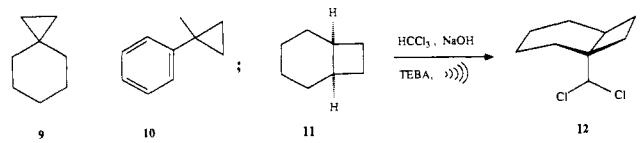
Scheme II



9 10 11 12

in the major isomer **2a** is located at the endo position. No reaction with **1** took place if dichlorocarbene was generated by the Doering-Hoffmann method[12] ($HCCl_3$, $KOBu^t$). Under normal phase-transfer-catalysis conditions[4d] ($HCCl_3$, 50% NaOH, 5% TEBA, 9 h), a 15% yield of **2a** and **2b** was obtained (ratio = ca. 4:1). When bromoform in dichloromethane was used,[9b] the C-H insertion products **3a** and **3b** were formed in 27% yield. Thus, dichloro- and dibromocarbene insert *selectively* into the C-H bonds adjacent to the three-membered ring of **1** (Scheme I).[13]

Similarly, dichlorocarbene insertion into the C-H bonds at C2 or C4 of bicyclo[3.1.0]hexane (**4**) afforded **5a** and **5b** in 40% yield. The exo product **5b**[11] is favored in this reaction (endo:exo = 1:3), rather than the endo product found from **1** (endo:exo = 4:1)! In the corresponding dibromocarbene insertion reaction, **6a** and **6b** were formed in a ratio of 1:4.

The insertion reactions of dichlorocarbene with **7a**[14] and **7b** (ratio = 7:1) yielded 57% of **8a** and **8b**. The syn isomer **7b** was found to be more reactive than the anti isomer **7a** by gas chromatographic monitoring of the reaction progress. Like the formation of the endo isomer **2a** in the reaction of **1**, the insertion reaction with **7b** leads to the formation of endo **8b**[11] as the major isomer. In addition to **8a** and **8b**, as indicated by ^1H NMR, ^{13}C NMR, and GC-MS, five bis-insertion products were detected. These products obviously derive from two dichlorocarbene insertions into *one* secondary C-H bond at C2 and *one* at C5 each. The "double activation" caused by the presence of two cyclopropane rings might be responsible for the formation of the bis-insertion products.

It is interesting to note that only ca. 2% of insertion products was detected in the reaction of dichlorocarbene with spiro[2.5]-octane (**9**), while 1-methyl-1-phenylcyclopropane (**10**) did not react at all under ultrasonication. One possible explanation for the observed endo/exo selectivities in the reactions of **1**, **4**, and **7b** is that only those C-H bonds whose orbitals can effectively interact with the Walsh orbitals of three-membered rings can be inserted by the dihalocarbene. Inspection of molecular models[15] reveals that in **1** the "conjugations" of the σ orbitals of the endo C-H bonds at the α positions with the Walsh orbitals seem to be better than those with the exo C-H bonds. In **4**, the σ orbitals of the exo and endo C-H bonds seem to interact with the Walsh orbitals to roughly the same extent. However, due to steric interactions with the endo hydrogens at both C3 and C6, preferential attack of dihalocarbenes should take place from the exo side (see Figure 1). Thus, the reactions of compounds **1** and **4** differently favor ratios of endo to exo isomers. In contrast, the four C-H bonds adjacent to the cyclopropane ring in spiro compound **9** cannot take on geometries favorable for sufficient "conjugations" with the Walsh orbitals. Consequently, dichlorocarbene insertion products are formed only in very low yield (ca. 2%). Thus, the reactivity of **9** is comparable with that of cyclohexane under ultrasonication, which affords a dichlorocarbene C-H bond insertion product in trace amounts (Scheme II).

In stark contrast, in the reaction of dichlorocarbene with *cis*-bicyclo[4.2.0]octane (**11**),[16] containing a cyclo*butane* ring, the C-H insertion reaction took place at the *tertiary* C-H bonds at the bridgehead position to afford **12** in a yield of 90%. No products resulting from insertion into C-H bonds adjacent to the cyclobutane ring were found. This suggests that the interactions between the Walsh orbitals of cyclopropane rings and the orbitals of suitably positioned adjacent C-H bonds are quite different from those of cyclobutane rings. While some cyclopropane rings "activate" *adjacent* C-H bonds, the cyclobutane ring in **11** does not.

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Supplementary Material Available: Details of experimental procedures and spectral data of the products, including NOE spectra of **2a** and **5b** (23 pages). Ordering information is given on any current masthead page.

(11) For **2a**, **5b**, and **8b** the stereochemistry of the $CHCl_2$ group was ascertained by 1D NOE difference spectroscopy. The syn and anti cyclopropane methylenes were assigned on the basis of spin simulation treating the cyclopropane protons as a four-spin system.

(12) Doering, W. v. E.; Hoffmann, A. K. *J. Am. Chem. Soc.* **1954**, *76*, 6162-5.

(13) These results differ totally from the thermal decomposition of $EtOCON_3$ in the presence of **1**. The five urethanes found were thought to be derived from insertions of (ethoxycarbonyl)nitrene into the C-H bonds at the α, β, and bridgehead positions of the cyclopropane ring of **1** (ratio = 1.3:1:0.2, endo and exo isomer mixtures). See: Tadella, P. A.; Di Stazio, G.; Pellacani, L. *Gazz. Chim. Ital.* **1971**, *101*, 730-1.

(14) Simmons, H. E.; Blanchard, E. P.; Smith, R. D. *J. Am. Chem. Soc.* **1964**, *86*, 1347-56.

(15) 4-31G optimized geometries of **1** and **4** were taken from the following: Wiberg, K. B.; Bonneville, G.; Dempsey, R. *Isr. J. Chem.* **1983**, *23*, 85-92.

(16) Brinker, U. H.; König, L. *Chem. Lett.* **1984**, 45-8.

Vibrational Raman Optical Activity of Proteins

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Although vibrational Raman optical activity (ROA) was first observed nearly 20 years ago,[1,2] its widespread application has been hampered by a lack of sensitivity with studies restricted to favorable samples.[3-5] We recently reported a major breakthrough in ROA instrumentation based on backscattering with CCD detection[6] which we have now developed sufficiently (by graduating to a *backthinned* CCD and employing an $f/4.1$ single-stage spectrograph fitted with an ion-etched holographic grating and a holographic edge filter) to render most biological molecules in aqueous solution, in particular proteins, accessible to ROA studies.[7] Preliminary results indicate that ROA provides a valuable new perspective on protein conformation complementing that obtained from its sibling technique, vibrational circular dichroism (VCD).[8]

Peptide and protein ROA spectra covering ~1100–1500 cm^{-1} obtained at an early stage of instrument development were presented and discussed previously.[9,10] The new instrument extends the range to ~600–1750 cm^{-1} and provides ROA spectra with much higher signal-to-noise ratios. Some typical protein ROA spectra are collected in Figure 1, with the tripeptide L-

(1) Barron, L. D.; Bogaard, M. P.; Buckingham, A. D. *J. Am. Chem. Soc.* **1973**, *95*, 603-605.

(2) Hug, W.; Kint, S.; Bailey, G. F.; Scherer, J. R. *J. Am. Chem. Soc.* **1975**, *97*, 5589-5590.

(3) Barron, L. D. In *Vibrational Spectra and Structure*; Bist, H. D., Durig, J. R., Sullivan, J. F., Eds.; Elsevier: Amsterdam, 1989; Vol. 17B, pp 343-368.

(4) Nafie, L. A.; Zimba, C. G. In *Biological Applications of Raman Spectroscopy*; Spiro, T. G., Ed.; Wiley: New York, 1987; Vol. 1, pp 307-343.

(5) Diem, M. In *Vibrational Spectra and Structure*; Durig, J. R., Ed.; Elsevier: Amsterdam, 1991; Vol. 19, pp 1-54.

(6) Barron, L. D.; Hecht, L.; Hug, W.; MacIntosh, M. J. *J. Am. Chem. Soc.* **1989**, *111*, 8731-8732.

(7) Hecht, L.; Barron, L. D.; Gargaro, A. R.; Wen, Z. Q.; Hug, W., to be published.

(8) Pancoska, P.; Yasui, S. C.; Keiderling, T. A. *Biochemistry* **1989**, *28*, 5917-5923.

(9) Barron, L. D.; Gargaro, A. R.; Wen, Z. Q. *J. Chem. Soc., Chem. Commun.* **1990**, 1034-1036.

(10) Barron, L. D.; Gargaro, A. R.; Hecht, L.; Wen, Z. Q.; Hug, W. In *Laser Applications in Life Sciences*; Akhmanov, S. A., Poroshina, M. Y., Koroteev, N. I., Toleutaev, B. N., Eds.; SPIE: Bellingham, WA, 1991; Proc. SPIE 1403, pp 66-75.

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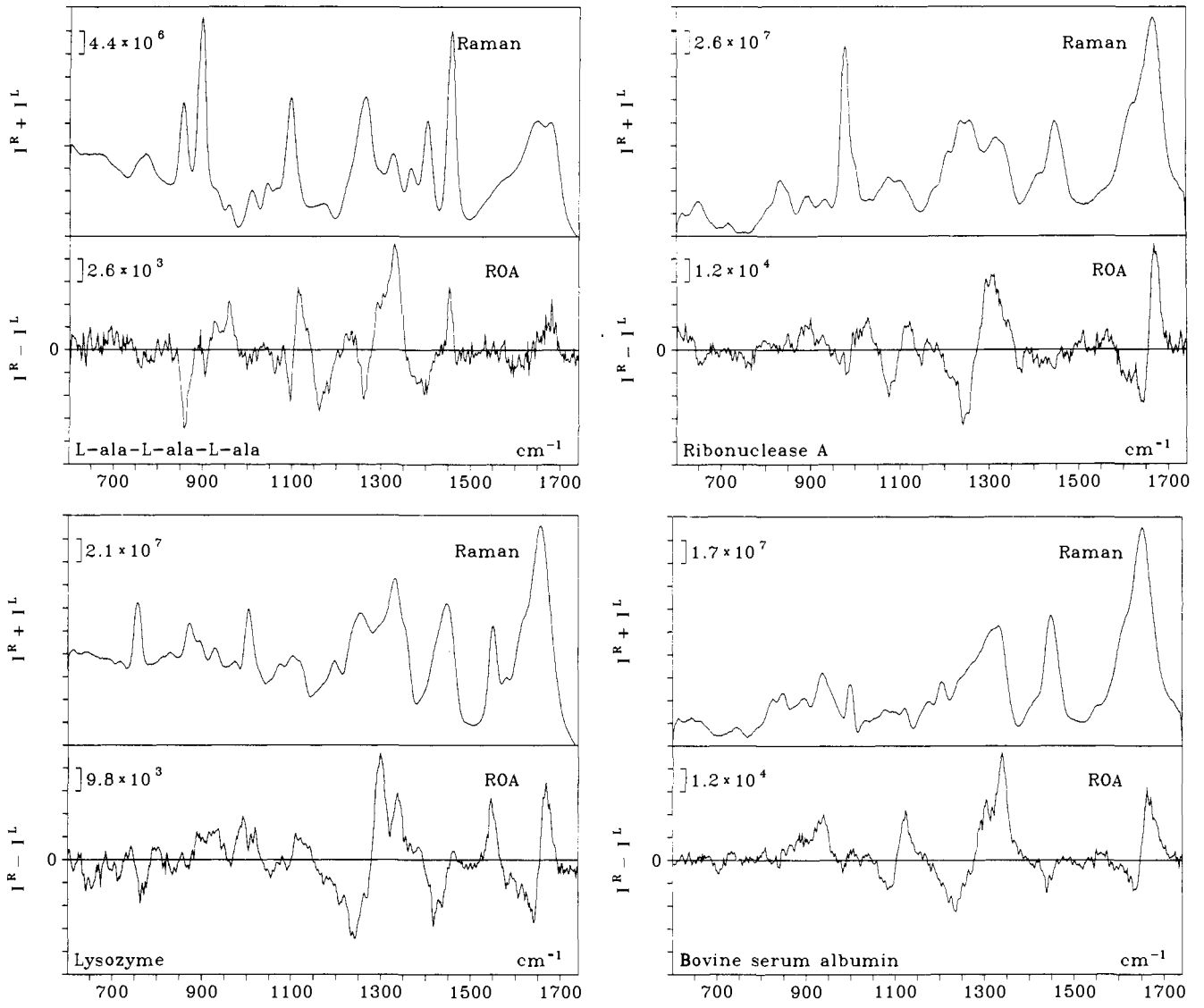

Figure 1. The backscattered Raman and ROA spectra of L-alanyl-L-alanyl-L-alanine, ribonuclease A, lysozyme (hen egg white), and bovine serum albumin in subsaturated aqueous solution (~150 mg/mL, filtered and centrifuged). The backgrounds, which are of similar intensity to the Raman spectra at these concentrations, have been subtracted from the Raman spectra. Experimental conditions: laser wavelength, 514.5 nm; laser power, 600 mW; spectral band width, ~10 cm^{-1}; recording time for the tripeptide, 3 h, and for the proteins, ~10 h. All samples were purchased from Sigma and used without further purification (apart from prolonged exposure to the laser beam to reduce fluorescence).

alanyl-L-alanyl-L-alanine included for reference.

Vibrations of the peptide backbone are usually associated with four main regions of the Raman spectrum:[11,12] the amide I region at ~1645–1680 cm^{-1} arising predominantly from C=O stretching; the amide III region at ~1230–1310 cm^{-1} involving N—H and C_α—H deformations; the C_α—N stretch region at ~1020–1150 cm^{-1}; and the C_α—C stretch region at ~870–950 cm^{-1}. Raman bands in the first two regions have been associated with α-helix, β-sheet, β-turn, and random coil structures; in the ~1020–1060-cm^{-1} part of the third region with β-sheet; and in the ~890–945-cm^{-1} part of the fourth region with α-helix.[11,12] ROA appears in all four regions, with the overall appearance for the three proteins being similar. There are also similarities with the tripeptide ROA spectrum, which itself is very much like that of the corresponding L-alanyl-L-alanine dipeptide.[7] This implies that relatively local interactions dominate peptide and protein ROA: in particular, the close similarity between the couplet observed in all three proteins and in the tripeptide (and also the dipeptide) in the amide I region suggests that, unlike VCD, a coupled oscillator mechanism is not significant here. There are some interesting differences in the other regions which might be diagnostic of the different proportions of α-helix, β-sheet, β-turn, and random coil content. For example, bovine serum albumin, which has zero β-sheet but high α-helix content,[13] has large positive ROA intensity in the α-helix part of the C_α–C stretch region but virtually none in the β-sheet part of the C_α–N stretch region, whereas ribonuclease A and lysozyme, which contain both α-helix and β-sheet,[13] show significant ROA features in both these parts of the two regions. Perhaps the most striking feature is the large positive ROA band in bovine serum albumin at ~1340 cm^{-1}: this region has been identified as one where "amide III" modes characteristic of reverse turns might be observed,[14] and bovine serum albumin does indeed contain many turns on account of the large number (17) of disulfide bridges[15] (on the other hand, CH_2 twisting vibrations in the aliphatic side chains have also been assigned to this region[16]).

(11) Chen, M. C.; Lord, R. C. *Biochemistry* **1976**, *15*, 1889–1897.
(12) Tu, A. T. In *Spectroscopy of Biological Systems, Advances in Spectroscopy*; Clark, R. J. H., Hester, R. E., Eds.; Wiley: Chichester, 1986; Vol. 13, pp 47–112.
(13) Carey, P. R. *Biochemical Applications of Raman and Resonance Raman Spectroscopies*; Academic Press: New York, 1982.
(14) Krimm, S. In *Biological Applications of Raman Spectroscopy*; Spiro, T. G., Ed.; Wiley: New York, 1987; Vol. 1, pp 1–45.
(15) Peters, T., Jr. In *Advances in Protein Chemistry*; Anfinsen, C. B., Edsall, J. T., Richards, F. M., Eds.; Academic Press: 1985; Vol. 37, pp 161–245.

Side-chain features also appear. It is striking how lysozyme shows a number of sharp-structured ROA features associated with tryptophan bands at ~1582, 1552, 1340, and 757 cm^{-1} (however, the positive ROA band at ~1340 cm^{-1} in lysozyme coincides in frequency with the strong positive ROA band in bovine serum albumin, which contains only one tryptophan residue, so it might not necessarily originate exclusively in tryptophan vibrations). There is also large negative ROA intensity associated with the side-chain CH_2 deformation band at ~1450 cm^{-1} in lysozyme and bovine serum albumin, but little in ribonuclease A.

Unfortunately we have not obtained ROA data on the important disulfide link in any of the proteins studied so far. The ROA is generally weak in the C-S stretch region[13] ~600-750 cm^{-1}, and measurements in the S-S stretch region[13] ~500-550 cm^{-1} are unreliable due to stray light. However, any ROA in these vibrations is likely to have a large isotropic component: isotropic ROA vanishes in backscattering, so measurements in forward scattering[17] might be necessary to detect disulfide ROA signals.

We have shown that protein ROA spectra can now be measured routinely and that they appear to contain new information about secondary backbone and side-group conformation. However, caution must be exercised in interpreting the observed features at this early stage, and we defer a detailed analysis until the ROA spectra of appropriate model peptides together with many more proteins have been acquired. Even at the rather coarse resolution used here to increase the intensity and hence reduce the acquisition time, much reproducible fine structure can be discerned (such as the steps on either side of the sharp negative ROA band at ~1240 cm^{-1} in lysozyme) which could contain subtle new information about conformations of the various structural elements. Higher resolution requires even better sensitivity, which would follow both from further instrument development and from ultrapurification of samples to reduce the background.

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(16) Chen, M. C.; Lord, R. C. *J. Am. Chem. Soc.* **1976**, *98*, 990-992.
(17) Barron, L. D.; Hecht, L.; Gargaro, A.; Hug, W. *J. Raman Spectrosc.* **1990**, *21*, 375-379.

Synthesis and Characterization of the First Main Group Oxo-Centered Trinuclear Carboxylate

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We report the synthesis and structural characterization of the first main group oxo-centered, trinuclear carboxylato-bridged species, namely, $[Ga_3(\mu_3\text{-}O)(\mu\text{-}O_2CC_6H_5)_6(4\text{-}Mepy)_3]GaCl_4\cdot 4\text{-}Mepy$ (**1**); 4-Mepy is 4-methylpyridine. Compound **1** is a main group example of a well-established class of complexes, referred to as "basic carboxylates" of the general formula $[M_3(\mu_3\text{-}O)(\mu\text{-}O_2CR)_6L_3]^+$, previously observed only for transition metals.[1,2]

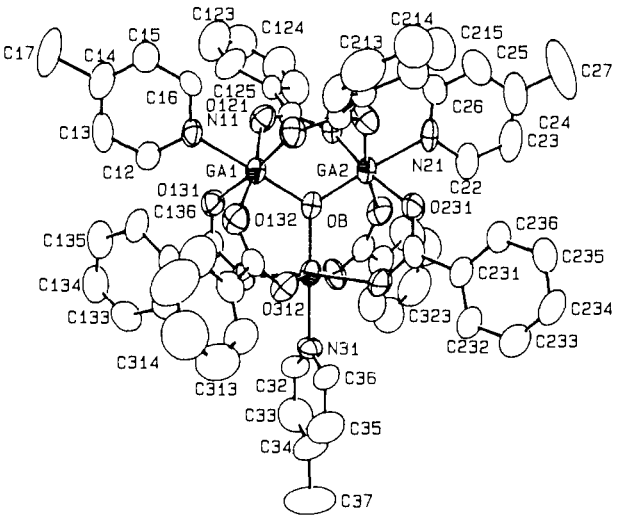

Figure 1. ORTEP drawing of the complex cation $[Ga_3(\mu_3\text{-}O)(\mu\text{-}O_2CC_6H_5)_6(4\text{-}Mepy)_3]^+$, showing 50% thermal ellipsoids and the atomic-labeling scheme. Pertinent average bond distances (Å) and angles (deg) are as follows: Ga-O(B), 1.874 (8); Ga-O(benzoates), 1.985 (6); Ga-N, 2.08 (0); Ga···Ga, 3.246 (9); C-C, 1.37 (2) Å; C-O, 1.25 (2); Ga-Ga-Ga, 60.0 (3); Ga-Ga-O(B), 30.0 (3); Ga-O(B)-Ga, 120.0 (9); O-C-O, 126 (1); O(B)-Ga-N, 177.6 (9); O-C-C, 117 (2).

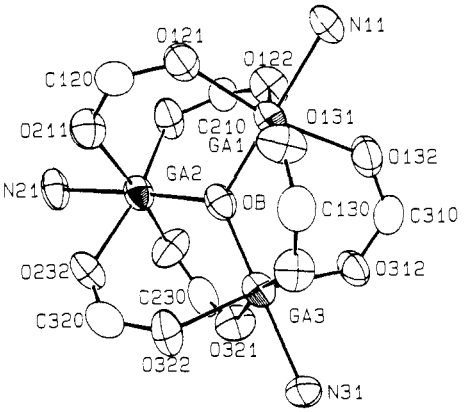

Figure 2. $Ga_3(\mu_3\text{-}O)(\mu\text{-}O_2C)_6N_3$ core of **1** showing the coordination sphere around the gallium atoms.

Compound **1** was prepared in the following manner. Under argon, a solution of Ga_2Cl_4 (1.25 g, 4.44 mmol) and $C_6H_5CO_2Na$ (1.28 g, 8.88 mmol) in 35 mL of 4-methylpyridine was stirred for 3 days at 25 °C. The mixture was filtered; the resulting light gray residue was washed with hexanes and recrystallized from 4-methylpyridine/hexanes to produce white microcrystalline **1** in 80% yield.[3] Colorless, prismatic crystallographic-quality crystals were obtained by diffusion of hexanes into a 4-methylpyridine solution of $[Ga_3(\mu_3\text{-}O)(\mu\text{-}O_2CC_6H_5)_6(4\text{-}Mepy)_3]GaCl_4\cdot 4\text{-}Mepy$ over a period of 1 week.[4]

[†] This work was done while the author held a National Research Council-NASA Research Associateship.
[‡] NASA Lewis Research Center.
[§] Cleveland State University.
[⊥] Purdue University.

(1) (a) Cotton, F. A.; Wilkinson, G. *Advanced Inorganic Chemistry*, 5th ed.; Wiley: New York, 1988; pp 466-467. (b) Cannon, R. D.; White, R. P. *Prog. Inorg. Chem.* **1988**, *36*, 195-298. (c) Mehrotra, R. C.; Bohra, R. *Metal Carboxylates*; Academic Press: London, 1983.
(2) (a) Cotton, F. A.; Shang, M.; Sun, Z. S. *J. Am. Chem. Soc.* **1991**, *113*, 6917-6922. (b) Poganiuch, P.; Liu, S.; Papaefthymiou, G. C.; Lippard, S. J. *J. Am. Chem. Soc.* **1991**, *113*, 4645-4651. (c) Jang, H. G.; Geib, S. J.; Kaneko, Y.; Nakano, M.; Sorai, M.; Rheingold, A. L.; Montez, B.; Hendrickson, D. N. *J. Am. Chem. Soc.* **1989**, *111*, 173-180.
(3) Analytical results calculated (found) for $C_{60}H_{51}Cl_4Ga_4N_3O_{13}$: C, 49.95 (49.58); H, 3.56 (3.78); Cl, 9.83 (9.6).
(4) A single crystal (0.5 × 0.4 × 0.38 mm) of **1** was sealed in a glass capillary for data collection. Diffraction data were collected at 20 °C on an Enraf-Nonius CAD-4 diffractometer with graphite monochromatized Mo Kα radiation (λ = 0.710 73 Å). A total of 5137 independent reflections with 2θ = 4-45° were collected. Crystal data: monoclinic, space group $P2_1$; a = 14.398 (2) Å, b = 17.638 (3) Å, c = 16.215 (2) Å, β = 113.15 (1)°; V = 3786 (2) Å^3; Z = 2; D_c = 1.347 g/cm^3; μ(Mo Kα = 16.01 cm^{-1}); R = 0.057 (R_w = 0.067) for 3776 reflections with $I > 3\sigma(I_o)$; GOF = 1.15.

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