Cobalt(III) Cage Complexes Linked to 4-Pyridyl: Models for Protein Modification

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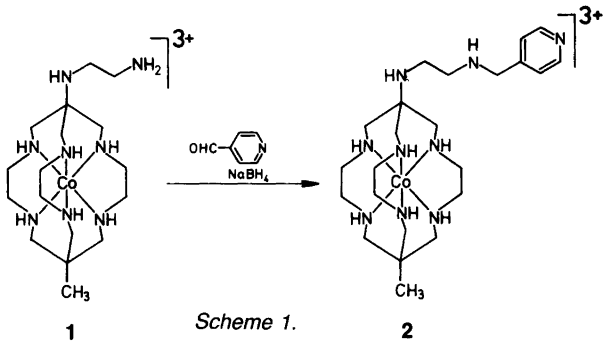
The complex ions 1-methyl-8-(4-(4-pyridyl)-3-aza-1-butylamino)-3,6,10,13,16,19-hexaazabicyclo[6.6.6]eicosanecobalt(III) ($[Co(H_3CsarNHCH_2CH_2NHCH_2py)]^{3+}$), **2** and (2-(4-pyridylmethylamino)ethylamineN^1)pentaamminecobalt(III) ($[(H_3N)_5Co(NH_2CH_2CH_2NHCH_2py)]^{+}$) were synthesized in aqueous buffer at ca. pH 9.5 by reductive alkylation with 4-formylpyridine and $NaBH_4$ from the parent pendant amine complexes 1-(2-aminoethylamino)-8-methyl-3,6,10,13,16,19-hexaazabicyclo-[6.6.6]eicosanecobalt(III) ($[Co(H_3CsarNHCH_2CH_2NH_2)]^{3+}$), **1**, and (1,2-ethanediamineN^1)pentaamminecobalt(III) ($[(H_3N)_5Co(NH_2CH_2CH_2NH_2)]^{3+}$), respectively. The 1-amino-8-methyl-3,6,10,13,16,19-hexaazabicyclo[6.6.6]eicosanecobalt(III) ($[Co(H_3CsarNH_2)]^{3+}$) complex only undergoes similar alkylation in non-aqueous conditions. The structure of the product, 1-methyl-8-(4-pyridyl-methylamino)-3,6,10,13,16,19-hexaazabicyclo[6.6.6]eicocanecobalt(III) tristrifluoromethane sulfonate monohydrate ($[Co(H_3CsarNHCH_2py)](O_3SCF_3)_3 \cdot H_2O$), was determined by X-ray diffraction [triclinic, $P\bar{1}$, a = 9.035(5), b = 14.171(5), c = 15.170(5) Å, α = 67.45(3), β = 88.43(4), γ = 85.02(4)°; Z = 2]. In the structure the complex ion adopts the ob_3 conformation, and the pyridylmethyl element is disordered. Concentration dissociation constants (aqueous solution, 25 °C, μ = 1.00 M) for the protonated products have been determined. The pyridyl units of the new complexes are available for coordination to another metal centre. It would seem probable that similar reductive alkylation of protein lysine residues could provide alternative ruthenation sites in metalloproteins.

Studies of electron-transfer reactions involving at least one redox partner of biological origin[1] have been confined to intermolecular reactions, but recently a growing number of studies involving intramolecular electron transfer over known distances in structurally well-defined systems have appeared.[1–6] For the intramolecular studies the added redox centre, mainly in the form of simple suitably modified metal complexes [Ru(II) or Cr(II)], have been chemically attached at a known site on the protein prior to reaction. In the majority of cases the successful strategy has involved coordination of a Ru(II)-amine moiety in a process referred to as 'ruthenation'. So far, this has taken place exclusively at His residues, owing to the great affinity of the Ru(II) centre for ligands which allow π-backbonding. Another significant property in favour of Ru is the relative robustness in both of its oxidation states, II and III. However, the scope of this strategy would be even greater if it were possible to perform similar attachments at other amino-acid residues. The provision would be that these residues were appropriately modified by introduction of ligating groups with sufficient affinity for the Ru(II) centre. An obvious candidate in this context would be the pyridyl segment. This study was initiated in order to provide an easy method (by way of a model study) for the introduction of the 4-pyridylmethyl group at Lys residues, with the prospect of subsequent ruthenation.

As simple models for the ε-amine of metalloprotein Lys residues various known cobalt(III) complex ions with pendant primary amines were selected. These complexes all carry positive charges, ensuring high solubility and thus allowing reactions to be carried out in water. The strategy is exemplified in Scheme 1 for the cage complex $[Co(H_3CsarNHCH_2CH_2NH_2)]^{3+}$, **1**. Reductive alkylation of the pendant terminal amine with 4-formylpyridine† and $NaBH_4$ may produce the 4-pyridylmethyl derivative **2** by analogy with the well-known modifications of Lys residues with formaldehyde or pyridoxal phosphate.[7,8]



1 *Scheme 1.* 2

† In all compounds having a $-CH_2py$ group the pyridine ring is substituted in the 4 (p) position.

Experimental

Commercial anhydrous trifluoromethanesulfonic (triflic) acid (3M Co.) was used without further purification. All other chemicals were of reagent grade or better. The cation-exchange resin employed, Dowex AG 50W-X2 (Bio-Rad) was 200–400 mesh. Dimensions of resin columns are given as diameter × length in mm. All routine evaporation of solvent was carried out at reduced pressure (ca. 20 Torr) on a Büchi rotary evaporator using a water aspirator and water bath (45 °C). Product purity was checked by chromatography on a Pharmacia FPLC instrument with a Mono S cation-exchange column. Visible absorption spectra in solution were recorded on a Perkin–Elmer Lambda 17 spectrophotometer with automatic disk data collection. The reflection spectra were measured by the diffusion method on a Perkin–Elmer Lambda 7 spectrophotometer with a reflection attachment. Acid concentration dissociation constants were determined at 25.0 °C and μ = 1.00 M ($NaClO_4$) as described by Mønsted and Mønsted.[9] The ^1H and ^{13}C NMR spectra were recorded on a modified 250 MHz Bruker HX-270 instrument in D_2O with tetramethylsilane as external reference, and chemical shifts δ are given in ppm relative to this standard. $[Co(H_3CsarNH_3)]Cl_4\cdot\frac{1}{2}H_2O$[10] and $[Co(H_3CsarNH_2CH_2CH_2NH_3)]Cl_5\cdot H_2O$[11] were prepared as described. Conversion of chloride salt into the corresponding trifluoromethanesulfonates (triflates), and the synthesis of $[(H_3N)_5Co(O_3SCF_3)](O_3SCF_3)_2$ was carried out by treating the chloride with anhydrous triflic acid according to Dixon *et al.*[12]

$[(H_3N)_5Co(NH_2CH_2CH_2NH_3)]Cl_4\cdot\frac{1}{2}H_2O$. 1,2-Ethanediamine (15.0 ml, 160 mmol) was placed in a beaker which was cooled in ice. 0.1 ml 30 % H_2O_2 and $[(H_3N)_5Co(O_3SCF_3)](O_3SCF_3)_2$ (7.1 g, 12 mmol) was rapidly added with vigorous stirring. The reaction was stopped after 2 min by addition of 65 ml ice-cold 6 M HCl. The mixture was diluted to 300 ml with water and adsorbed on a cation-exchange column (70×200, H^+ form). The column was washed with 1 l of 1 M HCl and the adsorbed complexes were eluted with initially 2 M, later 4 M, HCl. The elution gave three bands[‡] (violet, red and orange). The eluate containing the orange band was evaporated until crystals started to form, and the crystallization was completed by cooling in ice. The product was collected and recrystallized by leaving a solution in 6 M HCl in an ethanolic atmosphere. The resulting orange crystals were collected, washed with absolute ethanol and diethyl ether, and dried in vacuum over both P_4O_{10} and NaOH. Yield: 1.45 g (34 %). Anal. Found: C 6.7; H 7.0; N 28.3; Cl 41.1; Co 16.5. Calc. for $C_2H_{25}N_7Cl_4CoO_{1/2}$: C 6.75; H 7.08; N 27.54; Cl 39.83; Co 16.55. ^1H NMR: δ 2.9 (t, 2 H), 3.4 (t, 2 H) (CH_2); 3.8 (broad, NH_2). ^{13}C NMR: δ 38.7 (CH_2NH_3), 39.7 ($CoNH_2CH_2$).[13]

$[(H_3N)_5Co(NH_2CH_2CH_2NH_2CH_2pyH)]Cl_5$. $[(H_3N)_5Co(NH_2CH_2CH_2NH_3)]Cl_4\cdot\frac{1}{2}H_2O$ (344 mg, 0.97 mmol) was dissolved in 15.0 ml 0.2 M borate buffer solution, pH 11. The complete addition of 4-formylpyridine (504 mg, 4.71 mmol) was achieved by using 5.0 ml buffer solution. After addition of $NaBH_4$ (75 mg, 2 mmol) the solution was left with stirring and cooling in ice for 1½ h. The reaction mixture was diluted with buffer to 50 ml and rapidly adsorbed on 25 ml wet Dowex resin (Na^+ form) on a glass filter. The resin was washed with 1 M NaCl, then suspended in water and transferred to the top of a column of the same resin (35×250, H^+ form). Elution with 2–4 M HCl gave two orange bands of unreacted $[(H_3N)_5Co(NH_2CH_2CH_2NH_3)]^{4+}$ (first) and $[(H_3N)_5Co(NH_2CH_2CH_2NH_2CH_2pyH)]^{5+}$ (second). The second band was evaporated to a few millilitres and the product precipitated by addition of absolute ethanol. The resulting powder was collected, washed with absolute ethanol and diethyl ether, and dried in vacuum over both P_4O_{10} and NaOH. Yield: 217 mg (47 %). Anal. Found: C 19.9; H 6.4; N 23.3; Cl 37.3; Co 12.5. Calc. for $C_8H_{30}N_8Cl_5Co$: C 20.25; H 6.37; N 23.61; Cl 37.35; Co 12.42. ^1H NMR: δ 2.9 (t, 2 H), 3.6 (t), 4.7 (methylenes); 3.8 (broad, NH_2); 8.3 (d, 2 H), 8.9 (d. 2 H) (py). ^{13}C NMR: δ 38.7, 46.9, 49.7 (methylenes); 127.3, 142.2, 151 (py).

$[Co(H_3CsarNH_2CH_2CH_2NH_2CH_2pyH)]Cl_6\cdot 5H_2O$. $[Co(H_3CsarNH_2CH_2CH_2NH_3)]Cl_5\cdot H_2O$ (1.56 g, 2.54 mmol) was dissolved in 50 ml 0.2 M borate buffer solution, pH 11. 2.5 ml 1.0 M NaOH and 4-formylpyridine (1.0 g, 9.3 mmol) were added, followed by $NaBH_4$ (200 mg, 10.7 mmol), resulting in hydrogen production. After about 1 h 100 ml wet Dowex-resin (Na^+ form) were added. The resin was filtered, washed with 0.5 M NaCl and transferred to the top of a Dowex column (70×200, H^+ form). Elution with 2–4 M HCl gave two orange bands of unreacted $[Co(H_3CsarNH_2CH_2CH_2NH_3)]^{5+}$ (first) and $[Co(H_3CsarNH_2CH_2CH_2NH_2CH_2pyH)]^{6+}$ (second). Occasionally, the second band was so broadened that re-chromatography was warranted. The eluate containing the orange band was evaporated nearly to dryness and the product was precipitated by addition of absolute ethanol. The orange powder was collected, washed with absolute ethanol and diethyl ether, and dried in vacuum over both P_4O_{10} and NaOH. Yield: 1.29 g (62 %). Anal. Found: C 33.7; H 6.8; N 15.5; Cl 27.0; Co 7.2. Calc. for $C_{23}H_{58}N_9Cl_6CoO_5$: C 34.00; H 7.20; N 15.52; Cl 26.18; Co 7.25. ^1H NMR: δ 0.9 (s, 3 H, CH_3); 2.4–3.3 (m, 28 H), 4.6 (s, 2 H) (methylenes); 8.1 (d, 2 H), 8.9 (d, 2 H) (py). ^{13}C NMR: δ 20.3 (CH_3); 38.4, 48.8, 50.3 (pendant methylenes); 43.1, 61.1 (cap); 53.7, 55.6, 55.8, 55.9 (cage methylenes); 128.6, 143.0, 152.4 (py).

$[Co(H_3CsarNH_2CH_2pyH)]Cl_5\cdot\frac{1}{2}H_2O$. $[Co(H_3CsarNH_3)](H_3O)(O_3SCF_3)_5$ (3.47 g, 3.05 mmol) was suspended in 50 ml CH_3CN with 5 g of 3 Å molecular sieve. The suspension was stirred for 15 min, after which 4-formylpyridine (3.33 g, 31.1 mmol) was added, and the suspension became a

[‡] In some cases a second minor orange band, probably $[(H_3N)_5Co(NH_2CH_2CH_2NH_2)Co(NH_3)_5]^{6+}$, also appeared as a fourth band.

clear solution from which an orange substance precipitated during the next 20 min. At this point $NaBH_3CN$ (3 g, ca. 50 mmol) and 10 ml CH_3CN were added. The precipitate dissolved and the solution became intensely red. After about 1 h the reaction mixture was diluted to 400 ml with water, and 150 g wet Dowex resin (Na^+ form) were added. The resin was filtered, washed with water and transferred to a Dowex column (70×200, H^+ form). Elution with 1–4 M HCl gave one broad orange band, which was collected and the solution evaporated to dryness. The residue was dissolved in water and re-chromatographed. Elution revealed two bands: unreacted $[Co(H_3CsarNH_3)]^{4+}$ (first) and $[Co(H_3CsarNH_2CH_2pyH)]^{5+}$ (second). The eluate containing the second band was evaporated nearly to dryness and the product was crystallized by evaporation in the air. The orange crystals were washed with absolute ethanol and diethyl ether, and dried in vacuum over both P_4O_{10} and NaOH. Yield: 1.19 g (58 %). Anal. Found: C 37.5; H 6.6; N 16.7; Cl 26.5; Co 8.8. Calc. for $C_{21}H_{45}N_8Cl_5CoO_{1.5}$: C 37.66; H 6.77; N 16.73; Cl 26.46; Co 8.80.

$[Co(H_3CsarNHCH_2py)](O_3SCF_3)_3 \cdot H_2O$. $[Co(H_3CsarNH_2CH_2pyH)]Cl_5 \cdot \frac{1}{2}H_2O$ (2.68 g, 4.00 mmol) was dissolved in 5 ml water. Saturated $NaHCO_3$ was added until ca. pH 8. A solution of 5 g $NaO_3SCF_3 \cdot H_2O$ in 5 ml water was added and the mixture diluted to 20 ml with water. The orange solution was placed in a refrigerator (5 °C) overnight, when very fine yellow crystals slowly formed. The crystals were filtered, washed with ethanol and diethyl ether, and dried in vacuum over P_4O_{10} and NaOH; these crystals were used for the structure determination. Yield: 2.15 g (58 %). Anal. Found: C 31.0; H 4.6; N 12.0; Co 6.3; S 10–11. Calc. for $C_{24}H_{42}N_8F_9CoO_{10}S_3$: C 31.04; H 4.56; N 12.07; Co 6.35; S 10.36. ^1H NMR: δ 0.9 (s, 3 H, CH_3); 2.4–3.4 (m, 24 H), 3.8 (s, 2 H) (methylenes); 7.4 (d, 2 H), 8.5 (d, 2 H) (py). ^{13}C NMR: δ 20.2 (CH_3); 45.1 (pendant methylene); 42.7, 61.1 (cap); 54.1, 55.1, 55.2, 55.7 (cage methylenes); 124.5, 149.1, 151.0 (py).

Table 1. Crystal data.

Formula	$C_{21}H_{42}N_8F_9CoO_{10}S_3$
Formula weight/g mol^{-1}	928.76
Space group	Triclinic, $P\bar{1}$ (No. 2)
a/Å	9.035(5)
b/Å	14.171(5)
c/Å	15.170(5)
α/°	67.45(3)
β/°	88.43(4)
γ/°	85.02(4)
U/Å^3	1787.0(2)
Z	2
μ/cm^{-1}	7.54
F(000)	956

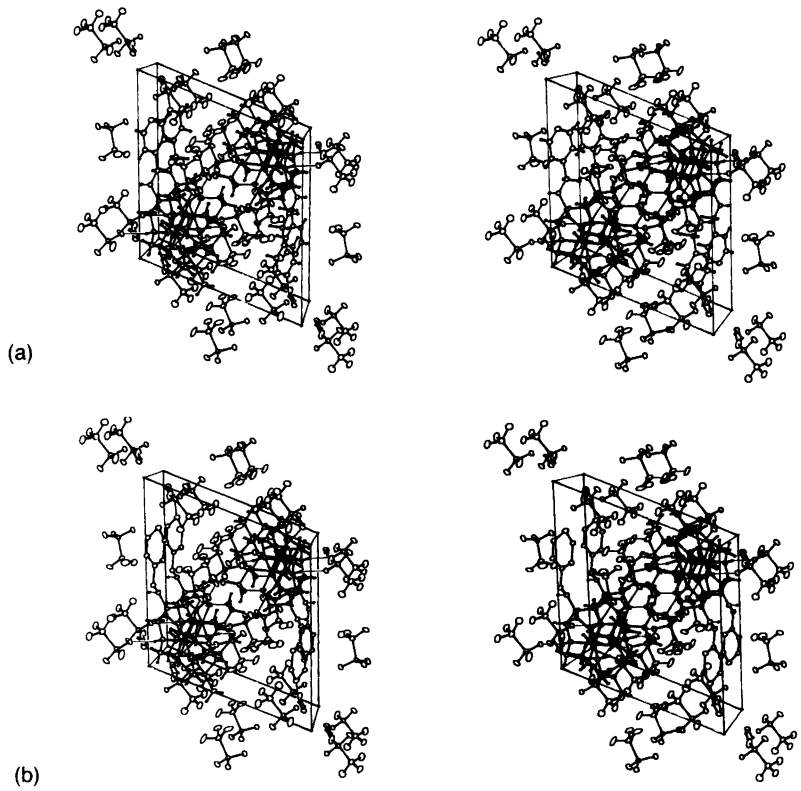


Fig. 1. Stereoscopic ORTEP drawings of the unit cell illustrating the two positions of the disordered pyridine ring populated 80 % (a) and 20 % (b).

Table 2. Positional and displacement parameters (in Å^2) for non-hydrogen atoms.

Atom	*x*	*y*	*z*	*B*	Atom	*x*	*y*	*z*	*B*
Co	0.21964(6)	0.74372(4)	0.24699(3)	1.094(9)	O10	0.2673(4)	0.9376(2)	0.9473(2)	2.51(7)
N1	0.3138(4)	0.8714(3)	0.2239(2)	1.59(7)	S1	0.7565(1)	0.50235(7)	0.28623(7)	1.63(2)
N2	0.0322(4)	0.7989(2)	0.2852(2)	1.45(6)	S2	0.7091(1)	0.73172(8)	0.44788(7)	1.59(2)
N3	0.2955(4)	0.6847(3)	0.3779(2)	1.56(7)	S3	0.2895(1)	1.15436(8)	0.06967(8)	2.01(2)
N4	0.4054(4)	0.6936(3)	0.2047(2)	1.70(7)	C22	0.7595(6)	0.4483(4)	0.1954(4)	3.3(1)
N5	0.1388(4)	0.8018(3)	0.1168(2)	1.63(7)	C23	0.6777(7)	0.7584(4)	0.5548(4)	3.4(1)
N6	0.1329(4)	0.6141(3)	0.2735(2)	1.67(7)	C24	0.3095(8)	1.2346(4)	0.1365(4)	4.1(1)
C1	0.1998(4)	0.8544(3)	0.3802(3)	1.54(8)	F1	0.8983(4)	0.4290(2)	0.1706(2)	4.08(7)
C2	0.2350(5)	0.6329(3)	0.1135(3)	1.79(8)	F2	0.6881(4)	0.5139(3)	0.1155(2)	5.24(8)
C3	0.3315(4)	0.8913(3)	0.3120(3)	1.53(8)	F3	0.6949(4)	0.3621(2)	0.2234(3)	5.60(9)
C4	0.0551(4)	0.8795(3)	0.3218(3)	1.42(7)	F4	0.6976(5)	0.6739(3)	0.6334(2)	5.15(9)
C5	0.2259(4)	0.7378(3)	0.4376(3)	1.56(8)	F5	0.5349(4)	0.7985(3)	0.5540(3)	5.9(1)
C6	0.4569(4)	0.8703(3)	0.1706(3)	1.84(8)	F6	0.7600(5)	0.8247(3)	0.5613(2)	5.9(1)
C7	−0.0770(4)	0.8312(3)	0.2038(3)	1.58(8)	F7	0.4176(4)	1.2976(3)	0.1011(3)	5.31(9)
C8	0.2809(5)	0.5710(3)	0.4182(3)	1.76(8)	F8	0.1875(5)	1.2923(2)	0.1359(3)	5.76(9)
C9	0.5235(4)	0.7626(3)	0.2018(3)	1.78(8)	F9	0.3403(6)	1.1767(3)	0.2288(3)	7.2(1)
C10	0.1400(5)	0.5527(3)	0.3800(3)	1.84(8)	O1	0.6000(3)	0.5143(2)	0.3064(2)	2.45(7)
C11	0.0066(4)	0.8747(3)	0.1125(3)	1.62(8)	O2	0.8440(4)	0.4267(3)	0.3623(2)	3.05(8)
C12	0.2009(5)	0.5571(3)	0.2159(3)	1.98(8)	O3	0.8239(4)	0.5982(2)	0.2393(2)	2.48(7)
C13	0.3861(5)	0.6762(3)	0.1149(3)	2.01(9)	O4	0.5949(3)	0.6646(2)	0.4530(2)	2.76(7)
C14	0.1125(5)	0.7222(3)	0.0792(3)	1.94(8)	O5	0.6908(3)	0.8314(2)	0.3718(2)	2.30(7)
C15	0.2430(6)	0.5768(4)	0.0455(3)	2.6(1)	O6	0.8586(3)	0.6830(2)	0.4589(2)	2.58(7)
C16	0.0919(6)	0.8735(4)	0.5242(4)	2.17(9)*	O7	0.2437(4)	1.2266(3)	−0.0226(2)	3.37(8)
C17	0.1390(5)	0.8804(3)	0.6197(3)	2.35(8)*	O8	0.1792(4)	1.0854(2)	0.1210(2)	2.39(7)
C18	0.0524(7)	0.8378(4)	0.6966(4)	2.3(1)*	O9	0.4354(4)	1.1052(3)	0.0747(4)	4.8(1)
C19	0.0911(7)	0.8450(5)	0.7799(4)	2.7(1)*	C16′	0.304(2)	0.922(2)	0.489(1)	2.0*
C20	0.2929(7)	0.9307(4)	0.7129(4)	2.6(1)*	C17′	0.207(2)	0.913(2)	0.591(1)	2.0*
C21	0.2612(6)	0.9272(4)	0.6250(4)	2.11(9)*	C19′	0.122(2)	0.869(2)	0.728(1)	2.0*
N7	0.1972(4)	0.9054(3)	0.4474(3)	2.17(8)	C20′	0.342(2)	0.942(2)	0.730(1)	2.0*
N8	0.2116(5)	0.8939(3)	0.7878(3)	2.58(8)	C21′	0.368(2)	0.948(2)	0.637(1)	2.0*

[a]Anisotropically refined displacement parameters, not marked with stars, are given in the isotropic equivalent form defined as:

$$B_{eq} = \frac{4}{3}[a^2\beta_{11}+b^2\beta_{22}+c^2\beta_{33}+2ab\cos(\gamma)\beta_{12}+2ac\cos(\beta)\beta_{13}+2bc\cos(\alpha)\beta_{23}].$$

Crystal structure determination of [Co(H_3CsarNHCH_2py)]-(O_3SCF_3)$_3$·H_2O. A summary of the crystallographic data is given in Table 1. Diffraction data were collected on a tabular yellow crystal with dimensions 0.05×0.25×0.40 mm with an Enraf–Nonius CAD4 diffractometer using ω/2θ scans and MoK_α radiation (λ = 0.71069 Å). The crystal was cooled to 110 ± 0.8 K in a stream of N_2 gas. The cell dimensions were determined from a least-squares fit of 18 reflections, well distributed in reciprocal space, with θ values in the range 14–20°. The maximum scan time was 90 s. Three reflections were measured every 2.8 h to check for crystal decay which, however, did not appear to take place to any significant degree. The intensity data were collected with θ varying between 1 and 30° for $-12\leq h\leq 12$, $-19\leq k\leq 19$ and $-21\leq l\leq 21$. A total of 15032 individual reflections were measured and reduced, by including corrections for Lorenz and polarization effects, but not for absorption, to values of $|F_o|$ and $\sigma(F_o)$ for 10414 unique reflections. 6402 reflections for which $I>3\sigma(I)$ were included in the final structure analysis. The structure was solved using SHELXS-86,[14] and refined with the SDP package.[15] Atomic scattering factors were taken from Ref. 16, except for hydrogen, for which the value was taken from Stewart *et al.*[17] Atomic coordinates for all atoms (except H39 and H42, the positions of which were calculated) and anisotropic thermal parameters for non-hydrogen atoms (except for C16–C21 and C16′, C17′, C19′ and C21′), a total of 601 parameters, were refined minimizing $\Sigma w(|F_o|-|F_c|)^2$, where $w^{-1} = \sigma^2(F_o) + 0.01\sigma F_o$.[2] The structure appeared to be disordered. The pyridylmethyl moiety occupied two sites, each populated to 80 and 20 %, respectively, but with the N atom essentially invariant. Figs. 1(a) and (b) are stereoscopic ORTEP[18] representations of the unit cell, displaying for each case the two different postions of the ring, as well as the crystal packing and the H-bonding pattern. The triflate anions were also disordered. It did not appear possible to make a model describing the disorder of these anions. Analysis of the surroundings of the fluorine atoms reveals that they form pockets in the structure and that they have only one van der Waals contact distance [F(2)–H(6) (positioned on C13) 2.45 Å]. Hence the positions of the fluorine atoms are determined only by the rotational barriers from the geminal oxygen atoms, and disorder should be expected. For each triflate anion two of the three oxygen atoms are involved in hydrogen bonding. As can be seen from the thermal parameters in Table 2, the oxygen atom positions are much more ordered than are the fluorine positions. The atomic parameters derived are listed in

Table 3. Selected interatomic distances (in Å) of the complex ion.

Co	N1	1.971(4)	C1	C3	1.545(5)
Co	N2	1.970(3)	C1	C4	1.537(5)
Co	N3	1.952(3)	C1	C5	1.544(5)
Co	N4	1.954(4)	C1	N7	1.457(7)
Co	N5	1.960(3)	C2	C12	1.553(5)
Co	N6	1.954(4)	C2	C13	1.548(7)
N1	C3	1.484(6)	C2	C14	1.543(6)
N1	C6	1.508(5)	C2	C15	1.521(8)
N2	C4	1.480(6)	C6	C9	1.489(6)
N2	C7	1.504(5)	C7	C11	1.496(5)
N3	C5	1.479(6)	C8	C10	1.496(6)
N3	C8	1.506(5)	C16	C17	1.560(8)
N4	C9	1.497(6)	C16	N7	1.442(7)
N4	C13	1.492(6)	C17	C18	1.352(7)
N5	C11	1.496(5)	C17	C21	1.356(8)
N5	C14	1.483(7)	C18	C19	1.36(1)
N6	C10	1.512(5)	C19	N8	1.372(8)
N6	C12	1.491(6)	C20	C21	1.392(9)
			C20	N8	1.292(7)

Table 4. Bond angles (in °) of the complex ion.

N1	Co	N2	91.6(1)	C4	C1	N7	112.0(3)
N1	Co	N3	90.7(1)	C5	C1	N7	108.0(3)
N1	Co	N4	87.1(1)	C12	C2	C13	108.8(3)
N1	Co	N5	90.2(1)	C12	C2	C14	110.4(3)
N1	Co	N6	177.7(1)	C12	C2	C15	109.6(4)
N2	Co	N3	92.5(1)	C13	C2	C14	109.2(3)
N2	Co	N4	177.9(1)	C13	C2	C15	109.6(4)
N2	Co	N5	86.5(1)	C14	C2	C15	109.3(4)
N2	Co	N6	89.7(1)	N1	C3	C1	110.8(3)
N3	Co	N4	89.2(1)	N2	C4	C1	111.0(3)
N3	Co	N5	178.6(1)	N3	C5	C1	111.3(3)
N3	Co	N6	87.3(1)	N1	C6	C9	108.7(3)
N4	Co	N5	91.9(1)	N2	C7	C11	108.1(3)
N4	Co	N6	91.7(2)	N3	C8	C10	107.8(3)
N5	Co	N6	91.8(1)	N4	C9	C6	109.6(3)
C3	N1	C6	113.7(3)	N6	C10	C8	108.6(3)
C4	N2	C7	114.5(3)	N5	C11	C7	108.0(3)
C5	N3	C8	113.6(3)	N6	C12	C2	110.3(3)
C9	N4	C13	115.2(3)	N4	C13	C2	111.1(3)
C11	N5	C14	114.7(3)	N5	C14	C2	110.9(4)
C10	N6	C12	113.6(3)	C17	C16	N7	115.9(5)
C3	C1	C4	109.3(3)	C16	C17	C18	117.1(5)
C3	C1	C5	109.4(3)	C16	C17	C21	121.4(4)
C3	C1	N7	107.5(4)	C18	C17	C21	121.6(5)
C4	C1	C5	110.4(3)	C17	C18	C19	117.6(6)
C18	C19	N8	122.8(5)	C1	N7	C16	116.7(4)
C21	C20	N8	123.8(6)	C19	N8	C20	117.2(5)
C17	C21	C20	117.1(5)				

Table 2, where the lesser populated positions are marked by a prime. After the final refinement cycle the agreement factors were $R = 0.060$ and $R_w = 0.076$ and the maximum ratio (Δ/σ) was 0.05. A final Fourier synthesis gave the highest and lowest residuals as 1.6 and -1.0 e Å^{-3}, respectively. Selected bond lengths and angles are listed in Tables 3 and 4. Fig. 2 is an ORTEP drawing of the cation. Tables of anisotropic thermal parameters, hydrogen positions and a listing of observed and calculated structure factor amplitudes are available from the authors on request.

Results and discussion

Syntheses and reactivity. $[(H_3N)_5Co(NH_2CH_2CH_2NH_3)]Cl_4$ was produced in 34% yield by addition of $[(H_3N)_5Co(O_3SCF_3)](O_3SCF_3)_2$ to anhydrous 1,2-ethanediamine (en), followed by acidification and chromatographic workup. This procedure is clearly superior to the older method,[19,20] which involves equilibration of $[(H_3N)_5Co(OH_2)]^{3+}$ with en in $(CH_3)_2SO$, producing a multitude of products and only little (6%) of the desired isomer, most probably owing to detrimental Co^{2+} catalysis.[21] The subsequent reductive alkylation of the primary aliphatic amines of $[(H_3N)_5Co(NH_2CH_2CH_2NH_3)]^{4+}$ and $[Co(H_3CsarNH_2CH_2NH_3)]^{4+}$ with 4-formylpyridine was easily achieved in aqueous borate buffer, with $NaBH_4$ as the reducing agent. In the reaction with $[(H_3N)_5Co(NH_2CH_2CH_2NH_3)]^{4+}$ some reduction of the metal centre, producing Co^{2+}, was inevitable. This side reaction is avoided with the Co(III) cage compounds, as the otherwise substitutionally labile Co^{2+} is efficiently trapped inside the cage and is readily reoxidised by atmospheric O_2. In this respect the cage compounds are better suited as primitive models for metalloproteins. Easy

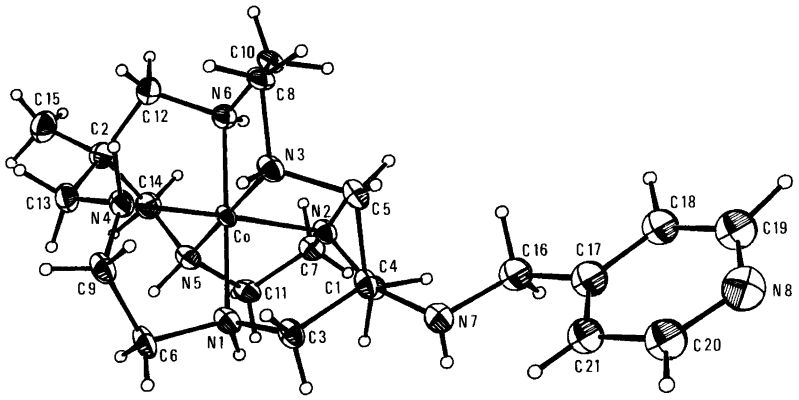


Fig. 2. ORTEP drawing of the Co(III) cage complex.

recovery of unreacted cage complex is also possible. In the reaction with $[Co(H_3CsarNHCH_2CH_2NH_3)]^{4+}$ no evidence of alkylation at the secondary amine was seen. This may be due to a combination of factors, such as steric hindrance and the generally lower reactivity of a secondary amine combined with very low basicity ($pK_a<2$, *vide infra*) in this case (*vide infra*). Such reduced reactivity was also clearly seen in attempts to alkylate $[Co(H_3CsarNH_3)]^{4+}$ (pK_a = 3.26)[22] in aqueous solution (under the same conditions as above), where no reaction took place. However, alkylation was achieved in anhydrous acetonitrile with molecular sieves and using BH_3CN^- as the reductant. In this respect the $[Co(H_3CsarNH_3)]^{4+}$ system is inadequate as a model for a protein Lys residue.

Structure. The crystal structure determination established the identity of the $[Co(H_3CsarNHCH_2py)]$ $(O_3SCF_3)_3 \cdot H_2O$ complex. The Co(sar) structural element is very similar to that of other Co(III)(sar) complexes,[10,23-27] with no unusual interatomic distances (Co–N distances average to 1.96 Å) or angles (Tables 3 and 4). However, the conformational features of this segment warrant comment. The cage may be viewed as being derived from the tris(1,2-ethanediamine) element capped at either trigonal face. As for the other cage ligand complexes (sarcophagine[10] and sepulchrate[28]), this gives rise to different conformational isomers owing to the orientations of the $-C(CH_2-)_3$ caps and of the C–C vectors of the five-membered '1,2-ethanediamine' (en) rings. In the present case the complex adopts, at least in the solid state, the D_3ob_3 conformation with all the en rings oblique (*ob*) to the pseudo-D_3 (cap–cap) axis. Among the structures of Co(III)(sar)derivatives (seven) published so far, the lel_3 conformation (en ring parallel to the cap–cap axis) dominates,[10,24-27] with only two reported cases of the ob_3.[10,23] Molecular mechanics calculations[10,29] for the sar complexes have indicated that the energy difference between the different conformational isomers is quite small,

Table 5. Hydrogen bonding distances (in Å).[a]

N1	(H25) ··· O8	(1)	2.990(4)
N2	(H26) ··· O6	(2)	3.000(4)
N3	(H27) ··· O4	(1)	2.913(5)
N4	(H10) ··· O1	(1)	2.879(4)
N5	(H11) ··· O10	(3)	2.845(4)
N6	(H12) ··· O3	(2)	2.897(5)
O10	(H41) ··· N8	(1)	2.785(6)
O10	(H42) ··· O9	(4)	2.741(5)

[a]Symmetry operations: (1) *x*, *y*, *z*; (2) *x*−1, *y*, *z*; (3) *x*, *y*, *z*−1; (4) 1−*x*, 2−*y*, 1−*z*.

but there is disagreement with respect to the relative stabilities. Several effects, such as hydrogen bonding, crystal packing and nature of substituents, may influene the solid-state structure. It has been argued that strong hydrogen bonding involving the coordinated secondary amine H atoms should favour lel_3[26,30] and that bulky substituents on the apical amine group should favour ob_3.[30] Here, extensive hydrogen bonding (Table 5) is seen between O atoms of the triflate ions and coordinated amine H atoms. Even so, it would seem that, provided the theory holds, the effect of the apical aminopyridylmethylgroup must dominate the issue in this case.

Spectral properties. Spectral data are given in Table 6. The marked difference in the position of the d–d bands in the reflection spectra of $[Co(H_3CsarNH_2CH_2pyH)]Cl_5 \cdot \frac{1}{2}H_2O$ (469 and 343 nm) and $[Co(H_3CsarNHCH_2py)](O_3SCF_3)_3 \cdot H_2O$ (444 and 320 nm) seems to be a consequence of lel_3/ob_3 conformational differences,[31] the latter compound being found to adopt the ob_3 conformation in the crystals. In aqueous solution all the cage compounds gave virtually the same spectra, which are similar to the reflection spectrum of solid $[Co(H_3CsarNH_2pyH)]Cl_5 \cdot \frac{1}{2}H_2O$. It is therefore likely that the sar-cage elements of all these com-

Table 6. Spectral (0.1 M $HClO_4$) and acid dissociation [25.0 °C, μ = 1.00 M ($NaClO_4$)] data.

Compound	λ_{max}/nm, ε_{max}/M^{-1} cm^{-1}	pK_1	pK_2	pK_3
$[(H_3N)_5Co(NH_2CH_2CH_2NH_3)]Cl_4 \cdot \frac{1}{2}H_2O$	478, 52; 343, 42[a]	8.07[b]		
$[(H_3N)_5Co(NH_2CH_2CH_2NH_2CH_2pyH)]Cl_5$	480, 64; 341, 57	4.12	5.9	
$[Co(H_3CsarNH_2CH_2CH_2NH_3)]Cl_5 \cdot H_2O$	471, 140	≈1	9.72	
	474, 146;[e] 344, 124[e]			
$[Co(H_3CsarNH_2CH_2CH_2NH_2CH_2pyH)]Cl_6 \cdot 5H_2O$	471, 152	<2	4.4	7.9
	474, 157;[e] 343, 143[e]			
$[Co(H_3CsarNH_3)]Cl_4 \cdot \frac{1}{2}H_2O$	472, 137; 344, 111[c]	3.26[d]		
$[Co(H_3CsarNH_2CH_2pyH)]Cl_5 \cdot \frac{3}{2}H_2O$	471, 152	<2	5.77	
	474, 160;[e] 343, 134[e]			
	469;[f] 343[f]			
$[Co(H_3CsarNHCH_2py)](O_3SCF_3)_3 \cdot H_2O$	471, 152			
	474, 160;[e] 343, 134[e]			
	444;[f] 320[f]			

[a]Literature values (0.1 M HCl):[19] 481, 63; 342, 51. [b]Literature value (0.1 M NaCl):[20] 7.52. [c]Literature values (0.1 M HCl):[10] 472, 139; 345, 123. [d]Ref. 22. [e]In 6.0 M $HClO_4$. [f]Reflection data.

pounds are predominantly lel_3 in solution and that $[Co(H_3CsarNHCH_2py)](O_3SCF_3)_3 \cdot H_2O$ changes its conformation from ob_3 to lel_3 on dissolution.

Another interesting observation is the change in the position of the charge transfer (CT) bands upon acidification, revealing the otherwise obscured second d–d band as a distinct band in the spectrum. A similar, although weaker, effect was also seen with $[(H_3N)_5Co(NH_2CH_2CH_2NH_3)]^{4+}$ as already reported by Ogino,[20] who proposed an explanation involving intramolecular interaction between the pendant amine and one of the coordinated ammonia ligands. However, for the cage complexes of this study such interaction would seem to be obviated for steric reasons.

Acid dissociation properties. By comparing the pK_a values in Table 6 and literature values for 4-CH_3pyH^+ (pK_a = 6.11),[32] $CH_3NH_3^+$ (pK_a = 10.72)[32] and 4-$(CH_3NH_2CH_2)$-pyH^{2+} (pK_{a1} = 3.38, pK_{a2} = 8.48)[33] it is evident that the pK value of the pyridylmethylammonium derivative (secondary ammonium) is generally ca. 2 pK units lower than that of the parent primary ammonium ion. The effect on pK_a for the pyridinium ion depends on whether the pyridylmethylamine derivative is protonated on the amine or not. If the 4-(aminomethyl) substituent is more basic (protonated at higher pH) than the pyridine segment then the pK_a of the pyridinium ion is reduced by about 2 pK units relative to that for the 4-methylpyridinium ion. When the 4-(aminomethyl) substituent is more acidic (protonated at lower pH) than the pyridine segment, the pK_a value found is close to that of the 4-methylpyridinium ion. On this basis all of the acid dissociation constants may be associated with protonation/deprotonation, mainly at a specific amine. In this way the pK_a values associated mainly with deprotonation at the pyridinium moiety are 4.12 for $[(H_3N)_5Co(NH_2CH_2$-$CH_2NH_2CH_2pyH)]^{5+}$, 4.4 for $[Co(H_3CsarNHCH_2CH_2NH_2$-$CH_2pyH)]^{5+}$ and 5.77 for $[Co(H_3CsarNHCH_2pyH)]^{4+}$. For three of the cage compounds the apical ammonium groups have $pK_a < 2$, and qualitatively this agrees with the observed effect of pH on the positions of the charge-transfer bands in strongly acidic solution.

Conclusion

One purpose of this study was the investigation of some simple models designed to resemble the expected reactivity of a protein Lys residue in relation to developing a viable method for the introduction of the pyridylmethyl group at the pendant amine. The ultimate aim is to enable attachment of the Ru(II)-amine moiety for electron-transfer studies of redox-active metalloproteins as stated in the introduction. The pK_a of protonated Lys in proteins has been estimated[34] to be 10.4. Based on a comparison of pK_a values alone, the $[Co(H_3CsarNHCH_2CH_2NH_3)]^{4+}$ ion (pK_a 9.72) represents a much better model than the $[Co(H_3CsarNH_3)]^{4+}$ ion (pK_a = 3.26). The former complex readily succumbed to reductive alkylation with 4-formylpyridine in aqueous borate buffer, whereas the latter did not. The relatively mild reaction conditions employed should be compatible with many metalloproteins, and hence it would be expected that the same modification may also be carried out on such systems, with the Lys residues being the most likely targets. Preliminary experiments have provided spectral confirmation that the pyridyl segment is indeed incorporated when the procedure is applied to parsley plastocyanin,[35] which contains seven Lys residues. In order to perform intramolecular electron-transfer studies in a simple way it is necessary to be able to carry out the modification selectively at a single site in each protein molecule or, failing this, to separate the isomers formed.

The pyridylmethyl-modified Co(III) cage complexes may also be coordinated to the $(H_3N)_5Ru(II)$ unit to give heterobinuclear systems, and this has been achieved. Unlike other Co–Ru binuclear complexes reported so far,[5,36] these systems should also be stable for the Co(II) state, owing to the efficient trapping inside the sar-cage structure. As far as the ability to exist in two inert oxidation states is concerned, the Co(II)/(III) cage systems appear to be as efficient as the Ru(II)/(III) system without a cage ligand. This property was explored by Conrad and Scott, who recently attached the $[Co(H_2NsarNH_2)]^{3+}$ complex successfully to various pendant carboxylates of cytochrome-c by the carbodiimide coupling procedure.[6] Viewed on this background some of the cages holding terminal amines dealt with here may also find use in a similar fashion. Relating the present results to the carbodiimide coupling procedure the $[Co(H_3CsarNHCH_2CH_2NH_2)]^{3+}$ complex would be expected to be even more reactive than both the $[Co(H_2NsarNH_2)]^{3+}$ and $[Co(H_3CsarNH_2)]^{3+}$ systems, based on pK_a. Alternatively, cages carrying pendant carboxylate groups could also be candidates for attachment at the Lys amine through the carbodiimide procedure.

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