Thermodynamics of Protonation and Copper(II) Complex Formation of α-Aminomalonic Acid†

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The thermodynamic functions (ΔG°, ΔH°, and ΔS°) for the protonation of α-aminomalonic acid were determined in aqueous solution (25 °C, I = 0.1 mol dm^{-3} NaCl). The basicity constants and enthalpy changes were evaluated from potentiometric and calorimetric data for both the primary amino and the carboxylate groups. On the basis of the thermodynamic changes a closed structure is hypothesized with the protonated amino nitrogen hydrogen-bonded to both the carboxylate groups to form two five-membered rings. The protonation of the CO_2^- groups provokes opening of the rings. A relatively high stability constant for complexation with Cu^{2+} was determined, however in a narrow range of low pH. The co-ordination to the copper(II) occurs both with nitrogen and the two oxygen donor atoms, to form two five-membered chelate rings.

The thermodynamic functions relative to the protonation of asymmetric amino dicarboxylic acids, such as aspartic and glutamic acids, have been determined in aqueous solution together with their co-ordinating ability towards heavy metal ions.[1] By contrast, there have been few studies on protonation and complex formation of symmetric aminomalonic acids containing a primary amino group symmetrically positioned between the carboxylic groups.[2]

In two recent papers[3,4] we reported thermodynamic and spectroscopic studies on the protonation and complex formation with copper(II) of (3-aminopropyl)malonic acid NH_2-$CH_2CH_2CH_2CH(CO_2H)_2$ (H_2apmal) containing the primary amino group in the δ position of the malonic moiety. This synthetic compound was found to be resistant to decarboxylation even under acidic conditions and when co-ordinated to copper(II), unlike α-amino-α-alkylmalonic acids co-ordinated to cobalt(III).[5]

In view of our interest in the establishment of correlations between chemical structure and thermodynamic properties for this class of compounds, we wished to extend our studies to α-aminomalonic acid. The aminodiacid compound considered is a symmetric dicarboxylic acid having the primary amino group very close to the malonate moiety, $NH_2CH(CO_2H)_2$ (H_2amal). In an earlier report of Schwarzenbach *et al.*,[2] the acid–base behaviour and complexing ability of α-aminomalonic acid towards Zn^{II}, Ca^{II}, Mg^{II}, and Ba^{II} was described but only two basicity constants were evaluated at 20 °C by pH-metric measurements. In this paper we report additional information on the ligand structure at different pH values obtained by potentiometry and calorimetry at 25 °C. The complexation of copper(II) ions with α-aminomalonic acid was also investigated by the pH-metric method.

Results and Discussion

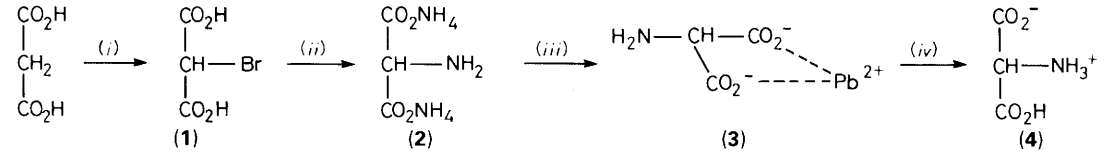
Synthesis.—α-Aminomalonic acid was synthesized in four steps (Scheme). The preparation of 2-bromomalonic acid (**1**) was accomplished with a fair yield by the Conrad and Reinbach procedure[6] and the remaining three steps by the Lutz sequence[7] to give the desired product (**4**).

† *Non-S.I. unit employed:* cal = 4.184 J.

The ^{13}C n.m.r. spectrum of compound (**4**) (Figure 1) reveals the presence of a signal at 46.7 p.p.m. due to the CH resonance and two signals at 183.8 and 180.4 p.p.m. attributed to the two symmetric carboxylic groups. Potentiometric analysis of the purity and elemental analysis are consistent with the proposed structure with one water for each α-aminomalonic acid molecule.

Protonation Studies.—The thermodynamic changes (ΔG°, ΔH°, and ΔS°) for the protonation of α-aminomalonate ($amal^{2-}$) in aqueous solution are reported in Table 1. For comparison the thermodynamic functions of $apmal^{2-}$ and glycinate ($glyO^-$) ions are included.

The first equilibrium concerns the protonation of the primary amino group, the second and third concern the two anionic carboxylate groups. The value of $\log K_1$ is similar to that of glycine,[1] but slightly lower due to the presence of two carboxylate groups in the α position instead of one as in glycine. The first enthalpy change is lower than that of glycine, aspartic and glutamic acids,[8] and $apmal^{2-}$.[4] Moreover the ΔS_1° value of $amal^{2-}$ is the highest of the series considered. All this can be explained by assuming, at the first step of protonation, a structure with two five-membered closed rings due to an internal neutralization of charges which involves both carboxylate groups. The formation of this compact structure with the associated decrease in net charge provokes a large liberation of water molecules which is responsible for the low $-\Delta H_1^{\circ}$ and high ΔS_1° values.[9] This assumption is supported by the energy-minimized structure[10] depicted in Figure 2. As far as the protonation of the first carboxylate is concerned, the thermodynamic quantities are in agreement with the above hypothesis of symmetrical molecular compactness. The $\log K_2$, corresponding to protonation of the first CO_2^- in the malonate moiety, is lower than that of free malonate and its alkyl derivatives[11] or the γ-aminoalkyl derivative as in $Hapmal^-$.[4] Furthermore an exothermic heat effect is observed, making $-\Delta H_2^{\circ}$ positive and very close to that of glycine[1] and the glycine-like aspartic and glutamic acids.[8] A different behaviour was observed for simple malonate[12] and its δ-aminoalkyl derivative,[4] the protonation of which leads to an endothermic heat effect. Generally the crude protonation of carboxylate groups is a slightly endothermic process,[8] but for some amino acids, like glycine and aspartic acid for which an electrostatic interaction between NH_3^+ and CO_2^- occurs, the



Scheme. (*i*) Br_2, OEt_2; (*ii*) 20% NH_3(aq), MeOH; (*iii*) $Pb(O_2CMe)_2$, $MeCO_2H$ (pH 4.5); (*iv*) $PbBr_2$, H_2S(g)

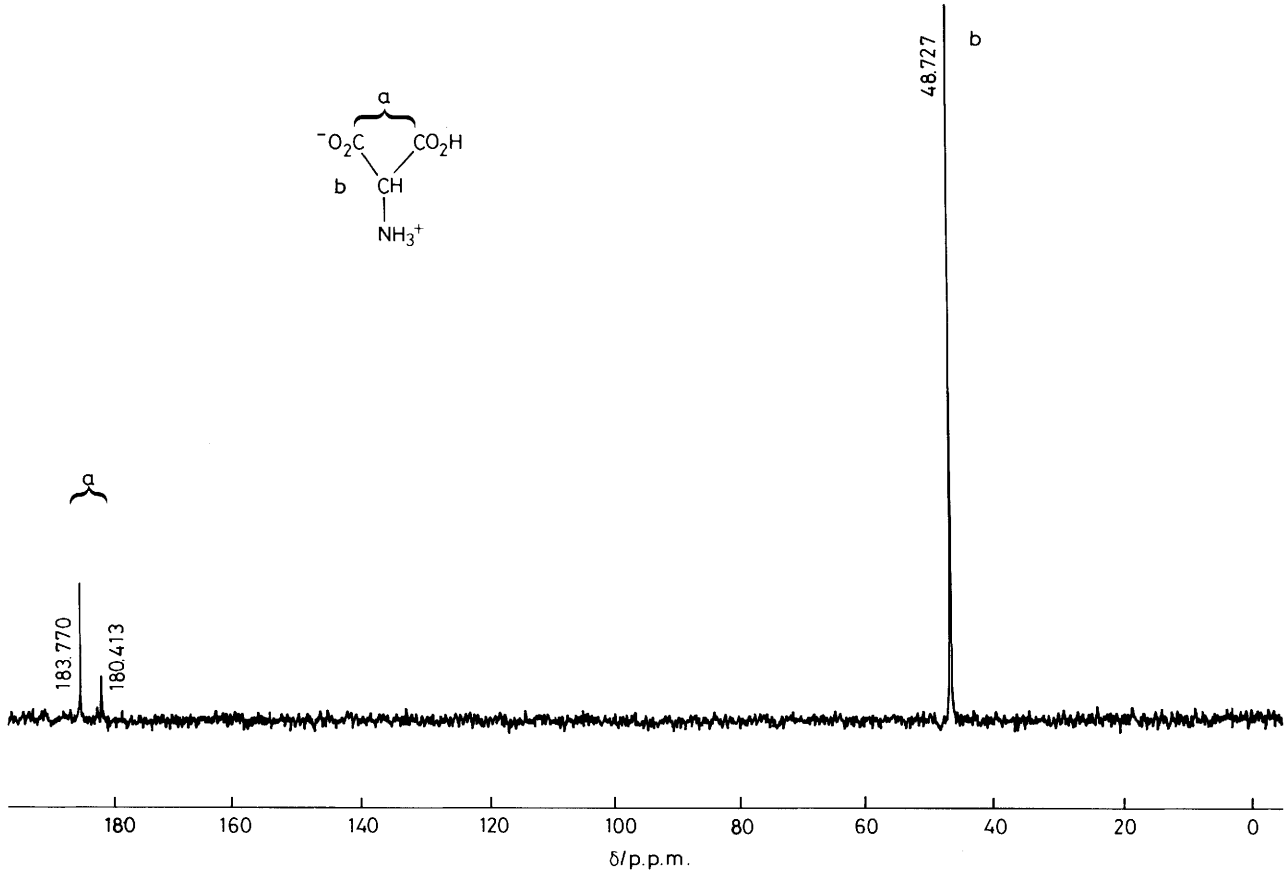


Figure 1. Carbon-13 n.m.r. spectrum of H_2amal in D_2O at 25 °C

Table 1. Stepwise thermodynamic functions for the protonation of $amal^{2-}$, $apmal^{2-}$, and glycinate ($glyO^-$) ions at 25 °C in 0.1 mol dm^{-3} NaCl. Values in parentheses are the estimated standard deviations of the last significant figure

Reaction step	log *K*	$-\Delta G^\circ$/kcal mol^{-1}	$-\Delta H^\circ$/kcal mol^{-1}	ΔS°/cal K^{-1} mol^{-1}	Ref.
$amal^{2-} + H^+ \rightleftharpoons Hamal^-$	9.216(4)	12.57(1)	8.40(9)	14.0(3)	This work
$Hamal^- + H^+ \rightleftharpoons H_2amal$	2.938(7)	4.01(1)	0.92(1)	10.37(3)	
$H_2amal + H^+ \rightleftharpoons H_3amal^+$	1.84(2)	2.51(3)	0.14(2)	8.0(1)	
$apmal^{2-} + H^+ \rightleftharpoons Hapmal^-$	10.448	14.24	12.23	6.7	4
$Hapmal^- + H^+ \rightleftharpoons H_2apmal$	4.863	6.63	−0.57	24.1	
$H_2apmal + H^+ \rightleftharpoons H_3apmal^+$	2.522	3.44	−0.10	12.0	
$glyO^- + H^+ \rightleftharpoons gly$	9.57	13.05	10.6	8.0	1
$gly + H^+ \rightleftharpoons Hgly^+$	2.36	3.22	1.0	8.0	

protonation of the carboxylate group also determines the opening of the ring, with a larger hydration of the two protonated (NH_3^+, CO_2H) groups. This leads to an exothermic contribution. Thus, during the protonation of the first CO_2^- group in $Hamal^-$, which is electrostatically linked to NH_3^+, the molecule loses its highly symmetrical compact structure and one of the two carboxylic groups is freed. Hence the diprotonated molecule is more hydrated. Breaking of an electrostatic interaction and the hydration of the molecule are exothermic processes which overcome the slightly endothermic effect of protonation of the CO_2^- group. The enthalpy change for the second protonation step in $Hapmal^-$, where no interaction between NH_3^+ and CO_2^- groups occurs, is endothermic.[4]

The third and last step of protonation of the second CO_2^- in H_2amal has a lower log K_3 than that of simple malonate and its alkyl or aminoalkyl derivatives.[4,11] The enthalpy change is close to zero indicating a lower influence of the protonated and hydrogen-bonded amino group. The calculation of the $-\Delta H_3^\circ$ value was possible only for a low degree of

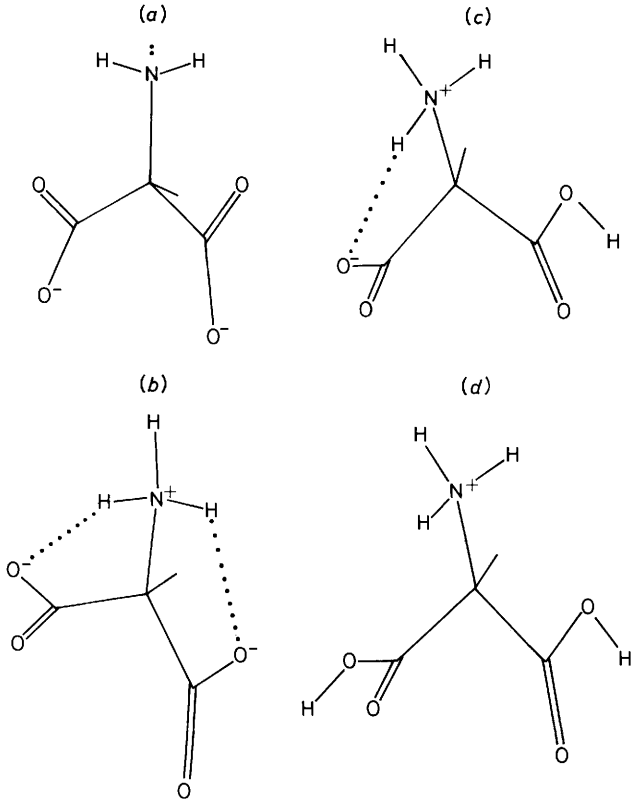


Figure 2. Molecular model of H_2amal in its minimum-energy conformation at different states of protonation: (*a*) unprotonated form ($amal^{2-}$); (*b*) monoprotonated form ($Hamal^-$); (*c*) diprotonated form (H_2amal); (*d*) triprotonated form (H_3amal^+). All minimizations were carried out following the gradient convergence and were continued until the root-mean-square derivative was below 0.01 kJ $Å^{-1}$

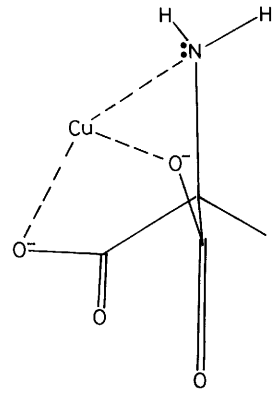


Figure 3. Molecular model of the [Cu(amal)] complex

protonation by titrating the H_2amal with 0.5 mol dm^{-3} hydrochloric acid to give low pH values. The ΔS_3° of $amal^{2-}$ is close to ΔS_2° indicating the same protonation mechanism in both steps. This means that we can exclude any interaction between carboxylic groups in the malonate moiety of α-aminomalonic acid, unlike in $apmal^{2-}$.

Copper(II) *Complex Formation.*—α-Aminomalonic acid is able to form a single stable complex with copper(II) of stoicheiometry [Cu(amal)]. This species is formed only under acidic conditions, its percentage is low (maximum 20%), and its existence is limited to a narrow pH range. The complete neutralization of charges in the complex-formation reaction $amal^{2-} + Cu^{2+} \rightleftharpoons$ [Cu(amal)], which renders the complex species completely neutral, may be a consequence of the lack of solubility of the complex in water, as occurs for the uncharged simple complex species in the copper(II)–aspartic acid system.[13] The relatively high stability constant (Table 2) suggests that co-ordination to Cu^{II} occurs through the amino nitrogen and both carboxylate groups (Figure 3). This hypothesis arises from the fact that simple glycine[14] and glycine-like compounds,[13] in which an amino nitrogen and only one carboxylate group are bound to the copper(II) ion, show lower stability. An analogous simple [Cu(apmal)] complex species was not found previously[4] because the amino group is too far from the malonic moiety to be involved in the co-ordination.

Table 2. Copper(II) complex-formation stability constants with $amal^{2-}$, $apmal^{2-}$, and $glyO^-$ ions at 25 °C in 0.1 mol dm^{-3} NaCl

Reaction	log β	Ref.
$Cu^{2+} + amal^{2-} \rightleftharpoons$ [Cu(amal)]	9.85(2)*	This work
$Cu^{2+} + Hapmal^- \rightleftharpoons$ [Cu(Hapmal)]$^+$	4.11	4
[Cu(Hapmal)]$^+ + Hapmal^- \rightleftharpoons$ [Cu(Hapmal)$_2$]	2.93	
[Cu(Hapmal)]$^+ + apmal^{2-} \rightleftharpoons$ [Cu(Hapmal)-(apmal)]$^-$	6.62	
$Cu^{2+} + glyO^- \rightleftharpoons$ [Cu(glyO)]$^+$	8.15	1

* Value in parenthesis is the standard deviation.

Experimental

Syntheses.—*2-Bromomalonic acid* (**1**). Bromine (16.0 g, 0.1 mol) was added dropwise to a well stirred and cooled mixture of malonic acid (10.4 g, 0.1 mol) in diethyl ether (100 cm^3). Bromine reacts rapidly and the solid malonic acid promptly dissolves. The diethyl ether was distilled off under reduced pressure and the residue kept over potassium hydroxide in a vacuum desiccator until hydrobromic acid was completely absorbed. The resulting material was hygroscopic. Yield: 80%. M.p. 112—140 °C (decomp.).

Diammonium 2-aminomalonate (**2**). Solid compound (**1**) (18.3 g, 0.1 mol) was dissolved in methanol (100 cm^3), and ammonia (20% v/v solution, 100 cm^3) in aqueous methanol (50% v/v) was added dropwise with external cooling. The mixture was stirred overnight at room temperature and then the solvent distilled off under reduced pressure. The solid residue was washed with methanol and used without attempting to separate ammonium bromide; m.p. 238 °C.

Lead(II) *2-aminomalonate* (**3**). The solid residue (10 g), containing compound (**2**), was dissolved in water (60 cm^3), the acidity adjusted with acetic acid to pH 4.5, then treated with an aqueous solution of lead(II) acetate (0.2 mol). The mixture was stirred overnight. The residue was collected by filtration and washed with distilled water.

2-Aminomalonic acid (**4**). The white solid residue (**3**) (10 g), containing lead(II) bromide, was suspended in distilled water (25 cm^3) in a Pyrex gas washing bottle. Hydrogen sulphide was passed through the solution for 3 h. The lead(II) sulphide was filtered off and washed with distilled water. The filtrate was concentrated under reduced pressure at 30 °C. The precipitate was collected by filtration and then treated with ethanol to yield (35%) α-aminomalonic acid as its monohydrate, m.p. 109—110 °C (decomp.) (Found: C, 26.00; H, 5.15; N, 10.00%. Calc. for $C_3H_5NO_4 \cdot H_2O$: C, 26.30; H, 5.15; N, 10.20%).

Reagents.—A CO_2-free sodium hydroxide solution was prepared, stored, and standardized as described elsewhere.[15]

Table 3. Potentiometry: experimental details for the protonation and copper(II) complex formation of $amal^{2-}$ at 25 °C in 0.1 mol dm^{-3} NaCl

	T_L/mmol	T_M/mmol	T_{H^+}/mmol	c_T/mol dm^{-3}	pH range	Points *
Protonation						
	0.3408	—	1.2076	0.1740	2.39—10.39	173
	0.3416	—	1.2092	0.1733	2.33—10.27	167
	0.4796	—	1.4852	0.1740	2.33—9.46	155
Copper(II) complex formation						
	0.3686	0.1842	1.7892	0.1741	2.11—2.39	76
	0.7489	0.2284	2.5498	0.1740	2.05—2.08	18
	0.6153	0.4921	2.2826	0.1741	2.06—2.08	16

T_L = Initial amount of ligand, T_M = initial amount of copper(II) ions, T_{H^+} = initial amount of hydrogen ions, and c_T = sodium hydroxide titrant concentration.
* Number of points from titration curve.

Table 4. Calorimetry: experimental details and results for the protonation of $amal^{2-}$ in 0.1 mol dm^{-3} NaCl at 25 °C

T_L/mmol	T_{H^+}/mmol	c_T/mol dm^{-3}	Points[a]	pH range	Reaction step	$-\Delta H^{\circ}$[b]/ kcal mol^{-1}
0.2569	−0.0070	0.1052	33	10.46—7.74	$amal^{2-} + H^+ \rightleftharpoons Hamal^-$	8.45(9)
0.0766	−0.0204	0.1052	27	10.37—3.05	$amal^{2-} + H^+ \rightleftharpoons Hamal^-$	8.34(9)
					average	8.40(9)
0.7452	0.7093	0.5625	30	3.49—1.93	$Hamal^- + H^+ \rightleftharpoons H_2amal$	0.92(1)
					$H_2amal + H^+ \rightleftharpoons H_3amal^+$	0.14(2)

T_L = Initial amount of ligand, T_{H^+} = initial amount of hydrogen ions (negative values refer to hydroxide ions), and c_T = hydrochloric acid titrant concentration.
[a] Number of points from titration curve. [b] Values in parentheses are standard deviations.

Stock solutions of 0.1 mol dm^{-3} NaCl were prepared from sodium chloride (Suprapur, Merck) and used without further purification as the ionic medium for the potentiometric and calorimetric measurements.

A stock copper(II) nitrate solution was prepared from the corresponding solid salt (C. Erba, RPE grade product) and the content of copper(II) ions was analyzed gravimetrically.

Potentiometric Measurements.—Potentiometric titrations were carried out according to a previously described procedure[16] using a digital Radiometer PHM-84 potentiometer equipped with a Ross glass electrode (Orion, model 81-01) and a Ross reference electrode (Orion, model 80-05) together with a Metrohm Multidosimat piston burette. All the accessories were connected to an Olivetti M20 computer which automatically controlled the stepwise titrations by means of a BASIC program.

For the determination of the protonation constants the glass cell, thermostatted at 25 °C, was filled with *ca.* 100 cm^3 of 0.1 mol dm^{-3} NaCl containing a weighed amount of H_2amal and a known large excess of hydrogen ions. Sodium hydroxide titrant solution was delivered stepwise by the piston burette under a nitrogen atmosphere to avoid contamination by CO_2.

For the determination of the stability constants, the cell was filled with *ca.* 100 cm^3 of 0.1 mol dm^{-3} NaCl containing a known amount of the ligand and aqueous copper(II) nitrate solution at different molar ratios and an excess of hydrogen ions. These solutions were titrated stepwise with sodium hydroxide solution until precipitation occurred.

The titration point data (voltage and volume of titrant added) were printed out on paper and automatically stored on a floppy disk for further processing.

Table 3 shows the experimental details both for protonation and copper(II) complex formation of the $amal^{2-}$ compound. The SUPERQUAD program,[17] on the Olivetti M28 computer, was used to calculate the protonation and copper(II) complex-formation constants.

Calorimetric Measurements.—Calorimetric titrations were carried out as reported previously[18] using a titration calorimeter (Tronac, model 1250) operating in the isothermal mode. A 0.1 mol dm^{-3} hydrochloric acid solution (2.50 cm^3) was delivered by the Gilmont burette at a rate of 0.0833 cm^3 min^{-1} into a stainless-steel reaction vessel (25 cm^3) thermostatted at 25 °C in a water-bath controlled by a precision temperature controller (PTC-40, Tronac Inc.). The titration vessel was filled with a known amount of Na_2(amal) containing a slight excess of sodium hydroxide solution dissolved in a total volume of 25 cm^3 of 0.1 mol dm^{-3} NaCl. Two experiments were performed to record the heats of protonation relative to the primary amino nitrogen at different concentrations of ligand.

To check the heats of reaction relative to the two carboxylate groups, a titration using 0.5 mol dm^{-3} HCl was carried out in order to reach low pH values where the CO_2^- groups are protonated in relatively higher quantities. The experimental details, with the associated results, are reported in Table 4.

The enthalpy changes were computed with the FITH program previously described running on a M24 Olivetti computer.[18] Correction for the heats of dilution was made under the same experimental conditions and the heat of ionization of water was taken from the literature.[19]

The titration experiments were controlled by a North-Star CCP 930 computer running the THERMAL program, connected to a M24 Olivetti computer for the 'data files' transfer.

Molecular Mechanics Computations.—The molecular mechanics computations were carried out using the MacroModel package version 1.5[9] implemented on a VAX 11/750 computer connected to a Digital VT 241 graphic terminal. They comprised an all-atom field set up for distance-dependent dielectric electrostatics. This functions as a crude approximation of polarization effects. The force field used was that reported by Keiner *et al.*[20] The total energy (contributions from bond length, valence angle, and torsion angle deformations, non-

bonding, electrostatic, and hydrogen-bond interactions) was minimized by block-diagonal and full-matrix Newton–Raphson procedures.[21]

Spectroscopic Measurements.—The ^{13}C n.m.r. spectrum was recorded on a Bruker AC200 spectrometer in D_2O solvent using $SiMe_4$ as internal standard.

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