2-Diazo-3,3,3-trifluoropropionyl chloride: Reagent for photoaffinity labeling

(Wolff rearrangement/thioesters)

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ABSTRACT 2-Diazo-3,3,3-trifluoropropionyl chloride has been synthesized from trifluorodiazoothane and phosgene. Its derivatives are acid stable, can be used to label enzymes, and undergo photolysis with substantially less rearrangement than do derivatives of other known diazoacetyl reagents designed for photoaffinity labeling. In particular, the diazotrifluoropropionyl thioester of methyl N-acetylcysteine undergoes photolysis in methanol with about 40% insertion into the —OH bond of the solvent; by contrast, photolysis of other diazoacetyl thiosteres gives substantially quantitative Wolff rearrangement. The trifluoro compounds hold promise for the photoaffinity labeling of thiols.

Photoaffinity labeling was initiated with the photolysis of diazoacetyl chymotrypsin, prepared from the enzyme and p-nitrophenyl diazoacetate (1, 2). Subsequently, this and other photolabile reagents have been applied (3-14) to a wide variety of problems in biochemistry and biology. (For reviews, see refs. 15-18.) Diazocetates and diazomalonates (6, 7), however, suffer from the disadvantages that they are not acid stable, and that photolysis leads in large measure to the products of the photochemical analog of the Wolff rearrangement (2, 19). Furthermore, attempts to use the diazoesters of thiols, either with glyceraldehyde-3-phosphate dehydrogenase† or model compounds (20), have led only to products of the photochemical Wolff rearrangement or to other products that are not informative from the point of view of photoaffinity labeling. In this paper, we report the synthesis and photolysis of diazotrifluoropropionyl derivatives that are not subject to these limitations.

2-Diazo-3,3,3-trifluoropropionyl chloride is easily prepared, and is purified by vacuum distillation; p-nitrophenyl diazo trifluoropropionate is a crystalline solid that can be purified by sublimation. Various diazotrifluoropropionyl derivatives (including that of chymotrypsin) can be made from these acylating reagents; ethyl diazotrifluoropropionate is stable at room temperature in 1 M hydrochloric acid! All of the diazo derivatives, however, readily undergo photolytic decomposition.

Furthermore, photolysis of ethyl diazotrifluoropropionate in methanol gives insertion into the —OH bond of the solvent to the extent of about 85%, and only about 15% rearrangement. [The product B, separated by vapor phase chromatography and identified by its nuclear magnetic resonance (NMR) and mass spectra, results from transesterification and then insertion into the solvent.] We have obtained similar results with other model compounds.

The results obtained with the diazotrifluoropropionyl ester of methyl N-acetylcysteine are even more significant, since (in

Abbreviation: NMR, nuclear magnetic resonance.

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‡ L. J. Crane, unpublished results.

Contrast to results with thiol derivatives of other photoaffinity reagents) photolysis does not lead exclusively to rearrangement. When the thioester is illuminated at 350 nm, the mixture of products contains about 60% of the thioether, D, which results from rearrangement, but also includes about 40% of the thioester, E, which arises from insertion of the carbene, formed on photolysis, into the —OH bond of the solvent. This result suggests that diazotrifluoropropionates may prove useful in the photoaffinity labeling of sulphydryl compounds.

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\begin{align*}
\text{CF}_2\text{CN},\text{CO}_2\text{H}_2 & \xrightarrow{\text{hr} \text{CH}_2\text{OH}} \text{CF}_2\text{CHOCH}_2\text{CO}_2\text{CH}_3 \quad (75\%) \quad \text{A} \\
+ \text{CF}_2\text{CHOCH}_2\text{CO}_2\text{CH}_3 & \quad (8\%) \quad \text{B} \\
+ \text{CF}_2\text{CHOCH}_2\text{H}_2\text{CO}_2\text{CH}_3 & \quad (15\%) \quad \text{C}
\end{align*}
\]

Scheme 1

Photolysis at short wavelengths (254 nm) leads initially to the same products, but the thioester, E, is rapidly destroyed by the light, so only small amounts can be isolated, and then only with difficulty. Prolonged photolysis at 254 nm eventually destroys even the thioether, D. Both D and E, however, are stable for long periods of time to light of long wavelength (350 nm).

EXPERIMENTAL

Preparations. 2,2,2-Trifluorodiazoothane was prepared from 27 g of trifluoroethylamine hydrochloride by the procedure of Gilman and Jones (21). A solution of this diazo compound in 300 ml of dichloromethane was treated at room temperature with 35 g of finely powdered dipotassium hydrogen phosphate and 15 ml of phosgene in 100 ml of methylene chloride. The mixture was allowed to warm to room temperature, was stirred overnight, and was then decanted through glass wool; the methylene chloride was evaporated and the residue was distilled at 50–60°/137 mm (18 kPa). Infrared (liquid film) peaks were
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at: 2165, 1750 (broad), 1135 (broad) cm⁻¹. Exact mass of the
35Cl molecular ion: Calculated, 171.9651; found, 171.9662.

Crude undistilled acid chloride from 6.75 g of trifluoro-
ethylamine hydrochloride was stirred in dichloromethane and
tetrahydrofuran with 4.0 g of anhydrous sodium p-nitro-
phenoxy for 24 hr. After the mixture had been filtered and the
solvent evaporated, the residue was chromographed on
neutral alumina (100 g, Woelm grade V) and eluted with ca-
bon tetrachloride. The ester, which came through with the
solvent front, crystallized on evaporation of the solvent; it
was sublimed at 50–70°/10⁻³ mm (0.13 Pa) and recrystallized
from cyclohexane–lignin; mp 63°. Infrared (KBr) peaks were at
2165, 1740 cm⁻¹. Elemental analyses for C, H, and N agree
with theory to ±0.2%.

The ethyl ester was prepared from 1.0 g of distilled diazo-
trifluoropropionyl chloride and 0.5 g of methanol with 0.75 g of
quinoline. The mixture was allowed to stand overnight; the
ester was extracted into ether and washed with acidified brine
and then with bicarbonate solution. After the solvent had been
evaporated, the ester was chromatographed on grade III alu-
mina in petroleum ether and then distilled at 66.5/75 mm (10
kPa). Infrared (liquid film) peaks were at 2165, 1730 cm⁻¹.
Elemental analyses for C, H, and N agreed with theory to ±0.2%.

2-Diazoo-3,3,3-trifluoro-N-acetylcysteine methyl ester was
prepared (although in very poor yield) from 0.575 g of N-
acetylcysteine methyl ester (22, 23), 0.560 g of 2-diazoo-3,3,3-
trifluoropropionyl chloride, and 0.348 g of 2,6-lutidine in 30
ml of tetrahydrofuran overnight at room temperature. The pale
yellow crystals obtained from methylene chloride–hexane were
sublimed at 85°/0.1 mm (13 Pa); mp 96–97°. Analysis for C,
H, and N were within 0.2% of theory. The compound shows a
strong diazo band at 2092 cm⁻¹ and UV and NMR spectra
consistent with the expected structure.

Diazotrifluoropropionyl chymotrypsin was prepared by al-
lowing 2.5×10⁻⁵ M enzyme to react with 5.5×10⁻⁵ M p-
nitrophenyl diazotrifluoropropionate at pH 5.95 at 30° for 1
hr. It proved to be 93% inactivated but reactivated completely
on standing at pH 7.0 at room temperature for 10–12 hr. The
diazoacetyl enzyme solution has also been purified by passage
over Sephadex G-25. (This preparation has been repeated by
Dr. Richard Hudson in our laboratories.)

Photolysis of Ethyl Diazotrifluoropropionate. The photo-
lolysis of 15–30 mg of ethyl diazotrifluoropropionate was car-
ried out for 200–500 min in a Srinivasan-Rayonet reactor with
RPR 2537 Å lamps in 0.1–0.3 ml of methanol. After photolysis,
the products were separated on a 120×0.31 cm column of 5%
SF-1265 on Chromosorb G/AW/DWCX at 60° using a Hew-
lett-Packard HP5750 gas chromatograph. Alternatively, the
methanol was evaporated, and the products were separated on a
360×0.31 cm column on 15% Carbowax 20M on Chromosorb
P at 115°. The products of photolysis consisted of 75% or more
of A (insertion into the solvent), 8% or less of B (ester exchange
and insertion into the solvent), and about 15% of C (rere-
narrangement). The structural assignments of A, B, and C are
derived from (a) their NMR spectra, (b) their mass spectra, and
(c) a transesterification experiment. The NMR spectrum of A
shows δ 1.21 (t, J = 7.0 Hz, 3 H), 3.47 (s, 3 H, ethyl OCH3), 4.04

Fig. 1. UV spectra of 7×10⁻⁵ M 2-diazo-3,3,3-trifluoropropionyl
N-acetyl cysteine methyl ester at zero time and after photolysis with
3500 Å lamps for 24 hr.

(q, JF-H = 7.0 Hz, 1 H), 4.22 (q, J = 7.0 Hz, 2 H, ester methylene
group). The spectrum of B shows δ 3.57 (s, 3 H, ether methyloxy),
3.86 (s, 3 H, ester methyloxy), 4.16 (q, JF-H = 6.5 Hz, 1 H), and
that of C shows δ 1.26 (t, J = 7.0 Hz, 3 H), 3.70 (m, 2 H, di-
astereotopic ether methylene group), 3.84 (s, 3 H, ester meth-
yloxy), 4.24 (q, JF-H = 6.5 Hz, 1 H).

The mass spectra are dominated by the ions obtained by the
McLafferty rearrangement (24, 25). In analogy with the mass
spectrometry of the esters of methoxyacetic-acid (26) the ether
groups of A, B, and C are lost, presumably as the corre-
sponding aldehydes. Thus A, a methyl ether, lost CH2O to yield
an ion of mass 156.0904 (calculated for CH3F3O2, 156.0982),
while B, also a methyl ether, lost CH2O, and C, an ethyl ether,
lost CH3CHO to yield ions of measured mass 142.02707 and
142.02885, respectively; calculated for CH3F3O2, 142.02417.
The formation of the products of the McLafferty rearrange-
ment unambiguously confirms the NMR assignment of A as an
ethyl ester, and of B and C as methyl esters, since these groups
survive in the ion beam. The transesterification experiment was
conducted by heating B in completely deuterated methanol
and 1% sulfuric acid. The NMR peak at δ 3.4 in CD3OD as
solvent disappears, while that at δ 3.14 is unchanged; these peaks
correspond to those at δ 3.86 and 3.57 in CDCl3. This experi-
ment, too, confirms the structural assignments made above.

Photolysis of Methyl 2-Diazo-3,3,3-trifluoropropionyl-
N-acetylcysteine. Photolysis of 3–4 mg of methyl 2-diazo-
3,3,3-trifluoropropionyl-N-acetylcysteine, dissolved in 100 µl
of dry methanol, was conducted in quartz tubes in a Srinivas-
an-Rayonet reactor with either RPR 2537 Å or RPR 3500 Å
lamps. Gas chromatographic analysis of 2 µl samples was con-
ducted with a Hewlett-Packard 5750 gas chromatograph on
a 180×0.31 cm column packed with 3% Dexsil 400 GC/
Chromosorb W/HP 80/100. About 20 injections yielded
enough material to obtain 1H Fourier transform NMR spectra
(about 2000 transients).

Photolysis with 3500 Å Lamps for 18 hr led to the disap-
pearance of the diazo band at 256 nm, whereas the intensity of
the thioester band decreased (Fig. 1) by 60% but then held
constant. Gas chromatographic analysis of the product led to
two well-separated compounds, D and E, in the ratio 6.4 (re-
tention time, 6.2 and 6.8 min; 40 ml/min helium carrier gas;
temperature programmed 110°–170° at 20°/min). Mass:charge
ratio for D was 317.05337; for E, 317.05483; calculated for

† A different preparation of this ester was reported at the American
Chemical Society Middle Atlantic Regional Meeting, Fall, 1974, by
A. Zampini and R. A. Shepard (Medicinal Chemistry and Bio-
chemistry Section, Paper No. 36).

‡ NMR resonances, δ, given in parts per million downfield from
tetramethylsilane. For peaks, s = singlet; d = doublet; t = triplet; q
= quartet; m = multiplet.
FIG. 2. Comparison of the 100 MHz $^1$H NMR spectra of compound D (the thioether produced by rearrangement of the 2-diazo-3,3,3-trifluoropropanoyl thioester of N-acetyl cysteine methyl ester) and compound E (the thioester produced by insertion into the solvent). The double peaks at high resolution arise because the products are mixtures of diastereomers.

$C_{19}H_{14}F_3NO_5$: 317.05449. The critical region of the 100 MHz $^1$H NMR spectra is shown in Fig. 2. Compound D shows two tightly spaced methoxy signals at $\delta$ 3.78 and 3.81; the further, barely resolved doubling of the signals in both spectra presumably arises because each structural formula represents two diastereomers. The spacing between the methoxyl signals from E ($\delta$ 3.62 and 3.71) and comparison of the spectra with those for the products of the photolysis of ethyl diazotrifluoropropionate identifies E as the methyl ether methyl ester thioester shown in Scheme 2. This structural assignment is confirmed by UV spectroscopy; E shows the strong absorption at 236 nm typical of thioesters (27-29), whereas A shows only the end absorption of thioesters (30, 31). Both D and E are stable to prolonged irradiation at 350 nm.

Photolysis with 2537 Å Lamps for 125 min completely eliminated the absorptions of both the thioester and diazo functions. When, however, the photolysis was carried only partially to completion, both UV and gas chromatographic analyses showed that 3-8% of E was present; on further irradiation with 2537 Å lamps, the thioester is rapidly destroyed. To isolate the insertion product, then, photolysis of the diazo-difluorothioiopropionate must be carried out at long wavelength.

Stability. A solution of ethyl diazotrifluoropropionate is stable for 24 hr in 1 M HCl. [Trifluorodiazoethane is also remarkably acid stable (32).]

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