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Dinuclear Calcium Complexes with Intramolecularly NH···O Hydrogen-Bonded Dicarboxylate Ligands

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A novel dinuclear calcium complex, $[Ca_2\{(2\text{-}OCO\text{-}3\text{-}CH_3C_6H_3NHCO)_2C(CH_3)_2\}_2(CH_3OH)_6]$ (**1**), was synthesized as a structural model of 8-coordinated Ca(II) ions in the double calcium-binding site of thermolysin. The complex has four NH···O hydrogen bonds between the amide NH and the carboxylate oxygen anion. Two types of bridging coordination of the carboxylate ligand to Ca(II) were found in **1**. The amide NH forms a strong NH···O hydrogen bond with the anionic oxygen of the two carboxylate oxygens. A ligand-exchange reaction between the dinuclear calcium complex and eight equimolar amounts of 2,4,6-trimethylbenzoic acid or $2\text{-}CH_3\text{-}6\text{-}t\text{-}BuCONHC_6H_3COOH$ indicates that the NH···O hydrogen bond prevents the dissociation of the Ca−O bond.

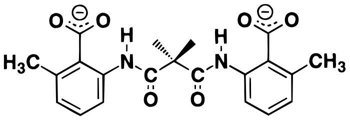
Introduction

A dinuclear calcium binding site was found in thermolysin as confirmed by crystallographic analysis,[1] which also revealed the binding sites of two other calcium ions and one zinc ion. A similar dinuclear Ca site was proposed for the C_2 domain of protein kinase C.[2] Three carboxylate ligands bridge between the two calcium ions in the dinuclear calcium site which consists of 8-coordinated and 6-coordinated Ca(II) ions. This dinuclear calcium binding site has been discussed as a cooperative coordination of the two metal ions.[3]

In these calcium-binding proteins, hydrogen bond networks were often found between carboxylate oxygen atoms and main chain amide NHs. Especially, two NH···O hydrogen bonds can be observed in the dinuclear calcium binding site of thermolysin using the Protein Data Bank. The hydrogen bonding networks are considered to provide the fixation of a carboxylate-ligand orientation and a suitable main-chain conformation in the calcium-binding loop.[4]

Various calcium complexes containing simple carboxylate ligands have been synthesized and characterized according to the classified structures of calcium−carboxylate complexes.[5−8] These complexes have a polymeric structure due to the bridging facility of the carboxylate oxygen atoms. However, only a few dinuclear calcium complexes were reported in terms of the synthesis and the structures.

Scheme 1



Dicarboxylate

This paper presents the chemical role of the NH···O hydrogen bond in the formation of a dinuclear calcium complex as well as in the dinuclear calcium binding site of thermolysin. A novel chelating ligand which contains NH···O hydrogen bonds and bulky groups at the ortho positions on benzoate is designed as shown in Scheme 1.

Experimental Section

The reagents used were of commercial grade unless otherwise stated. All organic solvents were dried over CaH_2 and distilled under an argon atmosphere before use.

$2\text{-}CH_3\text{-}6\text{-}t\text{-}BuCONHC_6H_3COOH$. To a THF solution (50 mL) of 2-amino-6-methylbenzoic acid (2.0 g, 13 mmol) were added dropwise triethylamine (2.7 mL, 19 mmol) and pivaloyl chloride (2.0 mL, 16 mmol) at 0 °C. After being stirred overnight at room temperature, the reaction mixture was concentrated under reduced pressure to give a brown oil. The oil was dissolved in ethyl acetate (200 mL) before water (30 mL) was added to the solution. The organic layer was successively washed with water, 2% HCl aqueous solution, water, and saturated NaCl aqueous solution and then dried over anhydrous sodium sulfate. After the concentration, the solution gave a colorless oil which was dissolved in hot *n*-hexane and cooled to room temperature. Slightly brownish crystals were formed and recrystallized from diethyl ether, yield, 1.43 g (46%): ^1H NMR (chloroform-*d*, δ) (270 MHz) 1.34 (s, 9H), 2.57 (s, 3H), 6.98 (d, 1H), 7.39 (t, 1H), 8.31(d, 1H), 10.12 (s,

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(1) Holmes, M. A.; Matthews, B. W. *J. Mol. Biol.* **1982**, *160*, 623−639.
(2) Shao, X.; Davletov, B. A.; Sutton, R. B.; Sudhof, T. C.; Rizo, J. *Science* **1996**, *273*, 248−251.
(3) Voordouw, G.; Roche, R. S. *Biochemistry* **1974**, *13*, 5017−5021.
(4) McPhalen, C. A.; Strynadka, N. C. J.; James, M. N. G. In *Calcium-Binding Sites in Proteins: A Structural Perspective*; McPhalen, C. A., Strynadka, N. C. J., James, M. N. G., Eds.; Academic Press: New York, 1991; Vol. 42, pp 77−144.
(5) Einspahr, H.; Bugg, C. E. *Acta Crystallogr., Sect. B* **1981**, *B37*, 1044−1052.
(6) Einspahr, H.; Bugg, C. E. *Metal Ions in Biological Systems*: *Calcium and Its Role in Biology*; Siegel, H., Ed.; Dekker: Basel, 1984; Vol. 17.
(7) Bahl, A. M.; Krishnaswamy, S.; Massand, N. G.; Burkey, D. J.; Hanusa, T. P. *Inorg. Chem.* **1997**, *36*, 5413−5415.
(8) Yun, J. W.; Tanase, T.; Lippard, S. J. *Inorg. Chem.* **1996**, *35*, 7590−7600.

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Table 1. Crystal Data Collection and Refinement Data for $[Ca_2\{(2\text{-}OCO\text{-}3\text{-}CH_3C_6H_3CONH)_2C(CH_3)_2\}_2(CH_3OH)_6]$ (**1**)

chemical formula	$C_{50}H_{72}O_{20}N_4Ca_2$
formula weight	1129.29
crystal system	monoclinic
a	12.526(4) Å
b	16.551(6) Å
c	14.750(4) Å
β	113.46(2)°
V	2805(1) Å^3
space group	*P*2$_1$/*n* (#14)
Z	2
D_{calc}	1.337 g cm^{-3}
μ (Mo Kα)	2.79 cm^{-1}
residual *R* value[a]	0.059
residual R_w value[b]	0.069

[a] $R = \sum||F_o| - |F_c||/\sum|F_o|$. [b] $R_w = [\sum w(|F_o| - |F_c|)^2/\sum wF_o^2]^{1/2}$.

1H). Anal. Calcd for $C_{13}H_{17}NO_3$: C, 66.36; H, 7.28; N, 5.95. Found: C, 66.31; H, 7.14; N, 5.95.

$(2\text{-}COOH\text{-}3\text{-}CH_3C_6H_3NHCO)_2C(CH_3)_2$. To a THF solution (50 mL) of 2-amino-6-methylbenzoic acid (3.5 g, 23 mmol) were added dropwise triethylamine (3.9 mL, 28 mmol) and dimethylmalonyl chloride (1.3 mL, 9.6 mmol) at 0 °C. After being stirred overnight at room temperature, the reaction mixture was concentrated under reduced pressure to give a brown oil. The oil was dissolved in ethyl acetate (150 mL), and water (50 mL) was added to the solution. The ethyl acetate layer was washed, and the crude product was purified by the same procedure as that described above ($2\text{-}CH_3\text{-}6\text{-}t\text{-}BuCONHC_6H_3\text{-}COOH$) to give a white powder, yield, 1.43 g (46%): ^{1}H NMR (270 MHz) ($Me_2SO\text{-}d_6$, *δ*) 1.51 (s, 6H), 2.39 (s, 6H), 7.06 (d, 2H), 7.34 (t, 2H), 7.82 (d, 2H), 9.99 (s, 2H). Anal. Calcd for $C_{43}H_{46}N_4O_{14}Cu_2$: C, 63.31; H, 5.57; N, 7.03. Found: C, 64.69; H, 5.37; N, 7.14.

$[Ca_2\{(2\text{-}OCO\text{-}3\text{-}CH_3C_6H_3NHCO\}_2C(CH_3)_2\}_2(CH_3OH)_6]$ (1). $(2\text{-}COOH\text{-}3\text{-}CH_3C_6H_3NHCO\}_2C(CH_3)_2$ (100 mg, 0.25 mmol) and $CaCO_3$ (25 mg, 0.25 mmol) were suspended in water (20 mL). The reaction mixture became a colorless solution and then was concentrated under reduced pressure to give a white residue. The residue was dissolved in methanol. After slow evaporation, the solution gave colorless plates, yield, 127 mg (25%). Anal. Calcd for $C_{48}H_{64}N_4O_{18}Ca_2$: C, 54.12; H, 6.06; N, 5.26. Found: C, 53.20; H, 5.13; N, 5.91.

Physical Measurements. ^{1}H NMR spectra were taken on a JEOL EX270 spectrometer in $Me_2SO\text{-}d_6$, acetonitrile-d_3, tetrahydrofuran-d_8, and chloroform-*d*. IR spectra were recorded on a JASCO FT/IR 8300 spectrometer. Samples were prepared as KBr pellets. Ion spray mass spectrometric analysis was performed on a Perkin-Elmer SCIEX API-III spectrometer which was operated in a negative ion mode.

X-ray Structure and Determination. A single crystal of $[Ca_2\{(2\text{-}OCO\text{-}3\text{-}CH_3C_6H_3NHCO\}_2C(CH_3)_2\}_2(CH_3OH)_6]$ (**1**) was sealed in a glass capillary for the X-ray measurement. The X-ray measurement was performed at 23 Å on a Rigaku AFC7R diffractometer equipped with a rotating anode X-ray generator. The radiation used was Mo Kα monochromated with graphite (0.710 69 Å). The basic crystallographic parameters are listed in Table 1. Unit-cell dimensions were refined by 19 reflections. These standard reflections were chosen and monitored with every 150 reflections and did not show any significant change. The structure was solved by heavy-atom Patterson methods and expanded with Fourier techniques using the teXsan crystallographic software package of the Molecular Structure Corp. The nonhydrogen atoms were refined anisotropically. Hydrogen atoms were placed on the calculated positions but not refined. The final cycle of full-matrix least-squares refinement was based on 3550 observed reflections ($I > 3.00\ \sigma(I)$) and 344 variable parameters. The final difference Fourier map showed no significant features.

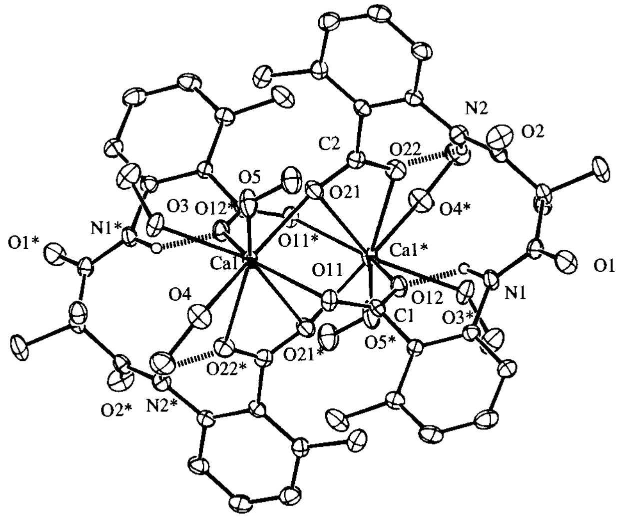

Figure 1. Molecular structure of $[Ca_2\{(2\text{-}OCOH\text{-}3\text{-}CH_3C_6H_3NHCO)_2C(CH_3)_2\}_2(CH_3OH)_6]$ (**1**). The 50% probability ellipsoid is shown.

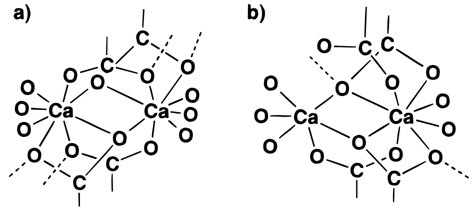

Figure 2. Schematic drawings of dinuclear sites in (a) $[Ca_2\{(2\text{-}OCO\text{-}3\text{-}CH_3C_6H_3NHCO\}_2C(CH_3)_2\}_2(CH_3OH)_6]$ (**1**) and (b) thermolysin. The coordinating oxygen atoms come from methanol in **1** and water for the dinuclear site in thermolysin. Dotted lines indicate NH···O hydrogen bonds from the amide NH groups.

Results and Discussion

Synthesis and Crystal Stucture. The dinuclear complex $[Ca_2\{(2\text{-}OCO\text{-}3\text{-}CH_3C_6H_3NHCO\}_2C(CH_3)_2\}_2(CH_3OH)_6]$ was synthesized by the reaction between $(2\text{-}COOH\text{-}3\text{-}CH_3C_6H_3\text{-}NHCO\}_2C(CH_3)_2$ and calcium carbonate in water and then crystallized in methanol as shown in eq 1. The reaction proceeded smoothly.

$$2CaCO_3 + 2(2\text{-}COOH\text{-}3\text{-}CH_3C_6H_3NHCO)_2C(CH_3)_2 + 6CH_3OH \rightarrow [Ca_2\{(2\text{-}OCO\text{-}3\text{-}CH_3C_6H_3NHCO\}_2C(CH_3)_2\}_2(CH_3OH)_6] + 2CO_2 + 2H_2O \quad (1)$$

Figure 1 shows the crystal structure of $[Ca_2\{(2\text{-}OCO\text{-}3\text{-}CH_3C_6H_3NHCO\}_2C(CH_3)_2\}_2(CH_3OH)_6]$ (**1**) determined by X-ray analysis. Table 2 lists the selected bond distances and bond angles for **1**, which crystallizes in the space group *P*2$_1$ (#14) (monoclinic) with $Z = 4$. The dicarboxylate ligand has a spiral structure to form a bridging structure. Figure 2shows the selected structure of the dinuclear calcium site in **1**. The Ca···Ca distance of 3.860(2) Å is similar to that of reported Ca dinuclear complexes.[7,9] Four carboxylate ligands bridge the two calcium ions. Two of them have a regular structure, and the other two form an anti carboxylate structure with *μ*-oxo type bridging due to the large Ca−O distances (2.350−2.431 Å). The four Ca−O bond distances are similar to the reported ones for 6-coordinate complexes.[7−9] Six methanol molecules coordinate outside of the dinuclear site. Although the COO plane is twisted from the benzene ring, the amide NH is directed toward one of the carboxylate oxygen atoms to form an NH···O hydrogen bond. The distance (ca. 2.72(2)−2.79(2) Å) between the amide N and the carboxylate oxygen atoms also supports the presence of the hydrogen bond. As listed in Table 2, the bond distances of C(1)−O(12) (1.260(6) Å) and C(2)−O(22) (1.266(6) Å) having

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(9) Carrell, C. J.; Carrell, H. L.; Erlebacher, J.; Glusker, J. P. *J. Am. Chem. Soc.* **1988**, *110*, 8651−8656.

Table 2. Selected Bond Distances, Angles and Torsion Angles of $[Ca_2\{(2\text{-}OCO\text{-}3\text{-}CH_3C_6H_3CONH)_2C(CH_3)_2\}_2(CH_3OH)_6]$ (**1**)

Bond Distances (Å)	
Ca(1)···Ca(1)*	3.859(2)
Ca(1)−O(3)	2.442(4)
Ca(1)−O(4)	2.518(4)
Ca(1)−O(5)	2.458(4)
Ca(1)−O(11)	2.403(4)
Ca(1)−O(12)*	2.429(4)
Ca(1)−O(21)	2.849(4)
Ca(1)−O(21)*	2.352(4)
Ca(1)−O(22)*	2.405(4)
C(1)−O(11)	1.243(6)
C(1)−O(12)	1.260(6)
C(2)−O(21)	1.247(6)
C(2)−O(22)	1.266(6)
Torsion Angles (deg)	
O(11)−Ca(1)−Ca(1)*−O(12)	7.3(1)
O(11)−C(1)−C(11)−C(16)	45.9(7)
O(12)−C(1)−C(11)−C(12)	43.3(7)
O(21)−C(2)−C(21)−C(26)	−47.7(7)
O(22)−C(2)−C(21)−C(22)	−44.0(7)
C(11)−C(12)−N(1)−C(17)	157.2(5)
C(21)−C(22)−N(2)−C(27)	−143.2(5)
Angles (deg)	
Ca(1)*−Ca(1)−O(3)	140.2(1)
Ca(1)*−Ca(1)−O(4)	145.7(1)
Ca(1)*−Ca(1)−O(5)	124.2(1)
O(21)*−Ca(1)−O(22)*	48.7(1)
Ca(1)*−Ca(1)−O(11)	73.4(1)
Ca(1)*−Ca(1)−O(12)*	66.6(1)
Ca(1)*−Ca(1)−O(22)*	85.2(1)
Ca(1)−O(21)*−Ca(1)*	95.3(1)
Ca(1)−O(12)*−C(1)*	138.5(3)
Ca(1)−O(21)*−C(2)*	83.9(3)
Ca(1)−O(22)*−C(2)*	104.7(3)
Ca(1)−O(11)−C(1)*	130.9(3)
Ca(1)−O(21)−C(2)	164.1(3)
Ca(1)−O(3)−C(31)	130.9(4)
Ca(1)−O(4)−C(41)	132.8(4)
Ca(1)−O(5)−C(51)	130.7(4)

an NH···O hydrogen bond are longer than those of C(1)−O(11) (1.243(6) Å) and C(2)−O(21) (1.247(6) Å). It turns out that the amide NH forms an NH···O hydrogen bond with the anionic oxygen of the carboxylate.[10] Intermolecular hydrogen bond incorporating carboxylate has been reported and is thought to play a role in consolidating the crystal structure.

Bahl et al. have reported the observation of a longer C−O bond distance for the bridging oxygen of carboxylate due to the higher coordination of that oxygen.[7] In the case of **1**, a shorter C(2)−O(21) bond is located at the bridging oxygen, whereas another longer C(2)−O(22) bond exists as a terminal ligand without bridging. On the other hand, in the other bridge carboxylate oxygens, the C(1)−O(11) bond distance is shorter and the other bond distance, C(1)−O(12), is longer. The longer one in **1** is incorporated as an anionic $C-O^-$ with the NH···O hydrogen bond. Thus, this type of carboxylate ligand still has anionic $C-O^-$ and C=O without complete conjugation. The combined data suggest that the strong NH···O hydrogen bond is formed between the amide NH and the $C-O^-$ anion oxygen.

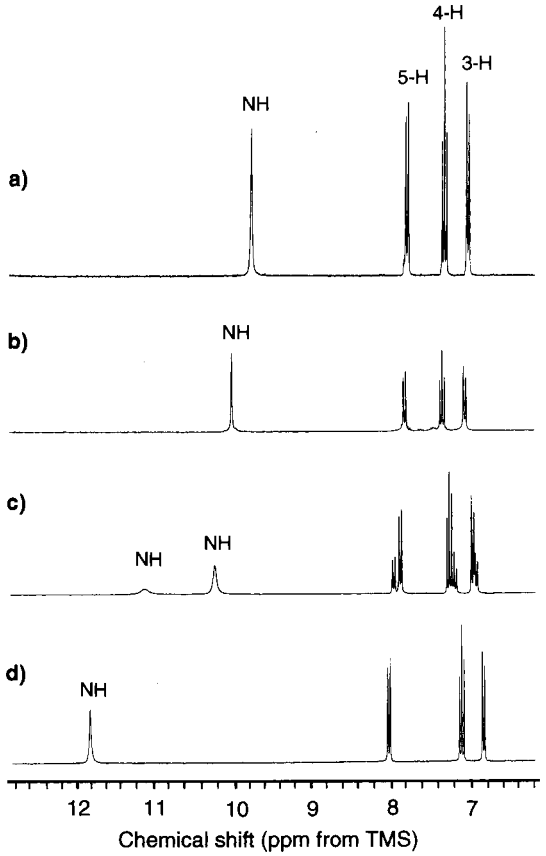

Figure 3. ^1H NMR spectra of (a) $2\text{-}CH_3\text{-}6\text{-}t\text{-}BuCONHC_6H_3COOH$, (b) $(2\text{-}COOH\text{-}3\text{-}CH_3C_6H_3NHCO)_2C(CH_3)_2$, (c) a solution mixed with the solution (a) and 8 equimolar amounts of $2\text{-}CH_3\text{-}6\text{-}t\text{-}BuCONHC_6H_3\text{-}COOH$, and (d) $[Ca_2\{(2\text{-}OCO\text{-}3\text{-}CH_3C_6H_3NHCO)_2C(CH_3)_2\}_2(CH_3OH)_6]$ (**1**) in $Me_2SO\text{-}d_6$ at 303 K.

Similar hydrogen bonds are observed in the calcium-binding site of thermolysin.[1,11] Figure 2b shows the dinuclear calcium binding site in thermolysin which was reported by Holmes et al.[1] This site has 8-coordinated and 6-coordinated geometry for the two calcium ions. Three carboxylate ligands bridge between them with Ca−O distances of 2.40−2.52 Å. The Ca···Ca distance in thermolysin was reported to be 3.8 Å, which is similar to that of **1**.

Although our model complex for a partial structure of the dinuclear site possesses coordinating methanol molecules instead of water molecules, the Ca−O bond distances are still similar. Actually, the hydroxyl groups of alcohol molecules coordinate in a fashion similar to the water molecules, as demonstrated for $CaBr_2$(α-D-fucose)$\cdot 3H_2O$.[12] Thus, the present synthetic complex **1** is one of the biologically relevant models for a dinuclear Ca(II) core with the 8-coordinated geometry, although the coordinating ligands beside the carboxylate ligand come from methanol molecules instead of water molecules in the thermolysin Ca(II) site.

IR and Raman Spectra. The NH bands of **1** were not analyzed because overlapped with the bands of methanol OH and water OH. Presumably, the NH···O hydrogen bond is relatively strong because it participates in a suitable six-membered ring. Actually, the strong NH···O hydrogen bond in $[Cu_2(2\text{-}CH_3\text{-}6\text{-}t\text{-}BuCONHC_6H_3COO)_4(CH_3OH)(H_2O)](H_2O)$, having a similar monocarboxylate ligand, was detected by the IR spectrum.[13]

Complex **1** exhibits two characteristic C=O Raman bands at 1465 and 1579 cm^{-1} in the solid state, which are assignable to C−O and C=O stretchings, respectively, in a bridging carboxylate. The difference ($\Delta\{\nu(C-O) - \nu(C=O)\}$) between the

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(10) Chen, X.-M.; Mak, T. C. W. *Inorg. Chem.* **1994**, *33*, 2444−2447.

(11) Matthews, B. W.; Weaver, L. H.; Kester, W. R. *J. Biol. Chem.* **1974**, *249*, 8030−8044.

(12) Cook, W. J.; Bugg, C. E. *Biochim. Biophys. Acta* **1975**, *389*, 428−435.

(13) Ueyama, N.; Yamada, Y.; Takeda, J.; Okamura, T.; Mori, W.; Nakamura, A. *J. Chem. Soc., Chem. Commun.* **1996**, 1377−1378.

two bands is 114 cm^{-1} which is relatively small compared with that (173 cm^{-1}) of a calcium complex, $[Ca\{OCO\text{-}2,6\text{-}(CH_3CONH)_2C_6H_3\}_4]^{2-}$, having a unidentate coordinating mode. [14] The smaller $\Delta\nu$ value in the bridging mode of the carboxylate bands has been reported by Nakamoto.[15]

The Ligand-Exchange Reaction. ^1H NMR spectra were measured to monitor the ligand-exchange reaction between calcium complexes and free carboxylic acid in $Me_2SO\text{-}d_6$ at room temperature. The amide NH signal of **1** shifts to 11.8 ppm from that (10.0 ppm) of the carboxylic acid. The large shift of 1.8 ppm is ascribed to NH···O hydrogen bonding between the amide NH and the carboxylate oxygen even in a polar solvent such as Me_2SO because the hydrogen bonding increases the acidity as the ^1H signal appears downfield. The ^1H NMR spectra in Figure 3 show the ligand-exchange reaction of **1** with the addition of 8 equimolar amounts of $2\text{-}CH_3\text{-}6\text{-}t\text{-}BuCONHC_6H_3COOH$ or 30 equimolar amounts of 2,4,6-trimethylbenzoic acid in $Me_2SO\text{-}d_6$ at 303 K. The results also indicate that $2\text{-}CH_3\text{-}6\text{-}t\text{-}BuCONHC_6H_3COOH$ or 2,4,6-trimethylbenzoic acid is unable to exchange with the carboxylate anion in **1**. Presumably, both carboxylic acids, $(2\text{-}COOH\text{-}3\text{-}CH_3C_6H_3NHCO)_2C(CH_3)_2$ and $2\text{-}CH_3\text{-}6\text{-}t\text{-}BuCONHC_6H_3COOH$, have similar pK_a's because the COOH groups in both acids possess almost the same environments. It is likely that the difference in Ca−O dissociation can be ascribed to the presence of a chelating effect produced by the two carboxylate groups in the ligand of **1**. Actually, the effect of the chelating dicarboxylate ligand on the high affinity to Ca(II) has been discussed for Ca(α-ethylmalonate).[16]

Conclusion

A novel dinuclear calcium complex was synthesized using a dicarboxylate ligand. The complex has a unique bridging site similar to the dinuclear calcium binding site in thermolysin. The dinuclear calcium complex is stabilized by the chelating effect and/or NH···O hydrogen bonding with the bulky dicarboxylate ligand.

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Supporting Information Available: X-ray crystallographic files, in CIF format. This material is available free of charge via the Internet at http://pubs.acs.org.

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(14) Yamada, Y.; Ueyama, N.; Okamura, T.; Nakamura, A. To be submitted.

(15) Nakamoto, K. *Infrared and Raman Spectra of Inorganic and Coordination Compounds*; John Wiley & Sons: New York, 1978.

(16) Zell, A.; Einspahr, H.; Bugg, C. E. *Biochemistry* **1985**, *24*, 533−537.