$R^2 = CH_3$), 110718-45-1; 8 ($R^1 = CH_3$), 110718-51-9; 8 ($R^1$ = Ph), 110718-59-7; 9, 110718-49-5; 10 ($R^1 = CH_3$), 110718-57-5; $CH_3C(OEt)$=NH·HCl, 2208-07-3; $PhCH_2C(OEt)$=NH·HCl, 5442-34-2; $CH_3CH_2CH_2C(OEt)$=NH·HCl, 2208-08-4; $(CH_3)_2CHC(OEt)$=NH·HCl, 52070-18-5; ethyl ortho formate, 122-51-0; 5-(2-methylphenyl)-1,3,4-thiadiazole-2-hydroxaminic acid chloride, 110825-31-5; 5-(2-methylphenyl)-1,3,4-thiadiazole-2-hydroxamine acid azide, 110718-50-8; butylamine, 109-73-9; benzylamine, 100-46-9; diethylamine, 109-89-7; morpholine, 110-91-8; ethylenediamine, 107-15-3.

# Specific Sequestering Agents for the Actinides. 16. Synthesis and Initial Biological Testing of Polydentate Oxohydroxypyridinecarboxylate Ligands[1]

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Chemical and biological similarities of plutonium(IV) and iron(III) suggested that octadentate ligands containing hydroxamate or catecholate functional groups, which are found in microbial iron chelating agents (siderophores), would be effective and relatively selective complexing agents for actinide(IV) ions. However, their usefulness for in vivo chelation of actinide(IV) is limited, because catechol and hydroxamate are such weak acids that the potential for octadentate binding of actinide(IV) cannot be achieved at physiological pH. The structurally similar monoprotic and more acidic 1-hydroxy-2(1*H*)-pyridinone (1,2-HOPO) group was, therefore, incorporated into multidentate ligands. Treatment of 1,2-dihydro-1-hydroxy-2-oxopyridine-6-carboxylic acid (**5**) with phosgene in THF solution gives the active ester poly[1,2-dihydro-1,2-dioxopyridine-6-carboxylate], which upon treatment with excess anhydrous dimethylamine gave a 60% yield of *N,N*-dimethyl-1,2-dihydro-1-hydroxy-2-oxopyridine-6-carboxamide (**6**). A similarly reactive intermediate was prepared from **5** and an equimolar amount of phosgene in *N,N*-dimethylacetamide. Combined in situ with 1,3-propanediamine, benzylamine, spermine, spermidine, 1,3,5-tris(aminomethyl)benzene, or desferrioxamine B and excess triethylamine, the latter intermediate gave the corresponding amides in isolated yields ranging from 16% to 60%. The free ligands, their Zn(II) complexes, and the ferric complex of 3,4,3-LIHOPO were administered to mice [30 μmol/kg intraperitoneally 1 h after Pu(IV)-238 citrate, kill at 24 h]. Net Pu removal [Pu excretion (treated) − Pu excretion (control)], expressed as percent of injected Pu, was as follows: Na salts and Zn(II) complexes, respectively, of 3-LIHOPO (54, 56), 3,4-LIHOPO (58, 60), 3,4,3-LIHOPO (73, 76); Na salts of MEHOPO (46), DFO-HOPO (78); Fe(III) complex of 3,4,3-LIHOPO (79). DFO-HOPO and 3,4,3-LIHOPO and its Zn(II) and Fe(III) complexes promoted significantly more Pu excretion than $CaNa_3$-DTPA (61% of injected Pu). Preliminary findings on the acute toxicity of the poly(HOPO) ligands and HOPO monomers are presented in an appendix. The biological data indicate strongly that the aqueous solubility and relatively high acidity of the octadentate HOPO ligands 3,4,3-LIHOPO and DFO-HOPO allow them to form complete eight-coordinate complexes with Pu(IV) ion.

The affinity and specificity of the siderophores [Fe(III) chelators produced by microorganisms[2,3]], prompted us[4-7] and others[8] to synthesize macrochelating agents composed of their binding groups, hydroxamic acid or catechol. Such ligands should be medically useful for removal and carrying of metal ions with similar charge radius ratios [$e/r$ for Fe(III), Pu(IV), Ga(III), and In(III) is 0.465, 0.484, 0.375 and 0.417 e $nm^{-1}$, respectively[9]]. The coordination behavior of the tetravalent actinides [actinide(IV)] and Fe(III) are so similar that Pu(IV) can fit into the Fe(III) positions in mammalian iron transport and storage systems.[10,11] We have exploited that similarity by using the siderophore functional groups to construct sequestering agents specific for actinide(IV).[5,6,12-16]

The first such agents were based on enterobactin, a hexadentate ligand that coordinates to Fe(III) through the

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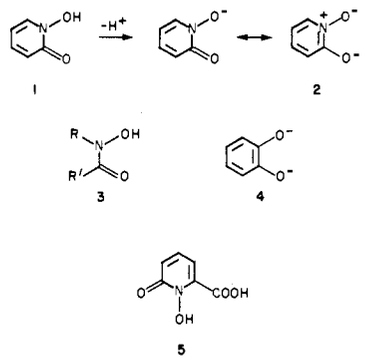

**Figure 1.** Structures of 1-hydroxy-2(1*H*)-pyridinone (1), the monoanion of 1 and its aromatic resonance form (**2**), a general hydroxamic acid (**3**), catechol dianion (4), and 1,2-dihydro-1-hydroxy-2-oxopyridine-6-carboxylic acid (**5**).

six hydroxyls of three catechol groups (cat.).[6,14-16] The structure of $[Th(cat.)_4]^{4-}$ crystals (prepared in 1 M NaOH) approaches the dodecahedral structure of many eight-coordinate metal ions;[1,17] therefore, ligands containing four catechols connected by alkyl chains of suitable lengths were expected to form eight-coordinate complexes with Pu(IV).[6,14-16] The most effective tetracatecholate ligands promoted prompt excretion of 70% of circulating Pu(IV) from mice[18,19] and 88% from dogs.[20] However, at pH 7.4, the weak acidity of the catechol hydroxyl groups and the eight-proton stoichiometry of the complexation reaction prevent the tetracatechoyl ligands from forming octadentate Pu(IV) or Ce(IV) complexes.[21] Thus, sequestering agents were sought with metal-binding groups similar to, but more acidic than, catechol so as to coordinate fully with actinide(IV) ions in dilute solution at pH 7.4.

The tris(hydroxamic acid) siderophore desferrioxamine B (DFOM) removes circulating Th(IV) and Pu(IV) from animals.[11,18] Crystals of tetrakis Th(IV) complexes with monohydroxamic acids (formed in 1 N NaOH) display an eight-coordinate geometry,[22] but synthesis of tetrakis hydroxamato ligands is difficult. Hydroxypyridinones are structural and electronic analogues of both hydroxamic acid and catechol (Figure 1); 1-hydroxy-2(1*H*)-pyridinone (1,2-HOPO, **1** and **2** in Figure 1) can be viewed as a cyclic hydroxamic acid, while as the anion, the zwitterionic resonance form is isoelectronic with catechol.[23] At least one siderophore is a HOPO derivative.[24,25] The cyclic hydroxamic acid groups of the HOPO are more acidic than aliphatic hydroxamic acids; the $pK_a$ is 5.8 for 1,2-HOPO and as low as 5.17 when there are electronegative substituents on the ring.[26] Insofar as chelation is concerned, the HOPO's have other advantages: They are monoprotic acids, which is important because the formation of metal complexes by these ligands is expontentially dependent on $H^+$.[23]

$$lLH_n + M^{m+} \rightarrow ML_l^{(m-nl)+} + nlH^+$$

$$K = \frac{[ML_l^{(m-nl)+}][H^+]^{nl}}{[LH_n]^l[M^{m+}]}$$

The practical effects are as follows. (i) The HOPO ligands should be soluble in all but strongly acidic aqueous media. (ii) Metal binding should be fast and effective in the pH range 5–8. (iii) The isomer possesses synthetic advantages in that the 6-carboxylic acid derived of **5** can be made in a straightforward manner,[23] and 1,2,6-placement of functional groups allows activation of the carboxylic acid and obviates the protection of the *N*-hydroxyl group (vide infra).

## Experimental Section

**Ligand Synthesis. Equipment.** Infrared spectra were obtained with a Perkin-Elmer Model 283 spectrophotometer. The NMR spectra were obtained on JEOL FX-90Q (90 MHz) or UCB-250 (250 MHz) spectrometers. The EI mass spectra were obtained with an AEI MS-12 instrument, and a Finnigan 4000 instrument provided CI mass spectra (with methane as a carrier). A Kratos MS-50 mass spectrometer using a xenon beam was used to acquire $^+$FAB mass spectra. Microanalyses for C, H, and N were performed for all new compounds by the Microanalytical Laboratory, Chemistry Department, University of California, Berkeley. The C, H, and N analyses were within 0.4% of the calculated values for all new compounds, unless otherwise noted in Table I.

The HPLC was performed on a Beckman-Altex Model 340 system with an Altex Ultrasphere ODS precolumn (4.6 mm i.d. × 40 mm) and column (4.6 mm i.d. × 150 mm) for analytical work. A similar 10 mm i.d. × 250 mm column was used for preparative chromatography. Unless otherwise stated, the mobile phase was a methanol–water gradient (0–100% MeOH over 10 min), with each component 0.025 M in formic acid (pH 2.8).

**Starting Materials and Reagents.** The 1,2-dihydro-1-hydroxy-2-oxopyridine-6-carboxylic acid[23] (**5**; 1,2-HOPO-6-COOH) and 1,3,5-tris(aminomethyl)benzene[14] (11; X = H) were prepared as previously described. Triethylamine, THF, and *N,N*-dimethylacetamide (DMAA) were purified by distillation under $N_2$ from sodium benzophenone ketyl, potassium benzophenone ketyl, and $P_2O_5$, respectively. Spermine (**9**; X = H) and spermidine (**10**; X = H) were obtained from The Ames Laboratories, Inc., Milford, CT. Desferrioxamine B (DFOM, **12**; X = +$MeSO_3H$) was a gift from Ciba-Geigy. Other organic reagents were purchased from Aldrich Chemical Co. and were used as received.

Solutions of phosgene in THF were prepared by bubbling the gas into a weighed quantity of anhydrous solvent cooled in an ice bath until the desired mass was dissolved. The titer of this solution was determined by reacting an aliquot with excess aniline in THF and quantifying the resulting carbanilide by HPLC.

**Reaction of 5 with Phosgene in THF.** A dry 10-mL flask with a serum stopper and magnetic stirrer was flushed with $N_2$ and charged with 5 mL of THF and 0.155 g (1.00 mmol) of **5**. To this white suspension was added via syringe 0.26 mL of 3.29 M phosgene in THF. After the mixture was stirred at room temperature for about 10 min, the solid completely disappeared, yielding a clear, light yellow mixture. Over the course of an additional 15 min, solid again appeared. Aliquots were withdrawn from the reaction mixture at intervals for examination by IR. After about 1 h at room temperature, volatile components were removed from the reaction mixture in vacuo. The IR and mass spectra of the light yellow, amorphous residue were obtained.

**Reaction of 5 with Phosgene and DMAA.** A dry, three-neck 50-mL flask with a serum stopper, $N_2$ inlet, and magnetic stirrer was cooled in an ice bath, flushed with $N_2$, and charged with 10 mL of DMAA and 0.53 mL of 3.79 M phosgene/THF. After 5 min, a solution of 0.310 g (2.0 mmol) of **5** was added, and the mixture became yellow. Next was added 0.40 g (4.0 mmol) of

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triethylamine, and the yellow color intensified. The cooling bath was removed, and the reaction mixture was stirred at room temperature for 30 min. Precipitated $Et_3N$·HCl was removed by filtration under $N_2$. Volatiles were removed from the amber filtrate in vacuo. The residue was examined by IR, NMR, and mass spectroscopy.

A similar reaction was attempted with a stoichiometric amount of DMAA in THF solvent. However, most of the added **5** remained undissolved, even after a 14-h reaction period.

This reaction was also examined by dissolving **5** in DMAA and treating the resulting solution with phosgene and, subsequently, $Et_3N$. Samples were withdrawn at each stage and sealed in capillary tubes for CI mass spectroscopy.

***N,N'*-Dimethyl-1,2-dihydro-1-hydroxy-2-oxopyridine-6-carboxamide (1,2-HOPO-6-CONMe$_2$).** A three-neck 25-mL flask with a magnetic stirrer, subsurface gas inlet, and gas outlet was charged with 10 mL of THF and 0.60 g (3.9 mmol) of **5**. Phosgene gas was bubbled in until the suspended starting material had dissolved. After a period of 15 min at 25 °C, volatiles were removed from the mixture in vacuo. The viscous residue was dissolved in THF, and this solution was added to 20 mL of THF saturated with dimethylamine. Additional gas was bubbled into the solution during the addition to ensure an excess. The resulting suspension was filtered to remove $[CH_3NH]Cl$, and solvent and excess amine were removed on a rotary evaporator. The residue was dissolved in 7 mL of water, and this solution was applied to a 1.45 cm i.d. × 17 cm long column of AG 50-X8 ion-exchange resin in the $H^+$ form and eluted with water. The HCl appeared first, followed by a light yellow fraction that gave a red color when treated with ferric ion. Removal of water from the latter fraction left 0.42 g (60%) of light yellow product, mp 165–167 °C [lit.[23] mp 167.5–168 °C].

**General Procedure for the Preparation of Ligands in DMAA.** A dry 250-mL three-neck round-bottom flask equipped with a magnetic stirrer, gas inlet, addition funnel, and thermometer was flushed with $N_2$ and charged with 3.10 g (20 mmol) of **5** and 50 mL of DMAA. The solution was cooled in an ice bath and then treated dropwise with 21 mmol of phosgene in THF solution. The resulting mildly exothermic reaction evolved some gas and produced a bright yellow reaction mixture. The addition of 8.71 g (86 mmol) of $Et_3N$ also gave an exothermic reaction, and a white precipitate was formed. After being stirred for 30 min at 5–100 °C, this suspension was treated with 16 mmol of the selected amine starting material (**7–12**). The resulting mixture was stirred overnight at room temperature. The reaction mixture was filtered to remove precipitated $[Et_3NH]Cl$, and solvent was removed from the filtrate on a rotary evaporator. The viscous, amber residue was partitioned between 50 mL each of chloroform and dilute aqueous $NH_3$ (pH 10). The chloroform extract (containing nonpolar byproducts) was discarded; the aqueous phase was concentrated to about 20 mL. This solution then was chromatographed on a 2.5 cm i.d. × 20 cm long column of AG 50-X8 ion-exchange resin in the $H^+$ form and eluted with water. The HCl eluted first, followed immediately by an acidic (pH 1.5) yellow fraction that gave a bright red-orange precipitate upon reaction with $Fe^{3+}$. This fraction contained essentially only **5**, and about 0.6 g (20%) could be recovered upon evaporation. The pH of the eluant then began to rise to about 3, and the color of the $Fe^{3+}$ spot test became brown-red. This signaled elution of the product-containing fraction, which was usually contaminated with small amounts of **5**, as well as byproducts. The mono- and bis[1,6-dihydro-1-hydroxy-6-oxopyridinyl-2-carbonyl]amines (**6–8**, **12**) were recrystallized from water; the tris and tetrakis compounds were hygroscopic glassy solids best purified by HPLC (see Table I).

**Biological Evaluation.** Under ether anesthesia, adult female mice were injected in a lateral tail vein with 0.2 mL of 0.004 M sodium citrate containing 9250 Bq of $^{238}Pu$(IV). One hour later, 30 μmol/kg of a ligand dissolved in isotonic saline at pH 7.1 ± 0.1 (120 μmol/kg of monomers) was injected intraperitoneally (ip) into groups of five mice, which were housed together in metabolism cages; they received water but no food, and were killed 24 h after the Pu injection. Groups of Pu-injected mice, given saline instead of a ligand, were killed at 1 or 24 h to define, respectively, the amount and distribution of Pu at the time of ligand administration and at the end of the experiment. Details of sample collection, preparation, radioactivity measurement, and data reduction have been published.[18,19]

The Zn(II) complexes were prepared by reacting stoichiometric quantities of $ZnCl_2$ and free ligand. The Fe(III) complex with 3,4,3-LIHOPO was prepared by adding excess $FeCl_3$ to the free ligand and filtering and washing the resulting precipitate. Suspensions were sonicated, and the pH was adjusted to 7.1. The complexes were rich suspensions at the concentrations used for toxicity testing. The Zn(II)–3,4-LIHOPO complex was in solution, and the others were thin suspensions at the concentration used to study Pu removal.

The procedure used to obtain preliminary information on acute toxicity of ligands (7-day survival after ip injection of 100–1000 μmol/kg in pairs of mice), and the detailed results are presented as supplementary material. Acutely toxic ligands are noted by asterisks in Table II.

## Results

**Synthetic Procedures. Nomenclature.** A system of abbreviated nomenclature was devised for the synthetic poly(catechoylamide) ligands based on CAM as an acronym for catechoyl amide groups.[4] The numbers in the prefix (separated by commas) indicate the number of methylene groups in the connecting chains. This system has been adapted to the HOPO-1,2 ligands, which are isostructural with the bis-, tris-, or tetrakis(catechoylamide) ligands,[27] by the use of LIHOPO, an acronym, for linear hydroxypyridinone. The abbreviations are shown in Table I.

**Products.** The yields and characterizations of the products are summarized below and in Table I.

**Physical Properties.** The mono- and bis(HOPO)'s are white to pale yellow solids. While not necessarily crystalline, they can be easily isolated as freely flowing powders with sharp melting points. As the number of pendant HOPO's is increased, however, the solids become progressively more hygroscopic, glassy in nature, and difficult to characterize. The most distinctive feature of their NMR spectra is the set of three doublets in the aromatic region, arising from the HOPO ring protons. Their IR spectra display a strong band at ca. 1650 $cm^{-1}$ due to overlapping amide and ring carbonyl absorptions.[28] A ring stretching band at 1570 $cm^{-1}$ is also observed. The $^+$FAB mass spectra are particularly useful for confirming the structure of these ligands. They display intense ($M^+ + H$) peaks as well as weaker ones resulting from successive losses (up to three) of 16 amu (oxygen) for each HOPO moiety. Fragments from cleavage of the carbonyl carbon–amide nitrogen bond are also observed occasionally.

**Chemical Properties.** The HOPO's show moderate to great solubility in water. They are weakly acidic, and the pH of a saturated aqueous solution is typically about 3. They form strong complexes with metal ions such as Th(IV)[22] and Fe(III)[22,23,27] and less so with Zn(II)[30] (vide infra). The orange-red complexes of ferric ion with **6**[23] and **8**[27] have been characterized.

**Reaction of 5 with Phosgene.** Due to the low solubility of **5** in THF, the starting solution displayed only weak carbonyl absorptions at 1720 and 1628 $cm^{-1}$. These were not observable in the first sample of the reaction mixture taken 15 min after addition of the phosgene solution. Bands at 1805 (sh, $COCl_2$), 1800 (s), 1755 (m), 1690 (s), and 1595 (m) $cm^{-1}$ were observed. After 2 h at 25 °C,

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**Table I.** 1,2-Dihydroxy-6-oxopyridinecarboxylate Ligands (As Produced in Schemes II or III)

| product[a] | yield, % | mp, °C | molecular formula (FW) | MS | NMR[f] |
|---|---|---|---|---|---|
| $(CH_3)_2NX$ **6** | 60 | 165–168 | $C_8H_{10}N_2O_3$[b] (182.18) | 182 ($M^+$, 1), 166 (88), 122 (49), 72 (92)[g] | 2.95 (s, 3, $NCH_3$), 3.05 (s, 3, $NCH_3$), 4.77 (br s, 1, NOH), 6.29 (dd, 1, $J$ = 1.6, 7.03, pyr-3- or 5-H), 6.59 (dd, 1, $J$ = 1.6, 9.22, pyr-5- or 3-H), 7.39, (dd, 1, $J$ = 7.03, 9.22, pyr-4-H)[h] |
| $C_6H_5CH_2NHX$ **7** | 34 | 129–130 | $C_{13}H_{12}N_2O_3$[b] (244.27) | 259 ($M^+$ + Na, 3), 245 ($M^+$ + H, 100), 229 (79)[i] | 4.40 (br s, 1, $NCH_2$), 4.47 (br s, 1, $NCH_2$), 6.34 (d, 1, $J$= 6.2, pyr-3- or 5-H), 6.57 (d, 1, $J$ = 7.8, pyr-5- or 3-H), 7.32 (m, 6, $C_6H_5$ and pyr-4-H), 9.37 (br t, 1, $J \approx$ 7, CONH)[h] |
| $XHN(CH_2)_3NHX$ **8** (3-LIHOPO) | 50 | 206–207 dec | $C_{15}H_{16}N_4O_6$[b] (348.32) | 349 ($M^+$ + H, 100), 333 (61), 317 (15)[i] | 1.76 (q, 2, $J$ = 6.5, $CH_2CH_2CH_2$), 3.26 (dt, 4, $J$ = 4.2, 6.5, $NHCH_2CH_2$), 6.30 (dd, 2, $J$ = 1.6, 6.5, pyr-3- or 5-H), 6.56 (dd, 2, $J$ = 1.6, 8.8, pyr-5- or 3-H), 7.39 (dd, 2, $J$ = 6.5, 8.8, pyr-4-H)[h] |
| $XNH(CH_2)_3N(X)(CH_2)_4NHX$ **9** (3,4-LIHOPO) | 34[j] | 130–135 dec | $C_{25}H_{28}N_6O_9$[c] (556.53) | 557 ($M^+$ + H, 100), 541 (65), 525 (19), 509 (4)[i] | 1.55 (br m, 6, $CH_2CH_2CH_2$), 3.2–3.4 (br m, 8, $CONCH_2$), 6.23 (m, 3, pyr-3- or 5-H), 6.55 (~d, 3, $J \approx$ 8, pyr-5- or 3-H), 7.39 (~t, 3, $J \approx$ 8, pyr-4-H), 8.74 (~t, 2, $J \approx$ 7, CONH)[h] |
| $[XNH(CH_2)_3N(X)(CH_2)_2]_2$ **10** (3,4,3-LIHOPO) | 15[j] | 135 dec | $C_{34}H_{38}N_8O_{12}$[d] (750.72) | 773 ($M^+$ + Na, 5), 751 ($M^+$ + H, 86), 735 (100)[i] | 1.73 (br m, 8, $CH_2CH_2CH_2$), 2.8–3.7 (br m, 12, $CONCH_2$), 6.18 (m, 4, pyr-3- or 5-H), 6.53–7.19 (47), 7.03 (22)[d] (~d, 4, $J \approx$ 7, pyr-5- or 3-H), 7.38 (~t, 4, $J \approx$ 7, pyr-4-H), 8.75 (m, 2, CONH)[h] |
| $1,3,5\text{-}(NHX)_3C_6H_3$ **11** (MEHOPO) | 17[j] | 135 dec | $C_{27}H_{24}N_6O_9$[e] (576.32) | 577 ($M^+$ + H, 100), 561 (50) 545 (31)[i] | 4.41 (s, 3, $CONCH_2$), 4.47 (s, 3, $CONCH_2$), 6.36 (dd, 3, $J$ = 1, 6.9, pyr-3- or 5-H), 6.57 (dd, 3, $J$ = 1, 8.5, pyr-5- or 3-H), 7.19 (s, 3, $C_6H_3$), 7.37 (dd, 3, $J$ = 6.9, 8.5, pyr-4-H), 9.35 (~t, 3, $J \approx$ 5, CONH)[h] |
| $XHN[(CH_2)_5N(OH)C(O)\text{-}(CH_2)_2CONH]_2(CH_2)_5N(OH)C\text{-}(O)CH_3$ **12** (DFOHOPO) | 56 | 141–143 | $C_{31}H_{51}N_7O_{11}$[b] (697.79) | 720 ($M^+$ + Na, 23), 698 ($M^+$ + H, 100), 682 (48)[i] | 1.14–1.86 (m, 18, $CH_2CH_2CH_2$), 2.08 (s, 3, $COCH_3$), 2.44 (t, 4, $J$ = 5.9, $NHCOCH_2$), 2.74 (t, 4, $J$ = 5.9, $NOHCOCH_2$), 3.16 (t, 4, $J$ = 6.4, $CONHCH_2$), 3.59 (t, 6, $J$ = 6.9, $CONOHCH_2$), 6.71 (d, 2, $J$ = 8.0, pyr-3- and 5-H), 7.46 (t, 1, $J$ = 8.0, pyr-4-H)[k] |

[a] X = 1,6-Dihydro-1-hydroxy-6-oxopyridinyl-2-carbonyl. [b] Anal. C, H, N. [c] Anal. $C_{25}H_{28}N_6O_9 \cdot 1.5H_2O$. [d] Anal. $C_{34}H_{38}N_8O_{12} \cdot 0.9H_2O$. [e] Anal. $C_{27}H_{24}N_6O_9 \cdot H_2O$ Calcd: C, 54.55; H, 4.41; N, 14.14. Found: C, 54.60; H, 4.70; N, 12.91. [f] $\delta$ (ppm) downfield from $Me_4Si$. [g] EI. [h] In $Me_2SO$-$d_6$. [i] +FAB. [j] Isolated by HPLC. [k] In $CD_3OD$.

the shoulder at 1805 and the band at 1755 $cm^{-1}$ had weakened; after 14 h, they had essentially disappeared. The spectrum of the nonvolatile residue was almost identical with that of poly[1,2-dihydro-1,2-dioxopyridine-6-carboxylate],[23] i.e., 1790 (s), 1690 (s), and 1595 (s).

The CI mass spectrum of the reaction mixture showed peaks indicative of a chlorinated product [176 (32), 174 ($M^+$ + H, 100), 160 (9), 158 ($M^+$ + H − O, 32), 132 (20), and 130 ($M^+$ + H − $CO_2$, 67); M = $C_6H_4ClN$] and, in the less volatile fractions, **5** [156 ($M^+$ + H)]. No ($M^+$ + H) peak at 182 amu indicative of **14** was observed.

**Reaction of 5 with Phosgene in DMAA.** Both the NMR and IR spectra of the nonvolatile portion of the reaction mixture filtrate were obscured by signals from residual $[Et_3NH]Cl$ and DMAA. However, an IR absorption at 1640 (br s) $cm^{-1}$ was observed. Pyridinone H's were apparent in the $^1H$ NMR at 6.6–6.8 ppm. The strongest peaks in the mass spectrum were attributable to **5**; no ($M^+$ + H) peak at 225 amu indicative of **18** was observed. Likewise, no ($M^+$ + H), ($M^+$ + 29), or ($M^+$ + 41) peaks attributable to **18** were observed in the CI mass spectrum of reaction mixtures containing **5**, DMAA, and phosgene, with or without $Et_3N$.

**Ligand Potency for Pu Removal.** Excretion of Pu and the distribution of the retained Pu in the tissues of mice given one injection of a HOPO ligand are shown in

**Table II.** Removal of $^{238}$Pu(IV) from Mice by Poly(HOPO-1,2) Ligands and Their Zinc and Ferric Complexes

| | | percent of injected $^{238}$Pu ± SD at 24 h[a,b] | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | tissues | | | | | excreta | |
| | no. of mice | liver | skeleton | kidneys | residual soft tissue | body content | urine | feces and GI contents |
| HOPO-1,2 ligands[c] | | | | | | | | |
| Fe(III)-3,4,3-LIHOPO[d] | 5 | 4.6 ± 1.2[g] | 7.4 ± 0.8 | 0.3 | 1.7 ± 0.3 | 14 ± 2.0 | 40 | 46 |
| DFOHOPO | 5 | 5.1 ± 2.2 | 6.0 ± 0.5 | 0.1 | 2.3 ± 0.5 | 13 ± 2.9 | 19 | 68 |
| Zn(iI)$_2$-3,4,3-LIHOPO[d]** | 5 | 4.0 ± 0.8 | 9.6 ± 0.6 | 0.3 | 2.5 ± 0.6 | 16 ± 1.7 | 21 | 63 |
| 3,4,3-LIHOPO* | 5 | 8.9 ± 1.7 | 7.5 ± 0.7 | 0.2 | 1.6 ± 0.6 | 18 ± 1.7 | 24 | 57 |
| Zn(II)-3-LIHOPO[d]** | 5 | 13 ± 3.9 | 13 ± 2.2 | 0.6 | 5.5 ± 1.5 | 33 ± 3.4 | 14 | 54 |
| 3,4-LIHOPO* | 5 | 18 ± 4.8 | 9.9 ± 3.6 | 0.6 | 5.8 ± 1.3 | 34 ± 9.2 | 7.9 | 58 |
| Zn(II)-3-LIHOPO[d]** | 5 | 5.5 ± 0.8 | 16.4 ± 2.4 | 0.4 | 13 ± 2.8 | 35 ± 5.5 | 5.2 | 59 |
| 3-LIHOPO* | 5 | 8.7 ± 1.2 | 17 ± 2.8 | 1.4 | 11 ± 0.8 | 38 ± 4.4 | 8.7 | 53 |
| MEHOPO** | 5 | 18 ± 6.3 | 17 ± 2.5 | 1.8 | 10 ± 1.8 | 47 ± 9.4 | 9.6 | 44 |
| 1,2-HOPO-6-$CO_2H$ | 5 | 52 ± 4.6 | 24 ± 5.1 | 1.2 | 6.7 ± 1.7 | 84 ± 4.0 | 7.0 | 9.1 |
| 1,2-HOPO-6-$CONMe_2$ | 5 | 56 ± 6.3 | 27 ± 5.5 | 1.4 | 6.2 ± 1.2 | 90 ± 7.0 | 4.5 | 5.1 |
| comparator ligands[e] | | | | | | | | |
| 3,4,3-LICAM(C) | 25 | 11 ± 5.2 | 11 ± 2.7 | 2.6 ± 0.8 | 4.8 ± 2.3 | 29 ± 8.2 | 49 | 22 |
| 3,4,3-LICAM(S) | 15 | 25 ± 5.3 | 6.6 ± 1.0 | 0.9 ± 0.2 | 3.1 ± 0.5 | 36 ± 4.7 | 62 | 2.4 |
| $CaNa_3$-DTPA | 10 | 16 ± 2.8 | 11 ± 1.2 | 0.5 ± 0 | 3.8 ± 1.5 | 30 ± 4.2 | 63 | 7.0 |
| DFOM | 5 | 19 ± 13 | 20 ± 11 | 1.8 | 4.5 ± 1.4 | 45 ± 25 | 40 | 15 |
| $^{238}$Pu-injected controls[f] | | | | | | | | |
| kill at 1 h | 13 | 30 ± 7.4 | 24 ± 4.4 | 2.7 ± 1.0 | 37 ± 7.1 | 93 ± 2.7 | 1.1 | 4.8 |
| kill at 24 h | 36 | 53 ± 6.7 | 29 ± 6.2 | 1.9 ± 0.6 | 8.8 ± 1.8 | 92 ± 4.2 | 4.2 | 3.7 |

[a] SD = $[\Sigma dev^2/(n-1)]^{1/2}$. No SD is shown for kidney or excreta of unreplicated test groups, because samples for five mice were pooled for Pu analysis. For statistical comparisons, $n$ is the total number of test or control mice shown, except for kidney, for which $n$ is the number of replicate experimental groups. See footnotes *e* and *f*. [b] Polyfunctional ligands were administered (30 μmol/kg ip) at 1 h, and mice were killed at 24 h after iv injection of plutonium(IV)-238 citrate. Dosage of DFOM was 50 μmol/kg, and of the two monomers, 120 μmol/kg. [c] Single (*) or double (**) asterisks indicate death within 7 days after ip injection of 1000 or 500 μmol/kg of test ligand, respectively. [d] Finely divided suspension. [e] $CaNa_3$-DTPA, two groups;[19] DFOM;[18] 3,4,3-LICAM(C), five groups include results for five new mice;[19] 3,4,3-LICAM(S), three groups include new results for five mice.[18,19] [f] Controls: 1 h, two groups;[19] 24 h, seven groups run contemporaneously with tests of HOPO ligands. [g] Underlined means were compared, by using the $t$ test,[29] with the appropriate means for mice given $CaNa_3$-DTPA, and the Pu content was significantly less, $p < 0.01$.

Table II; data are expressed as percent injected Pu (% ID) and are arranged in order of decreasing ligand effectiveness. For comparison, Table II includes data for (i) two effective tetracatechoylate ligands, sulfonated 3,4,3-LICAM(S)[18,19] and carboxylated 3,4,3-LICAM(C),[19] (ii) two clinically accepted metal removal agents, diethylenetriaminepentaacetic acid ($CaNa_3$-DPTA) and DFOM,[18,19] (iii) 1 and 24 h Pu-injected controls. Little Pu was removed [net Pu removal (% ID) = Pu excretion (treated, % ID) − Pu excretion (control, % ID)] by 120 μmol/kg of the monomer 1,2-HOPO-6-$COMe_2$. The more acidic monomer, 1,2-HOPO-6-$CO_2H$, increased Pu excretion to twice the control output—the best result for any monomeric ligand except disulfocatechol (Tiron).[19] One hour after injection of Pu (the time when ligands are injected) about 37% ID is present in the residual soft tissue (soft tissues other than liver and kidneys, Table II).[18] The Pu in those soft tissues, which is mainly in contained blood and extracellular fluid (ECF), is approximately equal to the amount circulating. All the poly(HOPO) ligands chelated the Pu circulating at 1 h and diverted it to excretion. In addition, amounts ranging from 10% to 68% and 25% to 83% of the Pu (assumed to be deposited at that time in skeleton and liver, respectively) were removed and excreted. Nearly all the Pu in kidney at 1 h (due mainly to contained blood, ECF, and urine) was removed by the tris- and tetrakis(HOPO) ligands. Compared with the 24-h controls, significant reductions ($p < 0.01$)[29] of the Pu content of the whole body, skeleton, and liver were obtained with all the poly(HOPO) ligands and of the Pu in residual soft tissues with tris- and tetrakis(HOPO) ligands, except MEHOPO. All the poly(HOPO) ligands, especially the trimers and tetramers, reduced the Pu content of kidney below the 24-h control level.

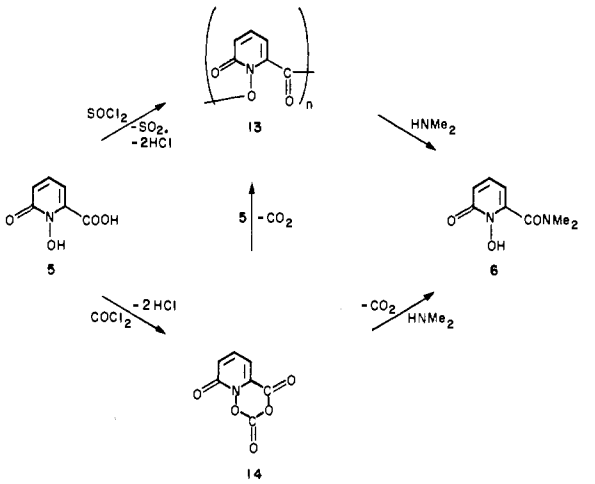
The variability of the DFOM data preclude useful statistical comparisons, however, 30 μmol/kg of the tetrakis(HOPO) ligands reduced liver Pu to 20–50%, the skeleton Pu to 30–48%, the kidney Pu to 6–17%, and the whole body Pu to 29–40% of the respective levels achieved with 50 μmol/kg of DFOM.

Removal of Pu by the tetrakis(HOPO) ligands was superior to an equimolar amount of $CaNa_3$-DTPA. The Pu content of mice given a tetrakis(HOPO) ligand was significantly less ($p < 0.01$) in whole body and liver (4/4 cases), in skeleton (3/4 cases), and in residual soft tissues (2/4 cases); kidney Pu was reduced to 25–75% of the level achieved with $CaNa_3$-DTPA. Those results are highlighted in Table II.

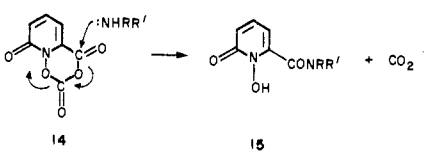
The effectiveness of the linear bis- and tris(HOPO) ligands and their Zn(II) complexes, which reduced the Pu content of the body to 33–38% ID, was about the same as the linear tetracatechoylates and $CaNa_3$-DTPA. The benzene-centered trimer MEHOPO was the least effective poly(HOPO) ligand, presumably because the configuration of its charge cavity does not conform to the geometry required by Pu(IV). Even so, Pu removal by MEHOPO (46% ID) was greater than for its tris(catechoylamide) analogue, MECAM (28% ID).[19] Removal of Pu from the body and liver was significantly better ($p < 0.01$) after treatment with a tetrakis(HOPO) ligand than either of the tetracatechoylate ligands; removal of Pu from skeleton and residual soft tissues was about the same for all six tetrameric ligands.

Kidneys of the five mice in each study group were pooled for Pu analysis, so statistical comparisons cannot be made between individual groups. However, some studies have been replicated (1-h and 24-h controls, $CaNa_3$-DTPA and the two tetracatechoylate ligands), and the mean of mean ± SD kidney Pu of those replicate groups is shown in Table II. If the mean Pu content of kidneys for the four groups given tetrakis(HOPO) ligands are combined ($n = 4$), the grand mean is 0.22 ± 0.1% ID, significantly less ($p < 0.01$)

Scheme I



Scheme II



than for either the 24-h controls or the mice given one of the comparator ligands.

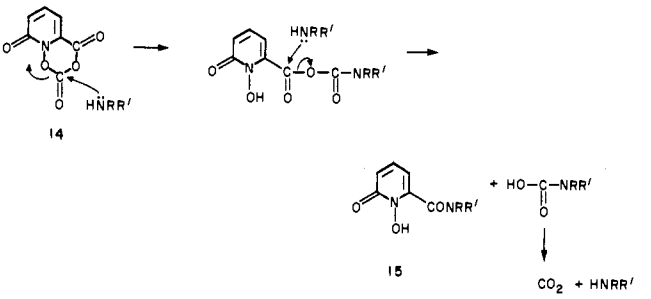
## Discussion

**Synthetic Strategies.** The title compounds and the corresponding catechoylamide ligands upon which they are modeled are synthesized by amidation of a polyamine with an activated carboxylic acid derivative. The latter bears the hydroxyl group(s) whose oxygens are ultimately the donor atoms in the metal ion–ligand complex. Thus two major considerations in the preparation of such ligands are protection/deprotection of the hydroxyl groups and activation of the carboxylic acid. These are accomplished in the catechol derivatives through etherification,[5,6,14-16] esterification,[5] or metal–ion complexation[5] of the aromatic hydroxyls, followed by activation of the carboxylic acid via conversion to an acyl chloride[5,6,14-16] or *N*-hydroxysuccinimide ester.[30] After amidation, hydroxyl-protecting groups are cleaved by hydrolysis (esters and metal ion complexes), hydrogenolysis (benzyl ethers), or treatment with $BBr_3$ (methyl ethers). Although the yields are good, the additional steps required in the protection → activation → deprotection sequence are disadvantageous.

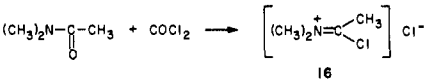
Esters of 1-hydroxy-2(1*H*)-pyridinone show high reactivity in nucleophilic reactions, and this class of activated esters has been used in peptide synthesis.[31] We hypothesized that a 1,2-HOPO bearing a carboxylic acid side chain should form an activated inter- or intramolecular ester and that subsequent reaction of such an intermediate with an amine should (by analogy with peptide synthesis) simultaneously form the desired amide and regenerate the nitroxyl group. Reaction of **5** with excess thionyl chloride did form the polyester, and reaction of this sparingly soluble intermediate suspended in THF with excess dimethylamine yielded the amide **6** (Scheme I).[23] However, when that reaction was tried with polyamines, no significant amount of the desired product could be isolated, apparently due to poor mass transfer. A monomeric, soluble active ester was sought, via reaction of phosgene with **5** to the cyclic anhydride **14**. However, the carboxylic

(31) Paquette, L. A. *J. Am. Chem. Soc.* **1965**, *87*, 5186–5190.

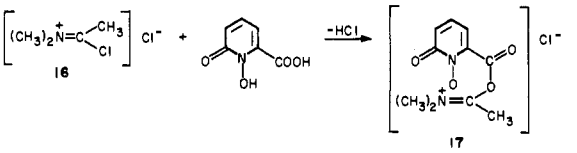
Scheme III



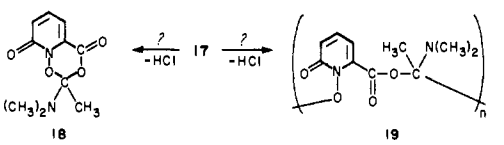
Scheme IV



Scheme V



Scheme VI



acid **5**, which is only slightly soluble in THF, also gave the polyester as a final product.[23]

Since solubility seemed to be a major problem in the synthesis of polyfunctional ligands, a better solvent than THF was sought. The reaction was carried out in DMAA with a stoichiometric amount of phosgene, and a 50% yield of the desired product **8** was obtained. Since phosgene reacts with *N*,*N*-disubstituted amides to form the immonium chloride **16** (Scheme IV),[32] a reaction of the intermediate with **5** to produce an imino ester salt (**17**; Scheme V) may occur. No unequivocal spectral evidence was observed for the monomeric, cyclic intermediate **18**. In any case, reaction of an amine nucleophile with **17**, **18**, or **19** would lead to regeneration of DMAA, regeneration of the nitroxyl group, and formation of an amide (Scheme VI).

Whatever the exact mechanism, the essential feature of these systems is the formation of an active ester with the pyridinone nitroxy group either directly (**13**), or via the intermediacy of a carbonyl group (**14**) or its functional equivalent (**18**, **19**). This active intermediate reacts with primary and secondary amines to yield amides (Scheme VII). Since unreacted material can be hydrolyzed back to **5** and recovered, an excess can be efficiently used to ensure complete reaction with polyamine substrates such as those described herein.[33] In addition this system should prove a good substrate for other nucleophiles and lead to additional 6-substituted 1-hydroxy-2(1*H*)-pyridinones.

(32) Martin, G.; Martin, M. *Bull. Soc. Chim. Fr.* **1963**, 1637–1646.

(33) Since esters of hydroxamic acids are easily hydrolyzed and **5** can be readily recovered, the three hydroxamic acid hydroxyls in desferriferrioxamine B (**12**; X = H) were not protected. Instead, 4.2 mol of **5** per mol of substrate was added to form the amide–tris(ester). The ester groups were hydrolyzed during the normal course of the aqueous alkaline workup to yield **12** and recovered **5**.

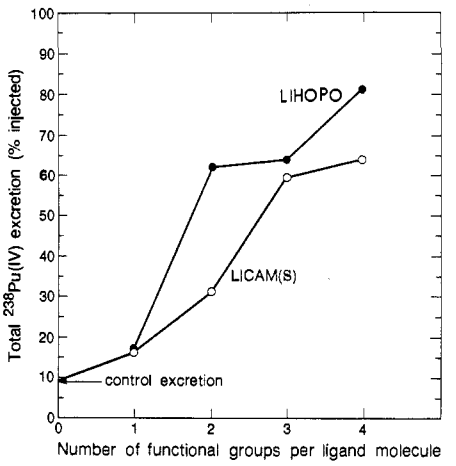
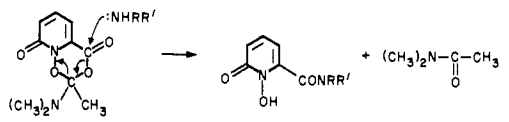



**Figure 2.** $^{238}$Pu excretion (urine + feces + GI contents) promoted in mice by structuraly analogous linear poly(sulfocatechoylamide) ligands [LICAM(S)—Tiron, 3-LICAM(S), 3,4-LICAM(S), 3,4,3-LICAM(S); structures published[18,19]] or poly(HOPO) ligands [HOPO—1,2-HOPO-6-$CO_2H$, 3-LIHOPO, 3,4-LIHOPO, 3,4,3-LIHOPO; structures shown in Table I] as related to number of functional groups per molecule.

**Scheme VII**



**Effectiveness for Pu Removal.** The great stability of the Pu(IV)–3,4,3-LIHOPO complex is demonstrated by the displacement of Fe(III) by Pu(IV): The similar efficiency of DFO-HOPO for Pu removal from mice suggests that its Pu(IV) complex is also very stable. The pH independence of the HOPO ligands in the pH range of mammalian tissue fluids is shown by the low Pu content of the kidneys at 24 h in mice given those ligands.

The effectiveness of the Zn(II) and Fe(III) complexes of 3,4,3-LIHOPO is impressive, inasmuch as both were given as finely divided suspensions, and only a small fraction of the nominal dosage of 30 μmol/kg was likely to have been immediately available to react with Pu. Injection of a soluble ligand produces a prompt peak plasma concentration, which declines rapidly as free ligand is excreted.[34] The poly(HOPO) ligands, like their poly(catechoylamide) analogues, effectively remove Pu from the circulation at dosages lower than 30 μmol/kg.[35-37] Slow dissolution of these sparingly soluble ligands and maintenance of an effective plasma level over an extended time may have contributed to their effectiveness.

The in vivo results of the catechol ligands compared with the HOPO ligands closely parallel their relative metal binding properties. Removal of Pu by the sulfocatechol ligands increased in series as: mono < bis < tris = tetrakis,[19] as shown in Figure 2. Since Pu(IV) ion is expected to be eight-coordinate, the absence of any increase in efficacy of the tetrakis ligand relative to the tris was initially surprising. However subsequent solution thermodynamic studies have shown[21] that at neutral pH only three catechol groups will deprotonate and bind to Pu(IV). Thus, the Pu(IV) complex with the tris(sulfocatechoylamide) ligand has essentially the same structure as that with the analogous tetrakis ligand. In contrast, the greater acidity of the HOPO ligands (and their one proton per ligating group stoichiometry versus two protons for catechols) allow ligands with HOPO groups to form eight-coordinate complexes that are stable in the physiological pH range[23,27] and more strongly binding than the hexadentate tris ligands. This, in turn, should lead to a greater selectivity of Pu(IV) relative to Fe(III), since the latter forms six-coordinate complexes with these ligands. As can be seen from the Pu removal data in Figure 2, the efficacy of the HOPO ligands increases in the series: mono < bis < tris < tetrakis as anticipated from the potential that this ligand has for octadentate binding. The fact that DFO-HOPO is at least as effective as octadentate 3,4,3-LIHOPO indicates that all three hydroxamate groups, as well as the oxohydroxypyridinecarboxylate group, of the DFOM derivative are binding. This also explains the enormous increase in Pu removal as one goes from the hexadentate DFOM (Pu removal 46% ID) to its octadentate derivative DFO-HOPO (Pu removal 77% ID).

**New Directions.** On an equimolar basis, DFO-HOPO and Fe(III)-3,4,3-LIHOPO promoted more Pu excretion and remove more newly deposited Pu from mouse tissues than any ligand tested in our mouse system, and both are candidates for extended biological investigation. These ligands have now been found to be effective in amounts lower than the standard test dosage. They are orally active. DFO-HOPO is as effective and Fe(III)-3,4,3-LIHOPO is more effective than an equimolar amount of $CaNa_3$-DTPA when injected 24 h after the Pu injection.[37] The same tests are in progress with 3,4,3-LIHOPO.

**Potential Applications.** The HOPO ligands are soluble in water, acidic without the addition of solubilizing groups, and therefore effective for metal binding at physiological pH. They have high binding constants for Fe(III)[28] and Pu(IV) and should be effective ligands for removal and/or carrying of other metal ions with similar coordination properties. For removal of Pu(IV) from mice, the poly(HOPO) ligands are more effective than their catechol analogues, and octadentate DFO-HOPO is markedly more effective than hexadentate DFOM.

## Conclusions

The properties of the HOPO ligands make them potentially useful pharmaceutical chelating agents. The Pu(IV) complexes with 3,4,3-LIHOPO and DFO-HOPO are very stable, and those ligands are the most effective Pu removal agents yet prepared. The toxicity of the Na salts of the linear poly(HOPO) ligands at high dosage (believed to be caused by removal of iron from kidney and liver cells) can be avoided by reducing the dosage or administering the Fe(III) complex. Addition of one HOPO group to DFO produced a low toxicity octadentate ligand with an effectiveness for Pu removal equal to the linear HOPO tetramer 3,4,3-LIHOPO and significantly greater than $CaNa_3$-DTPA.

**Acknowledgment.** This work was supported by the Director, Office of Energy Research, Office of Basic Energy Sciences, Chemical Sciences Division, U.S. Department of Energy, under Contract Number DE-AC03-76SF00098, and The National Institute of Environmental Health Sciences, Grant ES02698.

**Registry No.** **5**, 94781-89-2; **6**, 94781-90-5; **7** (X = 1,2-HOPO-6-CO), 110874-34-5; **8** (X = 1,2-HOPO-6-CO), 97570-39-3; **9** (X = 1,2-HOPO-6-CO), 110874-35-6; **9** (X = H), 71-44-3; **10** (X = 1,2-HOPO-6-CO), 110874-36-7; **10** (X = H), 124-20-9; **11** (X =

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(35) Volf, V. *Int. J. Radiat. Biol. Relat. Stud. Phys., Chem. Med.* **1986**, *49*, 449–462.
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**Supplementary Material Available:** Detailed methods and results of preliminary toxicity screening of HOPO ligands (2 pages). Ordering information is given on any current masthead page.

# Design, Structure–Activity, and Molecular Modeling Studies of Potent Renin Inhibitory Peptides Having N-Terminal $N^{in}$-For-Trp (Ftr): Angiotensinogen Congeners Modified by $P_1$-$P_1'$ Phe-Phe, Sta, Leu$\psi$[CH(OH)$CH_2$]Val or Leu$\psi$[$CH_2$NH]Val Substitutions[⊥1]

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A structure–conformation–activity investigation of several angiotensinogen (ANG) based inhibitors of human renin modified by either Phe-Phe, Sta, Leu$\psi$[$CH_2$NH]Val, or Leu$\psi$[CH(OH)$CH_2$]Val at the $P_1$-$P_1'$ cleavage site and $P_5$ Trp($N^{in}$-For) (Ftr) was performed. Specifically, Ac-Ftr-Pro-Phe-His-Phe-Phe-Val-Ftr-$NH_2$ (1) provided a potent ($K_I = 2.7 \times 10^{-8}$ M) $P_1$-$P_1'$ Phe-Phe substituted renin inhibitor to initiate these studies. Substitution of the $P_1$-$P_1'$ Phe-Phe in compound 1 by Sta effected a 1000-fold increase in biological potency for the resultant octapeptide Ac-Ftr-Pro-Phe-His-Sta-Val-Ftr-$NH_2$ (10; $K_I = 6.7 \times 10^{-11}$ M). Kinetic analysis of compound 10 showed it to be a competitive inhibitor of human renin catalyzed proteolysis of human ANG. Chemical modifications of the compounds 1 and 10 were evaluated on the basis of comparative human plasma renin inhibitory activities ($IC_{50}$ values) in vitro. Carboxy-terminal truncation studies on compound 10 showed that the $P_2'$ Val and $P_3'$ Ftr residues could both be eliminated without significant loss (ca. 10-fold) in renin inhibitory activity as exemplified by the pentapeptide Ac-Ftr-Pro-Phe-His-Sta-$NH_2$ (12; $IC_{50} = 3.8 \times 10^{-9}$ M). In addition, the corresponding $P_1$-$P_1'$ Leu$\psi$[CH(OH)$CH_2$]Val and Leu$\psi$[$CH_2$NH]Val derivatives of compound 12 were potent renin inhibitors: Ac-Ftr-Pro-Phe-His-Leu$\psi$[CH(OH)$CH_2$]Val-$NH_2$ (13; $IC_{50} = 3.1 \times 10^{-10}$ M) and Ac-Ftr-Pro-Phe-His-Leu$\psi$[$CH_2$NH]Val-$NH_2$ (14; $IC_{50} = 2.1 \times 10^{-8}$ M). The structure–conformation–activity properties of the N-terminal Ftr substitution of these human renin inhibitors was examined by (1) comparative analysis of several analogues of 1 and Ac-Ftr-Pro-Phe-His-Sta-Ile-$NH_2$ (17; $IC_{50} = 1.0 \times 10^{-10}$ M) having $P_5$ site modifications by Trp, His, D-Ftr, and D-His, (2) deletion of the N-terminal Ftr residue from compounds 12 and 17, to provide Ac-Pro-Phe-His-Sta-Ile-$NH_2$ (16; $IC_{50} = 3.1 \times 10^{-8}$ M) and Ac-Pro-Phe-His-Sta-$NH_2$ (15; $IC_{50} = 5.6 \times 10^{-6}$ M), and (3) computer modeling and dynamics studies of compounds 1 and 17 bound to CKH-RENIN, a simulated human renin model, which were focused on identifying potential intermolecular interactions of their common $P_5$-$P_2$ sequence, Ac-Ftr-Pro-Phe-His, at the enzyme active site. Finally, the human renin specificity of selected congeners of compound 10 were determined by comparison to porcine kidney renin in vitro.

Renin is an aspartic acid protease (EC 3.4.99.19) that catalyzes the first and rate-limiting step of the enzyme cascade that exists for the biosynthesis of angiotensin II[2-4] (Figure 1). Renin selectively cleaves its polypeptide substrate, angiotensinogen (ANG), to effect the formation of the decapeptide intermediate, angiotensin I. Angiotensin I is then cleaved by angiotensin converting enzyme (ACE) to yield the biologically active octapeptide product, angiotensin II. Angiotensin II possesses multiple bioactivities (e.g., vasoconstriction, aldosterone, and catecholamine secretion) at various target tissues, and the renin–ACE cascade has been implicated to have a physiological role for blood pressure and electrolyte homeostasis. In addition, this pathway apparently is involved in pathophysiological states related to various forms of hypertension.[5,6] Therefore, inhibition of renin may be therapeutically important in the development of novel antihypertensive agents.[7-11]

[⊥] Dedicated to The Upjohn Company in honor of its 100th anniversary, 1886–1986.

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(1) Symbols and abbreviations are in accord with the recommendations of the IUPAC–IUB Commission on Biochemical Nomenclature (*Eur. J. Biochem.* **1984**, *158*, 9). All optically active amino acids are of the L variety unless otherwise specified. Additional abbreviations used are: Ftr, Trp($N^{in}$-For); Sta, (3*S*,4*S*)-4-amino-3-hydroxy-6-methylheptanoic acid; Leu$\psi$[CH(OH)$CH_2$]Val, (2*S*,4*S*,5*S*)-4-hydroxy-2-isopropyl-7-methyloctanoic acid; and Leu$\psi$[$CH_2$NH]Val, (*S*)-4-methylpentyl-L-valine. In accord with nomenclature described by Schechter and Berger (*Biochem. Biophys. Res. Commun.* **1967**, *27*, 157), $P_n$-$P_n'$ refer to the side-chain positions of the peptide substrate, whereas $S_n$-$S_n'$ refer to the subsite on the enzyme that binds the corresponding side chain of the substrate. Other abbreviations are referenced as used in text.

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0022-2623/88/1831-0018$01.50/0 