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Hypervalent iodine reagents: Thiol derivatization with a tetrafluoroethoxy coumarin residue for UV absorbance recognition

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A new hypervalent iodine reagent (**5**) based on 1,2-dihydro-3,3-dimethyl-1,2-benziodoxole containing a 4-methyl-7-tetrafluoroethoxycoumarin unit as a specific UV absorber was prepared and fully characterized, including X-ray crystal structural analysis. The high reactivity of this compound towards thiols has been exploited for the selective tagging of several targets with the UV chromophore coumarin, including *e.g.* glutathione.

Keywords: Hypervalent iodine • Fluoroalkyl • Thiol • UV absorbance • Coumarin

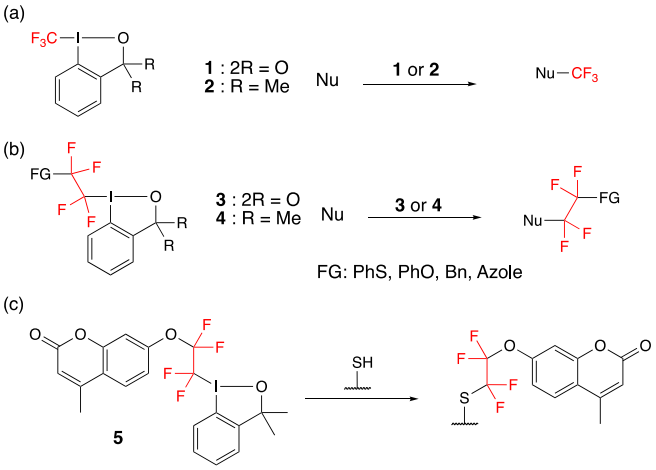
Introduction

Thiols play an important role in several biologically relevant compounds,[1] and offer the opportunity of tagging specific functionalities via reaction of such mercaptans using different reagents,[2] e.g. hypervalent iodine reagents. Indeed, Abegg *et al.* described recently the utility of alkynyl benziodoxolones for chemical proteomics applications.[3] In 2006, our group developed a new family of hypervalent iodine compounds **1** and **2** (**Scheme 1**[a])[4] which showed a very good reactivity towards a large variety of both carbon- and heteroatom-centered nucleophiles,[5] in particular thiols.[6] In fact, thiols were among the first nucleophiles that were trifluoromethylated by use of corresponding hypervalent iodine reagents.[6] Many aromatic and aliphatic mercaptans reacted in moderate to excellent yields furnishing the corresponding trifluoromethylthioethers under very mild conditions in DCM or MeOH at -78 °C for 1 h. Biologically relevant compounds such as protected 1-thio-β-D-glucose and cysteine were also successfully trifluoromethylated.[6-7] Moreover, the trifluoromethylation of thiols occurs also under acqueous conditions, as demonstrated by the trifluoromethylation of Coenzyme A using an excess of electrophilic CF_3 reagent.[8]

More recently, our group investigated the synthesis of new fluoroalkyl reagents in order to transfer not only trifluoromethyl but also functionalized tetrafluoroethyl groups -CF_2CF_2-FG (FG = PhS, PhO, Bn, azoles, etc.).[9] Owing to their excellent stability, these new hypervalent iodine reagents are anticipated to find future applications in the discovery of new drugs, the modification of existing lead structures, and in the design of new functional materials. In fact, these reagents have already shown interesting reactivity with a variety

of nucleophiles (**Scheme 1**[(b)]). Recently, selected examples of these new reagents were used in the synthesis of quaternary α-perfluoroalkyl lactams[10] or in a copper-catalyzed fluoroalkylation–cyclization sequence of alkenes and alkynes.[11]

We therefore designed a new reagent, bearing a UV absorber moiety as functional group linked to the tetrafluoroethyl bridge, which is able to tag thiol substrates *via S*-perfluoroalkylation reaction. Both UV absorbance and NMR spectroscopy can be used for the detection of the coumarin moiety once it has been transferred. We previously reported a similar proof of concept involving *e.g.* a pyrene-containing fluoroalkylation reagent.[9,12]



Scheme 1. [(a)]Electrophilic trifluoromethylation using reagents **1** and **2**. [(b)]Electrophilic tetrafluoroalkylation. [(c)]Transfer of UV absorber moiety using hypervalent iodine tetrafluoroalkylation reagent **5**.

Here, we describe the synthesis of a new hypervalent iodine reagent (**5**) modified by a 4-methyl-7-tetrafluoro-ethoxycoumarin residue and report its reactivity towards thiols (**Scheme 1**[(c)]). We focused our study on the labeling of selected biologically relevant molecules as model targets. The new shelf-stable reagent was fully characterized and offers a new way to label cysteine residue *e.g.* in peptides, as demonstrated by the specific example of glutathione (*vide infra*).

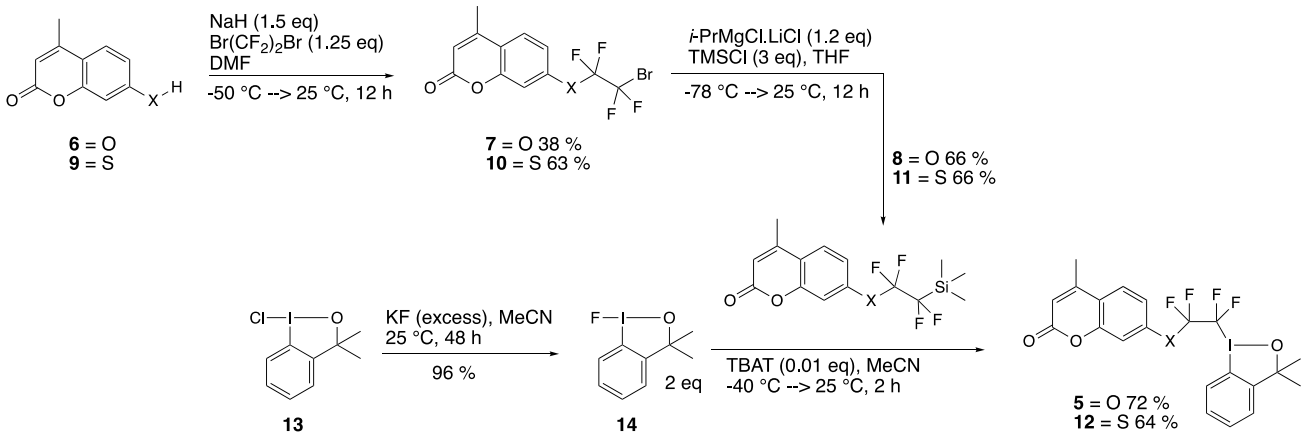
Results and Discussion

Synthesis and reactivity of hypervalent iodine reagents.

Using experimental conditions recently developed in our group,[9] 7-(2-(3,3-dimethyl-1λ^3-benzo[*d*][1,2]iodaoxol-1(3*H*)-yl)-1,1,2,2-tetrafluoro-ethoxy)-4-methyl-2*H*-chromen-2-one (**5**) was synthesized in three steps from commercially available **6**. Firstly, deprotonation of the phenol position with sodium hydride followed by the reaction with 1,2-dibromotetrafluoroethane provided bromide **7**. Subsequent reductive silylation under Barbier conditions afforded fluoroalkylsilane **8**.[13] Finally, the Umpolung reaction of **8** was carried out with fluoroiodane **14**, derived from 1-chloro-3,3-dimethyl-1,2-benziodoxole (**13**),[4b] in the presence of TBAT (1 mol%) affording reagent **5**. The procedure was also reproduced from mercaptan **9** to give compound **12** (**Scheme 2**). Despite the better yield for the first step thanks to the higher acidity of **9**, reagent **12** was obtained in lower purity as compared to **5**. Reagent **5** can be stored as pure material for several hours at ambient temperature or at -20 °C for several months without any detectable decomposition and was exclusively used for all subsequent tagging experiments of thiol substrates. However, reagent **5** is less stable in solution, as we observed partial decomposition in $CDCl_3$ after 24 h. It is important to mention its solubility, which seems odd at first sight. It is soluble in common chlorinated solvents ($CHCl_3$ and DCM), slightly soluble in polar aprotic solvent (DMF, MeCN and THF), but only sparingly soluble in polar protic solvents (MeOH, water).

The X-ray crystallographic analysis of iodane **5** (**Figure 1**) confirmed the structural assignment and revealed values of bond lengths and angles in the expected ranges. Indeed, we can observe an I1-C10-C11-O2 torsion angle of 62°, which indicates a gauche relationship between the iodine and oxygen atoms linked by the tetrafluoroethyl bridge, as previously observed in similar hypervalent iodine tetrafluoroethyl reagents.[9]

Based on results previously obtained in *S*-trifluoromethylation reactions (*vide supra*), we hoped reagent **5** would display a comparable reactivity as the parent compound and initially investigated its reactivity towards aromatic and aliphatic thiols. The desired products (**15a-d**) were obtained in moderate to good isolated yields (61-80%) by adopting the previously reported conditions. 2-Mercaptobenzoxazole reacted after stirring at 25 °C for 2 hours to form the expected product **15e** in 35% yield. Biologically interesting compounds such as protected thiopyranose and cysteine were subjected to the same reaction conditions. After 1 h at -78 °C, product **15f** and **15g** were collected in 54% and 80% yield, respectively. These results confirmed the very good reactivity of thiol substrates with the new hypervalent iodine reagent. Moreover, this tetrafluoroalkylation methodology was applied to the derivatization of selected drugs and steroids. For example, Captopril®, which is an angiotensin-converting enzyme (ACE) inhibitor,[14] used for the treatment of hypertension, was fluoroalkylated to provide product **15h** in 68% isolated yield. Thiocholesterol was also studied but was found to give the corresponding derivative **15i** only in low yield (12%).



Scheme 2. Synthesis of hypervalent iodine reagents **5** and **12**

These mild experimental conditions for tetrafluoroalkylation were really efficient for thiol substrates soluble in DCM. Some products (**15b,e,f,h**) were isolated in lower yields for no obvious reasons. Indeed, disulfide formation was not observed because the reactions were run at low temperature (78 °C) and full conversion of reagent **5** occured in all cases, as monitored by ^{19}F NMR. One can imagine that a slight excess of **5** could improve the yield.[6]

However, the main aim is to apply this reagent to label different biomolecules, usually non-soluble in chlorinated solvents. Thus, we first

focused on the tetrafluoroalkylation of non-protected 1-thio-β-D-glucose, a metabolite of glucose.[15] 1-Thio-β-D-glucose, obtained after the hydrolysis of the commercially available compound 1-thio-β-D-glucose tetraacetate using sodium methoxide,[16] was fluoroalkylated in a DCM/DMF (3:2) mixture for 1h at -78 °C and an additional hour at 25 °C to afford product **15i** (55%). These conditions could also be used for 4-thiouracil[17] to give the desired product **15k** in 28% yield (11% after recrystallization in MeOH). The lower yields can be explained by the low solubility of the reagent **5** in the solvent mixture DCM/DMF.

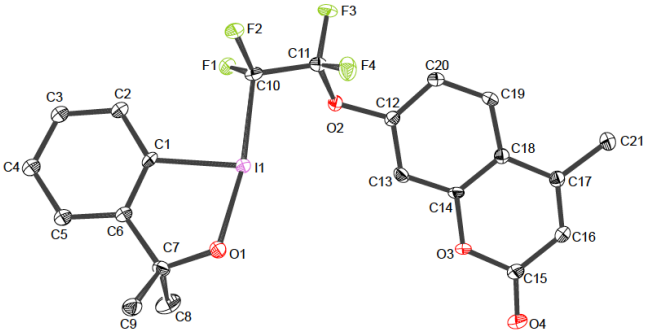
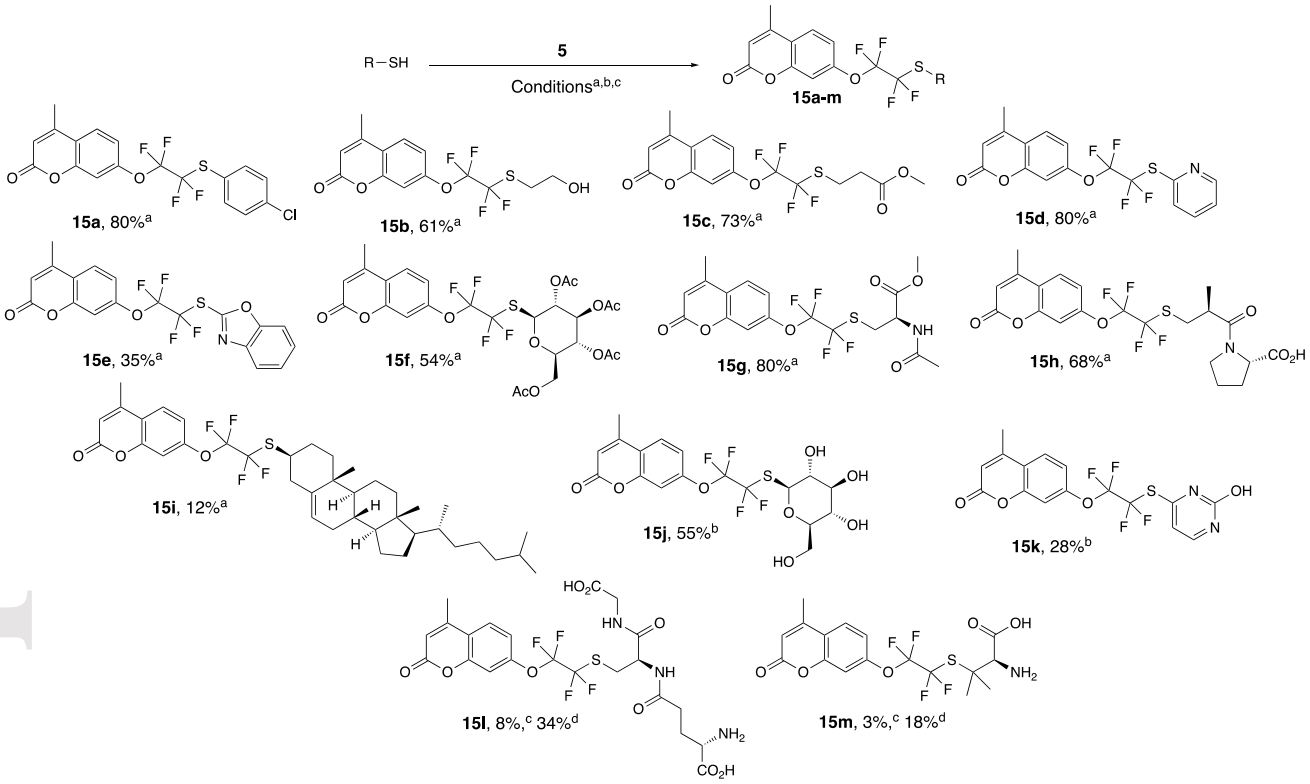


Figure 1. ORTEP drawing of reagent **5** (50 % probability for thermal ellipsoids with hydrogen atoms omitted for clarity). Selected bond lengths [Å]: C10-I1 2.295 (3), I1-O1 2.117 (2), C1-I1 2.136 (3); bond angles [°]: C10-I1-O1 170.60 (11), C1-I1-C10 91.50 (12), C1-I1-O1 79.43 (11); torsion angles [°]: C6-C1-I1-O1 12.3 (2), C2-C1-I1-C10 -8.6 (3), I1-C10-C11-O2 62.1 (3).[‡]



Scheme 3. Tetrafluoroalkylation of thiols transferring 4-methyl-7-tetrafluoroethoxycoumarin moiety. [a] **5** (1 eq, 0.05-0-1 M in DCM), R-SH (1 eq in DCM), -78 °C, 1 h (+ 2 h at 25 °C for **15e**). [b] **5** (1 eq, 0.05-0-1 M in DCM), R-SH (1 eq in DMF), -78 °C, 1 h then 25 °C, 1h. [c] **5** (1 eq in DMF), R-SH (1 eq) and $NaHCO_3$ (2 eq for **15m** and 3 eq for **15l**) in water, 25 °C, 12 h. Isolated as a trifluoroacetate salt. [d] Yields were determined by ^{19}F NMR using benzotrifluoride as an internal standard.

Finally, we successfully labeled cysteine residues by transferring the coumarin moiety of reagent **5**. The tripeptide glutathione (GSH) is the thiol compound present in the highest concentration in cells of all organs and fulfills many physiological functions.[18] Before we tried the reaction with reagent **5**, GSH was first reacted with iodine hypervalent trifluoromethylation reagent **2** in order to find suitable conditions. GSH was solubilized in aqueous basic solution (NaOH[19] or pyridine[20]) and treated with reagent **2** to afford trifluoromethylated GSH in quantitative yield (crude) after 12 h at 25 °C. These conditions were adopted for the reaction with **5**, however, no desired product was observed, probably due to the lack of solubility of the reagent. Promising results were observed when a solution of **5** (1 eq.) in DCM was added to a solution of GSH (1 eq.) and 1,1,3,3-tetramethylguanidine (TMG, 3 eq.) in MeOH. Stirring for 12 h at room temperature and 3 hours at 50 °C was necessary to convert all of the reagent, under which conditions the desired product **15l** was observed in 20% conversion, as determined by ^{19}F NMR spectroscopy using benzotrifluoride as an internal standard. The addition of TMG is essential as it leads to the formation of a salt soluble in organic solvents.[21] Better results (28% conversion) were obtained when a solution of **5** (1 eq.) in hot acetone that was added to a solution of GSH (1 eq.) in aqueous $NaHCO_3$ (3 eq). In the end, a further improvement was achieved using DMF instead of acetone, thus leading to 34% conversion. The crude product was purified by RP-HPLC to provide tetrafluoroalkylated glutathione **15l** (8% after isolation). Finally, these conditions were also used to label penicillamine, a pharmaceutical of the chelator class.[22] However, this substrate showed only low conversion and the corresponding product **15m** could be isolated in only 3% yield. Since the latter two products are isolated in form of their TFA-salt, the low isolated yields could partly be explained by the loss of crude material during filtration and purification.

Detection by UV absorbance and ^{19}F NMR

The coumarin moiety is generally used in fluorescence tagging.[23] This is particularly the case for coumarins containing an electron-donating group in position 7, such as 7-methoxy-4-methylcoumarin (λ_{ex} = 321 nm, λ_{em} = 385 nm in methanol).[24] The wavelengths of maximum absorbance of reagent **5** are λ_{abs} = 268 nm and λ_{abs} = 309 nm in ethanol. Unfortunately, when **5** was irradiated at these two wavelengths, no fluorescence could be observed. This is most likely due to the presence of the tetrafluoroethyl fragment attached to the oxygen atom strongly reducing delocalization of the oxygen lone-pairs into the coumarin backbone. For the same reason, no fluorescence was observed for *S*-perfluoroalky products **15**. We therefore decided to analyze the UV absorbance of the selected compounds **15h**, **15i**, **15j** and **15l**. Two absorbance maxima were observed at wavelengths λ_{abs} = 269 nm and λ_{abs} = 309 nm at a concentration of 10^{-4} mol.L^{-1}, as for the reagent **5**. As expected, at the same concentration, no absorbance was observed in the UV/visible range for the respective starting materials Captopril®, thiocholesterol, 1-thio-β-D-glucose and glutathione, consistent with the fact that none of these thiols contain a conjugated system. Thus, tagging them with the 4-methylcoumarin moiety allows to detect them easily by UV spectroscopy in up to micromolar concentrations. This specific chromophore owns two characteristic main λ_{abs}, but particularly the one at λ_{abs} = 309 nm distinguishes it from that of common aromatic compounds absorbing between 220 to 285 nm. The molar absorbance coefficients ε were calculated using classic methods, by measuring the absorbance of each sample at several concentrations at maximum absorbance wavelengths λ_{abs} = 269 nm (268 nm for **5**) and λ_{abs} = 309 nm and extrapolating the values using the Beer-Lambert law.[25] Values of ε for each compounds range from 9500 to 12100 L.mol^{-1}.cm^{-1} at 269 nm and from 7950 to 9100 L.mol^{-1}.cm^{-1} at 309 nm (Table **1**). The ε values of **5** are slightly higher than for the other compounds, probably due to the additional benzene ring. The values are consistent with the literature but lower than for 7-methoxy-4-methylcoumarin (ε = 13410 at λ_{abs} = 321 nm in methanol) and are likely to reflect the electron-withdrawing effect of the tetrafluoroethoxy group. More information about measurements and calculations of UV absorbance are presented in the supporting information.

In addition to UV spectroscopy, the new products can be easily detected by ^{19}F NMR spectroscopy. In fact, for **15a-e** and **15k**, the ^{19}F NMR spectrum consists of a pair of triplets around -85.0 ppm for the difluoromethylene group linked to the oxygen atom and -90 ppm for the one linked to the sulfur atom with a coupling constant ($^3J_{FF}$) of 6.0 Hz. The spectrum of reagent **5** is almost identical with a triplet at -84.3 ppm and another triplet at -97.5 ppm, due to the interaction with the iodane atom.

Table 1. Molar absorbance coefficients ε of **5**, **15h**, **15i**, **15j** and **15l** at λ = 268-269 nm and λ = 309 nm.

	λ_{max} (nm)	ε (L/mol/cm)	λ_{max} (nm)	ε (L/mol/cm)
Reagent **5**	268	12067	309	9081
15h	269	9632	309	8121
15i	269	9499	309	7957
15j	269	9734	309	8101
15l	269	9556	309	8135

The two carbohydrate derivatives **15f** and **15j** give rise to a pair of ddd around -86 ppm and -91 ppm because the two fluorine atoms in each pair are diastereotopic. This is also true for compounds **15h** and **15m** for which, however, a quadruplet or a multiplet, respectively, is observed around -85 ppm for the O-CF_2-group and a doublet of triplets ($^2J_{FF}$ = 220-230 Hz and $^3J_{FF}$ = 6-7 Hz) between -86 ppm and -92 ppm for the other two fluorine atoms.

Conclusions

We have synthesized a new hypervalent iodine reagent **5** containing a 4-methyl-7-tetrafluoroethoxycoumarin functionality, which can be transferred to thiols under very mild conditions. Some of the new materials derive from biologically relevant compounds and have been characterized by UV absorbance and by fluorine NMR spectroscopy. The syntheses and applications of further hypervalent iodine reagents, containing a linker between chromophore and tetrafluoroethyl chain (*e.g.*: PEG), for the tagging of functional groups amenable to optical spectroscopic methods are still in progress in our laboratory.

Supplementary Material

Supporting information for this article is available on the WWW under http://dx.doi.org/10.1002/MS-number. It collects experimental protocols and characterization data for new compounds, crystallographic details and copies of ^{1}H, ^{13}C, ^{19}F NMR spectra.

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Note

‡ CCDC-1486206 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.

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