Synthesis of Dihydroxyacetone Phosphate(and Isosteric Analogues) by Enzymatic Oxidation; Sugars from Glycerol

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2: Procedure as for the synthesis of **1**; $[(Ph_3P)_2)Pd(N_3)_2]$ is used in place of $[(dppe-Pd(N_3)_2]$ to furnish deep red crystals stable in air and in about 10% yield. FAB-MS: *m/z* (%): 4673.8 (15) $[M^+ - Cl]$, 4215.5 (40) $[M^+ - Cl - PPh_3 - Au]$, 4177.5 (100) $[M^+ - 2Cl - PPh_3 - Au]$, 3916.8 (80) $[M^+ - 2Cl - 2PPh_3 - Au]$, 3721.7 (50) $[M^+ - 2Cl - 2PPh_3 - 2Au]$, 3685.3 (35) $[M^+ - 3Cl - 2PPh_3 - 2Au]$, 3421.2 (20) $[M^+ - 3Cl - 3PPh_3 - 2Au]$.

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[4] Crystal data for **1**: *C2/c*, $a = 2953.8(8)$, $b = 1862.4(3)$, $c = 2921.8(6)$ Å, $\beta = 107.85(2)^\circ$, $Z = 4$, $R = 0.058$ for 6928 independent reflections with $I > 3\sigma(I)$ and 383 refined parameters (Enraf-Nonius CAD4 diffractometer, SDP-Plus, $Mo_{K\alpha}$ radiation, ω Scans, $3^\circ \leq \theta \leq 26^\circ$. Empirical absorption correction, solution of structure by direct methods (SHELXS 86 [10]), SDP [11]) refinement with anisotropic temperature parameters for the metal, Cl, and P atoms; H atoms were not included. The residual electron density in a final difference Fourier Synthesis could not be attributed to a solvate molecule. A test refinement with a Au atom in the center of the cluster resulted in a higher *R* value of 0.071 and the isotropic temperature factor increased from 2.15×10^4 to 6.72×10^4 pm^2. Further details of the crystal structure investigation may be obtained from the Fachinformationszentrum Karlsruhe, D-76344 Eggenstein-Leopoldshafen, on quoting the depository number CSD-57681.
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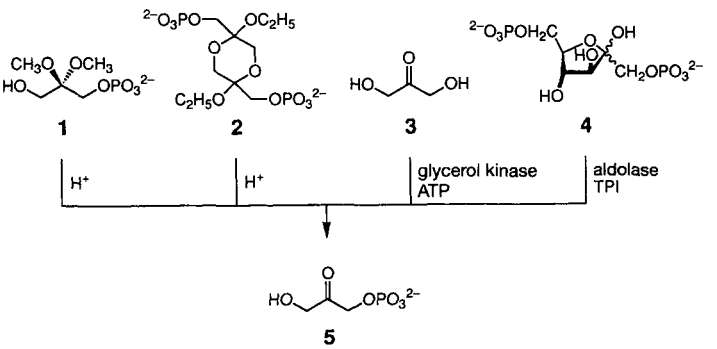
Synthesis of Dihydroxyacetone Phosphate (and Isosteric Analogues) by Enzymatic Oxidation; Sugars from Glycerol**

Wolf-Dieter Fessner* and Gudrun Sinerius

Enzyme-catalyzed aldol additions are currently of great interest because of their broad potential for application in asymmetric syntheses.[1] In particular, the dihydroxyacetone phosphate (DHAP) dependent aldolases, which are available as a complete stereochemical set for the generation of all four possible types of diastereomeric DHAP–aldehyde adducts (D-fructose 1,6-bisphosphate aldolase = FruA, [EC 4.1.2.13]; D-tagatose 1,6-bisphosphate aldolase = TagA, [EC 4.1.2.-]; L-fuculose 1-phosphate aldolase = FucA, [EC 4.1.2.17]; L-rhamnulose 1-phosphate aldolase = RhuA, [EC 4.1.2.19]),[2] are characterized by preparative efficiency, high chiral induction for two stereocenters, and a broad substrate tolerance for the aldol acceptor component. In addition, the FucA and RhuA enzymes have the capacity for an almost complete kinetic differentiation of racemic 2-hydroxyaldehydes.[3] Problematic is the fact that DHAP **5**, the essential donor component, is commercially too expensive for preparative syntheses[4] and relatively unstable in solution. The published procedures for its chemical synthesis (acid hydrolysis of the dimethyl acetal **1**, eight steps from 3-chloro-1,2-propanediol,[5] overall yield 17%; hydrolysis of the dimeric diethyl acetal **2**, three to five steps from dihydroxyacetone, phosphorylation by diphenyl chlorophosphate,[6a] phosphoroxy chloride,[6b] or *O,O'*-dibenzyl *N,N*-diethyl phosphoramidite,[6c] up to 50% overall yield) and enzymic synthesis (phosphorylation of dihydroxyacetone **3** by glycerol kinase/ATP, 83%;[7] cleavage of fructose 1,6-bisphosphate **4** by FruA/TPI (TPI = triosephosphate isomerase), ca. 100%[8]) have serious disadvantages with respect to expenditure (number of steps, cost of reagents) and purity of product. Here we describe a novel enzymic procedure that generates **5** practically quantitatively and in high chemical purity from inexpensive starting materials and its use in situ with different DHAP aldolases in a coupled reaction with aldehydes. This procedure also proved adaptable to the synthesis of DHAP analogues modified at the phosphate group, which are likewise accepted by the aldolases.



In the metabolism of several bacteria L-glycerol 3-phosphate (L-**6**) is oxidized to DHAP by a flavine-dependent glycerol phosphate oxidase (GPO, [EC 1.1.3.21]).[9] In contrast to the oxidation mediated by glycerol phosphate dehydrogenase [EC 1.1.1.8], a separate cofactor regeneration step[10] is not necessary because the reduced cofactor $FAD(H_2)$ remains tightly bound to the enzyme and is rapidly reoxidized by elemental oxygen with liberation of hydrogen peroxide. Since the latter can be used for the indirect quantification of glycerol in optical tests, GPO is a common and inexpensive enzyme. However, its obvious application to the preparative synthesis of **5** had to the best of our knowledge not been realized previously. We have found that the oxidation of L-**6** indeed proceeds smoothly and practically without competing side reactions if the buildup of a considerable stationary concentration of the coproduct H_2O_2, known to destruct enzymic activities, is suppressed by added catalase (cat, [EC 1.11.1.6]; Scheme 1). GPO exclusively oxidizes the L enantiomer,[11] which preparatively is best obtained by enzymic phosphorylation of glycerol.[7] This latter reaction, however, cannot be coupled with the oxidase reaction because of the low oxygen tolerance of glycerol kinase.

At higher concentrations **5** acts as a competitive GPO inhibitor. Out of the seven different commercial GPO preparations from at least three different organisms that we have inves-

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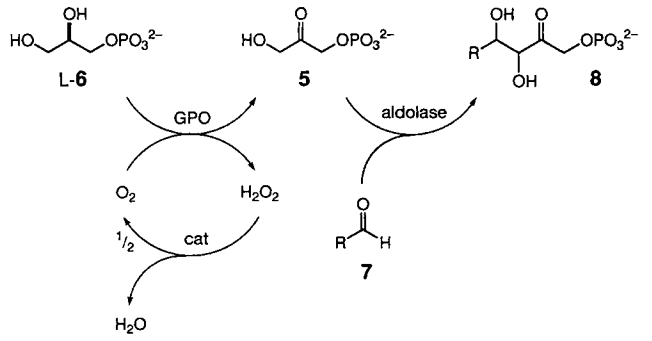
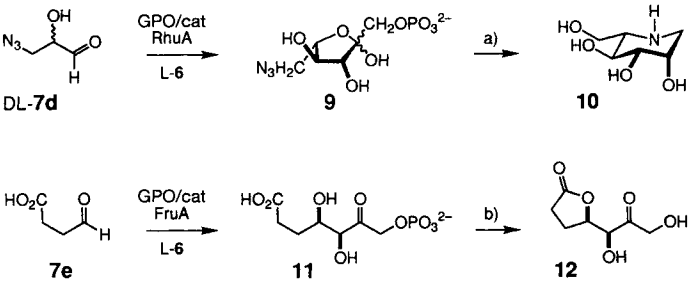


Scheme 1. Multienzymic synthesis of ketose-1-phosphates **8** from L-glycerol 3-phosphate (L-**6**).

tigated[12] the ones from *Streptococcus* species showed the lowest product inhibition (residual activity ca. 20% at 100 mM **5**). At this concentration preparative reactions can thus be run up to roughly 90–95% conversion to furnish **5** without further contaminants.[13] These particular enzyme preparations also displayed the highest stability in solution ($t_{1/2} \geq 3$ d at 25 °C; 2 U mL^{-1}),[14] which makes immobilization unnecessary, and only minimal sensitivity to higher concentrations of substrate (0.5 M), organic cations (e.g., cyclohexylammonium salts, 1.0 M), and inorganic phosphate (0.1 M; residual activity ≥ 70% in each case), as well as the highest tolerance against H_2O_2 ($t_{1/2} \approx 1.5$ h at 150 mM). On the preparative scale, a sufficient oxygen supply at room temperature could be easily provided by shaking or mechanical stirring without noticeable enzyme deactivation. Scheme 1 suggested further that oxygenation could be sustained by careful addition of H_2O_2, which was indeed verified experimentally.

Rather unexpectedly, the oxidative generation of **5** could be smoothly coupled to its consumption by enzyme-catalyzed addition to aldehydes **7** (Scheme 1). This not only prevents inhibition of the GPO by **5** and thus assures rapid and quantitative conversion of L-**6**, but also suppresses almost completely any decomposition of the labile **5** since it does not accumulate. Neither the (limited) occurrence of H_2O_2 nor the application of oxygen-saturated solutions—even at an elevated oxygen partial pressure of up to 5 bar—led to the feared deactivation of the several tested DHAP aldolases from rabbit muscle (FruA) and *E. coli* (FruA, FucA, RhuA). The coupled reactions were preferably run at slightly acidic pH to assure that **5** is sufficiently stable and to maintain a high conversion rate. Compared to the established procedures[6–8] the GPO method usually furnishes adducts **8** of higher purity and in equal or higher yields. An interesting observation is that when the cyclohexylammonium salt of **6** is used, some particular aldol adducts (e.g. L-**8e**) crystallize in high yield and purity already upon concentration of the crude product solution, which significantly simplifies the work-up.

In spite of the sensitivity of aldehydes for air oxidation, this anticipated competing reaction was negligible under the usual reaction conditions; we interpret this inter alia as resulting from hydrate formation in aqueous solution. Simple (acetaldehyde, **7a**) and hydroxylated aliphatic aldehydes (glycolaldehyde, **7b**; L-glyceraldehyde, **7c**) were transformed smoothly into the known ketose phosphates[2a] (Table 1). In a one-flask reaction starting from L-**6** and racemic 3-azido-2-hydroxypropanal **7d** only the L-configurated aldehyde was converted by the RhuA as expected, owing to efficient kinetic control.[3a] The resulting pure adduct **9** was transformed into the non-naturally configurated alkaloid 1-deoxy-L-mannojirimycin **10** by dephosphorylation and hydrogenolysis (overall yield 60%; Scheme 2).[15]



Scheme 2. Chemoenzymic syntheses of 1-deoxy-L-mannojirimycin (**10**) and chiral γ-lactones like **12**. a) Acid phosphatase [EC 3.1.3.2], H_2, Pt/C; b) H_3O^+.

Particularly advantageous is the GPO reaction in combination with FruA-catalyzed conversions of anionic aldol acceptors, because in this case the thermodynamic relationships are far more favorable than those involving **4**, which is most often applied as the starting material.[1c] Thus, succinic semialdehyde **7e** and L-**6** gave adduct **11** in good yield (81%; only 40% from **4**[16]). Acidic conditions effected a cyclization furnishing the optically pure γ-lactone **12** (Scheme 2; RhuA provides *ent*-**11**/**12**, see Table 1) which, because of its differentiated funtionalization, is of interest as a potential precursor to a range of biologically active compounds.

Table 1. Aldol reactions coupled with the GPO-catalyzed, in situ generation of DHAP and its analogues [a].

Aldehyde	Donor	Aldolase	Product	Yield [%]
–[b]	L-**6**	$FruA_{eco}$	D-fructose 1,6-P_2 (**4**)	95
acetaldehyde (**7a**)	L-**6**	RhuA	5-deoxy-L-*threo*-pentulose 1-P (**8a**)	70
glycolaldehyde (**7b**)	L-**6**	$FruA_{rab}$	D-*threo*-pentulose 1-P (**8b**)	84
glycolaldehyde (**7b**)	L-**6**	RhuA	L-*threo*-pentulose 1-P (**8c**)	96
glycolaldehyde (**7b**)	L-**6**	FucA	D-*erythro*-pentulose 1-P (**8d**)	93
L-glyceraldehyde (**7c**)	L-**6**	RhuA	L-fructose 1-P (**8e**)	85
L-glyceraldehyde (**7c**)	L-**6**	FucA	L-tagatose 1-P (**8f**)	86
3-azido-2-hydroxypropanal (**7d**)	L-**6**	RhuA	L-6-azido-6-deoxy-L-fructose 1-P (**9**)	70
succinic semialdehyde (**7e**)	L-**6**	$FruA_{rab}$	(4*R*,5*S*)-6-oxo-4,5,7-trihydroxyheptanoic acid 7-P (**11**)	81
succinic semialdehyde (**7e**)	L-**6**	RhuA	(4*S*,5*R*)-6-oxo-4,5,7-trihydroxyheptanoic acid 7-P (*ent*-**11**)	82
L-glyceraldehyde (**7c**)	DL-**13**	RhuA	2-deoxy-L-*arabino*-hept-3-ulose 1-phosphonate (**19**)	[c]
L-glyceraldehyde (**7c**)	DL-**15**	RhuA	1-deoxy-L-fructose 1-phosphoramidate (**20**)	[d]
L-glyceraldehyde (**7c**)	DL-**17**	RhuA	1-deoxy-L-fructose 1-phosphorothioate (**21**)	[c]

[a] Indices: eco = *Escherichia coli*, rab = rabbit muscle; P = orthophosphoric ester. Yields refer to isolated material purified by ion exchange and uniform according to ^{1}H NMR spectroscopy and thin-layer chromatography. [b] Formation of D-glyceraldehyde 3-phosphate by triosephosphate isomerase. [c] 1:1 Mixture with D-**13** or D-**17**, respectively. [d] Not determined.

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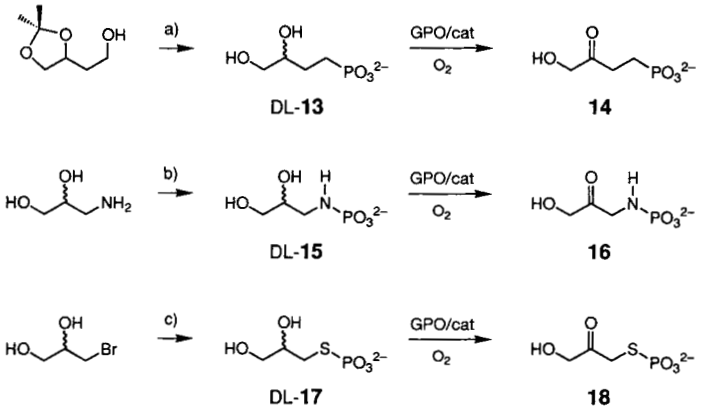
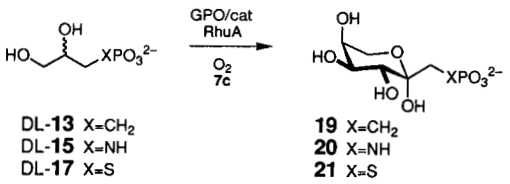
The positive results with the GPO method suggested a possible extension to the preparation of suitable DHAP analogues. Earlier work employing FruA from rabbit muscle had shown that modification of the free hydroxymethylene moiety in **5** generally leads to a complete loss of activity, whereas phosphonate **14**, which is derived from isosteric replacement of the phosphorylated subunit in **5**, is converted in enzymic assays at 10% of the rate of the reaction of **5**.[17] Ketone **14** had been accessible previously only by a multistep route of low efficiency,[18a,d] and its preparative use restricted to the assay scale.[18b] However, phosphonate **13**,[18c,e] isosteric to diol **6**, is readily obtained by the route illustrated in Scheme 3, as are the corresponding phosphoramidate and phosphorothioate analogues **15**[19] and **17**, respectively.



Scheme 3. Chemoenzymic syntheses of isosteric DHAP analogues. a) Ph_3PBr_2/dimethylformamide (DMF), $P(OEt)_3$, Me_3SiBr, H_2O; b) ref. [19]; c) aq. Na_3PO_3S (pH 8).

Indeed, upon treatment with GPO all three compounds were converted into the corresponding DHAP analogues which, for practical reasons (hydrolytic instability[19] of **15**–**18**), were directly converted in situ by coupled aldol additions into the sugar derivatives **19**–**21** (Table 1). As expected only up to 50% of the



racemic precursors **13** and **17** was converted. Thus, the adducts were characterized only as mixtures with remaining starting material (Table 2). The analysis of the reaction of **15**/**16** was complicated by competing rapid phosphoroamidate hydrolysis and potentially competing oxidative side reactions and subsequent reactions (plausibly via enamine tautomers[20]). For a more detailed investigation of the latter system and to facilitate product isolation the synthesis of enantiomerically pure L-analogues is highly desirable.

In the last two years the GPO procedure has completely replaced the competing methods for DHAP generation for almost all types of applications in our labs. In particular, the extension of the product spectrum through the use of DHAP analogues[21] is an advantage of this procedure.

Table 2. NMR data of selected compounds [a].

12: ^{1}H NMR (pH 1.0): δ = 2.40–2.52 (m, 3-H), 2.70 (m, 2-H), 4.51 (d, 5-H), 4.54 (d, 7-H_a), 4.65 (d, 7-H_b), 5.12 (dt, 4-H), $J_{3,4}$ = 6.0, $J_{4,5}$ = 2.3, $J_{7a,7b}$ = 19.5; ^{13}C NMR (pH 1.0): δ = 23.14 (C-3), 28.71 (C-2), 66.40 (C-7), 76.47 (C-4), 81.73 (C-5), 181.84 (C-1), 211.60 (C-6)

13: ^{1}H NMR: δ = 1.42–1.80 (m, 1-, 2-H), 3.52 (dd, 4-H_a), 3.62 (dd, 4-H_b), 3.70 (m, 3-H), $J_{2,4a}$ = 6.7, $J_{2,4b}$ = 3.75, $J_{4a,4b}$ = 12.0; ^{13}C NMR: δ = 24.90 (d, C-1), 27.55 (d, C-2), 65.49 (s, C-4), 72.99 (d, C-3); $J_{C\text{-}1,P}$ = 133.1, $J_{C\text{-}2,P}$ = 2.7, $J_{C\text{-}3,P}$ = 16.0; ^{31}P NMR: δ = 24.26

14: ^{1}H NMR: δ = 1.68 (m, 1-H), 2.67 (m, 2-H), 4.73 (s, 4-H); ^{13}C NMR: δ = 25.88 (d, C-1), 32.61 (d, C-2), 67.31 (s, C-4), 203.05 (d, C-3), $J_{C\text{-}1,P}$ = 131.6, $J_{C\text{-}2,P}$ = 4.1, $J_{C\text{-}3,P}$ = 13.2; ^{31}P NMR: δ = 24.58

17: ^{1}H NMR: δ = 2.80 (ddd, 1-H_a), 2.90 (ddd, 1-H_b), 3.61 (dd, 3-H_a), 3.69 (dd, 3-H_b), 3.90 (ddd, 2-H), $J_{1a,1b}$ = 13.5, $J_{1a,P}$ = 11.2, $J_{1b,P}$ = 12.0, $J_{1a,2}$ = 6.7, $J_{1b,2}$ = 4.8, $J_{2,3a}$ = 6.0, $J_{2,3b}$ = 4.5, $J_{3a,3b}$ = 11.7; ^{13}C NMR: δ = 32.80 (s, C-1), 64.38 (s, C-3), 72.56 (s, C-2); ^{31}P NMR: δ = 16.78

18: ^{1}H NMR [b]: δ = 3.04 (d, 1-H_H), 3.60 (s, 3-H_H), 3.64 (d, 1-H_K), 4.56 (s, 3-H_K), $J_{1,P(H)}$ = 13.5, $J_{1,P(K)}$ = 11.0, ketone : hydrate = 6 : 1; ^{13}C NMR: δ = 36.06 (s, C-1_K), 65.14 (s, C-3_K), 210.75 (s, C-2_K); ^{31}P NMR: δ = 15.55 (K)

19: ^{1}H NMR: δ = 1.70 (m, 2-H), 1.98 (m, 1-H); ^{13}C NMR: δ = 22.25 (d, C-1), 32.58 (s, C-2), 62.69 (s, C-3), 69.89, 70.46, 71.03 (3s, C-4, -5, -6), 109.36 (s, C-7), $J_{C\text{-}1,P}$ = 133.8; ^{31}P NMR: δ = 25.36

21: ^{1}H NMR: δ = 2.98 (dd, 1-H_a), 3.22 (dd, 1-H_b); ^{13}C NMR: δ = 36.95 (s, C-1), 63.81 (s, C-6), 69.30, 69.75, 70.34 (3s, C-3, -4, -5), 98.96 (s, C-2); ^{31}P NMR: δ = 16.77

[a] NMR spectra were recorded in D_2O at pH 7.0 after ion exchange giving the Na^+ salts. ^{1}H NMR data (400.1 MHz) are referenced relative to sodium 3-trimethylsilyl-[$^2H_4$]propionate (TSPNa) (δ = 0.00), ^{13}C NMR (100.6 MHz) to CH_3CN (δ = 1.30), and ^{31}P NMR (80 MHz) to D_3PO_4 (δ = 0.00); coupling constants in Hz. [b] Indices: H = hydrate, K = ketone.

Experimental Procedure

To a solution containing **6** (as its bis(cyclohexylammonium) salt; 370 mg, 1.0 mmol) and L-glyceraldehyde **7c** (110 mg, 1.2 mmol) in 10 mL of oxygen-saturated water at pH 6.8 was added 70 U of GPO, 1000 U of catalase, and 50 U of RhuA. The mixture was shaken at 20 °C under an oxygen atmosphere at 100 Upm. Conversion was monitored by enzymic assay for equivalents of DHAP produced, as well as by ^{1}H and ^{31}P NMR spectroscopy. After the solution was filtered through charcoal, the pH was adjusted to 7.5 by addition of 1.0 M cyclohexylamine in ethanol, and the solution was concentrated to dryness by rotary evaporation at $\leq$20 °C in vacuo. The solid residue was taken up in 0.5 mL of water, and the resulting solution was filtered. Anhydrous ethanol was added (2.5 mL) followed by anhydrous acetone until a faint turbidity remained. Recrystallization at 4 °C furnished the bis(cyclohexylammonium) salt of L-fructose 1-phosphate **8e** as colorless needles; chemical yield 370 mg (85%).

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[11] a) In analogy with biochemical literature we have retained the stereonotation for **6** which is defined by its relationship to L-glyceraldehyde 3-phosphate. b) As evident from enzymic assays, the GPO activity is only marginally affected by D-**6** and the isomeric glycerol 2-phosphate. This suggests that either the racemate or common commercial mixtures of 1(2)-monophosphates could also be utilized instead of enantiomerically pure L-**6** as inexpensive alternatives.
[12] Commerical glycerol phosphate oxidases from *Streptococcus, Pediococcus*, and *Aerococcus* as well as from an unspecified microorganism were employed as provided by Asahi, Boehringer Mannheim, Sigma, and Toyobo.
[13] **5** can be stored at −78 °C either as the product solution adjusted to pH 3–4 or as lyophilized salts, the composition of which can be predetermined by selection of the precursor salt of L-**6**.
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[21] The technique also seems to be applicable for the regiospecific introduction of isotopic labels from suitably labeled glycerol precursors.

Neutral Gold(I) Fluoride Does Indeed Exist**

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and Helmut Schwarz*

Dedicated to Professor Heinrich Nöth on the occasion of his 65th birthday

The chemistry of gold and its compounds still receives considerable attention.[1] This interest is not only caused by the potential catalytic activity of gold; it also reflects the fascinating coordination chemistry of gold(I) compounds.[2] Although several gold halides in various oxidation states are described in the literature,[1, 3] there is no unambiguous experimental evidence for the existence of the neutral AuF.[4] The only *indirect* characterization of gaseous AuF is based on the analysis of emission spectra of species generated in the etching of gold films in O_2/CF_4 and O_2/SF_6 rf glow-discharge plasmas.[5] Although the title of that contribution[5] implies that evidence for the existence of AuF had been found, in the discussion of the experimental results the authors leave no doubt that besides AuF the diatomic molecules AuO, AuO^+, and AuF^+ cannot be definitively ruled out as the emitting species, and the hope for additional investigations is explicitly expressed. Actually, it has been stated recently that "there is still no evidence for the existence of a definitively characterized gold(I) fluoride."[4b] Recent theoretical calculations, using a correlated relativistic approach, predict solitary AuF as a stable entity with a calculated bond dissociation energy of 59 kcal mol^{-1}.[6] Here for the first time, we will provide evidence for the existence of this long-sought molecule, and we also will report an estimate of the bond dissociation energy of AuF.

Neutralization–reionization mass spectrometry (NRMS) has been established as a versatile tool in the study of elusive molecules in the gas phase.[7] The inability to generate the corresponding bulk materials in the condensed phase often does not reflect an intrinsic instability of the neutral species; rather, the "non-existence" is due to facile bi- or termolecular reactions, such as isomerization, disproportionation, or solvolysis, which prevent experimental detection and characterization of the molecule in solution or in a matrix. Recently, NRMS has been successfully applied to the study of many unusual organometallic molecules, none of which is viable in the condensed phase.[7, 8]

Upon collisional activation[9] of AuF^+ with oxygen as a stationary gas, the formation of Au^+, corresponding to the loss of the fluorine ligand, is the only singly charged product observed (Fig. 1). Due to the high ionization energy of fluorine (IE(F) = 17.4 eV, IE(Au) = 9.2 eV)[16] as well as the reduced kinetic energy of the resulting fragment ion, the intensity of the signal corresponding to the fluorine cation was below the detection limit. In addition to atomic Au^+, dications are formed by charge stripping of AuF^+ in collision with oxygen. The observation that the doubly charged Au^{2+} gives rise to a signal about 25 times more intense than that of the AuF^{2+} dication indicates that the lifetime of AuF^{2+} is short; most likely upon charge stripping an electron is removed from the σ bond of AuF^+, thus making the incipient AuF^{2+} quite susceptible to fragmentation.[17] By means of high-resolution translational energy loss spectroscopy (HRTELS)[18] we ascertained that excited electronic states of AuF^+ do not contribute to the cations originating from electron impact ionization (EI) of AuF_3; this is evidenced by the absence of peaks at the high-energy side, which would result from superelastic collisions of excited AuF^+ in an HRTELS experiment (inset in Fig. 1).

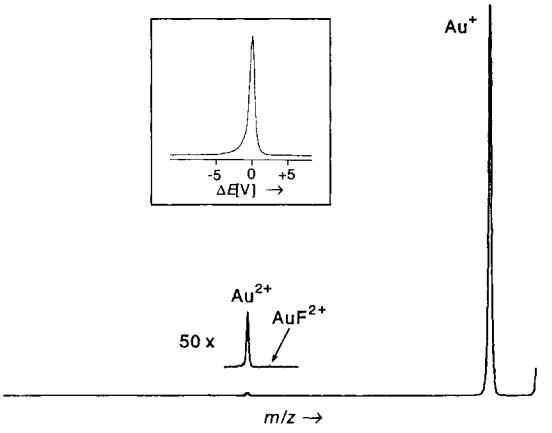

Fig. 1. Collisional activation spectrum of mass-selected AuF^+ with oxygen (70% *T*). The inset shows the HRTEL spectrum of AuF^+ with oxygen (50% *T*); see text and [9] for details.

If AuF^+ is subjected to a NRMS experiment, a distinct recovery signal is observed, which corresponds to the reionized neutral AuF molecule (Fig. 2). This provides for the first time un-

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