Synthesis of Water-Soluble Undecagold Cluster Compounds of Potential Importance in Electron Microscopic and Other Studies of Biological Systems

Paul A. Bartlett,*[1a,b] Barr Bauer,[1a] and S. J. Singer[1c]

Contribution from the Department of Chemistry, University of California, Berkeley, California 94720, and the Department of Biology, University of California at San Diego, La Jolla, California 92093. *Received February 3, 1978*

Abstract: The synthesis of an amino-substituted triarylphosphine ligand, 4,4',4''-phosphinidynetri(benzenemethanamine), has allowed the preparation of some water-soluble undecagold clusters, $(Ar_3P)_7Au_{11}X_3$ (**9a**, X = I; **9b**, X = CN). These compounds have been characterized by comparison with the corresponding tris(4-methylphenyl)phosphine clusters and by their ultracentrifuge sedimentation behavior. The water-soluble tricyano undecagold cluster **9b** is stable in aqueous solution, whereas the triiodo cluster **9a** decomposes to an octakis(triarylphosphine)enneagold complex. Because of their extraordinary heavy atom density, these compounds may be useful as high-resolution electron-dense labels for immunoelectron microscopy, as well as in other studies of biological ultrastructure and analysis.

Introduction

A variety of techniques for the study of biological systems employ macromolecules that are derivatized with heavy metal compounds. In electron microscopic studies of biological ultrastructure, for example, antibody molecules,[2] lectins,[3] hormones,[4] transfer RNA,[5] and other molecules can be used as labels to detect and localize the complementary molecular structures to which they specifically bind, provided that these labels are rendered sufficiently "electron dense" to be visible as individual molecules in the transmission electron microscope (TEM). The first and still most generally useful method for accomplishing this is to covalently couple the protein ferritin to the antibody or other labeling molecules.[6] Ferritin is a uniquely electron-dense protein, whose molecular structure consists of a protein shell of about 120 Å outer diameter surrounding an inner core of insoluble ferric hydroxide–phosphate. This core contains more than 2000 iron atoms per ferritin molecule, and this permits *single* ferritin molecules to be easily visualized by the TEM.[7] Because of this fact, ferritin-conjugates of antibodies and other molecules allow the TEM localization of their complementary structures. Although this approach provides the highest resolution, general TEM labeling procedures currently available, the large size of the ferritin molecule and the intervening protein ligand limit the resolution to the order of 200–300 Å.

There are, however, ultrastructural problems for which an electron-dense labeling reagent with a potential resolution of the order of 50 Å or less would be very useful. Antibodies and other proteins have been derivatized with monometallic compounds of uranium[8] and mercury,[9] for example, but such conjugates do not have enough electron scattering power to be distinguished as individual molecules on biological specimens in the TEM, and only with difficulty in the high-resolution scanning electron microscope (SEM).[10] What is required as an electron-dense labeling reagent is a stable, water-soluble, small molecule which contains a large number of heavy atoms and which is capable of being covalently attached to proteins under mild conditions. There are no naturally occurring proteins analogous to ferritin which fit this description, and synthetic agents are therefore called for.

It should also be added that there are many potential applications for such molecules in addition to biological ultrastructure studies. For example, they might be used to increase the sensitivity of a recently introduced alternative to radioimmunoassay techniques, which employs antigens derivatized with monometallic compounds.[11]

Transition metal cluster compounds[12] are a class of molecules which might provide the required electron-dense labeling reagents. For example, a number of undecagold clusters, $[(aryl)_3P]_7Au_{11}X_3$ (X = I, SCN, CN), have been prepared by sodium borohydride reduction of the triarylphosphine gold(I) complexes.[13] These clusters are among the largest and most electron dense known, with a structure comprised of a central core of 11 gold atoms encased in a hydrophobic sheath of the triarylphosphine and anionic ligands.[14] In the crystalline state, x-ray diffraction analysis shows the size of this core to be about 8.2 Å in diameter, making it an attractive candidate for our purposes. We have synthesized some water-soluble derivatives of the undecagold cluster and have studied their stability and mode of decomposition in aqueous solution. In this connection, we prepared a triarylphosphine ligand which may be of broad utility in synthesizing other water-soluble and easily derivatized metal complexes. In addition, two previously unknown tris(4-methylphenyl)phosphine undecagold clusters have been prepared as comparison compounds.

Results

Synthesis of Ligand. The scheme below outlines the synthesis of the ligand tris[*p*-(aminomethyl)phenyl]phosphine (*Chemical Abstracts* name: 4,4',4''-phosphinidynetri(benzenemethanamine)). Lithiation of the protected *p*-bromobenzaldehyde derivative **1** and condensation with phosphorus trichloride afford the phosphine triacetal **3** in 67% yield after chromatographic purification. The crystalline trialdehyde **4** is obtained after removing the protecting groups in aqueous acid. Addition of methoxyamine to the formyl groups and reduction of the resulting tris(oxime ether) **5** with borane–THF[15] gives the desired phosphine triamine in 38% overall yield from **1**. This material is purified and stored as the tris(*p*-toluenesulfonic acid) salt, **6**.

Synthesis and Purification of Undecagold Clusters. Reduction of chloroauric acid with 2,2'-thiodi(ethanol) ($HOCH_2CH_2SCH_2CH_2OH$, "thiodiglycol") in ethanol[16] and complexation with tris(tosylate) **6** gives the tetrachloride **7** on precipitation with acetone. Although the deprotonated, neutral gold(I) chloride complex cannot be extracted readily from aqueous solution into organic solvents, the corresponding iodide **8a** is extracted by methylene chloride after neutralization. This relatively unstable, crystalline material is reduced with 1 mol/mol of sodium borohydride in ethanol to give a red-brown solution which shows the characteristic UV-visible absorption maxima of the undecagold complexes. Conventional purifi-

0002-7863/78/1500-5085$01.00/0

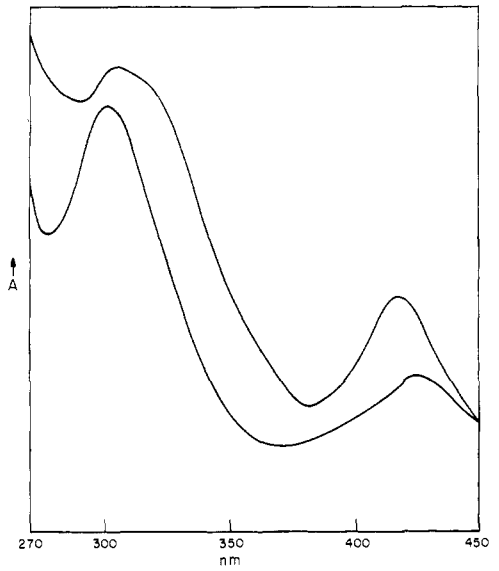


Figure 1. Upper curve, $[(p\text{-}CH_3C_6H_4)_3P]_7Au_{11}I_3$ in ethanol; lower curve, $[(p\text{-}NH_2CH_2C_6H_4)_3P]_7Au_{11}I_3$ in 0.2 M NH_4HCO_3.

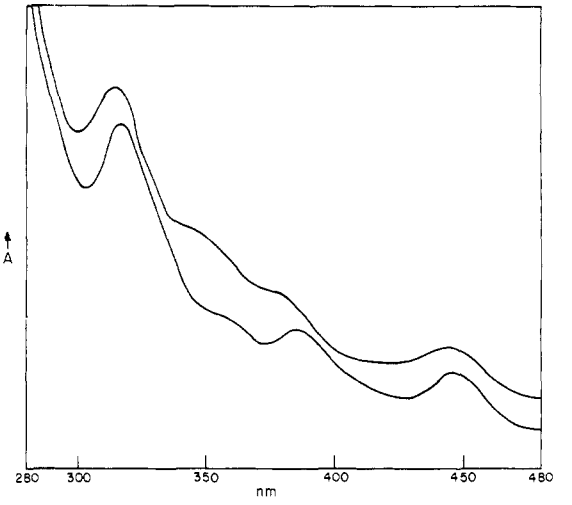
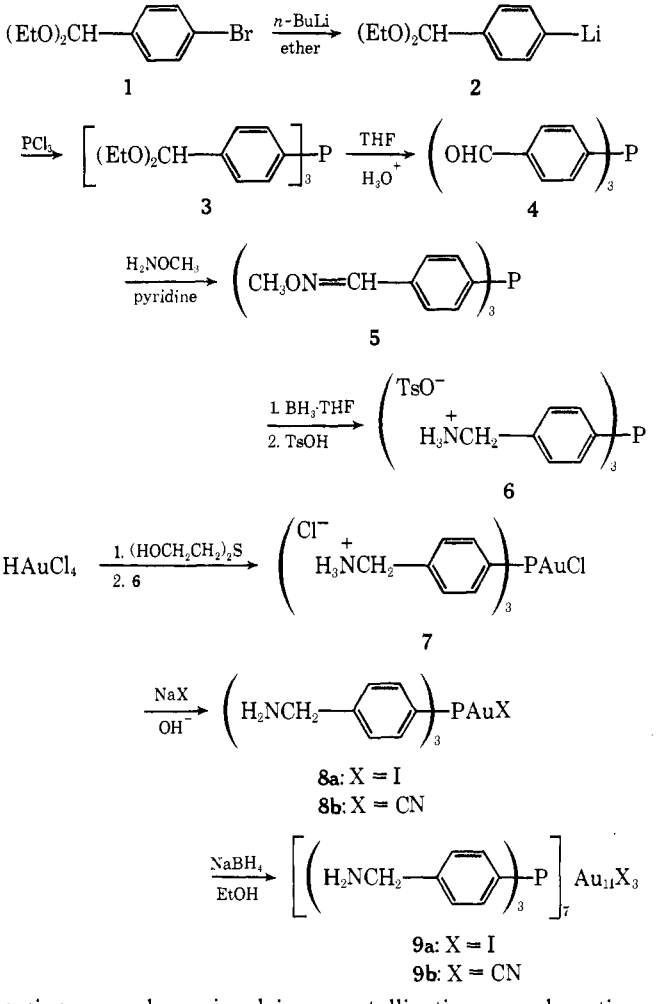
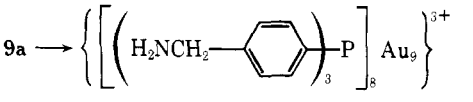


Figure 2. Upper curve, $[(C_6H_5)_3P]_8Au_9(NO_3)_3$ in ethanol; lower curve, $[(p\text{-}NH_2CH_2C_6H_4)_3P]_8Au_9(HCO_3)_3$ in 0.2 M NH_4HCO_3.



cation procedures involving crystallization or adsorption chromatography are precluded by the highly charged, hydrophilic nature of this cluster, arising from the 21 peripheral amino groups. The mixture may be fractionated on the basis of size, however, by gel filtration chromatography on Bio-Gel P-6, using neutral ammonium bicarbonate or potassium phosphate buffer. This procedure cleanly separates the high molecular weight, colored materials from small monomeric contaminants. Moreover, the high molecular weight material is resolved into a red band, comprised of the desired undecagold cluster (**9a**), and a faster moving brown band, of unknown higher molecular weight compounds. The undecagold cluster was characterized by the similarity of its UV–visible spectrum with that of triiodoheptakis[tris(4-methylphenyl)phosphine]-undecagold (Figure 1), and on the basis of its sedimentation behavior, which is discussed below.

After purification, the complex **9a** is relatively unstable, decomposing on standing in aqueous solution for a day at room temperature or upon lyophilization. On the basis of UV–visible spectral changes (Figure 2), it appears that this decomposition involves conversion of the heptakis(phosphine)undecagold cluster to the octakis(phosphine)enneagold complex, **10.**



10

Clusters of the latter type are produced by borohydride reduction of triarylphosphine gold(I) complexes with noncoordinating counterions (e.g., Ph_3PAuNO_3),[17] suggesting that decomposition of **9a** to **10** involves dissociation and subsequent loss of the iodide ligand in aqueous solution. Consistent with this interpretation is the fact that decomposition is slowed, albeit not prevented, by the addition of excess potassium iodide to aqueous solutions of **9a.**

Although the iodide ligand was desirable because of its electron density, a more stable complex is required. Among several counterions investigated (including thiocyanate and *N,N*-diethyldithiocarbamate), cyanide proved to be the most successful. Neutralization of the ethanolic solution of **7,** addition of **1** equiv of sodium cyanide, and direct reduction with sodium borohydride give a red-brown solution containing the tricyanoundecagold cluster **9b.** The same material can be prepared more simply by dissolving aurous cyanide in an ethanolic solution of the tris(tosylate) **6,** neutralizing the ammonium groups with sodium hydroxide, and reducing as usual. The undecagold cluster **9b** is purified by chromatography (Bio-Gel P-6, 0.05 M K_2HPO_4), and appears to be stable indefinitely in the cold or after lyophilization. The electronic spectrum of this material is similar to that of tricyanoheptakis[tris(4-methylphenyl)phosphine]undecagold (Figure 3). Upon standing at 4 °C, the complex precipitates from the chromatography fractions as a dark red, poly(phosphate salt), which can be redissolved in neutral or alkaline solution only

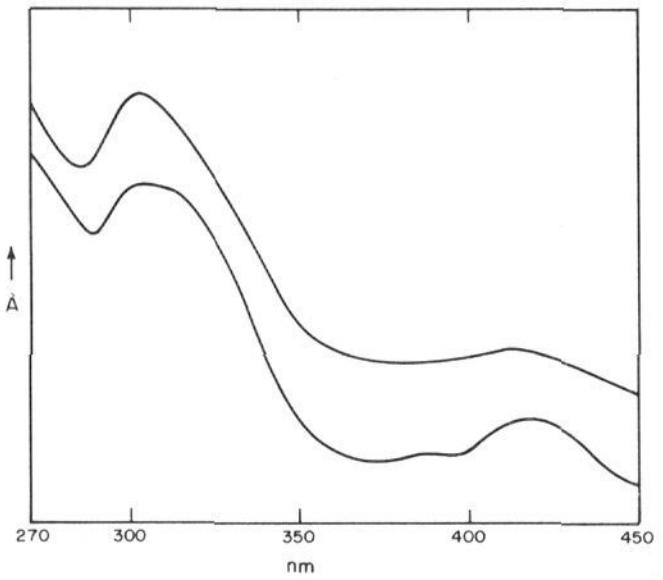


Figure 3. Upper curve, $[(p\text{-}CH_3C_6H_4)_3P]_7Au_{11}(CN)_3$ in ethanol; lower curve, $[(p\text{-}NH_2CH_2C_6H_4)_3P]_7Au_{11}(CN)_3$ in 0.05 M K_2HPO_4.

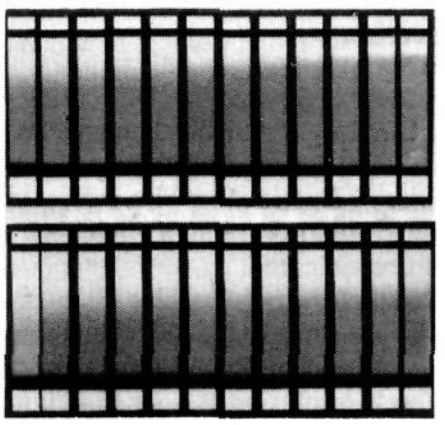
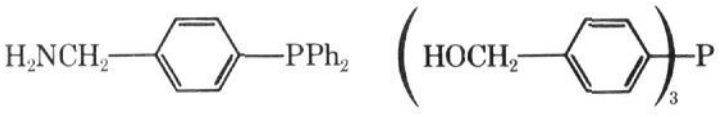


Figure 4. Sedimentation velocity experiment with the triiodo cluster compound **9a** in 0.2 M NH_4HCO_3, in a Beckmann Model E analytical ultracentrifuge at 68 000 rpm. The moving boundary was photographed with UV absorption optics. Successive pictures from right to left were taken at 4-min intervals after reaching full speed. Sedimentation proceeds from top to bottom in each frame.

with difficulty. The red precipitate does dissolve readily in dilute acid, and remains soluble when neutralized in the absence of phosphate: λ_{max} (0.05 M potassium phosphate, pH 4) 316, 424 nm; (dilute K_2CO_3, pH 8) 305, 420 nm. Using the extinction coefficients of the analogous tris(4-methylphenyl)-phosphine cluster, a molecular weight around 6000 is calculated for the precipitated complex, as expected for a salt with 12–14 phosphate counterions.

Sedimentation Behavior. By virtue of the density of the undecagold core, these water-soluble gold clusters sediment quite spectacularly in the ultracentrifuge. Figure 4 depicts the boundary movement in a sedimentation velocity experiment with the triiodo cluster **9a** in 0.2 M NH_4HCO_3 during 2 h at 280 000 ×g. The S^{W}_{20} value was found to be 2.4 S; this, together with a molecular weight of 5000, leads to a calculated partial specific volume, $\bar{v}$, of 0.54 and a radius of 10 Å, on the assumption that the molecule is an unhydrated sphere obeying Stokes' law. In a sedimentation equilibrium experiment at 120 000*g* with the tricyano complex **9b,** in 0.05 M KH_2PO_4, the introduction of the calculated molecular weight of 4700 leads to a $\bar{v}$ of 0.55, corresponding to a Stokes' radius of 10 Å for this derivative as well. From the crystallographic data reported for triiodoheptakis[tris(4-fluorophenyl)phosphine]-undecagold,[14c] a van der Waals radius of 10.5–11.0 Å is calculated for the nearly spherical molecule, measuring from the central gold atom to the fluorine and adjacent hydrogen atoms. The sedimentation characteristics of the water-soluble derivatives are therefore quite consistent with their presumed structures.

Other Ligands. Two other ligands were prepared and briefly investigated. Reaction of the lithio acetal **2** with chlorodiphenylphosphine,[18] and subsequent elaboration as in the scheme above, gave the monamino-substituted ligand **11.** The phosphine triol **12** was obtained from the trialdehyde **4** by sodium borohydride reduction. The gold(I) iodide adducts of these ligands were prepared and reduced in the usual manner; however, the complexes so obtained were insoluble in neutral aqueous solution and were not studied further.



11 **12**

Discussion

The presumed structure of the water-soluble clusters is depicted in Figure 5, in analogy to those reported for $[Ph_3P]_7Au_{11}(SCN)_3$,[14a] $[(4\text{-}ClC_6H_4)_3P]_7Au_{11}I_3$,[14b] and $[(4\text{-}FC_6H_4)_3P]_7Au_{11}I_3$.[14c] From the structural data published for

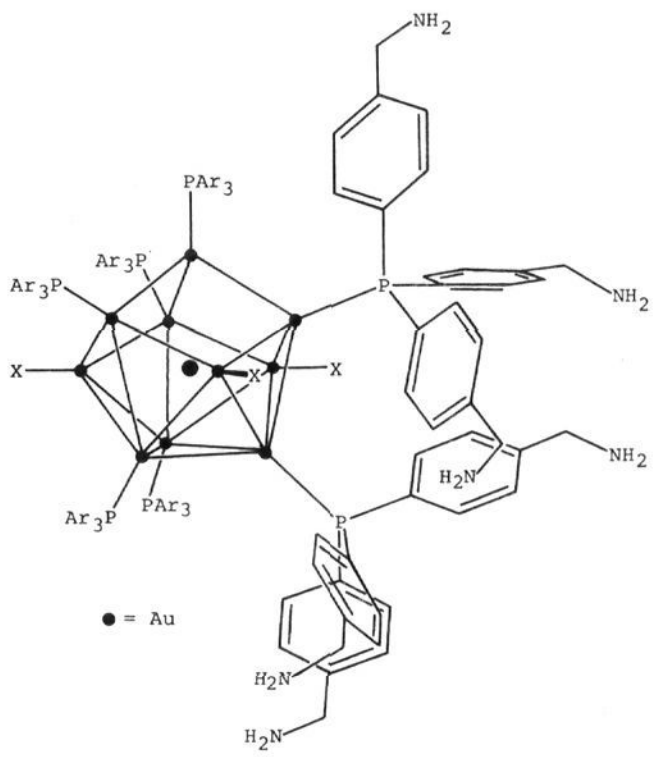


Figure 5. Structure of the undecagold clusters, in analogy to ref 14c. **9a,** X = I; **9b,** X = CN.

the latter compound, a diameter of 8.2 Å is calculated for the central core of 11 gold atoms, using an atomic radius of 1.44 Å for Au.[19] The distance from the central Au atom to a peripheral amino group in **9** is less than 12 Å. For electron microscopic purposes, the intrinsic resolution of such a cluster compound is therefore about 25 Å, compared to 120 Å for ferritin. For the cluster compound to be used as an electron-dense label, however, a number of additional problems have to be overcome. Covalently linked conjugates between the cluster and a protein or other molecule have to be formed, presumably through the use of some small bifunctional coupling reagent that reacts with amino groups in aqueous buffers near neutral pH. Self-coupling of the cluster compound could be minimized in such conjugation procedures by using, for example, toluene-2,4-diisocyanate[20] or a coupling reagent with different specificities at each end. Furthermore, if advantage is to be taken of the cluster's high resolving power to prepare electron-dense conjugates with protein reagents such as antibodies or lectins, these large proteins will first have to be fragmented into much smaller pieces while retaining their specific binding capacities.[21] Alternatively, highly specific, synthetic labels will have to be developed for the same purpose. These problems, together with electron microscope studies of the cluster compounds, will be addressed further in the near future.

Experimental Section

^{1}H NMR spectra were recorded in $CDCl_3$ using tetramethylsilane as internal standard, and are reported in parts per million on the δ scale as follows: chemical shift (multiplicity, integrated intensity, coupling constants, assignment). Unless otherwise indicated, distillations involved bulb-to-bulb distillation using a Kugelrohr oven, at the temperature and pressure indicated.

Tris[4-(diethoxymethyl)phenyl]phosphine (3). *p*-Bromobenzaldehyde diethyl acetal[23] (**1**) was prepared from the aldehyde with triethyl orthoformate, ethanol, and *p*-toluenesulfonic acid in 90% yield, bp 63–64 °C (0.05 Torr). A solution of 8.7 g (34 mmol) of the acetal in 50 mL of anhydrous ether was stirred under nitrogen at 0 °C and treated with 25 mL of a 1.4 M *n*-butyllithium/hexane solution (1.05 equiv). After 30 min at room temperature, the solution was brought back to 0 °C and treated with 0.98 mL (11 mmol) of PCl_3 in 25 mL of ether, added over a 3-h period. After 1 more h at room temperature, the mixture was filtered, diluted with ether, washed with saturated $NaHCO_3$ and brine, dried (K_2CO_3), and concentrated to give 6.1 g (95% crude yield) of phosphine **3** as a yellow syrup.

This material could be purified by chromatography on Florisil (10% ether/petroleum ether) (67% yield) and distillation (105 °C, 0.01 Torr): ^{1}H NMR δ 1.23 (t, 6), 3.59 (q, 4), 5.50 (s, 1), 7.1–7.7 (m, 4); IR (film) 1050, 1100, 2850, 2900 cm^{-1}.

Anal. ($C_{30}H_{45}O_6P$) C, H, P.

4,4',4''-Phosphinidynetri(benzaldehyde) (4). A solution of 13.3 g (23.4 mmol) of chromatographed triacetal **3** and 50 mL of 2 N HCl in 200 mL of THF was stirred at 60 °C for 15 min. The mixture was diluted with water and extracted with ether, and the organic layer was washed with saturated $NaHCO_3$ and brine, dried ($MgSO_4$), and concentrated to give the crude trialdehyde as a yellow syrup. This material was purified by recrystallization from ether, affording 5.1 g (63% yield) of **4** in two crops: mp 115–115.5 °C; ^{1}H NMR δ 7.5 (t, 2, J = 7.5 Hz, H-ortho), 7.9 (dd, 2, J = 2 and 7.5 Hz, H-meta), 10.0 (s, 1); IR (film) 805, 825, 1165, 1200, 1560, 1590, 1700 s, 2920, 2980 cm^{-1}.

Anal. ($C_{21}H_{15}O_3P$) C, H, P.

4,4',4''-Phosphinidynetri(benzaldehyde) Tris(*O*-methyloxime) (5). A sample of noncrystalline trialdehyde **4** which had been prepared from 47 mmol of *p*-bromobenzaldehyde diethyl acetal (**1**) without purification of intermediate **2** was dissolved in 45 mL of dry pyridine with 4.35 g (42 mmol) of methoxyamine hydrochloride and stirred at 4 °C for 3 h. The mixture was diluted with ether and washed with 2 N HCl until the pyridine was removed, with water, aqueous $NaHCO_3$, and brine, dried (K_2CO_3), and concentrated to a cloudy, orange syrup. This material was purified by chromatography on Florisil (7% ether/hexane), affording 3.47 g (51% overall yield from **1**) of the tris(oxime ether) **5**: ^{1}H NMR δ 3.97 (s, 3), 7.0–7.8 (m, 4), 8.03 (s, 1); IR (film) 826, 847, 926, 1053, 1605 (C=N), 2800, 2950 cm^{-1}. A sample was further purified for analysis by distillation (160 °C, 0.025 Torr).

Anal. ($C_{24}H_{20}N_3O_3P$) C H, N, P.

4,4',4''-Phosphinidynetri(benzenemethanammonium) Tris(4-methylbenzenesulfonate) (6). A solution of 7.4 g (17.0 mmol) of tris(oxime ether) **5** and 30 mL of dry tetrahydrofuran was treated with 140 mL of a 1 M borane–THF solution at 0 °C under nitrogen in a 1-L round-bottom flask. This mixture was brought to room temperature cautiously (exothermic) and stirred at reflux for 3 h. After cooling to 0 °C, the excess hydride was quenched with 50 mL of water (*Caution:* vigorous hydrogen evolution and foaming) and 50 mL of 20% NaOH, and the resulting mixture was again heated for 1 h. Finally, 40 mL of concentrated HCl and 60 mL of water were added and the boron complexes were completely hydrolyzed by heating for 1 h. After cooling, the mixture was washed with ether, neutralized with 30 g of NaOH in 100 mL of water, and extracted with three portions of methylene chloride. This organic layer was washed with alkaline brine, dried (K_2CO_3), and concentrated to give 5.3 g (89% yield) of the triamine as a semisolid: ^{1}H NMR δ 1.53 (s, 2), 3.83 (s, 2), 7.22 (AB q, 4). The tris(tosylate) salt was prepared with 9.5 g (1.05 equiv) of *p*-toluenesulfonic acid in 100 mL of hot water and recrystallized from absolute ethanol to give 10.9 g (74% overall yield from the tris (oxime ether) of **6** as white needles, mp 320 °C dec.

Anal. ($C_{42}H_{48}O_9N_3PS_3$) C, H, N, P, S.

Iodo[tris(4-methylphenyl)phosphine]gold. To a solution of 1.38 g (2.58 mmol) of tri(*p*-tolylphosphine)gold(I) chloride[23] in 10 mL of acetone was added 460 mg (3.1 mmol) of NaI in 3 mL of acetone. The mixture was diluted with water and centrifuged, and the precipitate was washed with three portions of water. This material was stirred in 20 mL of acetone with another 200-mg portion of NaI, and partitioned between methylene chloride and water. The organic layer was dried (K_2CO_3) and concentrated, and the residue was recrystallized from absolute ethanol to give 1.40 g (87% yield) of the iodide complex in two crops: mp 233–234 °C; ^{1}H NMR δ 2.42 (s, 3), 7.1–7.7 (ABX, 4, $J_{H_{ortho}-P}$ = 12.5, $J_{H_{meta}-P}$ = 2.5, $J_{H_o-H_m}$ = 8 Hz). An anlaytical sample was prepared by recrystallization from absolute ethanol, mp 232.5–234.0 °C.

Anal. ($C_{21}H_{21}AuIP$) C, H, I.

Triiodoheptakis[tris(4-methylphenyl)phosphine]undecagold. To a stirred suspension of 1.0 g (1.59 mmol) of the gold(I) iodide complex in 43 mL of absolute ethanol was added 60 mg (1.59 mmol) of sodium borohydride over 15 min. The dark coffee-colored mixture was stirred for 2 h, poured into 1.6 L of hexane, and allowed to stand overnight. After filtration and evaporation, the red-brown residue was dissolved in 7 mL of CH_2Cl_2 and filtered to remove insoluble, white material. The flask containing the filtrate was set in an atmosphere saturated with hexane, and crystallization of the components of the mixture proceeded over several days. This crystallization could be monitored by TLC (10% ethyl acetate/benzene, silica gel), compound (R_f): undecagold cluster (0.76); $(tolyl)_3P$ (0.70); $(tolyl)_3PAuCl$ (0.65); and an unidentified component (0.53). After crystallization of the major portion of the R_f 0.70 and 0.53 materials, the undecagold cluster was obtained as red prisms. Another recrystallization from CH_2Cl_2/hexane gave 125 mg of material: mp 208–210 °C dec; UV (absolute EtOH) λ 305 (log ϵ 4.87), 417 (4.55).

Anal. Calcd for $C_{147}H_{147}Au_{11}I_3P_7$: C, 37.72; H, 3.17; Au, 46.35; I, 8.14; P, 4.63. Found: C, 37.74; H, 3.32; Au, 45.9 (as residue); I, 7.95; P, 4.47.

Tricyanoheptakis[tris(4-methylphenyl)phosphine]undecagold. A suspension of 121 mg (0.54 mmol) of AuCN and 165 mg (0.54 mmol) of tri(*p*-tolyl)phosphine in 40 mL of absolute ethanol was stirred at room temperature until almost all of the AuCN had dissolved (about 1 h). The solution was filtered and treated with 21 mg (0.55 mmol) of $NaBH_4$, resulting in an opaque brown mixture which slowly clarified to a ruby red over a 5-h period. After quenching with acetone and evaporating, the residue was chromatographed on a 1.5 × 35 cm column of Biobeads S-X-1, eluting with chloroform, to give 51 mg of red, microcrystalline material: mp 180–181 °C dec; UV (absolute EtOH) λ 303 (log ϵ 5.00), 415 (4.50).

Anal. Calcd for $C_{150}H_{147}Au_{11}N_3P_7$: C, 41.18; H, 3.39; Au, 49.52; N, 0.96; P, 4.96. Found: C, 41.13; H, 3.49; Au (as residue), 49.0; N, 0.90; P, 4.93.

Octakis(triphenylphosphine)enneagold Trinitrate. This material was prepared according to the published procedure,[17a] and was recrystallized from CH_2Cl_2/hexane: mp 229–230 °C dec (lit. 230 °C dec; UV (95% ethanol) λ 314 (log ϵ 4.78), 352 (s), 443 (4.21).

Iodo[4,4',4''-phosphinidynetri(benzenemethanamine)]gold (8a). A solution of 173 mg (0.44 mmol) of $HAuCl_4 \cdot 3H_2O$ and 170 mg (1.4 mmol) of 2,2'-thiodi(ethanol) in 1 mL of 95% ethanol was kept at room temperature until colorless. A 346-mg sample (0.40 mmol) of the tris(tosylate) **6** was added, and the mixture was stirred until homogeneous. After 2 drops of concentrated HCl was added, the complex was precipitated by diluting into 75 mL of acetone. The white solid was isolated by centrifugation, redissolved in 2 N HCl, reprecipitated, and washed with acetone. The tetrachloride (**7**) so obtained was stirred in 10 mL of absolute ethanol with 400 mg (2.7 mmol) of NaI, the NaCl was removed by filtration, and the filtrate was evaporated at reduced pressure. This residue was partitioned between CH_2Cl_2 and aqueous NaOH (containing some NaI), and the organic layer was dried (K_2CO_3) and evaporated to give 230 mg (86% crude yield) of purple, gelatinous material: ^{1}H NMR δ 1.65 (s, 2), 3.95 (s, 2), 7.3–7.8 (m, 4). The unstable complex was dissolved in $CHCl_3$, filtered, diluted with CCl_4, and allowed to stand at 4 °C as crystallization slowly proceeded. By this means, 92 mg (34% yield) of colorless crystals was obtained, mp 175–185 °C dec.

Anal. ($C_{21}H_{24}AuIN_3O$) C, H, Au (residue), I.

Triiodoheptakis[4,4',4''-phosphinidynetri(benzenemethanamine)]undecagold (9a). A solution of 100 mg (0.15 mmol) of the gold(I) iodide complex **8a** in 4 mL of absolute methanol was treated with 5.7 mg (0.15 mmol) of $NaBH_4$. After 1 h at room temperature and 12 h at 4 °C, the red-brown solution was evaporated to a gum and chromatographed in 0.2 M NH_4HCO_3 on Bio-Gel P-6 (200–400 mesh, 2.7 × 80 cm, 10 mL/h), affording an approximately 10^{-5} M

solution of the purified complex: UV (0.2 M NH_4HCO_3) λ 302, 425 nm (see Figure 1).

Upon standing in the dark for 10 days at 4 °C, the complex had decomposed completely to the enneagold cluster: UV λ 317, 357 (s), 385, 447 nm (see Figure 2).

Tricyanoheptakis[4,4',4''-phosphinidynetri(benzenemethanamine)]undecagold (9b). A suspension of 80 mg (0.36 mmol) of AuCN and 311 mg (0.36 mmol) of the tris(tosylate) **6** in 10 mL of 95% ethanol was stirred for 1 h, when almost all of the AuCN had dissolved. The mixture was filtered, neutralized with KOH, and treated with 14 mg (0.36 mmol) of $NaBH_4$. After 1 h, the ruby-red solution was quenched with 0.1 mL of acetone, concentrated at room temperature, and chromatographed in 0.05 M K_2HPO_4 on Bio-Gel P-6 (100–200 mesh, 4 × 50 cm column, 1.2 mL/min), affording an approximately 10^{-4} solution of purified complex: UV (0.05 M K_2HPO_4) λ 303, 420 nm (see Figure 3). Upon standing at 4 °C, this solution deposited a sparingly soluble, dark red, amorphous solid; the supernatant was lyophilized for storage of the remaining material.

Acknowledgment. Support for this research was provided by the National Institutes of Health through Grants GM-15971 and GM-21612. We also thank Professor John Hearst for the sedimentation-equilibrium measurements.

References and Notes

(1) (a) University of California, Berkeley; (b) National Institutes of Health Postdoctoral Fellow, 1972–1973; (c) University of California, San Diego.
(2) S. J. Singer and A. F. Schick, *J. Biophys. Biochem. Cytol.*, **9**, 519 (1961).
(3) G. L. Nicolson and S. J. Singer, *Proc. Natl. Acad. Sci. U.S.A.*, **68**, 942 (1971).
(4) L. Jarett and R. M. Smith, *J. Supramol. Struct.*, **6**, 45 (1977).
(5) M. Wu and N. Davidson, *J. Mol. Biol.*, **78**, 1 (1973); N. Davidson, *Proc., Electron Microsc. Soc. Am.*, **32**, 302 (1974); L. Angerer, N. Davidson, W. Murphy, D. Lynch, and G. Attardi, *Cell*, **9**, 81 (1976).
(6) S. J. Singer, *Nature (London)*, **183**, 1523 (1959).
(7) J. L. Farrant, *Biochim. Biophys. Acta*, **13**, 569 (1954).
(8) L. A. Sternberger, E. J. Donati, and C. E. Wilson, *J. Histochem. Cytochem.*, **11**, 48 (1963).
(9) F. A. Pepe and H. Finck, *J. Biophys. Biochem. Cytol.*, **11**, 521 (1961); P. A. Kendall, *Biochim. Biophys. Acta*, **97**, 174 (1965).
(10) J. Wall, J. Langmore, M. Isaacson, and A. V. Crewe, *Proc. Natl. Acad. Sci. U.S.A.*, **71**, 1 (1974).
(11) M. Cais, S. Dani, Y. Eden, O. Gandolfi, M. Horn, E. E. Isaacson, Y. Joseph, Y. Saar, E. Slovin, and L. Snarsky, *Nature (London)*, **270**, 534 (1977).
(12) R. B. King, *Prog. Inorg. Chem.*, **15**, 287 (1972).
(13) F. Cariati and L. Naldini, *Inorg. Chim. Acta*, **5**, 172 (1971).
(14) (a) M. McPartlin, R. Mason, and L. Malatesta, *Chem. Commun.*, 334 (1969); (b) V. G. Albano, P. L. Bellon, M. Manassero, and M. Sansoni, *ibid.*, 1210 (1970); (c) P. L. Bellon, M. Manassero, and M. Sansoni, *J. Chem. Soc., Dalton Trans.*, 1481 (1972); (d) see also D. M. P. Mingos, *ibid.*, 1163 (1976).
(15) H. Feuer and D. M. Braunstein, *J. Org. Chem.*, **34**, 1817 (1969).
(16) B. M. Sutton, E. McGusty, D. T. Walz, and M. J. DiMartino, *J. Med. Chem.*, **15**, 1095 (1972).
(17) (a) F. Cariati and L. Naldini, *J. Chem. Soc., Dalton Trans.*, 2286 (1972); (b) P. L. Bellon, F. Cariati, M. Manassero, L. Naldini, and M. Sansoni, *Chem. Commun.*, 1423 (1971).
(18) G. P. Schiemenz and H. Kaack, *Justus Liebigs Ann. Chem.*, 1480 (1973).
(19) W. B. Pearson, "Lattice Spacings and Structures of Metals and Alloys", Pergamon Press, Oxford, 1957.
(20) See, for example, A. F. Schick and S. J. Singer, *J. Biol. Chem.*, **236**, 2477 (1961).
(21) S. J. Singer, *Immunochemistry*, **12**, 615 (1975).
(22) A. E. Tschitschibabin, *Chem. Ber.*, **37**, 188 (1904).
(23) D. I. Nichols, *J. Chem. Soc. A*, 1216 (1970).

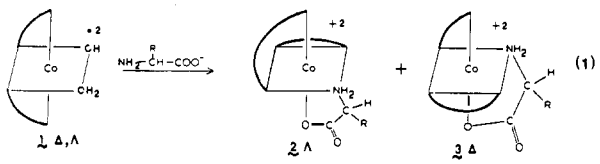
Amino Acid Mediated Asymmetric Transformation and Catalytic Asymmetric Transformation of the α-Triethylenetetraminecobalt(III) Moiety

Robert C. Job

Contribution from the Department of Chemistry, Colorado State University, Fort Collins, Colorado 80523. *Received April 1, 1977*

Abstract: A high degree of asymmetric transformation is realized upon treatment of racemic α-dichlorotriethylenetetraminecobalt(III) chloride (**5**) with (*S*)-proline and triethylamine in refluxing methanol and ethanol when up to 94.5% of the cobalt appears in the products as $\Delta(+)_{436}$-β_2-[triethylenetetraminecobalt(III) (*S*)-prolinate]$^{2+}$. A similar asymmetric transformation is observed when racemic **5** is treated with a symmetrical amino acid, α,α-aminomethylmalonic acid, and triethylamine in refluxing methanol with only catalytic amounts of various chiral acids to produce $\Lambda(-)_{436}$-β_2-[triethylenetetraminecobalt(III) (*R*)-aminomethylmalonate]$^{2+}$ (the purity of which was proven by a resolution via cation-exchange chromatography).

In recent years considerable research has been directed toward the synthesis and isolation of tetraminocobalt(III)-amino acid complexes. A typical reaction between a racemic tetraminocobalt(III) moiety and an α-amino acid is shown in eq 1 to produce two amino acid complexes. Because product isolation could be greatly simplified, a desirable and useful case of this type of synthesis would be one in which the unwanted isomer is not produced. This situation could be brought about in two ways: (1) a kinetic differentiation wherein the incoming amino acid reacts preferentially with either the Λ or Δ form of the tetramine; (2) a thermodynamic differentiation in which equilibrium is established between the two products and the more stable one predominates. A kinetic resolution involving **1** and glutamic acid had been proposed and subsequently disproven.[1,2] Little stereospecificity was found in the synthesis of β_2-[trien-Co (*S*)-Pro]$^{2+}$ [3] from β-[trien-Co(OH)(OH_2)]$^{2+}$ and (*S*)-proline under kinetically controlled conditions.[4] Kinetic differentiation has been claimed in the complexation of **1** with the N-terminus of chiral dipeptides, but no evidence is presented to preclude thermodynamic equilibration upon hydrolysis of the peptide linkage to produce **2** and **3**.[5] Neither has a thermodynamic differentiation been conclusively demonstrated for an amino acid complex. However, Busch has presented conditions under which the ethylenediamine groups in



1 Δ,Λ 2 Λ 3 Δ

0002-7863/78/1500-5089$01.00/0