2. Criddle, W. J.; Ellis, G. P. *Spectral and Chemical Characterization of Organic Compounds*, 2nd ed.; John Wiley & Sons: Chichester, 1980; Chapter 2.
(21) Skoog, D. A. *Principles of Instrumental Analysis*, 3rd ed.; CBS College Publishing: Philadelphia, 1985; p 349.
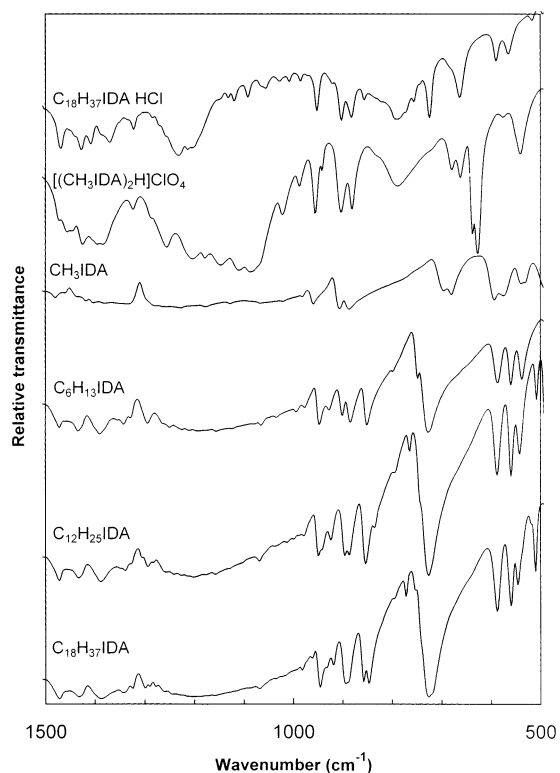
Figure 1. Infrared spectra, 2000−500 cm⁻¹, of solid methyl-, hexyl-, dodecyl-, and octadecyl-*N*-iminodiacetic acids and solid hydrogenbis(methyl-*N*-iminodiacetic acid) perchlorate.

band just below 1200 cm^{-1} which may be assigned as the ν(O−H) stretching vibration. In the case of methyl-*N*-iminodiacetic acid, the ν(O−H) stretching band seems to be shifted toward an even lower wavenumber, 698 cm^{-1}, the lowest value reported so far. This may indicate that the hydrogen bonding in methyl-*N*-iminodiacetic acid is somewhat stronger than that in the alkyl-*N*-iminodiacetic acids with longer alkyl chains. Somewhat higher ν(O−H) stretching wavenumbers are found for hydrogenbis(methyl-*N*-iminodiacetic acid) perchlorate, 789 cm^{-1}, and *n*-octadecyl-*N*-iminodiacetic acid hydrochloride, 794 cm^{-1}, as well as for ethylenediaminetetraacetic acid, 802 cm^{-1} (Figure S2).[6]

Crystal Structures. Description of the Crystal Structures of Alkyl-*N*-iminodiacetic Acids. The crystal structures of the methyl-, *n*-hexyl, and *n*-dodecyl-*N*-iminodiacetic acids have been determined crystallographically, while it has only been possible to accurately determine the unit cell parameters of *n*-octadecyl-*N*-iminodiacetic acid; the crystallographic data are summarized in Table 2. All compounds form colorless crystals, with those of methyl-*N*-iminodiacetic acid as blocks, and those of *n*-hexyl-*N*-iminodiacetic acid as prisms or octahedrons, while the crystals of *n*-dodecyl- and *n*-octadecyl-*N*-iminodiacetic acid are very thin and fragile flakes. The single-crystal X-ray diffraction study has shown that the alkyl-*N*-iminodiacetic acids crystallize in their zwitterionic form with one carboxylic acid group deprotonated and the nitrogen protonated into an ammonium ion. The molecules are oriented with the iminodiacetic acid groups in the *a*−*b* plane and the alkyl chain parallel with the *c*-axis. This causes the length of the *c*-axis to increase linearly with increasing alkyl chain length according to $c(\text{Å}) \approx 13.3 + 2.4n$, where *n* is the number of carbons in the saturated linear alkyl chain.
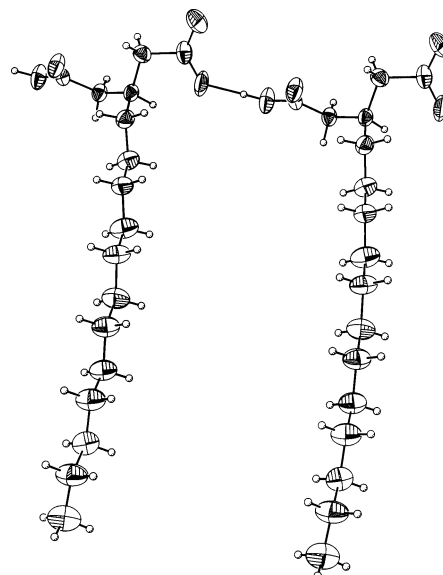

Figure 2. Structure of zwitterionic *n*-dodecyl-*N*-iminodiacetic acid, polymer form.

Table 3. Hydrogen Bond Distances (Å) and Angles (deg) of Alkyl-*N*-iminodiacetic Acids (C$_n$H$_{2n+1}$−IDA, *n* Is Number of Carbon Atoms in the Aliphatic Chain) Obtained from the Crystallographic Study

compound	D−H···A	d(D−H)	d(H···A)	d(D···A)	∠(DHA)
CH$_3$−IDA	N(1)−H(6)···O(2)#1	0.98(4)	2.03(4)	2.857(4)	141(3)
	O(4)−H(44)···O(2)#2	1.08(6)	1.45(6)	2.480(4)	157(4)
C$_6$H$_{13}$−IDA	N(1)−H(16)···O(1)#1	1.10(5)	1.72(5)	2.782(5)	160(4)
	O(4)−H(44)···O(2)#1	1.08(−)^a	1.42(−)	2.464(4)	160
C$_{12}$H$_{25}$−IDA	N(1)−H(16)···O(1)#1	1.08(6)	1.75(6)	2.792(5)	162(4)
	O(4)−H(44)···O(2)#2	1.08(−)^a	1.41(−)	2.455(6)	160
(CH$_3$−IDA)$_2$-	N(1)−H(6)···O(7)#2	0.95(5)	2.16(5)	2.930(8)	137(4)
HClO$_4$	O(2)−H(22)···O(2)#3	1.228(3)^b	1.228(3)	2.456(6)	180.000(1)
	O(4)−H(44)···O(1)	1.00(2)	1.57(2)	2.558(5)	173(5)

^{a} The position of the proton is estimated from the ν(O−H) stretching vibration frequency, and the relationship between the O(−H)···O distance, O−H bond distance, and ν(O−H) stretching vibration frequency in ref 6. ^{b} The position of the proton is forced to be in the center of symmetry, while in reality the proton is equally distributed ca. 1.1 Å from one of the carboxylic oxygens according to the ν(O−H) stretching vibration frequency.

The growth of the crystals into thin and brittle flakes directly reflects the layered lamellar buildup of the structure. The basic building block is a polymer chain of zwitterionic units linked together with an exceptionally short and strong intermolecular hydrogen bond between two carboxylic groups, one protonated and one unprotonated, $d(\text{O}(-\text{H})\cdots\text{O}) = 2.455-2.480$ Å (Figure 2 and Table 3). The proton in this strong hydrogen bond was localized in methyl-*N*-iminodiacetic acid giving an O−H bond distance of 1.08(6) Å, and an O−H···O angle of 157(4)° (Table 3). The very strong hydrogen bond in the methyl-*N*-iminodiacetic acids is further supported by the very low wavenumber of the ν(O−H) stretching frequency, 698 cm^{-1} (Figure 1). The results from methyl-*N*-iminodiacetic acid are perfectly in line, as an extreme point, with the correlation between O(−H)···O distances, O−H bond distances, and ν(O−H) stretching frequencies given by Bertolasi et al.[6] It has, however, not been possible to localize the hydrogen position in the strong hydrogen bond in *n*-hexyl- and *n*-dodecyl-*N*-iminodiacetic acid. The O(−H)···O distances close to 2.46 Å and the very low wavenumbers of the ν(O−H) stretching frequency, ca. 720 cm^{-1}, show that the O−H bond distance in these compounds
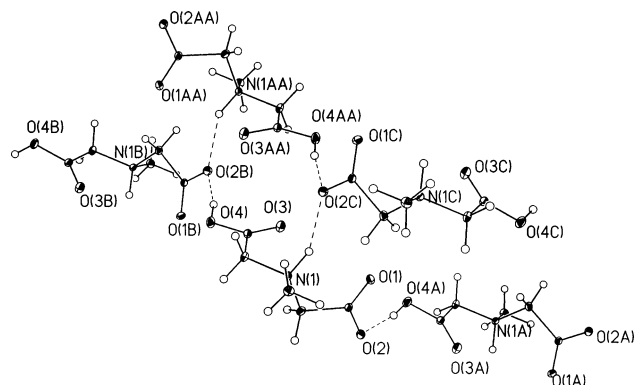
Figure 3. Structure of *n*-hexyl-*N*-iminodiacetic acid, packing model seen along the *c*-axis. The hydrogen bonds are marked with dashed lines.

is very close to that observed in methyl-*N*-iminodiacetic acid, and the carboxylic acid hydrogen position has been placed accordingly.

The hydrogen bond in the solid alkyl-*N*-iminodiacetic acids is among the shortest and strongest ever observed and is of the (−)CAHB (negative charge assisted hydrogen bond) type according to the classification made by Bertolasi et al.[6] The bond order in the two parts of the hydrogen bond can be estimated from the O−H bond distance and/or the ν(O−H) stretching vibration using a formula given by Bertolasi et al.[6] These bond orders have been calculated to ca. 0.65 and 0.35 for the O−H and H···O bonds, respectively, which is in total agreement with previous observations and calculations.[6]

The polymer chains of zwitterionic iminodiacetic acid units are cross-linked via other relatively strong hydrogen bonds between an ammonium group and an oxygen in a carboxylate group in a neighboring polymer chain of alkyl-*N*-iminodiacetic acid to form a two-dimensional network stabilizing the lamellar sheet structure, d(N(−H)···O) = 2.782−2.857 Å (Figure 3, Table 3). It is possible to distinguish the carboxyl hydroxo and carbonyl oxygen atoms in the carboxylic acid groups from the C−O bond distances, because they are significantly shorter in the carbonyl groups than in the carboxyl hydroxo groups (Tables S3, S5, and S7).

The alkyl chains are packed in such a way that two adjacent alkyl chains in a polymer chain are closer together in a "pair" to give space for another interdigitating "alkyl chain pair" coming from the opposite direction in the double sheet (Figure 4). The "paired" alkyl chains have a dumbbell shape, clearly seen in dodecyl-*N*-iminodiacetic acid (Figure S3), to optimize the van der Waals forces, as the distance between two nitrogen anchors is longer than the ideal van der Waals distance between two alkyl chains. A mismatch between the imino-*N*-diacetic acid and the alkyl chain areas causes a tilted structure (Figures 5 and S4).[3] By viewing the alkyl chains along the *a*-axis, perpendicular to the polymer chain, we find that the alkyl chains are arranged with every other chain coming from above and below, respectively (Figures 5 and S4).

The very strong hydrogen bonds along the alkyl-*N*-imino-diacetic acid polymer chain, the hydrogen bonds cross-linking these polymer chains, and the very efficient packing of the alkyl chains at van der Waals distance make the structure strong and rigid in the *a*−*b* plane (Figures 2 and 4). The forces parallel with the *c*-axis between two neighboring bilayers are due to weak van der Waals interactions (Figure 4). The crystal growth
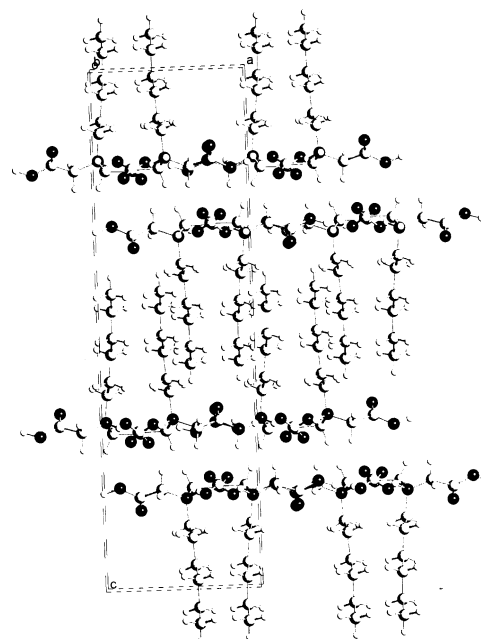

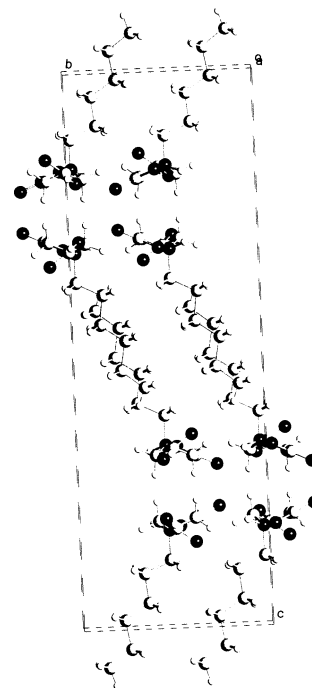**Figure 4.** Structure of *n*-hexyl-*N*-iminodiacetic acid seen along the *b*-axis.

Figure 5. Structure of *n*-hexyl-*N*-iminodiacetic acid seen along the *a*-axis.

directions are certainly in the *a*−*b* plane. In the alkyl-*N*-iminodiacetic acids with a short alkyl chain, the stabilization of the layers is weaker, and no tendency to form flake-shaped crystals is observed. It shall, however, be stressed that the principal buildup of the crystals of alkyl-*N*-iminodiacetic acids is maintained also for analogues with short alkyl chains. The atomic parameters and selected bond distances and angles are summarized in Tables S2−S7.

Hydrogenbis(methyl-*N*-iminodiacetic Acid) Perchlorate. The hydrogenbis(methyl-*N*-iminodiacetic acid) perchlorate crystallizes as rhombohedrally shaped colorless crystals in the triclinic space group $P\bar{1}$ (No. 2). The principal buildup of the structure is similar to the methyl-*N*-iminodiacetic acid but with
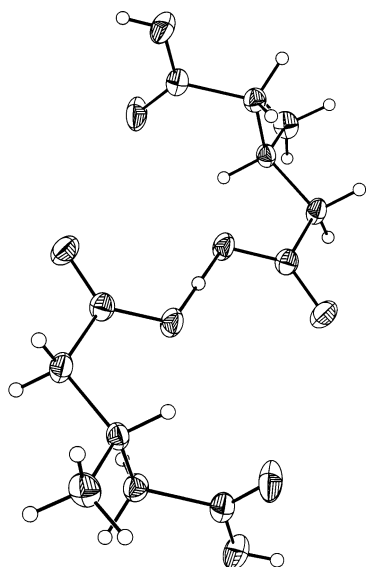
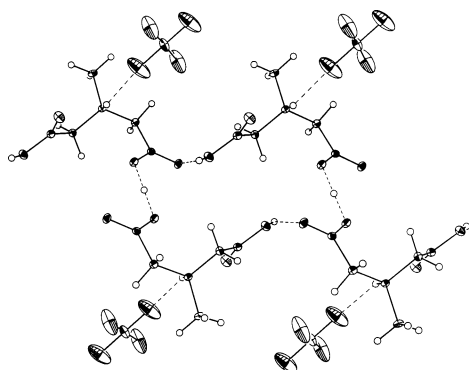**Figure 6.** Structure of hydrogenbis(methyl-*N*-iminodiacetic acid) perchlorate, dimeric form.

Figure 7. Structure of hydrogenbis(methyl-*N*-iminodiacetic acid) perchlorate, packing model seen along the *c*-axis. The hydrogen bonds are marked with dashed lines.

Figure 8. pH titrations of alkyl-*N*-iminodiacetic acids in aqueous solutions. The arrows show the titration direction. (a) 0.4 mmol dm^{-3} of *n*-octadecyl-*N*-iminodiacetic acid (no supporting electrolyte), (b) 0.4 mmol dm^{-3} of *n*-octadecyl-*N*-iminodiacetic acid ($I = 0.1$), (c) 1.0 mmol dm^{-3} of dodecyl-*N*-iminodiacetic acid (no supporting electrolyte), (d) 1.0 mmol dm^{-3} of dodecyl-*N*-iminodiacetic acid ($I = 0.1$), (e) 20 mmol dm^{-3} of hexyl-*N*-iminodiacetic acid ($I = 0.1$), (f) 10 mmol dm^{-3} of methyl-*N*-iminodiacetic acid ($I = 0.1$).

formation of dimers held together with a very strong hydrogen bond between the carboxylate groups in two zwitterionic units sharing an extra proton, $d(O(-H)\cdots O) = 2.456(6)$ Å (Figure 6 and Table 3). The position of the proton is forced to be at the center of symmetry, while in reality the proton is equally distributed ca. 1.08 Å from one of the carboxylic oxygens according to the $\nu(O-H)$ stretching vibration frequency and the correlation given by Bertolasi et al.[6] The hydrogen bond linking the two zwitterionic methyl-*N*-iminodiacetic acid units is indeed very strong as seen by the very small wavenumber of the $\nu(O-H)$ stretching vibration, 789 cm^{-1} (Figures 1 and S2b). The hydrogen bond distance $O(4)-H(44)\cdots O(1)$ linking the dimers is fairly short and strong, 2.558(5) Å (Table 3). Furthermore, there is a relatively weak hydrogen bond between the protonated nitrogen and a perchlorate oxygen, $d(N(-H)\cdots O) = 2.930(8)$ Å, holding the layers of hydrogenbis(methyl-*N*-iminodiacetic acid) dimers and the interlaying perchlorate ions together in the three-dimensional lattice (Figure 7). All hydrogen bond distances are summarized in Table 3, and the atomic parameters and selected bond distances and angles are summarized in Tables S8 and S9.

Potentiometry. The pH titrations of the methyl-, *n*-hexyl-, *n*-dodecyl-, and *n*-octadecyl-*N*-iminodiacetic acid systems were straightforward, and the calculations of the acidic constants from
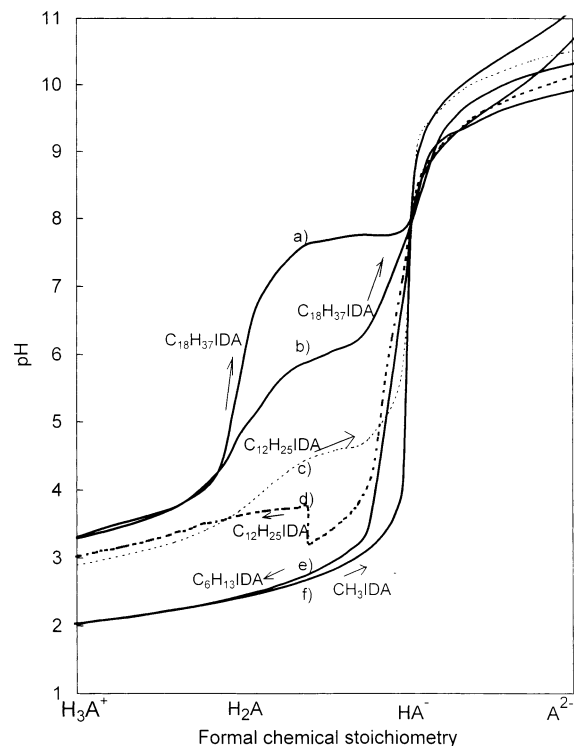
experimentally obtained pH titration functions did follow theory using the ion product of water at the applied ionic strength and medium, 0.1 mol dm^{-3} of sodium perchlorate, pK_w = 13.79.[22] However, some data points immediately before the second equivalence point had to be deleted for a good fit (Figure 8). The solutions of alkyl-*N*-iminodiacetic acids with short alkyl chain were clear before, during, and after the titrations, the *n*-dodecyl-*N*-iminodiacetic acid solutions were turbid up to ca. pH 7, but above the second equivalence point they were clear, while the *n*-octadecyl-*N*-iminodiacetic acid solutions were turbid throughout the titrations. The correct stoichiometry in the titrations with alkyl-*N*-iminodiacetic acids in aggregate form shows that the aggregates most probably consist of individual hydrated bilayers and that the mean size of the aggregates seems to increase with increasing alkyl chain length, thus increasing the mechanical stability of the bilayers.

The pH titrations of methyl-*N*-iminodiacetic acid from low pH with sodium hydroxide and from high pH with perchloric acid are in complete coherence. Also, the titrations of the *n*-hexyl-*N*-iminodiacetic acid system are also in coherence with the exception of a somewhat smeared-out region below the second equivalence point around pH 6. The acidic constants of the methyl- and hexyl-*N*-iminodiacetic acids are as those expected for an iminodiacetic acid,[23] pK_{a1} = 1.7 and pK_{a2} =

(22) Maeda, M.; Hisada, O.; Kinjo, Y.; Ito, K. *Bull. Chem. Soc. Jpn.* **1987**, *60*, 3233.
(23) *IUPAC Stability Constants Database*; Academic Software: Sourby Old Farm, Timble, Otley, Yorks, LS21 2PW, United Kingdom.

ARTICLES

Häggman et al.

2.3, and with a pK_{a3} value for ammonium group of 9.6 and 10.4 for methyl- and *n*-hexyl-*N*-iminodiacetic acid, respectively (Table 1).

The titrations with *n*-octadecyl-*N*-iminodiacetic acid with sodium hydroxide were performed at different ionic strength to study how an aggregated system is affected by the presence of salt. Titrations with acid have only been performed at the standard ionic medium in this study, 0.1 mol dm^{-3} of sodium perchlorate. The time to reach equilibrium is very long, ca. 2 h, and the reproducibility of the octadecyl-*N*-iminodiacetic acid system is lower than that for the other systems studied. The results show that the first acidic constant, K_{a1}, is very acidic and any value could not be determined. The pH value of the prepared solutions with the composition *n*-octadecyl-*N*-imino-diacetic acid hydrochloride, [C$_{18}$H$_{37}$—N(CH$_2$COOH)$_2$]·HCl, is equal to the logarithm of the molar concentration of the salt; thus, it behaves as a strong acid. The second acidic constant is substantially less acidic, pK_{a2} = 5.5−7.5 depending on the ionic strength, than that in the analogue monomer. The acidity of the ammonium group, K_{a3}, is in the same order of magnitude as that in the analogue monomer, and it is less sensitive to ionic strength than K_{a2} (Table 1). The slight ionic strength dependence on K_{a3} may indicate the same kind of intermolecular hydrogen bonding of the ammonium proton in *n*-octadecyl-*N*-imino-diacetic acid in aqueous suspensions in a similar way as found in the solid states of the alkyl-*N*-iminodiacetic acids (Table 1 and Figure 3).

As there is some kind of equilibrium/distribution between monomers and aggregates in the *n*-dodecyl-*N*-iminodiacetic acid system in water, it is not possible to determine any acidic constants as the concentrations of the monomeric and aggregate forms are not known, and it can be seen from the other systems that the acidic constants are strongly dependent on the state of aggregation. The titration function of *n*-dodecyl-*N*-iminodiacetic acid seems to be a mix of the titration functions of *n*-hexyl- and octadecyl-*N*-iminodiacetic acid (Figure 8). The obvious discontinuity in pH (Figure 8d) depends on partial precipitation of the zwitterionic *n*-dodecyl-*N*-iminodiacetic acid, seen as a significant turbidity. The precipitation in the titrations takes place at decreasing pH with decreasing concentration of alkyl-*N*-iminodiacetic acid.

Steady-State Fluorescence. III/I Ratios. When examining the ratio of the first and third vibrational bands of the pyrene monomer, III/I, in *n*-hexyl-*N*-iminodiacetic acid at pH$_{ie}$ ≈ 2.0, we find that the ratio is constant at 0.594(5) in the concentration range 1.5−150 mmol dm^{-3} (Figure 9), revealing that pyrene does not sense a polar environment different from that of water. It does not exclude the possibility of aggregate formation, but these then do not have sufficiently large hydrophobic dimensions to dissolve pyrene.

The III/I ratio of pyrene in neutral and alkaline solutions of *n*-dodecyl-*N*-iminodiacetate was examined. Up to ca. 1 mmol dm^{-3} of *n*-dodecyl-*N*-iminodiacetate, the III/I ratio is constant at 0.598(8) and thereafter is increasing up to 10 mmol dm^{-3}. Above 10 mmol dm^{-3} in the neutral solutions of *n*-dodecyl-*N*-iminodiacetate, the III/I ratio is constant at ca. 0.85, but in the alkaline *n*-dodecyl-*N*-iminodiacetate solutions, the pyrene emission is almost completely quenched. Thus, at neutral pH and higher concentrations than 10 mmol dm^{-3}, *n*-dodecyl-*N*-iminodiacetate seems to form hydrophobic aggregates large
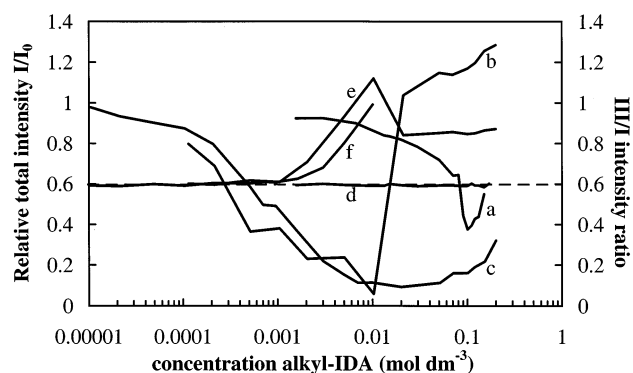

Figure 9. Relative total intensities, I/I_o (a−c), and III/I intensity ratios (d−f) from the pyrene fluorescence spectrum versus concentration of alkyl-*N*-iminodiacetic acid. (a) and (d) *n*-hexyl-*N*-iminodiacetic acid at isoelectric pH; (b) and (e) *n*-dodecyl-*N*-iminodiacetate in neutral pH solutions; (c) and (f) *n*-dodecyl-*N*-iminodiacetate in alkaline pH solutions. The III/I ratio of aqueous pyrene is inserted for comparison (dashed line). The arrows show corresponding y-axis.

enough to dissolve pyrene seen from the small but significant difference in the III/I ratio. In the alkaline *n*-dodecyl-*N*-iminodiacetate, the poor pyrene emission is not typical of that found in a well-defined hydrophobic moiety, for example, in micelles.

Changes in Total Emission Intensity. In the *n*-hexyl-*N*-iminodiacetic acid system, the fluorescence emission is decreasing with increasing concentration up to 100 mmol dm^{-3}, and thereafter it increases up to the solubility limit at ca. 150 mmol dm^{-3} at pH 2.0. In terms of the Stern−Volmer equation, there is linearity in I_o/I up to 80 mmol dm^{-3} of *n*-hexyl-*N*-iminodiacetic acid, suggesting a collisional quenching by the monomeric form. The intensity is slightly increasing above 100 mmol dm^{-3}, and the fine structure in the pyrene monomer spectrum is well developed, which may indicate that aggregates are formed. The negative slope from the Stern−Volmer equation has not a physical interpretation but indicates that the local environment is different and shielding the probe from quenching and enhancing probe emission.

When examining the intensity variations with concentration of *n*-dodecyl-*N*-iminodiacetate, we find that the intensity decreases up to 10 mmol dm^{-3} in both neutral and alkaline solutions and follows the Stern−Volmer equation for collisional quenching in this concentration range. The emission above 10 mmol dm^{-3} of *n*-dodecyl-*N*-iminodiacetate differs completely for the neutral and alkaline solutions. In neutral *n*-dodecyl-*N*-iminodiacetate, there is an abrupt jump to intensities higher than that for the unquenched aqueous pyrene, and a steadily increasing gain in emission intensity with concentration is seen. In alkaline *n*-dodecyl-*N*-iminodiacetate, the pyrene emission is almost completely quenched. Thus, at neutral pH and concentrations above 10 mmol dm^{-3}, *n*-dodecyl-*N*-iminodiacetate is mainly present in aggregate form, while it is impossible to determine the state of aggregation in alkaline dodecyl-*N*-iminodiacetate. The results from the steady-state fluorescence experiments are displayed in Figure 9.

Amounts of Alkyl-*N*-iminodiacetic Acid Possible To Dissolve in Water. The amounts of alkyl-*N*-iminodiacetic acid possible to dissolve in water at different pH values have been determined. It is possible to dissolve methyl-*N*-iminodiacetic acid in molar concentrations, in water at the isoelectric pH (2.0). On the other hand, only small amounts of methyl-*N*-imino-

Table 4. Fragmentation Pattern of Alkyl-*N*-iminodiacetic Acids (C_nH_{2n+1}–IDA) Obtained in MS; *n* Is Number of Carbon Atoms in the Aliphatic Chain[a]

fragment	CH₃–IDA	C₆H₁₃–IDA	C₁₂H₂₅–IDA	C₁₈H₃₇–IDA
$-\cdot COOH$	102(100)	172(100)	256(100)	340(100)
$-\cdot COOH-CO$	74(45)	144(2)	228(1)	312(1)
$-\cdot COOH-CO-CH_2O$	44(62)	114(2)	198(5)	282(6)
$-\cdot COOH-CO-\cdot OH$	57(54)	127(8)	211(2)	295(20)
$-\cdot COOH-COO$	58(14)	128(48)	212(5)	296(13)
$-C_{n-1}H_{2n-1}$	146(0)	146(62)	146(68)	146(55)
$-H_2O$	129(7)	199(6)	283(1)	367(5)

[a] Molar weight and height (in parentheses) are given.

diacetic acid, low mmol dm⁻³ range, are possible to dissolve in ethanol, thus giving a factor of around 1000 in difference between water and ethanol.

The solubility of *n*-hexyl-*N*-iminodiacetic acid at pH 2.04, close to the isoelectric point, is 0.15 mol dm⁻³, which at elevated temperatures approaches the molar range, reflecting the entropy effect. Addition of a half equivalent of sodium hydroxide (pH 2.59) increases the solubility to 0.23 mol dm⁻³. A highly viscous gel phase is formed at concentrations exceeding 2.5 mol dm⁻³ and at room temperature. This gel phase is maintained above pH 7 at similar concentrations.

The solubility of the neutral zwitterionic form of *n*-dodecyl-*N*-iminodiacetic acid is in the submillimolar range. As for *n*-hexyl-*N*-iminodiacetic acid, the amount of *n*-dodecyl-*N*-iminodiacetic acid possible to dissolve increases dramatically in solutions with neutral pH, ca. 1.4 mol dm⁻³, giving a stiff gellike phase.

The amounts of *n*-octadecyl-*N*-iminodiacetic acid possible to dissolve is in the submillimolar range in the entire pH range, reflecting the dominating hydrophobic effect present due to the long hydrocarbon chain. This is supported by the fact that the amounts possible to dissolve in ethanol are higher than those in water, while the opposite trend is observed for methyl-*N*-iminodiacetic acid as mentioned above.

The low solubility of the neutral zwitterionic form at pH$_{ie}$ was used at the purification of the synthesized material. The resulting products are stunningly pure as found from NMR and mass spectroscopy, and elemental analyses.

Mass Spectroscopy. The obtained fragmentation patterns are presented in Table 4 and Figure S7. In all mass spectra, the largest peak is obtained when one carboxylate radical is split off from the alkyl-*N*-iminodiacetic acid, and this peak is normalized and set to a value of 100. The fragmentation pattern of methyl-*N*-iminodiacetic acid is different in comparison to the other alkyl-*N*-iminodiacetic acids, probably due to the shorter hydrocarbon chain.

NMR Spectroscopy. ^{1}H NMR measurements were performed in the deuterated solvents dimethyl sulfoxide (Me₂SO-*d₆*), ethanol (C₂D₅OD-*d₆*), and/or water (D₂O-*d₂*). The spectrum of *n*-octadecyl-*N*-iminodiacetic acid is lacking several expected bands in water and in dimethyl sulfoxide as observed in the other alkyl-*N*-iminodiacetic acid systems, probably due to low solubility. Therefore, NMR data of *n*-octadecyl-*N*-iminodiacetic acid are only presented in the ethanol, the solvent with sufficiently high solubility of *n*-octadecyl-*N*-iminodiacetic acid. The lowest chemical shifts are observed in the aprotic solvent dimethyl sulfoxide, and the highest values are observed in the protic ethanol. These results do not follow the expected trend related to the polarity of solvents. Because of the very low

solubility of *n*-dodecyl- and *n*-octadecyl-*N*-iminodiacetic acids, ^{13}C NMR data are too noisy and weak to gain aggregational information. The ^{1}H NMR data are summarized in Table S1.

Discussion

The Crystal Structures. The short strong hydrogen bond, causing a polymerization of the alkyl-*N*-iminodiacetic acids in their zwitterionic form, has an amazing impact on self-aggregation, crystallization, and physical-chemical behavior as observed by several techniques. This hydrogen bond is of negative charge assisted hydrogen bonding ((−)CAHB) character[6] linking two molecules through a strong hydrogen bond resulting in a very short O−(H)···O distance, including a long O−H distance as found from the crystallographic study of alkyl-*N*-iminodiacetic acids, as well as from the extremely low wavenumbers of the ν(O−H) stretching vibration, ca. 720 cm⁻¹, as observed in the IR spectra (Figure 1). The alkyl chains in the alkyl-*N*-iminodiacetic acids are highly ordered, being parallel to each other in a polymer chain. The van der Waals forces between the alkyl chains are fairly strong as the alkyl chains are densely packed, giving them a dumbbell shape as the distance between the nitrogen anchors is longer than the ideal van der Waals distance between two alkyl chains (Figures 4 and S2).

It is evident that the primary ordering of polymer chains is made into two-dimensional layers by cross-linking hydrogen bonds. From the crystal structures, it is shown that the two-dimensional structure is bilayers where the alkyl chains from the layers are interdigitating each other and the zwitterionic headgroups make up the surfaces of a very well-ordered lamellar structure. The headgroups have the same structural form and size independent of the length of the alkyl chain. The thickness of the bilayer and the length of the *c*-axis are a direct function of the length of the alkyl chain (vide infra).

pH Dependence on Aggregation Studied by Steady-State Fluorescence. Aggregated structures of *n*-hexyl-*N*-iminodiacetic acid at isoelectric pH are probably formed in water above 100 mmol dm⁻³ when interpreting the increase in the total emission as shielding from quenching. However, the aggregates are not thick enough to dissolve pyrene in a hydrophobic environment because the III/I ratio does not shift to higher values. It is concluded that the *n*-dodecyl-*N*-iminodiacetic acid system is mainly monomeric up to ca. 1 mmol dm⁻³ where aggregation takes place in the neutral solution as found from the high emission intensity and the shifted III/I ratio at concentrations above 10 mmol dm⁻³, which is not observed for the alkaline *n*-dodecyl-*N*-iminodiacetic acid. In *n*-dodecyl-*N*-iminodiacetate in alkaline solution, there is an extremely quenched pyrene signal above 10 mmol dm⁻³, most possibly due to quenching by the completely deprotonated dodecyl-*N*-iminodiacetate. However, it is impossible to draw any definite conclusions as quenching might occur in both unaggregated and aggregated systems.

The fluorescence measurements show no synergy effects of an oligomerization leading to lowered critical aggregation concentrations. The observed critical aggregation concentrations, ca. 100 mmol dm⁻³ for *n*-hexyl-*N*-iminodiacetic acid at isoelectric pH and ca. 10 mmol dm⁻³ for neutral *n*-dodecyl-*N*-iminodiacetic acid, are reasonable relative to the critical micelle

concentration of sodium n-hexyl sulfate (0.42 mol dm^{-3}) and sodium n-dodecyl sulfate (8.2 mmol dm^{-3}).[24]

Protolytic Properties of Alkyl-N-iminodiacetic Acids in Water. The determination of the acidic constants of the alkyl-N-iminodiacetic acids in aqueous solution clearly shows two principal cases. First, the short-chained alkyl-N-iminodiacetic acids (methyl- and n-hexyl-N-iminodiacetic acid) are present predominantly as monomers in aqueous solution. The other clear principal case is the long-chained alkyl-N-iminodiacetic acids (n-octadecyl-N-iminodiacetic acid, and to some extent also n-dodecyl-N-iminodiacetic acid), which are present mainly as aggregates, and the monomer concentration is negligible. The alkyl-N-iminodiacetic acids behave in both cases as ordinary three-protonic acids in titrations with strong acid or base but with the exception that the kinetics to reach stable pH values in solutions containing aggregates are very slow. However, the results in the two cases are significantly different and will be discussed separately: (a) The acidic constants of the methyl- and n-hexyl-N-iminodiacetic acids (Table 1) are in coherence with literature data, respectively,[23] and the acidic constants are those expected for compounds with ammonium and acetic acid groups.[23] (b) The first acidic constant, K_{a1}, of n-octadecyl-N-iminodiacetic acid in aggregate form is more acidic than that in monomeric forms of alkyl-N-iminodiacetic acids and acts as a strong acid, while the second carboxylic acid proton, participating in the short strong hydrogen bond, acts as a much weaker acid than that in the analogue monomers (Table 1). This strongly indicates that the short strong hydrogen bonds linking the iminodiacetic acid units into polymer chains in the solid state are maintained in aggregates in aqueous systems. It is very difficult to protonate the carboxylate group participating in the short strong hydrogen bond, and the acidity of this group, K_{a1}, becomes very strong. The proton participating in the short strong hydrogen bond, K_{a2}, is very much stabilized, and its acidity properties decrease therefore substantially, ca. 10^5 times, in comparison to the corresponding proton in alkyl-N-iminodiacetic acids present as monomers (Table 1). Furthermore, the K_{a2} value is unusually dependent on ionic strength with the acidity increasing with increasing ionic strength. It is well known that the stability of colloids decreases with increasing ionic strength,[25] and the increasing acidity of the strongly hydrogen bound proton with increasing ionic strength is in line with this fact. The acidic properties of the ammonium group, K_{a3}, seem to be fairly independent of the state of aggregation of the alkyl-N-iminodiacetic acids (Table 1).

These results show that short strong hydrogen bonds can be present in aqueous systems if this hydrogen bond is supported by a very strong backbone structure, in this case in the form of long interdigitating alkyl chains and hydrogen bonds within the surfaces of the aggregates consisting of iminodiacetic acid groups. The shorter the alkyl chain is, the less stable the interdigitating bilayer becomes. There are weak indications of aggregate formation in n-hexyl-N-iminodiacetic acid, while it is obvious in the n-dodecyl- and n-octadecyl-N-iminodiacetic acid systems. The solubility of n-dodecyl- and n-octadecyl-N-iminodiacetic acid in water is very low, and/or very slow, and

ultrasonication is required to get manageable concentrations, then in aggregate form, for titration experiments. As the proton stoichiometry in the aggregates is in accordance with the weighed amounts of iminodiacetic acid, this strongly indicates that the aggregates are present as individual bilayers where the iminodiacetic acid/iminodiacetate surfaces are strongly hydrated. For the n-dodecyl-N-iminodiacetic acid system, the steady-state fluorescence studies show that the fraction monomer form increases with pH and increasing charge of the headgroup. This shows that there is a fine balance between hydration and formation of monomers and aggregate formation with its hydrogen bonds in the surface and the van der Waals forces between the densely packed alkyl chains. The van der Waals forces in the n-octadecyl-N-iminodiacetic acid system are sufficiently strong to maintain the aggregate form, while for n-hexyl-N-iminodiacetic acid the hydration is sufficiently strong to break up the aggregates into monomers. As short strong hydrogen bonds seem to exist in aqueous systems if they are supported by, for example, a rigid structure as in the present systems, it is reasonable to believe that they can also be present in other strong and rigid structures as, for example, biomolecules, if the structural backbone support is strong enough to resist structural breakup due to hydration effects.

Conclusions

The alkyl-N-iminodiacetic acids form exceptionally strong intermolecular hydrogen bonds between neutral zwitterionic units forming polymer chains in the solid state; the O($-$H)$\cdots$O distance is in the range 2.45$-$2.48 Å, the O$-$H bond distance is ca. 1.08 Å, and the ν(O$-$H) stretching vibration is ca. 720 cm^{-1}. A protonated dimer, hydrogenbis(methyl-N-iminodiacetic acid), as perchlorate salt, has been structurally characterized in the solid state. This structure has also a short strong hydrogen bond as the neutral zwitterions of the alkyl-N-iminodiacetic acids. The acidic properties of monomeric alkyl-N-iminodiacetic acids (methyl and n-hexyl) follow those expected with pK_a values of about 1.7, 2.3, and 9.6/10.4. However, when present as larger aggregates in aqueous solution (n-octadecyl- and partly n-dodecyl-N-iminodiacetic acids), the first acidic constant is very strong, behaves as a strong acid, and no acidic constant could be determined, while the second acidic constant decreases substantially, ca. 5 orders of magnitude. The acidity property of the ammonium group, K_{a3}, is independent of the state of aggregation. This strongly indicates that the lamellar structure observed in the solid state most certainly remains in aggregates in aqueous systems and that the proton in the extremely strong hydrogen bond has weak acidic properties. This also shows that short strong hydrogen bonds exist in aqueous systems if the hydrogen bond is supported by a strong backbone structure, as the long interdigitating alkyl chains are in bilayers of alkyl-N-iminodiacetic acid systems.

(24) Aniansson, E. A. G.; Wall, S. N.; Almgren, M.; Hoffmann, H.; Kielmann, I.; Ulbricht, W.; Zana, R.; Lang, J.; Tondre, C. *J. Phys. Chem.* **1976**, *80*, 905.
(25) Atkins, P. W. *Physical Chemistry*, 6th ed.; Oxford University Press: Oxford, 1998; Chapter 20.9.

Supporting Information Available: Crystallographic data in CIF format. Tables S1−S9 give the NMR data of the synthesized alkyl-*N*-iminodiacetic acids, the fractional atomic coordinates, and interatomic bond distances and angles of *n*-dodecyl-, *n*-hexyl-, and methyl-*N*-iminodiacetic acid and hydrogenbis-(methyl-*N*-iminodiacetic acid). Figure S1 shows the mid-infrared spectra of *n*-dodecyl-, *n*-hexyl-, and methyl-*N*-iminodiacetic acid and hydrogenbis(methyl-*N*-iminodiacetic acid) and *n*-octadecyl-iminodiacetic acid hydrochloride, and with the second deriva-tives in Figure S2. Figures S3−S6 show the structures of the *n*-dodecyl-, *n*-hexyl-, and methyl-*N*-iminodiacetic acids along a direction different from those in Figures 2−7. The fragmentation pattern in mass spectroscopy of solid methyl-, *n*-hexyl-, *n*-dodecyl-, and *n*-octadecyl-*N*-iminodiacetic acid is given in Figure S7 (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

JA021012I