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Synthesis of *N*-(2-Hydroxy-1-phenoxyacetyl)-prolylproline and Related Cation Binding and Molecular Mechanics Studies

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Abstract. The synthesis of *N*-(2-hydroxy-1-phenoxyacetyl)prolylproline **2** from 2-acetoxyphenoxyacetic acid is described. A first synthesis led to *N*-(2-acetoxy-1-phenoxyacetyl)prolylproline methyl ester **8b** that fragmented upon attempted ester hydrolysis with 1N NaOH. A second synthesis gave the corresponding benzyl ester **13**, which was converted to **2** by deacylation of the phenolic acetoxy group with pyrrolidine followed by hydrogenolysis of the ester. Cation binding by **2** in methanol and related molecular mechanics (MM) geometric optimizations are discussed.

Key words: prolylproline derivatives, cation binding, carboxy ionophore model, MM calculations

1. Introduction

We have synthesized and studied the properties of neutral ethanedioxydiamide ionophores for a number of years [1, 2]. However, many of the most useful ionophores contain carboxy and hydroxy groups that form internal hydrogen bonded rings upon metal cation binding [3]. These cation complexes are electrically neutral since the carboxyl group loses its proton upon binding. They are more effectively transported through membranes than are the cation complexes of neutral ionophores that must include an external counter anion [4]. We recently published the synthesis of *N*-(2-hydroxy-1-phenoxyacetyl)glycylglycine **1b** [5] that serves as a model for carboxy-containing ionophores such as Lasalocid [3a]. It was prepared by dilute NaOH saponification of the acetoxy ethyl ester **1a** without any complications. Compound **1b** is a moderate metal cation chelator in methanol and binds $Ba^{2+} \geq Ca^{2+}$ (K_{app} = 3.5–4.0 $\times$ 10^4 M^{-1}) > $Sr^{2+} \gg Li^+ > Na^+, K^+$. We now report the synthesis of *N*-(2-hydroxy-1-phenoxyacetyl)prolylproline **2**. By substituting the less flexible prolylproline as the peptide instead of glycylglycine, it was anticipated that **2** would more readily assume a pseudo-cyclic conformation that would lead to enhanced metal cation chelation. Indeed, MM modeling of **2** with

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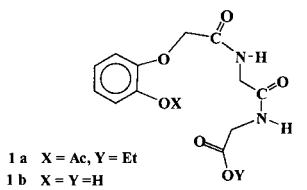
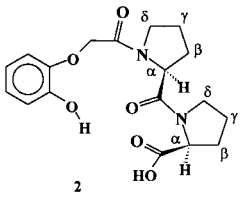
early versions of *PCModel* [6] showed that a hydrogen bond might form between the phenolic O*H* and the carboxyl C=O in the gas phase more readily than it does for **1b**.





2. Experimental

Solvents used were distilled from molecular sieves. Melting points were taken on a Thomas Hoover melting point apparatus and are uncorrected. Thin layer chromatography was done on E. Merck, Whatman, or Analtech HF_{254} silica gel sheets or plates using various solvent mixtures. Column chromatography was done using E. Merck chromatographic grade 40 μm silica gel. Organic solutions were dried over anhydrous $MgSO_4$. Solvents used were distilled from molecular sieves. Proton NMR spectra, in $CDCl_3$ or acetone-d_6 with TMS as the internal standard, were recorded in a Varian EM 360A (60 MHz) spectrometer at Ramapo College and on a GE QE Plus (300 MHz) spectrometer at Wyeth-Ayerst (formerly Lederle Laboratories), Pearl River, N.Y. ^{13}C NMR spectra were done on a GE QE Plus (75 MHz) at Wyeth-Ayerst. Infrared spectra were recorded on Perkin Elmer 1420 and Paragon 1000 PC FTIR spectrometers at Ramapo College and on a Nicolet FTIR spectrometer at Wyeth Ayerst. Mass spectra were done by Dr. M. Siegel at Wyeth Ayerst using a Kratos MS-50 and later a Micromass Platform 2 mass spectrometer (using CI or FAB techniques) or a Finnegan MAT90 mass spectrometer for the determination of exact mass by high resolution measurements.

2.1. 2-HYDROXYPHENOXYACETIC ACID **7a**

7a was prepared from catechol and chloroacetonitrile as previously described [5]. It was later purchased from Lancaster Chemical, Wyndham, NH.

2.2. ACETOXYPHENOXYACETIC ACID **7b**

2-Acetoxyphenoxyacetic acid **7b** was prepared from 2-hydroxyphenoxyacetic acid and acetic anhydride as previously described [5].

2.3. BOC-PROLYLPROLINE METHYL ESTER **5**

To a solution of Boc-L-proline (**3**, 4.3 g, 0.02 mol) in anhydrous tetrahydrofuran (40 mL, drawn by syringe from an Aldrich *Sure/Seal*™ bottle) in a 3-necked round

bottom flask fitted with a stirring bar, condenser, and nitrogen inlets and outlets was added CDI (3.2 g, 0.02 mol) with stirring under nitrogen. After 30 min, L-proline methyl ester hydrochloride (**4**, 3.31 g, 0.02 mol) and *N*-ethylmorpholine (NEM, 8.8 mL, 8.0 g, 0.07 mol) were added with stirring. The resulting mixture was stirred at rt for 24 h under nitrogen. After removal of some solid by vacuum filtration, the resultant filtrate was evaporated *in vacuo*. Ethyl acetate (40 mL) was added to the oily residue. The resultant solution was washed with 1N HCl (20 mL), saturated NaCl solution, dried over $MgSO_4$, and evaporated *in vacuo* to give the crude product as an oil (*ca.* 5 g). This was chromatographed on silica gel (50 g) using 2 : 1 EtOAc : low bp petroleum ether as the eluting solvent to give pure product as an oil (4.22 g, 0.013 mol, 65%); IR (neat, NaCl) 1747 (ester C=O), 1699 (*t*-BOC C=O), 1661 cm^{-1} (amide C=O); 300 MHz NMR ($CDCl_3$) δ 4.45, 4.50 (2t, 2, α-CH—C=O), 3.70 (s, 3, OCH_3), 3.30–3.70 (m, 4, δ-CH_2N), 2.00 (t, 8, proline β,γ-CH_2), 1.40, 1.42 (2s, 9, *t*-Boc CH_3); mass spectrum (CI NH_3) *m/z* (rel intensity) 327 (M + H, 16), 271 (M + 2H- *t*-Bu, 16), 227 (M+ 2H-*t*-Bu—CO_2, 40), 154 (100). It was later found that this compound has been previously synthesized in 70% yield in a similar coupling reaction using dicyclohexylcarbodiimide (DCC) by Pettit and Gupta [7]. Only TLC data were given. It was converted by these authors to Boc-prolylproline, which was characterized by ORD, mp, and elemental analysis.

2.4. PROLYLPROLINE METHYL ESTER TRIFLUOROACETATE **6**

Boc-prolylproline methyl ester (**5**, 3.0 g 0.0092 mol) in CH_2Cl_2 (32 mL) was reacted with trifluoroacetic acid (30 mL, 44.4 g, 0.39 mol, 42 equiv.) at rt for 3 h with stirring. The resultant solution was then evaporated *in vacuo* to give the product as a light brown oil (3.1 g, 0.0088 mol, 96%); IR (neat, NaCl) 1760 (methyl ester C=O), 1670 cm^{-1} (amide C=O); 60 MHz ^{1}H NMR ($CDCl_3$) δ 4.6–4.9 ((broad t, 2, α-CH–C=O), 4.4, 4.5 (2s, H_2N^+), 3.7 (s, 3, OCH_3), 3.3–3.8 (m, 4 δ-CH_2N), 2.0 (m, 8, prolyl β,γ-CH_2).

2.5. *N*-2-(HYDROXY-1-PHENOXYACETYL)PROLYLPROLINE METHYL ESTER **8b**

To 2-acetoxyphenoxyacetic acid (**7b**, 1.24 g, 0.0059 mol) in anhydrous tetrahydrofuran (20 mL) was added CDI (0.96 g, 0.0059 mol) with stirring under nitrogen. After 40 min, H-Pro-Pro-OMe trifluoroacetate (**6**, 2.0 g, 0.0058 mol) and *N*-methylmorpholine (NMM, 0.75 mL, 0.69 g, 0.0068 mol) were added with stirring. The reaction mixture was stirred under nitrogen at rt for 24 h, evaporated *in vacuo* to give an oil which was dissolved in ethyl acetate (30 mL), washed with 1N HCl (2 $\times$20 mL), saturated NaCl (10 mL), dried, filtered, and evaporated *in vacuo* to give an oil whose TLC (3 : 1 EtOAc : MeOH) gave several spots. The oil was purified by column chromatography (20 g silica gel with EtOAc as the eluting solvent) to

give the product as an oil (2.05 g, 0.0055 mol, 93%); TLC (3 : 1 ethyl acetate-low bp petroleum ether) one spot R_f = 0.64; IR (neat, NaCl) 3400–3550 (ArOH), 1740 (ester C=O), 1650 (amide C=O) cm^{-1}; 300 MHz NMR ($CDCl_3$) δ 6.80, 6.90 (m, 4, aryl), 4.62 (s, 2, OCH_2), 4.52–4.56, 4.72 (m, 2, N—CH-C=O), 3.40, 3.60, 3.85 (3 q, 4, δ-CH_2—N), 3.72 (s, 3, OCH_3), 2.10–2.30 (m, 8, proline β,γ-CH_2); mass spectrum (FAB) *m/z* (rel intensity) 753 (2M + H), 377 (37, M + H), 359 (100, M-18), 227 (100, M-150), 220 (70), 130 (12), 112 (13).

2.6. *N*-2-(ACETOXY-1-PHENOXYHYDROXY)PROLYLPROLINE METHYL ESTER **8a**

8b was converted to the free phenol methyl ester **8a**, during column chromatography on silica. Some **8b** was isolated without chromatography and gave the above ^{1}H NMR spectrum plus an extra peak at δ 2.05 (s, 3, O(C=O)CH_3).

2.7. ATTEMPTS TO CONVERT **8** TO *N*-(2-HYDROXY-1-PHENOXYACETYL)PROLYLPROLINE **2**

Attempted hydrolyses of **8a** or **8b** with 1N aqueous NaOH at rt for 10 min caused fragmentation to 2-hydroxyphenoxyacetic acid (**7a**, *ca* 60%); mp 134–136 °C, and prolylproline residues (presumed but not isolated).

2.8. BOC-PROLYLPROLINE BENZYL ESTER **11**

To a solution of Boc-L-proline (**3**, 0.48 g, 0.0022 mol) and L-proline benzyl ester hydrochloride (**10**, 0.54 g, 0.0022 mol) in dichloromethane (20 mL) at 0 °C was added diisopropylethylamine (0.7 μL, 0.51 g, 0.0025 mol) over 5 min. with stirring. After another 5 min BOP coupling reagent (1.1 g, 0.0025 mol) was added in one portion. The resultant mixture was stirred at rt for 2 h, quenched with water (30 mL), and extracted with dichloromethane (2 × 20 mL). The combined organic layer was washed with 10 mL portions of saturated $NaHCO_3$, 2% HCl, saturated NaCl, dried, filtered, and evaporated to give an oil. This oil was chromatographed on silica gel and eluted with 30–60% EtOAc in hexane to give the product as a colorless oil (0.81 g, 0.0020 mol, 91%); IR (NaCl) 1742 (Bn ester), 1697 (BOC C=O), 1652 (amide C=0) cm^{-1}; 300 MHz ^{1}H NMR ($CDCl_3$) δ 7.30 (s, 5, Ph), 5.15 (center of AB quartet, 2, OC*H*$_2$ Ph), 4.37, 4.50, 4.65 (m, 2, NC*H*C=O), 3.40, 3.60, 3.80 (m, 4, δ-CH_2N), 2.00 (m, 8, β, γ-prolyl CH_2), 1.40, 1.50 (2s, 9, Boc CH_3).

Compound **11** has been previously prepared by Deber *et al.* [8] as a syrup from Boc-L-Pro and L-Pro-OBn.HCl *via* a mixed anhydride method using isobutyl chloroformate. These authors converted **11** to Boc-ProPro-OH, which had a correct analysis. Spectral data was not published for either compound but was deemed appropriate. The related Boc-*D*-ProPro-OBn has been prepared *via* a DCC coupling and has a similar ^{1}H NMR spectrum to our sample of **11** [9].

2.9. PROLYLPROLINE BENZYL ESTER TRIFLUOROACETATE **12**

Boc-prolylproline benzyl ester (**11**, 1.19 g, 0.0030 mol) in CH_2Cl_2 (15 mL) was treated with trifluoroacetic acid (2 mL, 2.96 g, 0.026 mol, 8.8 equiv.) at rt with stirring for 4 h. The solution was then evaporated *in vacuo* to give a colorless liquid (**12**), which was directly used in the next step.

2.10. *N*-2(ACETOXY-1-PHENOXYACETYL)PROLYLPROLINE BENZYL ESTER **13**

To the above prepared **12** in CH_2Cl_2 (10 mL) at 0 °C was added diisopropylethylamine (1.2 mL, 0.87 g, 0.0086 mol, 2.9 equiv.) dropwise, followed by **7b** (0.62 g, 0.0030 mol). After 5 min EDC HCl (0.61 g, 0.0032 mol) and then 1-hydroxybenzotriazole (0.44 g, 0.0033 mol) was added. The mixture was stirred for 3 h, saturated $NaHCO_3$ was added, and the resultant mixture was partitioned. The aqueous layer was extracted with CH_2Cl_2 (2 × 10 mL) which was added to the organic layer. The combined organic layers were washed with saturated NaCl, dried, filtered and evaporated *in vacuo* to give an oil which was chromatographed on silica gel (25 g) using 2.5–7.5% MeOH in $CHCl_3$ to give **13** as a semisolid foam (1.40 g, 0.0028 mol, 96%); TLC (3 : 1 EtOAc : MeOH) one spot R_f = 0.82; IR (neat, NaCl) 1762 (Ar—OAc), 1745 (Bn ester), 1658 cm^{-1} (amide C=O); 300 MHz ^{1}H NMR ($CDCl_3$) δ 7.20, 7.00 (2 m, 1 : 3 H, O-aryl H), 7.35 (s, 5, Ph), 5.16 (center of AB quartet, 2, C*H*$_2$Ph), 4.71, (s, OCH_2 C=O, 4.67 (s, NC*H*C=O), 3.60, 3.70, 3.84 (m, 4, δ-CH_2N), 2.32 (s, 3, CH_3C=O), 1.92–2.1, 2.15–2.3 (2 m, 8, β, γ-CH_2N); mass spectrum (electrospray ionization) *m/z* (positive ions) 495.4 (M + H).

2.11. *N*-2-(ACETOXY-1-PHENOXYACETYL)PROLYLPROLINE **14**

A mixture of **13** (0.02 g, 0.04 mmol) and $Pd(OH)_2$/C (0.006 g) in MeOH (0.5 mL) was stirred under a double-layered hydrogen balloon for 10 h. The resultant mixture was suction filtered through a pad of Celite^{TM} with caution. The resultant filtrate was concentrated, chromatographed on silica gel, eluted with 5–20% MeOH in $CHCl_3$ and evaporated *in vacuo* to give **14** as a white solid (0.01 g, 0.025 mmol, 63%), mp 75–82 °C; TLC (20% MeOH in $CHCl_3$, $KMnO_4$ stain) mainly one spot R_f = 0.35 with slight impurities; IR (1% KBr) 3700–2800 broad with peaks at 3500, 2971 (CO_2H, CH), 1763 (Ar—OAc), 1654 (amide C=O) cm^{-1}; 300 MHz ^{1}H NMR ($CDCl_3$) δ 7.20, 7.00 (2 m, 1 : 3 H, O-aryl H), 4.65 (s, 2, OCH_2), 4.60 (s, 2, α-NCHC=O), 3.60, 3.70, 3.80 (3 m, 4, δ-prolyl CH_2), 1.90–2.35 (2 m, 8, β, γ-prolyl CH_2), 2.38 (s, 3, OAc).

2.12. *N*-2-(HYDROXY-1-PHENOXYACETYL)PROLYLPROLINE BENZYL ESTER **15**

A solution of **13** (1.4 g, 0.0028 mol) and pyrrolidine (1.2 mL, 1.02 g, 0.014 mol, 5.1 equiv.) in CH_2Cl_2 (20 mL) was stirred at rt for 1 h. The resultant solution was diluted with CH_2Cl_2 (10 mL), washed with 1 N HCl (2 × 20 mL), H_2O (2 × 20 mL), and saturated NaCl (20 mL) to give an organic layer which was concentrated *in vacuo* to give **15** as a thick syrup (1.34 g, 0.0029 mol if pure); IR (NaCl) 3500–2800 broad, 1741 (ester C=O), 1643 (amide C=O), 736, 700 (aryl) cm^{-1}; 300 MHz ^{1}H NMR ($CDCl_3$) δ 9.28 (s, 1, OH), 7.34 (s, 5, Ph), 7.27 (m, 3, aryl H), 6.97 (t, 1, aryl H), 5.15 (center of AB quartet, 2, C*H*$_2$Ph), 4.75, 4.66 (m, 4, OCH_2, α-NCHC=O), 3.84, 3.61, 3.58 (centers of 3 m, 4, δ-CH_2 prolyl H),1.87–2.5 (m, 8, β, γ-CH_2 prolyl H); ^{13}C NMR ($CDCl_3$) δ 172 (ester C=O), 169.8, 169.1 (2 amide C=O), 149.7, 135.7, 128.6, 128.3, 128.2, 125.2, 120.2, 120.0, 117.4, 72.0, 66.9, 58.9, 58.3, 46,8, 45.8, 45.6, 28.9, 27.9, 26.2, 25.0, 24.7, 24.6.

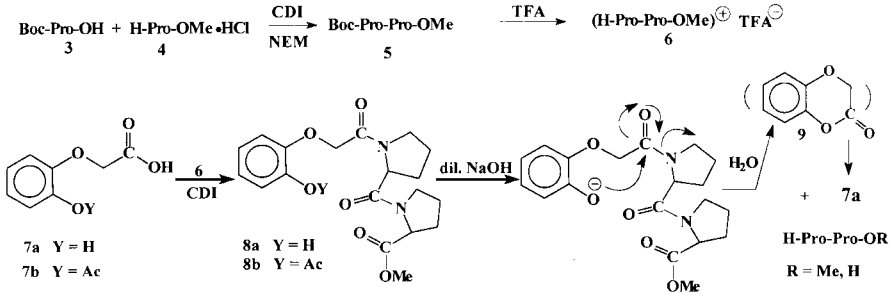
2.13. *N*-2-(HYDROXY-1-PHENOXYACETYL)PROLYLPROLINE **2**

A mixture of **15** (1.3 g, 0.0029 mol) and 10% Pd/C (0.067 g) in MeOH (20 mL) was stirred under a double layered hydrogen balloon for 1.5 h. The resultant mixture was suction filtered through a Celite^{TM} pad with caution (fire hazard: don't allow pad to become dry unless it is under an inert atmosphere) and concentrated *in vacuo* to give **2** as a colorless foam (1.02 g) which partially solidified upon cooling (0.77 g, 0.0021 mol, 75%): mp 75–80 °C; TLC (3 : 1 EtOAc : MeOH) one spot R_f = 0.15; IR (1% KBr) 3600–2700 broad with peaks at 3000, 2950 (ArOH, CO_2H, CH), 1748 (CO_2H C=O), 1655 (amide C=O) cm^{-1}; 300 MHz ^{1}H NMR (DMSO-d_6) δ 9.35, 9.20 (OH), ($CDCl_3$) 6.98, 6.81 (2 m, 3 : 1 H, O-aryl H), 4.77, 4.68 (m, 4, OCH_2, α-NCHC=O), 3.45, 3.58, 3.89 (3 m, 4, δ-prolyl CH_2), 2.00–2.40 (m, 8, β,γ-prolyl CH_2); mass spectrum calcd for $C_{18}H_{22}N_2O_6$ + H = 363.1550; found (FAB) M + H = 363.1556; (electrospray, positive ions) *m/z* (rel. intensity) 385 (6, M + Na), 363.4 (25, M + H), 344 (22, M—H_2O), 227 (50), 155 (19, OCH_2C(=O)Pro-C=O), 115(6), 113.9 (100); (negative ions) 723.3 (20, 2M-H), 362.3 (10, M), 361.4 (100, M—H), 167 (60), 113 (70).

3. Results and Discussion

3.1. SYNTHETIC PATHWAYS

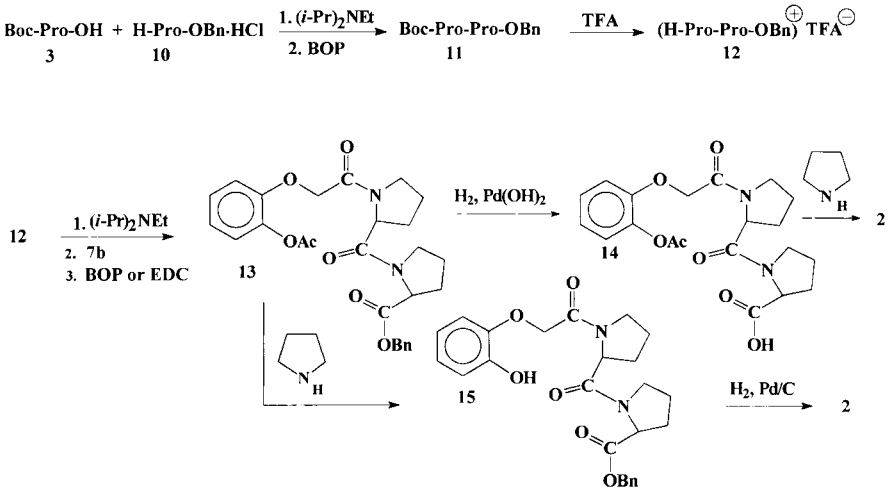
The first synthetic pathway (Scheme 1) started with the condensation of Boc-proline with proline methyl ester hydrochloride using carbonyldiimidazole (CDI) and *N*-ethylmorpholine (NEM) to give Boc-prolylproline methyl ester (**5**). Treatment of **5** with trifluoroacetic acid (TFA) removed the Boc group to give prolyl-proline methyl ester trifluoroacetate (**6**). Condensation of 2-acetoxyphenoxyacetic acid (**7b**) with CDI, **6**, and N-methylmorpholine (NMM) gave the acetoxy car-



Scheme 1.

bomethoxy ester **8b** [10]. The phenolic acetate was readily lost upon column chromatography to give the free phenol **8a**. Attempts to hydrolyze either **8a** or **8b** with dilute NaOH did not give the desired **2**. Instead fragmentation of the molecule gave **7a**, presumably *via* lactone **9**, and Pro-Pro (assumed, not isolated). Attempts to hydrolyze **8a** with 1N HCl gave erratic results. Several alternative approaches for ester conversion to acid were attempted using the more available **1a** as a model for **8**. Treatment of **1a** with iodotrimethylsilane [11] was not successful. Also unsuccessful were several attempts to hydrolyze **1a** with chymotripsin according to procedures of Bodansky [12a] or Walton [12b] or with pig liver esterase according to Moorlag *et al*. [13]. It is recognized that another methyl ester would be a more effective model since ethyl esters are hydrolyzed much more slowly. The ease of fragmentation of **8** as compared to **1** is presumably related to the former's more rigid structure which allows the facile addition of the phenoxy anion to the prolyl carbonyl as shown in Scheme 1. An attempt to use *tert*-butyldimethylsilyl as the blocking group for the phenolic OH in **7** instead of acetyl was also unsuccessful.

It was decided to keep the phenolic acetate approach but to use a benzyl ester at the carboxy end. This successful pathway started with the preparation of Boc-prolylproline benzyl ester **11** by the condensation of Boc-proline with proline benzyl ester HCl **10**, diisopropylethylamine and BOP (benzotriazolyl *N*-oxytridimethylamino-phosphonium hexafluorophosphate) coupling reagent [14a] (Scheme 2). Treatment of **11** with TFA gave Pro-Pro benzyl ester trifluoroacetate **12**, which was coupled with 2-acetoxyphenoxyacetic acid, diisopropylethylamine, and BOP reagent to yield the acetoxy benzyl ester **13**. Since this method gave hexamethylphosphoramide (HMPA) as an impurity that was hard to remove completely, EDC (1-(3-dimethylaminopropyl)-3-ethylcarbodiimide) [14b] was later used as the coupling reagent. Hydrogenation of **13** with a $Pd(OH)_2$ catalyst gave the acetoxy acid **14**. Surprisingly, the phenolic acetate of **14** is resistant to removal by acid promoted hydrolysis. It was removed with pyrrolidine according to the procedure of Mansson [15] to give **2**. Alternatively, removal of the phenolic acetate with pyrrolidine first to give the benzyl ester **15**, followed by hydrogenolysis of the ester, gave **2** in a better overall yield.



Scheme 2.

The structure of **2** is confirmed by a detailed analysis of its ^{1}H NMR and mass spectrum. See Experimental. The labeling of the prolyl hydrogens is according to Deber *et al.* [8], who previously synthesized **11**. It is useful in assigning ^{1}H NMR signals. The ^{1}H NMR spectra of the aryl protons in the acetoxy compound **8b**, **13**, and **14** have two absorptions at *ca* δ 7.2 (t, 1), 7.0 (m, 3). The ratio of the two absorptions is *reversed* in the free phenols **2**, **8a**, **15** which exhibit absorptions at *ca* δ 7.0 (m, 3), 6.8 (t, 1). It is postulated that the triplet (1H) is probably the hydrogen that is *para* to the hydroxy/acetoxy group. It is more shielded in the free phenols and is diagnostic for a free phenol in these compounds [16].

3.2. METAL CATION BINDING STUDIES

The binding of **2** with metal cations in methanol was studied with a previously described UV method [1c, 2b]. Addition of incremental amounts of concentrated solutions of Group II metal cation bromides to a dilute solution of **2** in anhydrous methanol gives shifts to longer wavelengths from the absorption maximum for **2** which occurs at 275 nm. From the calculated ΔA_{max} values, K_{app} values and the stoichiometry of binding can be calculated using the Scatchard plot method [2b]. The data are summarized in Table I.

Compound **2** is found to be a weaker binder for Ba^{2+} and Ca^{2+} [K_{app} 17,600 for Ba^{2+}, for Ca^{2+} 6,770) than the previously synthesized **1b** (K_{app} 40,000 for Ba^{2+}, 35,300 for Ca^{2+}) [5]. However **2** is more selective (Ba^{2+}/Ca^{2+} = 2.6 for **2** and 1.1 for **1b**). As was found for **1b**, the binding of Sr^{2+} with **2** is much weaker than that for Ba^{2+} or Ca^{2+} and the stoichiometry of binding is not 1 : 1. The reasons for the weak and more complicated Sr^{2+} binding of **1b** and **2** are not clear. For most of our previously synthesized neutral 1,2-dioxydiamide ionophores, such as **16** or **17**

Table I. Binding constants for **2** (5.73×10^{-5} M) in methanol

Salt (0.1 M)	UV λ nm[a]	ΔA_{max}	R^b	K_{app}	*n* (cation/ligand)	R^b
$CaBr_2$	282	0.120	0.995	$6.77 \pm 1.49 \times 10^3$	0.84	0.930
$BaBr_2$	285	0.0700	0.986	$17.6 \pm 2.78 \times 10^3$	0.89	0.962

[a] Wavelength of maximum change in absorbance upon complex formation. The values at 285 nm for Ca^{2+} or 282 nm for Ba^{2+} gave lower *R* values.
[b] Correlation coefficient in linear regression analysis.

(Figure 1) [1, 2], the ordering of metal cation binding is $Ca^{2+} > Sr^{2+} > Ba^{2+} \gg Mg^{2+}$. This ordering of metal cation binding is found for Troponin [17]. It has been rationalized using intramolecular 'cavity' fitting and other factors for compounds including **16–18** by Simon *et al*. [18].

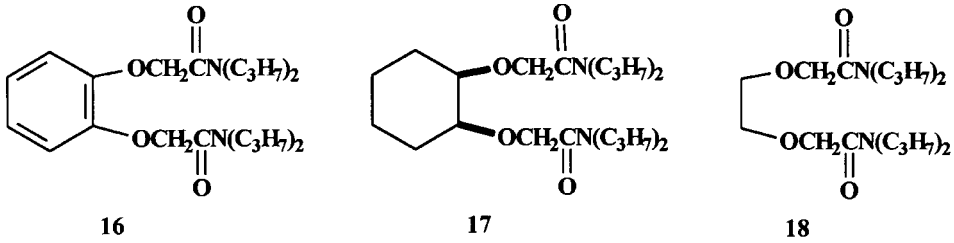


Figure 1.

3.3. MOLECULAR MECHANICS (MM) STUDIES

Originally, MMX (enhanced MM2) minimization calculations using *PCModel, V.6*, [6] predicted that **2** would more readily form a stable hydrogen bonded form between the phenolic OH and the carboxy carbonyl than does **1b**. It was postulated that such a *pseudo* cyclic form might make **2** a stronger metal cation binder than **1b** since cyclic chelators are usually stronger cation binders than acyclic ones [19]. The MMX and GMMX (global steric energy minimization search) parts of *PCModel, V.7* were applied to find the minimized energy conformations of the ligands. It was found that the hydrogen bonded form of **2** is not at the global minimum. It is less stable than other more open conformations. In comparison, **1b** spontaneously forms a hydrogen bond between the carbonyl closest to the aryl group and the N—H of the second amide. Thus **2** does not have a more favorable initial conformation for cation binding than **1b**. The ligand must reorganize its conformation to form the metal complex. It may be more relevant to consider the geometries of the Ba^{2+} and Ca^{2+} complexes of **1b** and **2**. Since such complexes have not yet been isolated, only hypothetical structures can be studied at this time. Using *PCModel V.7*, the GMMX modeling of such Ba^{2+} and Ca^{2+} complexes of **2** (Figure 2) and of **1b** (Figure 3), predicts them all to be symmetrical structures

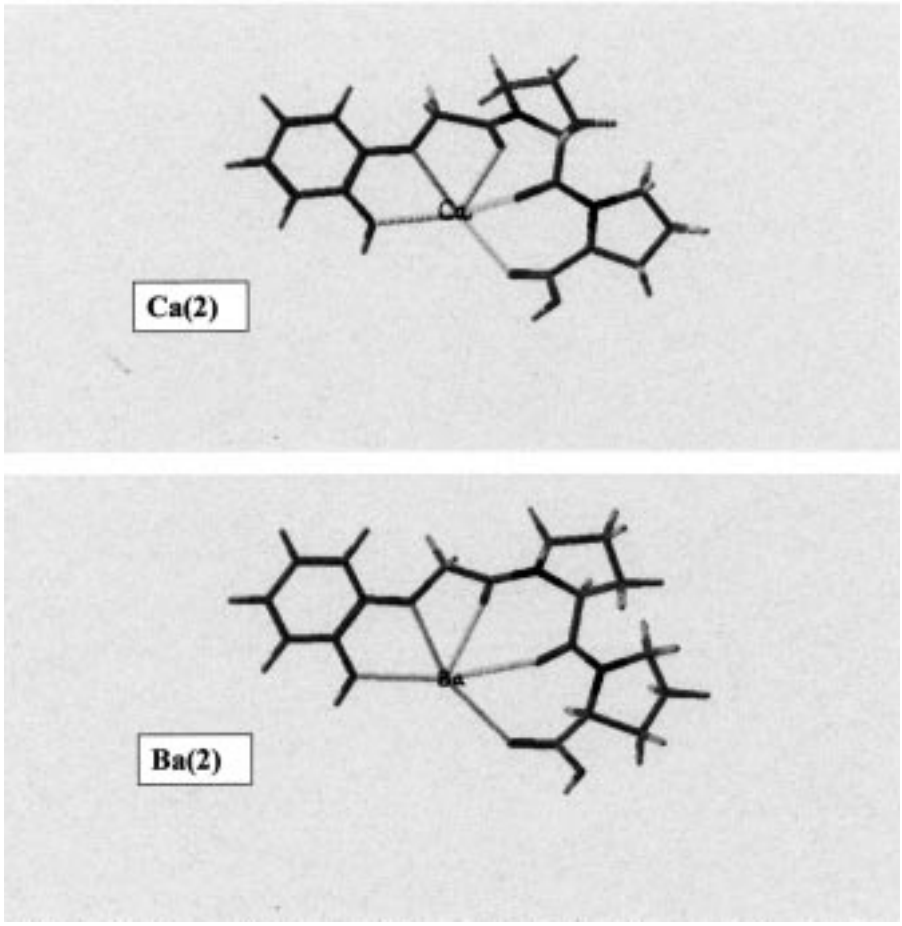


Figure 2. Ca^{2+} and Ba^{2+} complexes of **2**.

with all of the metal-oxygen bonds fairly equal in length. The Ca—O distances in **Ca (1b)**, 2.24–2.54 Å, are not that different from the sum of oxygen and Ca $^{2+}$ ionic radii (1.40 + 0.99 =2.39 Å). They are also similar to the Ca—O distances of 2.46 Å found in a single crystal X-ray analysis of the $CaBr_2$ complex of **18** [20]. Complexes **Ba (2)** and **Ca (2)** reproducibly form with one very long metal-oxygen bond (Ca—O = 6.42 Å, Ba—O = 6.51 Å) in the initial MMX energy minimizations. They become symmetrical after the global searches done with GMMX [21].

The structure determinations are based on a number of assumptions including penta-coordination of the metal cation. The geometric optimizations using MMX, initially used hexa-coordination for both Ca^{2+} and Ba^{2+} with a molecule of methanol added. However, the GMMX method does not currently work with the added methanol ligand and has to be done with penta-coordination. Omitting methanol as a ligand (penta-coordination of the ligands) gives similar metal-oxygen bond

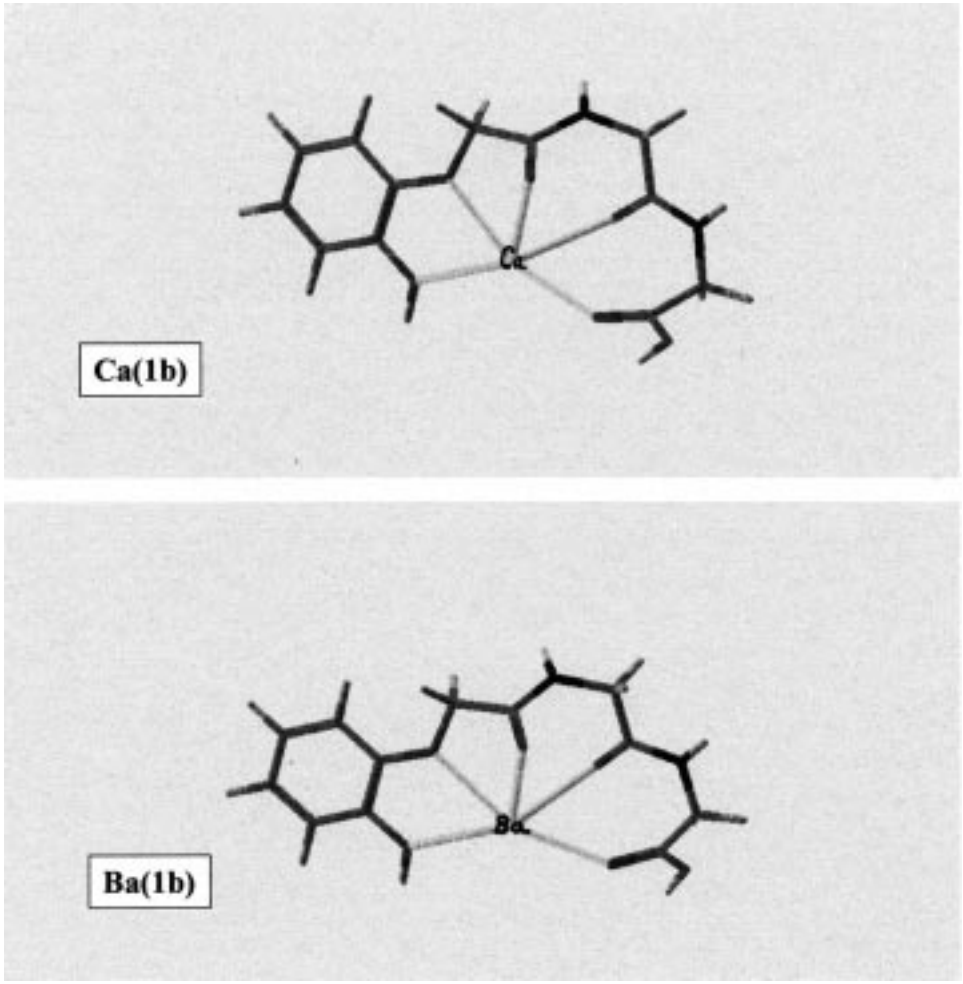


Figure 3. Ca^{2+} and Ba^{2+} complexes of **1b**.

distances. The penta-coordinate form allows space for a methanol ligand without changing the overall conformation of the complex.

The formation of covalent bonds between the ligand and metal cation is utilized. The simplified usage of such covalent bonding, instead of electrostatic and ionic bonding, has been successfully used by Hancock in MM calculations of the geometry and relative stabilities of metal cation complexes of 18-crown-6 [22]. The default dielectric constant of 1.5 was used, to fit with the assumption of covalent bonding in the gaseous state. It should be noted that the geometry of the complexes or ligands do not change if a dielectric constant of 33 is used. That is the value for methanol, which is the solvent used in the binding studies.

4. Conclusions

Ligand **2** is found to be a moderate metal cation binder with greater selectivity for binding Ba^{2+} rather than Ca^{2+} than is found for **1b** [5]. It is a weaker binder than is **1b**, in contradiction to early expectations. At this point, a more thorough study involving MM and semi-empirical methods will be undertaken to try to predict which structural modifications of **1b** might lead to enhanced metal cation binding. One such modification will involve the incorporation of six, rather than five, coordination sites in the molecule itself.

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21. We thank a referee for suggesting that we should do global conformational energy searches of our ligands and complexes. We were able to do this with the recent availability of *V 7* of *PCModel*. We hope to utilize this method in future research aimed at correlating our experimental metal cation binding data on various amide-containing ligands with MM and semi-empirical energy calculations.
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