Synthetic Communications: An International Journal for Rapid Communication of Synthetic Organic Chemistry
Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/lsyc20

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Published online: 21 Nov 2007.

To cite this article: Sui Xiong Cai & John F. W. Keana (1996): Design and synthesis of trifunctional perfluorophenyl azide-based photoactivatable reagents, Synthetic Communications: An International Journal for Rapid Communication of Synthetic Organic Chemistry, 26:4, 809-818

To link to this article: http://dx.doi.org/10.1080/00397919608086757

SYNTHETIC COMMUNICATIONS, 26(4), 809-818 (1996)

DESIGN AND SYNTHESIS OF TRIFUNCTIONAL PERFLUOROPHENYL AZIDE-BASED PHOTOACTIVATABLE REAGENTS

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Abstract: The novel trifunctional perfluorophenyl azide based photoactive compounds **3**, **6** (unstable), **10** and **13** are described. Diels-Alder reaction of maleimide **13** with diene-heteropolytungstate **14** produced the PFPA-HPT-maleimide trifunctional conjugate **15**.

Trifunctional reagents continue to enjoy wide application for the study of biological systems.[1] For example, a photoaffinity labeling reagent[2,3] generally consists of a ligand for binding to a receptor, a photoactivatable group for covalent labeling the receptor, and a radioactive label which facilitates the isolation and identification of the receptor. We[4,5] and others[6] have introduced perfluorophenyl azides (PFPAs) as a new class of efficient photolabeling agents[7-9] and photoactivatable crosslinking agents.[10] We have also described the design and synthesis of trifunctional reagents for the preparation of radioactive iodinated functionalized PFPAs.[11,12] Herein we report the preparation of novel trifunctional reagents **3**, **6** (unstable), **10**, **13** and **15** represented schematically as **1**.

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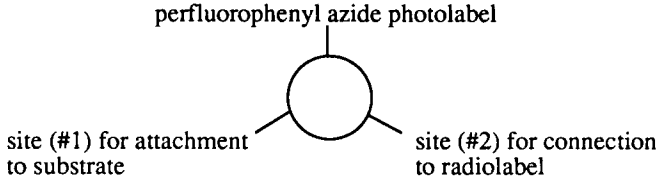
Reagent **1** carries a PFPA photoactivatable group together with a reactive group such as a carboxylic acid or a maleimide for attachment to a target molecule and an orthogonal reactive group such as a 1,3-diene or a primary amine through which a labeling reagent such as radioactive iodine may be attached.

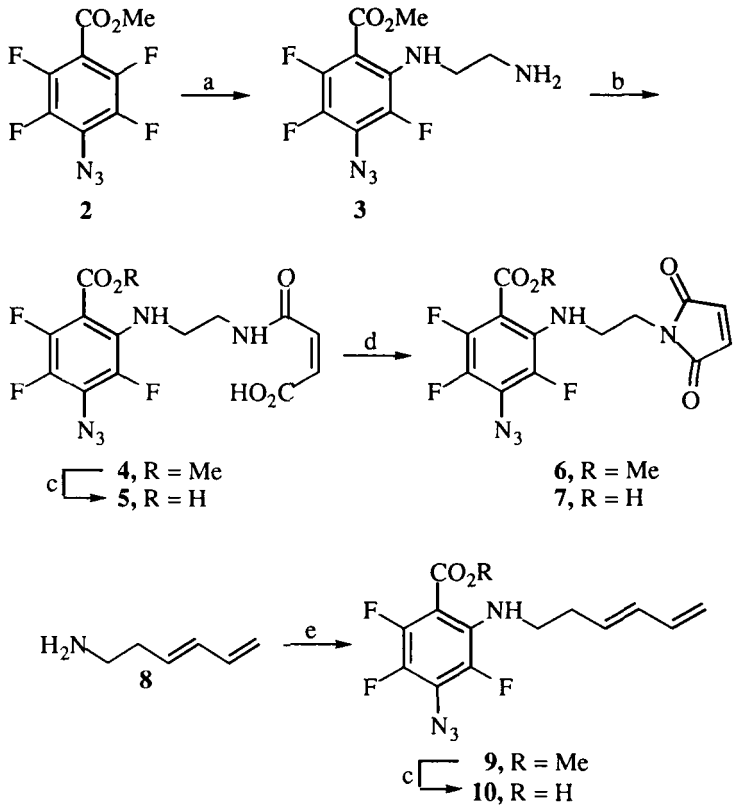


1

We first undertook the preparation of carboxylic acid **7** by way of the trifunctional PFPA **3** (Scheme 1). The carboxyl group of **7** was intended as a site for attachment to a substrate through acylation of an amino or hydroxy group. The maleimide group might be used to react with a thiol-containing reagent or a 1,3-diene-containing reagent by a Diels-Alder reaction. Also, owing to the o,o'-difluoro substitution surrounding the azide group in **7**, the desirable photochemical properties characteristic of other PFPAs is expected to be retained in derivatives of **7**.[12]

In the event, nucleophilic aromatic substitution of one of the fluorine atoms ortho to the activating carbonyl group of azide **2**[5] by ethylenediamine produced the amino PFPA ester **3** which was allowed to react with maleic anhydride to give maleamic acid **4**. Hydrolysis of ester **4** gave diacid **5** as a pale yellow solid. Cyclization of **4** to **6** or **5** to **7** was problematic. For example, when **4** was heated at 60 °C in $CDCl_3$ in the presence of acetic anhydride, cyclization was observed by ^1H NMR as evidenced by the transformation of the characteristic AB vinyl proton pattern (δ 6.244 and δ 6.397) to a singlet (δ 6.321). Pure maleimide **6** however could not be obtained even after carefully removing the solvent at 0 °C

Scheme 1



(a) $NH_2CH_2CH_2NH_2/CH_3CN$, 65 °C; (b) maleic anhydride/THF; (c) $NaOH/H_2O/MeOH$ then HCl; (d) $(CH_3CO)_2O/CDCl_3$, 60 °C; (e) **2**, CH_3CN, 65 °C.

under vacuum. Product **6** was likely destroyed by an intermolecular 1,3-dipolar cycloaddition reaction[13-15] between the azide group and the maleimide during evaporation of the solvent.

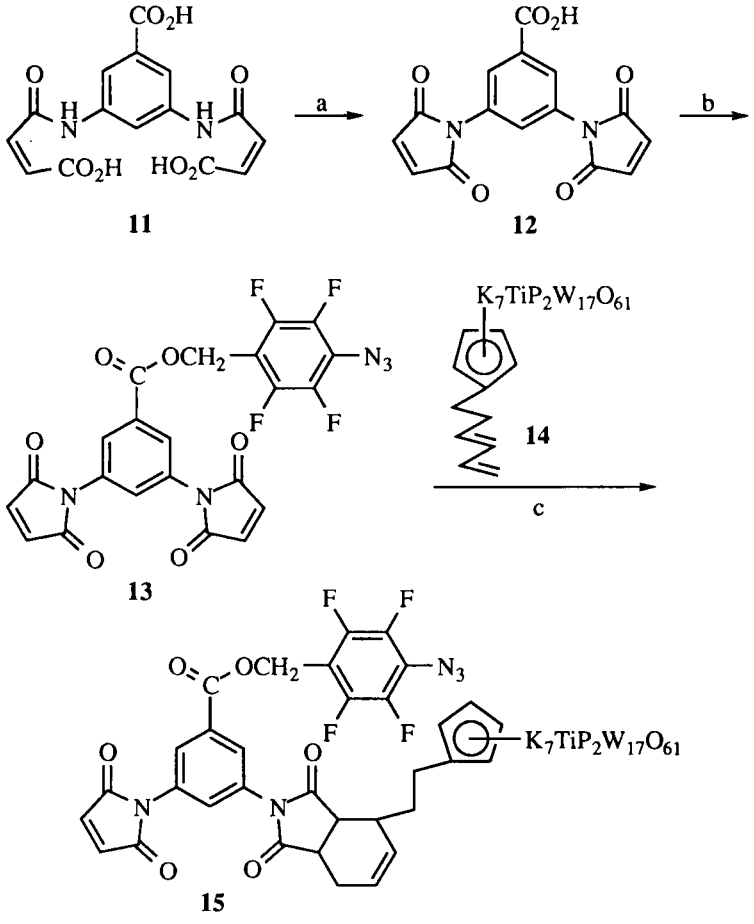
We next turned to the preparation of 1,3-diene functionalized PFPA **10**. The diene unit is designed to react with a dienophilic maleimide-containing reagent

such as a Dawson-type heteropolytungstate (HPT) maleimide[16,17] in a Diels-Alder reaction. In the event, reaction of diene amine **8**[18] with azide **2** produced diene azide **9** which was hydrolyzed to give acid **10** as a solid. Interestingly, **10** was stable to storage at room temperature, showing compatibility of the 1,3-diene group with the azide group. Comparing the stability of **10** and **6** suggests that a 1,3-diene unit is not as good a dipolarophile as a maleimide unit.

The 1,3,5-trisubstituted benzene structural motif of PFPA dimaleimide **13** (Scheme 2) was designed to separate the functional groups from each other, thus preventing intramolecular reactions. Cyclization of triacid **11**[17] under the conditions of Koechel *et al.*[19] gave dimaleimide **12** in 82% yield. Coupling of acid **12** with 4-azido-2,3,5,6-tetrafluorobenzyl alcohol[5] using 1-methyl 2-chloropyridinium iodide[20] as the coupling reagent gave ester **13** as a colorless solid in 30% yield.

To demonstrate the utility of these trifunctional reagents, one of the maleimide groups in **13** was used to react with HPT diene **14**.[17] HPT **14** was developed earlier in our laboratory as a small, highly electron dense label for the study of biological systems using electron microscopy.[16,17] By using a 4:1 excess of **13** to **14** the maleimide functionalized Dawson HPT-PFPA monoadduct **15** was obtained as a pale brown solid. HPT **15** was characterized by its ^{1}H NMR spectrum which showed three benzene ring protons (δ 7.629, 7.919 and 8.042), two maleimide vinyl protons (δ 7.117), four Cp ring protons (δ 6.516-6.682), two cyclohexene vinyl protons (δ 6.078-6.117), two benzylic protons (δ 5.543), as well as nine remaining aliphatic protons (δ 2.247-3.655). Elemental analysis of **15** gave expected H and N values, although carbon was somewhat high, possibly owing to the presence of traces of **13**. The maleimide group in **15** may serve as a site for further attachment of substrates containing a 1,3-diene or thiol functionality to produce new HPT conjugates.

Scheme 2



(a) 1. $(CH_3CO)_2O/CH_3CO_2Na$/90 °C; 2. H_2O. (b) $N_3C_6F_4CH_2OH$/1-Methyl-2-chloropyridinium iodide/Bu_3N/THF. (c) DMSO/60 °C.

In conclusion, the synthesis of trifunctional PFPA reagents **3**, **6**, **10**, **13** and **15** has been accomplished. These versatile reagents should be useful for the preparation of a variety of trifunctional photoreactive conjugates.

Experimental Section

General. ^1H NMR spectra were measured on a QE-300 NMR spectrometer in $CDCl_3$ unless otherwise specified. IR spectra were recorded on a Nicolet 5DXB FTIR spectrometer in $CDCl_3$. Melting points were obtained on a Thomas-Hoover apparatus and are uncorrected. Solvents were reagent grade unless otherwise specified. $MgSO_4$ was used to dry organic solutions. Microanalysis were performed by Desert Analytics, Tuscon, Arizona. All reactions involving azides were run under subdued light by wrapping flasks with aluminum foil.

Methyl 2-(2-Aminoethyl)amino-4-azido-3,5,6-trifluorobenzoate (3) A solution of 425 mg (1.70 mmol) of azide **2**[5] and 220 mg (3.66 mmol) of ethylenediamine in CH_3CN (13 mL) was heated at 65 °C for 6 h. It was diluted by $CHCl_3$ and extracted with 5% aqueous CH_3CO_2H (3 x 20 mL). The extract was neutralized by 5% aqueous Na_2CO_3 to pH = 8, then extracted with $CHCl_3$ (5 x 20 mL). The organic extract was dried and evaporated to leave 331 mg (67%) of a yellow liquid which solidified at room temperature. ^1H NMR, 2.948 (t, 2, J = 5.0), 3.44 (m, 2), 3.910 (s, 3). IR, 3000, 2126, 1693, 1638, 1586, 1538, 1507, 1482, 1463, 1438, 1333, 1256, 1212 cm^{-1}. The analytical sample of **3** was obtained as a solid by sublimation (0.05 mm/50 °C), mp 57-58 °C. Anal. Calcd for $C_{10}H_{10}F_3N_5O_2$: C, 41.53; H, 3.49; N, 24.21. Found: C, 41.61; H, 3.37; N, 23.93.

***N*-[2-(3-Azido-2,4,5-trifluoro-6-methoxycarbonylphenylamino)ethyl]-maleamic Acid (4)** To a solution of 843 mg (2.91 mmol) of amine **3** in dry THF (8 mL) was added a solution of 345 mg (3.57 mmol) of maleic anhydride in THF (10 mL). The solution was stirred for 40 min and evaporated. The residual solid

was stirred with ether (20 mL) for 2 h. The mixture was filtered and the solid was dried to leave 1.02 g (90%) of **4** as a pale red solid, mp 145-146 °C. ^{1}H NMR, 3.573 (s, 4), 3.929 (s, 3), 6.244 (d, 1, J = 12.9), 6.397 (d, 1, J = 12.9), 6.93 (bs, 1). IR, 2931, 2126, 1720, 1691, 1636, 1613, 1519, 1482, 1466, 1453, 1258 cm^{-1}. Anal. Calcd for $C_{14}H_{12}F_3N_5O_5$: C, 43.42; H, 3.12; N, 18.08. Found: C, 43.25; H, 3.09; N, 17.84.

***N*-[2-(3-Azido-2,4,5-trifluoro-6-carboxyphenylamino)ethyl]maleamic Acid (5)** A solution of 60 mg of ester **4** with 0.2 mL of 20% NaOH and 0.5 mL of MeOH and 1 mL of water was stirred at room temperature for 3.5 h. It was acidified with 2N HCl to pH <1. The precipitate was filtered and washed with water (2 x 1 mL), dried to give 46 mg (80%) of acid **5** as a pale yellow solid, mp 175 °C (dec.). ^{1}H NMR ($CDCl_3$ + DMSO-d_6), 3.2 (m, 4), 6.048 (d, 1, J = 12.8), 6.225 (d, 1, J = 12.8), 9.011 (mb, 1). Anal. Calcd for $C_{13}H_{10}F_3N_5O_5$•0.2 H_2O: C, 41.43; H, 2.78; N, 18.58. Found: C, 41.06; H, 2.64; N, 18.50.

2-(3,5-Hexadienyl)amino-4-azido-3,5,6-trifluorobenzoic Acid (10) A solution of 303 mg (3.12 mmol) of amine **8**,[18] 364 mg (1.46 mmol) of azide **2**[5] and 160 mg of Et_3N in CH_3CN (15 mL) was heated at 65 °C overnight. It was evaporated and the residue was dissolved in $CHCl_3$ (10 mL), washed with water (2 x 10 mL), dried and evaporated to leave a liquid. It was separated by preparative TLC (1:3 acetone-hexane) to give 201 mg (42%) of ester **9** (R = 0.70) as a liquid. ^{1}H NMR, 2.346 (q, 2, J = 6.60), 3.433 (m, 2), 3.880 (s, 3), 5.016 (d, 1, J = 10.20), 5.133 (d, 1, J = 16.8), 5.646 (m, 1), 6.099 (m, 1), 6.305 (m, 1), 6.972 (mb, 1). Ester **9** was hydrolyzed in a manner similar to ester **4** to give acid **10** as a yellow solid (76%), mp 80 °C (dec.). ^{1}H NMR, 2.377 (q, 2, J = 6.90), 3.476 (m, 2), 5.023 (d, 1, J = 9.96), 5.155 (d, 1, J = 16.87), 5.651 (m, 1), 6.131 (m, 1), 6.311 (m, 1). Anal. Calcd for $C_{13}H_{11}F_3N_4O_2$: C, 50.01; H, 3.55; N, 17.94. Found: C, 50.17; H, 3.45; N, 17.48.

3,5-Dimaleimidobenzoic Acid (12) A mixture of 1.28 g of acid **11**[17] and 0.51 g of sodium acetate in acetic anhydride (8 mL) was heated at 95 °C for 6 h, followed by addition of H_2O (15 mL) in an ice bath and stirred for 20 h. The mixture was filtered and dried to leave 0.94 g (82%) of **12** as a solid, mp >250 °C (lit[17]. mp >250 °C). ^1H NMR ($CDCl_3$ + DMSO-d_6), 6.527 (s, 4), 7.143 (s, 1), 7.565 (s, 2).

(4-Azido-2,3,5,6-tetrafluorobenzyl) 3,5-Dimaleimidobenzoate (13) A mixture of 45.0 mg (0.144 mmol) of acid **12**, 42.0 mg (0.190 mmol) of 4-azido-2,3,5,6-tetrafluorobenzyl alcohol,[5] 112 mg (0.437 mmol) of 2-chloro-1-methyl pyridinium iodide and 127 mg (0.686 mmol) of Bu_3N in THF (4 mL) and CH_2Cl_2 (4 mL) was stirred for 36 h. It was filtered and the filtrate was evaporated to dryness. The residue was separated by preparative TLC (2:3 acetone-hexane) to give 25 mg (30%) of **13** (R_f = 0.43) as a solid. ^1H NMR, 5.456 (s, 2), 6.897 (s, 4), 7.726 (t, 1, J = 1.8), 8.054 (d, 2, J = 1.8). IR, 2123, 1727, 1499, 1465, 1364 cm^{-1}. The analytical sample of **13** was obtained by crystallization (CH_2Cl_2-hexane) as colorless solid, mp 114-115 °C. Anal. Calcd for $C_{22}H_9F_4N_5O_6$: C, 51.27; H, 1.76; N, 13.59. Found: C, 50.83; H, 1.51; N, 13.73.

Maleimide and PFPA Functionalized Dawson HPT (15) A solution of 6.5 mg (12 mmol) of **13** and 14.6 mg (3.1 mmol) of diene Dawson HPT **14** (K^+ salt)[17] in DMSO-d_6 (0.5 mL) was heated at 60 °C under Ar for 6 h. It was precipitated by adding the solution to ether (20 mL) and then the mixture was centrifuged. The precipitate was washed with $CHCl_3$ (2 x 2 mL) and dried to leave an orange solid. The solid was dissolved in H_2O (3 mL), centrifuged and filtered to remove insoluble solids. The aqueous was lyophilized to leave 18.2 mg of an orange solid. It was redissolved in H_2O (2 mL) and centrifuged and the aqueous solution was lyophilized to leave 12 mg (73%) of **15** as a solid. ^1H NMR (D_2O), 2.246 (m, 2), 2.431 (m, 1), 2.709 (m, 2), 3.066 (m, 1), 3.378 (m, 1), 3.655 (m, 2), 5.543

(s, 2), 6.117 (m, 2), 6.538-6.681 (m, 4), 7.117 (s, 2), 7.629 (s, 1), 7.919 (s, 1), 8.042 (s, 1). Anal. Calcd for $C_{33}H_{22}F_4N_5O_{67}P_2W_{17}K_7Ti$: C, 7.70; H, 0.43; N, 1.36. Found: C, 10.13; H, 0.66; N, 1.70.

Acknowledgment. This work was supported by a grant from the National Institute of General Medical Sciences GM 27137.

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(Received in the USA 19 July 1995)