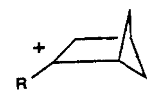
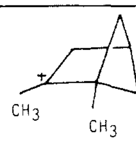
Table I. Comparison of Experimental and Calculated (IGLO)[a] ^{13}C NMR Chemical Shifts, ppm (vs TMS)

					
	classical			bridged	
ring positns	expt, R = CH_3[b] **6**	calcd, R = H[a] **2**	expt,[c] **7**	expt[b] **1**	calcd[a] **1**
1	81.6	80.6	*e*	*e*	158.5
2	322.0	334.2[d]	*e*	*e*	158.5
1, 2 av	201.8	207.4[d]	199.5	157.8	158.5
3	*e*	57.1	*e*	*e*	36.1
5	*e*	60.8	*e*	*e*	36.1
6	*e*	60.8	*e*	*e*	64.8
3, 5, 6 av	56.0	59.6	60.6	49.1	45.7
4	35.5	30.4	34.6	43.4	37.5

[a] IGLO method, DZ basis set on the MP2/6-31G* geometries (see ref 18). [b] Reference 7. [c] Reference 10. [d] These values would be lowered by methyl substitution, as in the experimentally examined systems. [e] Only average signals for these carbons are observed.

stable than **2** in nucleophilic media.[3]

Extensive calculational studies of carbocation potential energy surface have established the levels of theory needed for problems of this type.[5,13,14] Use of a CRAY XMP Computer and the GAUSSIAN 82 program[14] permitted MP2FULL/6-31G* (FULL = all electrons correlated) optimizations of **1** and **2**; 3-21G and 6-31G* optimizations were carried out as well. Frequency analysis at 6-31G* showed both **1** and **2** to be minima (no imaginary frequencies). MINDO/3 gives the same result, with a barrier of only 0.3 kcal/mol.[11] Whether or not this also is the case at the correlated levels (e.g., MP2) could not be established, but the potential energy surface between **1** and **2** evidently is quite flat.

The MP2FULL/6-31G* geometries (Figure 1) were then employed for MP3FC/6-31G* single point computations (by using the frozen core approximation, FC) on **1** (−232.92392 au) and **2** (−232.91673 au). As expected,[5,13,14] the relative stability of the bridged ion is underestimated at HF/3-21G and at HF/6-31G*, but the correlated MP2 and MP3 levels give quite similar results and favor **1** over **2** by 5.2 and 4.5 kcal/mol, respectively. After correction for the zero-point energy differences, the bridged form **1** is 4.0 kcal/mol more stable than the open isomer **2**. Kirmse's recent estimate of about 3 kcal/mol[3] is only slightly smaller, and Sorensen's results[10] also are in line. Theoretical estimates of the stabilization energy of the 2-norbornyl cation due to bridging (**3** vs **4**) are higher, in the 10–15 kcal/mol range, but are derived more arbitrarily.[5a] Stabilization energy estimates around 11 kcal/mol are obtained from gas-phase data.[16]

Experimental ^{13}C chemical shifts now provide a sensitive structure probe, since these values now can be calculated reliably for different structural models (e.g., **1** and **2**). The IGLO[17] results (DZ//MP2FULL/6-31G*) on **1** and **2** are compared with the experimental ^{13}C chemical shifts for the parent secondary **1** and two tertiary 2-methyl **6** and 1,2-dimethylbicyclo[2.1.1]hexyl **7** cations in Table I. As all three of the experimental systems are rapidly equilibrating at the temperatures investigated, only average signal positions of C(3), C(5), and C(6) are reported. C(1) and C(2) also either are identical (e.g., as in **1**) or averaged (e.g., as in **2**). IGLO clearly differentiates between these possibilities: For **1**, the experimental (157.8 ppm) and calculated (158.5 ppm) values are nearly identical but do not correspond to the IGLO chemical shift for **2** (207.4 ppm). However, the latter is in good agreement with the C(1)–C(2) average ^{13}C chemical shift found in **7**. For **6**, both C(1) and C(2) can be observed; note the close agreement between theory and experiment shown in Table I (also for C(4) and for the C(3), C(5), and C(6) averages).

Both on the basis of the calculated energies and the IGLO results, we conclude that the 2-bicyclo[2.1.1]hexyl cation prefers the symmetrically bridged **1** over the **2** structure. The energy difference, about 4 kcal/mol, appears to be lowered only slightly in solution (if at all), judging from Kirmse's 3 kcal/mol estimate[3] and that from the NMR line broadening experiments. This supports Kirmse's conclusion that "nucleophilic solvation does not appreciably effect the relative stabilities of bridged and open ions". The tertiary 2-methylbicyclo[2.1.1]hexyl cation **6** is classical and the 1,2-dimethyl analogue **7** equilibrates rapidly between two

7

equivalent classical tertiary structures. These results calibrate and demonstrate the reliability of similar evidence[5,16] indicating the 2-norbornyl cation to be a *symmetrically* bridged species with a *single sharp* energy minimum.

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Photoactivable Fluorophores. 3. Synthesis and Photoactivation of Fluorogenic Difunctionalized Fluoresceins[1,2]

Grant A. Krafft,* W. Randall Sutton, and
Richard T. Cummings

Department of Chemistry, Syracuse University
Syracuse, New York 13244-1200

Received August 17, 1987

The design and synthesis of new fluorescent probe molecules have been essential aspects in the continuing development and application of biophysical tracer methods.[3] In this paper we describe the synthesis and photoactivation of a new type of probe molecule, the photoactivable fluorophore (PAF), designed for tracer studies of molecular transport and diffusion in biological systems.

Photoactivable fluorophores are stable, nonfluorescent molecules with latent fluorescent characteristics that can be revealed by photoinduced processes.[4,5] Photoactivable fluorophores attached to biological macromolecules or other species of interest may be converted to ultimate fluorophores within a confined spatial region

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(1) Preliminary aspects of this work were presented at the 192nd American Chemical Society Meeting, Anaheim, CA, September 1986. Organic Abstract no. 264.

(2) Parts 1 and 2 in this series are in press in *Tetrahedron Lett.* (a) Cummings, R. T.; Krafft, G. A. *Tetrahedron Lett.* **1987**, *28*, 0000. (b) Cummings, R. T.; DiZio, J. P.; Krafft, G. A. *Tetrahedron Lett.* **1987**, *28*, 0000.

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(4) A number of photochemical reactions that generate fluorescent products have been reported. For an early review on the photochemical generation of fluorescence, see: Zweig, A. *Pure Appl. Chem.* **1973**, *33*, 389–410.

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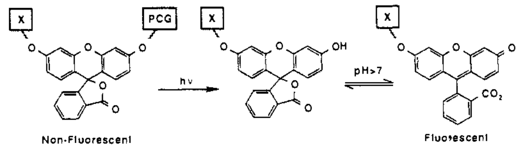
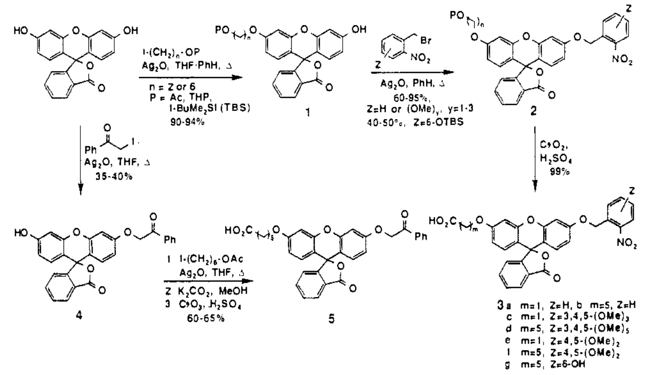


Figure 1. Fluorescein diether PAFs are nonfluorescent until irradiation removes the photocleavable group (PCG). The closed lactone isomer of the fluorescein monoether can tautomerize at nonacidic pH to the highly fluorescent xanthen-3-one tautomer. X is an auxiliary functional group that imparts particular solubility characteristics and/or covalent attachment capability.

Scheme I



by laser irradiation, permitting tracer differentiation of molecular species that are identical in all other respects. PAFs impart direct, real-time molecular surveillance capability in which the fluorescence is created only in a specified location and at a particular instant. Thus, PAFs offer a distinct advantage over conventional fluorophores which afford little opportunity for spatial or temporal control. These unique characteristics of PAFs have led to the development of a technique called fluorescence photoactivation and dissipation (FPD) by Ware and us,[6] which facilitates direct measurement of molecular transport and diffusion in solutions or in complex media.

The design of effective PAF molecules based on difunctionalized fluoresceins was motivated by the recognition that fluorescein can exist as either of two tautomeric forms: a fluorescent, xanthen-3-one form favored in nonacidic aqueous solution or a nonfluorescent, lactone form favored at acidic pH or in nonaqueous solvents. Constraint of fluorescein as its nonfluorescent lactone tautomer could be accomplished by dialkylation of the two phenolic oxygens, with one of the ether groups susceptible to a photocleavage reaction that would trigger lactone opening to the fluorescent xanthen-3-one tautomer, as illustrated in Figure 1. Further design considerations included the introduction of a reactive electrophilic group and a water soluble group to facilitate covalent attachment to biological macromolecules. These features could be incorporated as part of the second ether appendage or the lactone aryl group.

The synthesis of differentially substituted fluorescein diethers was accomplished by silver oxide mediated alkylation of one oxygen with a protected hydroxy-functionalized alkyl iodide in refluxing THF/benzene (1:3) in 90–94% yield, followed by a second silver oxide mediated alkylation with a substituted *o*-nitrobenzyl bromide electrophile in benzene to incorporate the photocleavable group, as outlined in Scheme I.[7,8] Cleavage of

Scheme II

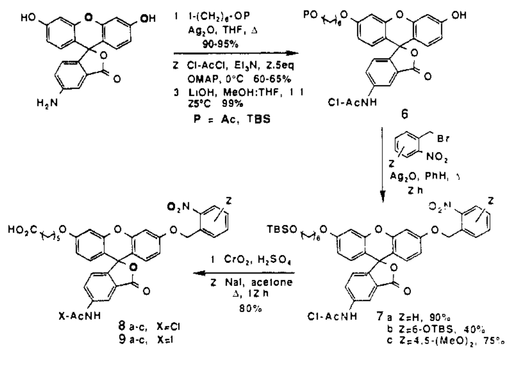


Table I. Photoactivation Quantum Yields

PAF	Φ^{10}	$\epsilon^{350\,\mathrm{nm}}$	rel fluor yield[10]
8a	0.26	293	121
8c	0.01	6050	84
3d	0.008	1100	15
5	0.07	47	6

the hydroxyl-protecting groups and Jones oxidation generated the carboxylate functionalized appendage, which imparted modest water solubility and permitted formation of amide linkages with amine residues of biological macromolecules, such as poly-L-lysine. The phenacyl ether derivative **5** was more difficult to prepare, requiring alkylation with phenacyl iodide and silver oxide in a minimum of THF as the first alkylation (35–40% yield) and reaction with 1-acetoxy-6-iodohexane and silver oxide as the second alkylation 65–70% yield).

Scheme II outlines the synthesis of trifunctionalized fluorescein derivatives containing a photocleavable *o*-nitrobenzyl ether, a polar alkyl carboxylate group, and an iodoacetamide group for attachment to protein amino or sulfhydryl groups. The first step in this synthesis is a silver oxide mediated alkylation of a phenolic ether of 5'-aminofluorescein with 1-(*tert*-butyldimethylsiloxy)-6-iodohexane or 1-acetoxy-6-iodohexane. Remarkably, this reaction does not result in competing nitrogen alkylation. Chloroacetylation of the 5'-amine and free phenolic oxygen and cleavage of the phenolic ester generates **6**, which is alkylated with substituted *o*-nitrobenzyl bromides and silver oxide to provide the chloroacetamides **7a–c**. The chloroacetamide group is inert to silver oxide under these conditions. Jones oxidation, followed by displacement of the chloride by iodide under standard Finkelstein conditions, generated the target PAFs **9a–c**, which were purified sequentially by flash chromatography, two recrystallizations, and finally by HPLC.[9] The 4,5-dimethoxy-2-nitrobenzyl substituted PAF (**9c**) was successfully attached to poly-L-lysine by alkylation in aqueous solution.

The quantum yields and relative fluorescence yields for photocleavage of several fluorescein diethers were measured and are presented in Table I.[10] The 2-nitrobenzyl ether **8a** cleaves with

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(7) Alkylation of the phenolic oxygens proved to be nontrivial, due to competing alkylation of the carboxylate oxygen of the open lactone tautomer. It was essential to carry out the second alkylation in a nonpolar solvent (benzene), to keep the monoether predominantly in the closed lactone form. Numerous syntheses of functionalized fluoresceins have been reported, but the desired products in these syntheses were the fluorescent carboxylate esters, obtained by alkylation under basic conditions. The isolation of *O,O'*-diethylfluorescein as a minor product in the reactions of sodium fluorescein with ethyl iodide in ethanol or fluorescein with quinoline and silver oxide in benzene has been reported. Miller, A. G. *Anal. Biochem.* **1983**, *133*, 46–57.

(8) All new compounds were fully characterized and gave satisfactory spectral data. Yields are given for isolated material purified to homogeneity by chromatography or recrystallization.

(9) This multiple step purification of final probe molecules was required, since contamination by the highly fluorescent monosubstituted fluoresceins or other fluorescent byproducts, even at low levels, renders the material sufficiently fluorescent to be of little use in FPD experiments which involve photoactivation of only a fraction of the PAF molecules present in a sample. All purified material was handled in the dark to avoid premature photoactivation.

(10) The photoactivation quantum yields were determined by measuring the fluorescence intensity of the monosubstituted fluorescein generated by photocleavage of 30 μM solutions (9:1 H_2O/THF, pH 8.2) at 2–13% conversion. Irradiation at 350 nm was conducted in a Rayonet photochemical reactor configured with a merry-go-round apparatus. Actinometry was accomplished by the photoaquation reaction of Reinecke's salt as described by Wegner and Adamson (Wegner, E. E.; Adamson, A. W. *J. Am. Chem. Soc.* **1966**, *88*, 394–404). The relative fluorescence yield is the normalized fluorescence intensity of a PAF solution of fixed concentration, irradiated for a fixed interval. This quantity is a combination of the photoactivation quantum yield and the extinction coefficient of the PAF at 350 nm.

the highest quantum yield (0.26) of the molecules evaluated. This is consistent with the results we obtained for the corresponding *o*-nitrobenzyl alcohols.[2a,11,12] Surprisingly, the 4,5-dimethoxy-2-nitrobenzyl derivative **8c** photoactivates less efficiently than expected, based on the quantum yield of the 4,5-dimethoxy-2-nitrobenzyl alcohol (0.07);[2a] however, the fluorescence yield for this derivative is still quite high due to its high molar absorptivity (ϵ = 6050 at 350 nm). The phenacyl ether cleavage also is reasonably efficient,[13] but this derivative **5** suffers from very low absorptivity (ϵ = 47 at 350 nm).

The fluorescein monoethers generated as ultimate fluorophores have emission characteristics (λ_{max} = 515 nm) that are virtually identical with fluorescein itself in aqueous solution at neutral to slightly basic pH. Thus, these molecules should be useful in biological environments where fluorescein or other functionalized fluoresceins have been used effectively.

The functionalized fluoresceins described in this paper are excellent PAFs, converting from nonfluorescent precursors to highly fluorescent ultimate fluorophores with reasonable efficiency. Furthermore, the functionality present in **9a–c** makes them ideal molecules for functionalization of biologically interesting macromolecules. FPD studies of molecular transport and diffusion that exploit the capabilities of these unique molecules are underway.

Acknowledgment. This work was supported by a grant from the National Institutes of Health, GM-33864. G. A. Krafft has been a fellow of the American Cancer Society, 1983–1986. R. T. Cummings is a Graduate Scholar-Fellow of Syracuse University.

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Highly Reduced Organometallics. 23. Synthesis, Isolation, and Characterization of Hexacarbonyltitanate(2–), Ti(CO)$_6^{2-}$. Titanium NMR Spectra of Carbonyltitanates[†1]

Kai Ming Chi, Scott R. Frerichs, Stephen B. Philson, and John E. Ellis*

Department of Chemistry, University of Minnesota
Minneapolis, Minnesota 55455

Received September 3, 1987

The relatively robust nature of the previously known low spin d^6 octahedral carbonyls, Mn(CO)$_6^+$, Cr(CO)$_6$, and V(CO)$_6^-$, suggested to us that it should be possible to isolate salts containing the isoelectronic dianion, Ti(CO)$_6^{2-}$.[2] We now report on two independent routes to this previously unknown species which contains titanium in its lowest known formal oxidation state of 2–.[3] Unambiguous characterization of Ti(CO)$_6^{2-}$ has been provided by elemental analyses, IR, ^{13}C, and Ti NMR spectra. Hexacarbonyltitanate(2–) may be regarded as a saturated and dianionic derivative of the extremely unstable neutral Ti(CO)$_6$ (dec ≥ –220 °C) which was observed by matrix isolation spectroscopy.[4] By comparison, the most stable salt of Ti(CO)$_6^{2-}$ obtained so far decomposes above 200 °C, more than 400° higher than the neutral parent! We believe the availability of Ti(CO)$_6^{2-}$ will be of substantial importance in the exploration of new areas of titanium chemistry.

Scheme I[a]

$$\mathrm{Ti(CO)_3(dmpe)_2} \xrightarrow{\text{i,ii}} \underset{\geq 80\%}{[\mathrm{K(2.2.2)}]_2[\mathrm{Ti(CO)_6}]}\ (\mathbf{1})$$

[a] Reagents and conditions: i. 2KC$_{10}$H$_8$ + 2(2.2.2) at –70 °C in THF under argon. ii. Remove argon, add CO, 12 h, and slowly warm to room temperature.

Scheme II[a]

$$\mathrm{TiCl_4(DME)} \xrightarrow{\text{i–iii}} \underset{30\text{–}40\%}{[\mathrm{K(15\text{-}C\text{-}5)_2}]_2[\mathrm{Ti(CO)_6}]}\ (\mathbf{2})$$

[a] Reagents and conditions: i. 6KC$_{10}$H$_8$, –70 °C, 5 h in DME. ii. Four 15-C-5 at room temperature, 2 h, under argon. iii. Cool to –60 °C, remove Ar, and add CO, 40 h.

Reductive carbonylations of either Ti(CO)$_3$(dmpe)$_2$,[5] dmpe = Me$_2$PCH$_2$CH$_2$PMe$_2$, or TiCl$_4$(DME), DME = 1,2-dimethoxyethane, by alkali metal naphthalenides yield isolable salts of Ti(CO)$_6^{2-}$ *provided* an appropriate crown ether or cryptand is present to effectively complex alkali metal cations. Although the latter appear to prevent formation or cause decomposition of Ti(CO)$_6^{2-}$ at low temperature in these syntheses, little is known concerning this interesting phenomenon, which is presently under examination. Cooper and co-workers have previously noted the ability of cryptands to stabilize Na$_2$[M(CO)$_5$] (M = Mo, W) in the solid state, but, in contrast to Ti(CO)$_6^{2-}$, these pentacarbonylmetallates(2–) are readily prepared in tetrahydrofuran (THF) or similar solvents in the absence of effective alkali metal complexants.[6]

Cold (–70 °C) THF solutions containing Ti(CO)$_3$(dmpe)$_2$ and 2 equiv of KC$_{10}$H$_8$ and cryptand (2.2.2) were mixed under an argon atmosphere followed by evacuation and addition of carbon monoxide at atmospheric pressure. Stirring was continued for 12 h while the mixture slowly warmed to room temperature. A finely divided orange-red solid was removed by filtration, washed with THF, dried, and then recrystallized from acetonitrile–THF at 0 °C. An 80% yield (1.2 g) of dark violet crystalline product (mp 216–217 °C with dec) was obtained. This material provided satisfactory elemental analyses (C, H, N) for the composition [K(2.2.2)]$_2$[Ti(CO)$_6$] (**1**).[7] This procedure is summarized in Scheme I. In the second procedure, cold (–70 °C) DME solutions

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[†] Dedicated to the memory of L. Mond and C. Langer who discovered the first binary metal carbonyl, Ni(CO)$_4$, nearly 100 years ago.

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