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PAPER

Design, synthesis and photoactivation studies of fluorous photolabels†

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Two fluorous diazirine photolabels were designed, synthesized and subjected to photoactivation studies. The photoactivation studies revealed an unexpected photoreaction when the fluorous tag was directly connected to the diazirine ring, leading to the formation of a fluorous alkene. The more efficient photolabel of the two was identified as a flexible precursor for target specific photoaffinity labels for fluorous proteomics by adding appropriate ligands depending on the target protein subset. As a proof of feasibility, mannose residues were added to the photolabel making it a potential photoaffinity label to tag proteins that bind mannose.

Introduction

In proteomics, fractionation techniques are widely applied and usually involve an affinity-based enrichment step prior to mass spectrometry analysis. Biotin is one of the most common affinity tags taking advantage of the very strong biotin–streptavidin interaction.[1] Immobilized metal ion affinity chromatography (IMAC),[2] lectin affinity chromatography[3] and enrichment of an alkyne-tagged proteome *via* the Huisgen 1,3-dipolar cycloaddition[4] are alternative affinity-based separations which have recently found broad use. Nevertheless, there is a dire need for additional fractionation techniques complementing the current ones that suffer from shortcomings such as impermeability, incomplete elution from affinity column[5] and complication of MS/MS spectral interpretation due to undesired fragmentation of the affinity tag.[6]

Fluorous tags have emerged as a useful purification tool in catalysis,[7] combinatorial chemistry,[8] natural product synthesis,[9,10] microarrays,[11] peptide labeling, peptide purification and proteomics.[12,13] Fluorous tags are commercially available, relatively inexpensive, highly inert to most reaction conditions and do not complicate MS/MS spectral interpretation due to their stability against fragmentation in the mass spectrometer.[13] Recently, the concept of fluorous proteomics has been introduced since fluorous tags have proven to be an effective enrichment moiety for peptides, proteins and small molecules of biological origin.[11–14]

Photoaffinity labeling has been a valuable biochemical tool for studying the interaction between a ligand and its receptor. Although there has been significant success in the use of fluorous tags in the enrichment of peptides[12,13] and proteins,[11] there has been limited research in the development of fluorous photoaffinity probes. Song and Zhang reported the synthesis of the first class of fluorous photolabeling agents, in which the photoreactive diazirine ring is incorporated on the fluorous tag (Fig. 1).[15] Meanwhile, Burkard and coworkers reported the design and synthesis of fluorinated photoaffinity probe attached to V-ATPase inhibitors.[16] Although these previous works showed the efficiency of fluorous probe–fluorous stationary phase interaction, its use in protein labeling experiments has not been reported thus far.

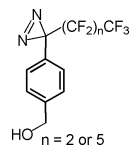

Fig. 1 Previously reported fluorous photolabel.[15]

Herein, we describe the design and synthesis of two diazirine-containing fluorous photolabels, **1** and **2** (Fig. 2). Photolabel **1**, which is closely related to the previously reported photolabel,[15] consists of a diazirine ring directly installed in the fluorous tag, while in probe **2**, the diazirine ring and the fluorous tag are apart from each other.

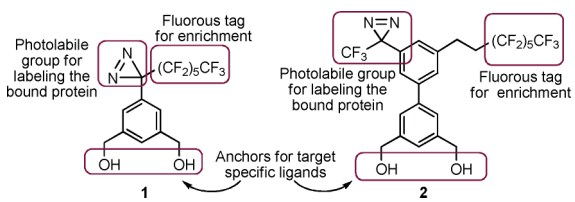

Fig. 2 Design of fluorous photolabels **1** and **2**.

Furthermore, we report photoactivation studies of diazirines **1** and **2**. These studies demonstrate that fluorous probe **1** unexpectedly eliminates to form a fluorous alkene as the major product when the fluorous chain is directly connected to the diazirine ring.

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These results suggest that probe **1** or a fluorinated photoaffinity probe design similar to the probe developed by Song and Zhang probably leads to deactivation upon UV irradiation, rendering it unavailable to tag the protein. In contrast, photolabel **2** with its fluorous tag remote from the diazirine ring undergoes photoactivation cleanly to the desired product. Therefore, photolabel **2** can serve as a flexible precursor for the synthesis of fluorous and target-specific photoaffinity labels by attaching suitable ligands to the benzyl alcohol groups. As proof of principle, the synthesis and characterization of potential target-specific, mannose-containing photolabel **3** is discussed.

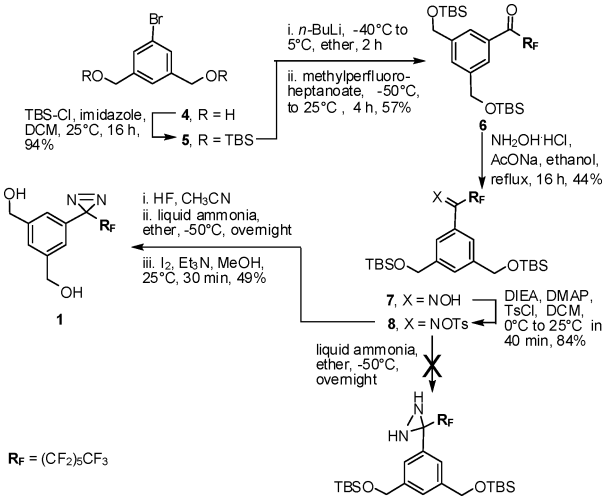
Results and discussion

Design of fluorous photolabels

In photoaffinity labeling, a ligand linked to a photoreactive group is bound non-covalently to a specific receptor in the proteome. Upon exposure to light, a covalent bond is formed between the photoreactive group and the receptor. The labeled protein is then separated from the unlabeled proteins using the affinity tag that the photolabel is equipped with. Analogously, the design of fluorous photolabels **1** and **2** includes the integration of three essential moieties in one molecule (Fig. 2): (a) alcohol functions for the attachment of ligands that specifically bind to the receptor of interest in a proteome mixture, (b) a diazirine ring for the photo-induced covalent cross linking between the photolabel and its receptors and (c) a fluorous tag for the straightforward isolation of the photolabeled targets from the proteome mixture. Two alcohol residues were incorporated in photolabels **1** and **2** to induct avidity effect by covalent attachment of two ligands.

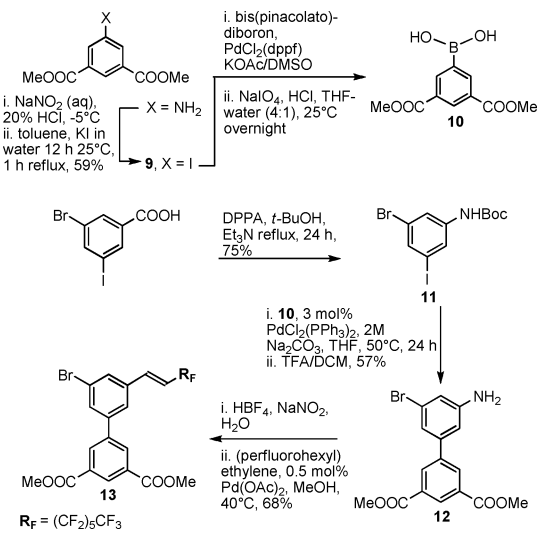
Synthesis of photolabels

For the synthesis of photolabel **1** (Scheme 1), diol **4** was protected as its *tert*-butyldimethyl silyl (TBS) ether **5**. Lithiation of **5** by *n*-butyllithium followed by the addition of methyl perfluoroheptanoate gave ketone **6** in moderate yield. Ketone **6** was converted to the corresponding oxime **7** and subsequently tosylated to the tosyl oxime **8** in good yield. The attempts to form the diaziridine *via* direct nucleophilic attack of tosyloxime **8** with ammonia failed. The bulky nature of the two TBS groups and the fluorous tag in **8** could be the possible reason for the unexpected failure. Thus a synthetic route was devised involving a TBS deprotection prior to the diaziridine ring formation. The tosyloxime **8** was treated with HF for TBS deprotection. Then, the cyclization step with ammonia yielded the desired diaziridine, which was oxidized using iodine to give the diazirine **1** in 49% yield over three steps.



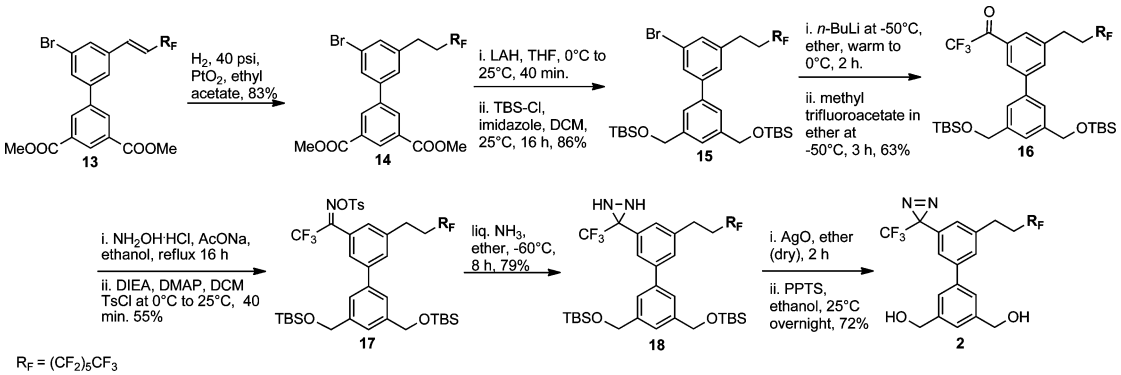
Scheme 1 Synthesis of photolabel **1**.

The synthesis of photolabel **2** began with the preparation of aryl iodide **9** through the diazo intermediate derived from dimethyl 5-aminoisophthalate. The aryl iodide **9** was subjected to Miyaura borylation followed by hydrolysis to give the boronic acid **10** (Scheme 2). In parallel, 3-bromo-5-iodobenzoic acid was refluxed with diphenyl phosphoryl azide (DPPA) and triethylamine to generate the corresponding aryl isocyanate, which underwent Curtius rearrangement with *t*-butyl alcohol to give the Boc-protected amine **11**. The boronic acid **10** was coupled with the iodide **11** *via* Suzuki cross-coupling reaction, followed by Boc deprotection under acidic condition to give amine **12** in good yield over two steps. Amine **12** was converted to its corresponding arenediazonium salt using sodium nitrite and HBF_4, which was followed by Heck coupling of the arenediazonium salt with (perfluorohexyl)ethylene to give the alkene **13**.[17]



Scheme 2 Synthesis of alkene **13**.

The next crucial step was the selective reduction of the electron-deficient carbon–carbon double bond of the aryl bromide **13** *via* catalytic hydrogenation. Alkenyl-substituted aryl bromides undergo debromination under the widely used hydrogenation conditions of hydrogen and palladium on charcoal (Pd/C). Catalytic hydrogenation of perfluorinated alkenyl-aryl bromides to the corresponding saturated aryl bromides has been previously reported to succeed with rhodium on charcoal (Rh/C) only at high hydrogen pressure of 50 bar.[17] Alternatively, alkenyl-substituted aryl bromides have been reported to be hydrogenated with Adam's catalyst (Pt_2O).[18] However, previous attempts to reduce perfluorinated alkenes under similar reaction conditions failed due to the electron-deficient double bond.[17] After exploring a number of reaction conditions, Pt_2O in ethyl acetate at 40 psi (2.75 bar) hydrogen pressure was found to provide the



Scheme 3 Synthesis of photolabel **2**.

maximal yield of **14**. Other combinations of solvent and hydrogen pressures resulted in the formation of debrominated product or the unreacted alkene. This catalytic hydrogenation is an interesting find due to the aforementioned importance of the molecules with fluorous tags and aryl-bromide residues that help in further derivatization. Using these optimized reaction conditions, alkene **13** was hydrogenated to the diester **14** in 83% yield. Diester **14** was subjected to LAH reduction and the resulting diol was protected as the TBS ether **15** in good yield. Compound **15** was reacted with *n*-butyllithium followed by methyl trifluoroacetate to furnish ketone **16** in moderate yield. Treatment of ketone **16** with hydroxylamine hydrochloride and sodium acetate followed by tosylation furnished tosyl oxime **17** in moderate yield. The tosyl oxime **17** was cyclized with liquid ammonia to the diaziridine **18** in good yield. Oxidation of the diaziridine **18** using AgO followed by TBS deprotection yielded the desired photolabel **2** in good yield (Scheme 3).

Photoactivation studies of photolabels 1 and 2

The photoactivation reactions were conducted in deuterated methanol with UV light irradiation (λ > 320 nm) and the reactions were monitored by ^{19}F-NMR at various time intervals. As expected from previous studies,[19,20] upon irradiation, diazirine **2** cleanly reacted within 5 min to methyl ether **19** and linear diazo compound **20** (Fig. 3). On further exposure to UV light, the linear diazo compound **20** was converted to the methyl ether

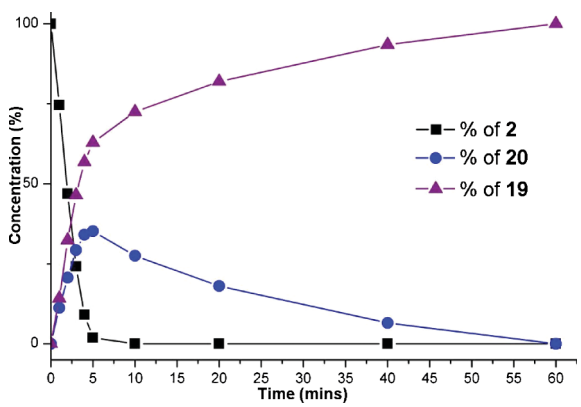


Fig. 3 Formation of **19** and **20** from **2** during the course of photoactivation in relative percent distribution, as estimated by ^{19}F-NMR.

19. ^{19}F-NMR and LCMS (Fig. 4, A—right and B—bottom) of the final sample indicated that ether **19** was the only major product. Further purification and analysis of the photo irradiated final sample confirmed the formation of one single product **19**. The photoactivation of photolabel **1**, which has structural similarity with the work reported by Song and Zhang,[15] was investigated next (Scheme 4). Close observation of the chemical shift of the trifluoromethyl group in ^{19}F-NMR (Fig. 4, A—left) over the course of the photoreaction suggested the decomposition of photolabel **1** into multiple products. HPLC analysis (Fig. 4, B—top) of the photoirradiated sample identified three distinct peaks. The two most abundant photoproducts were isolated by reverse phase HPLC and subjected to HRMS. The mass of one of the minor products matched the predicted photoactivation product, ether **21**. However, the major product had a mass corresponding to a molecular formula of $C_{16}H_9D_3F_{12}O_3$, which led to the conclusion that it was **21** – DF. Further characterization using 1D and 2D NMR (^{19}F-gCOSY, ^{19}F-NOESY, Fig. 5) indicated that the major photoactivation product with the mass equal to **21** – DF is the polyfluorinated alkene **22**. In recent literature, a combination of ^{19}F-gCOSY and ^{19}F-NOESY has proved to be an efficient method for structural analysis of perfluorinated straight chains.[21] ^{19}F-gCOSY shows the $^4J_{FF}$ correlations where as ^{19}F-NOESY shows both $^4J_{FF}$ and $^3J_{FF}$ correlations. By using these two techniques in tandem, sequencing of straight chain fluorous tag is made straightforward and reliable. As shown in Fig. 5, ^{19}F-NOESY of **22** gives $^4J_{FF}$ and $^3J_{FF}$ correlations while ^{19}F-gCOSY shows only the $^4J_{FF}$ correlations except for the vinyl fluorine due to its connection to a sp^2 carbon.[21] The third minor photoproduct was not characterized due to its very low availability.

It has been suggested that photoaffinity labeling is highly dependent on the generation of a reactive intermediate ideally undergoing clean insertion reactions. If the lifetime of the reactive intermediate is too long and the ligand–protein exchange rate is high, then the photolabel dissociates from the binding site and possibly attaches to parts of the protein other than the binding site or even other proteins, resulting in an unspecific labeling also termed pseudo-affinity labeling.[22] In order to avoid pseudo-affinity labeling, the photochemically generated intermediate should be highly reactive, very short lived and not susceptible to intramolecular rearrangements to much less reactive species.[22] The unexpected outcome of the photoreaction of probe **1** suggests that the predicted carbene-insertion reaction is not the major pathway and thus probe **1** has potential for pseudoaffinity labeling.[23] These

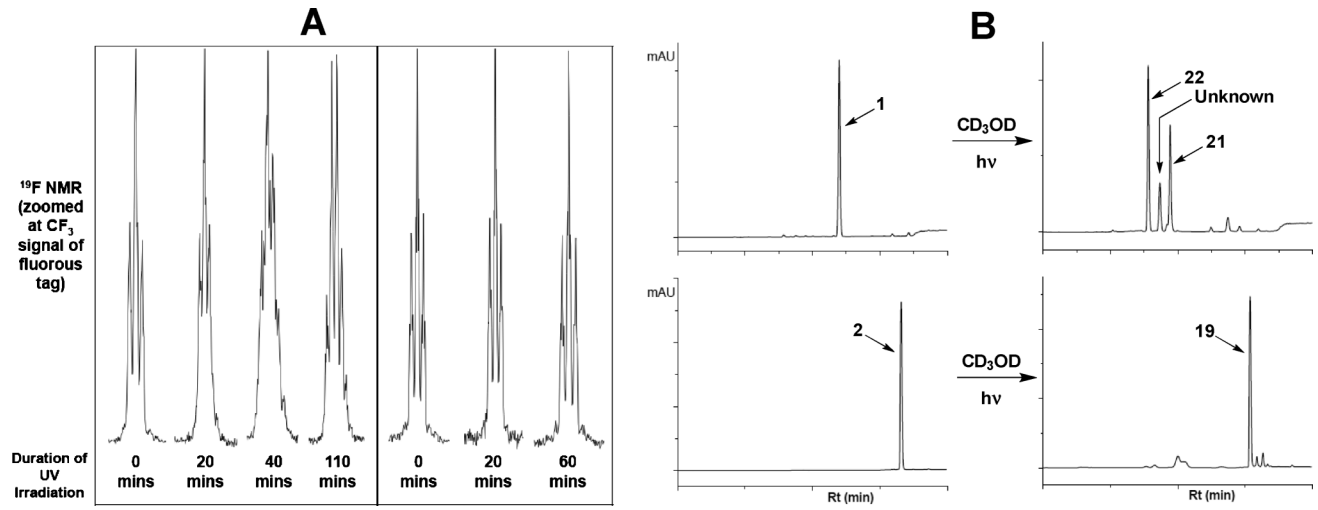
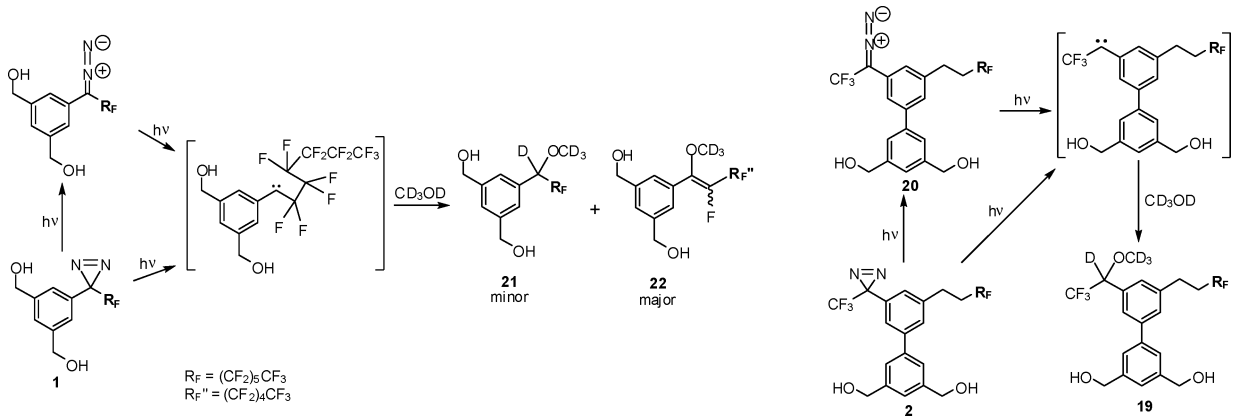


Fig. 4 (A) ^{19}F NMR (zoomed at CF_3 signal of fluorous tag) of **1** (left) and **2** (right) at different time intervals of UV irradiation (B) HPLC trace of **1** (top) and **2** (bottom) before (left) and after (right) UV irradiation.



Scheme 4 Photoactivation pattern of the photolabel **1** and **2**.

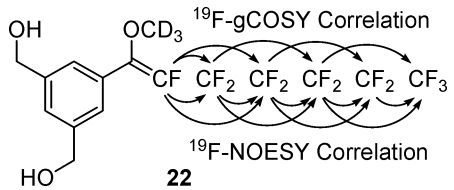


Fig. 5 The correlations observed with ^{19}F-gCOSY and ^{19}F-NOESY of photoproduct **22**.

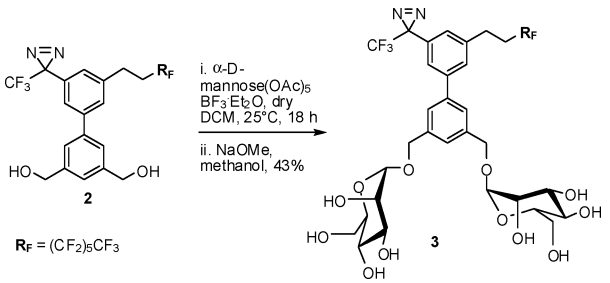
results clearly distinguish compound **1** from the conventional trifluoromethyldiazirine probe **2**.

Synthesis of mannose photoaffinity probe 3

The photoactivation studies suggest that photolabeling agent **1** and the previously reported photolabels (Fig. 1), in which the diazirine ring is directly incorporated on the fluorous chain, would possibly lead to poor and/or nonspecific protein labeling and consequently to erroneous or misleading data interpretations. Therefore, for deriving target specific photoaffinity probes, it is essential to use a predictable photolabel like **2**. Since proteins that bind to mannose residues occupy a central role in glycobiology,[24] we decided to assess the derivatization capacity of photolabel **2** using mannose ligands. To achieve glycosylation of the diol **2** with a glycosyl-donor like mannose pentaacetate, it is essential to use boron trifluoride etherate to activate the glycosyl donor. The glycosylation reaction yielded the desired bis-glycosylated product with the intact diazirine ring as the major product. Subsequently, the crude material was subjected to acetate deprotection using sodium methoxide and purified by preparative HPLC to obtain the desired fluorous photolabel **3** in moderate yield over two steps (Scheme 5). The coupling constants and 2D NMR indicated that the bis-glycosylation was diastereoselective, yielding the α-anomer exclusively. The alcohols in the photolabel can be further converted to amine and carboxylic acid groups as reported by Song and Zhang,[15] for efficient attachment of various ligands targeting different receptors.

Enrichment of photoaffinity probe 3 by FSPE

To evaluate the enriching ability of the photolabel using fluorous interactions a mixture of peptide along with the photolabel **3** was loaded on to a bed of fluorous silica gel in a pipette column (fluorous bed: 40 × 6 mm) (Fig. 6). Initially the loaded fluorous short column was flushed with water to elute out the peptide (Fig. 6, B) and followed by elution with methanol to get the



Scheme 5 Synthesis of fluorous photoaffinity label **3**.

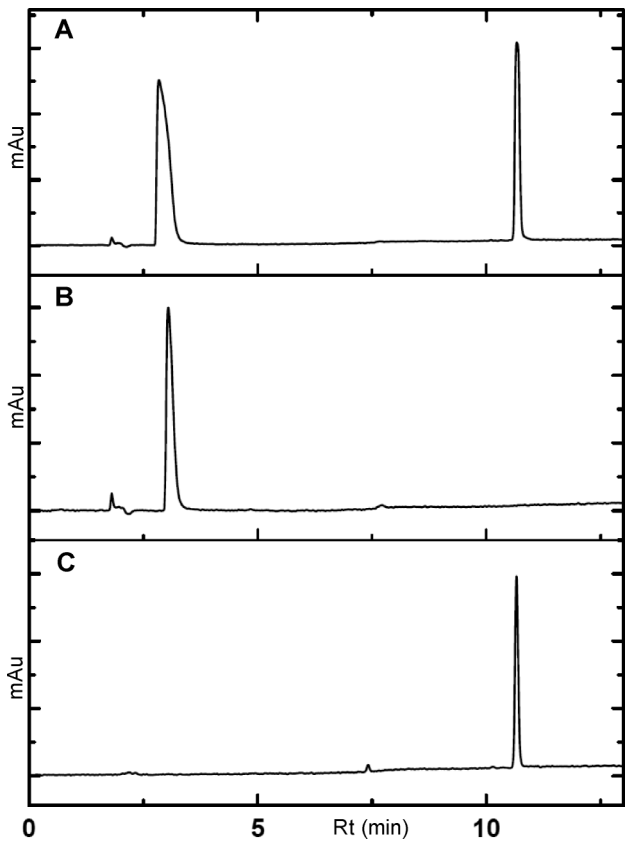


Fig. 6 Separation of fluorous photolabel **3** from unlabeled peptide using fluorous silica gel: HPLC trace of (A) mixture of peptide and photolabel **3** (B) fraction eluted by 100% water (C) fraction eluted by 100% methanol.

fluorous photolabel **3** (Fig. 6, C). These data show the potency of fluorous separation just by using a short plug of fluorous silica gel. Further it has already been demonstrated that short fluorous tags can be used for the efficient enrichment of peptides and proteins using fluorous derivatized silica gel or fluorous derivatized glass plates.[12,13,11] These previous reports coupled with the aforementioned enrichment data show that **3** and the derivatives of **2**, with different ligands, can prove to be efficient enrichment moieties in specific photolabeling of macromolecules of biological origin.

Conclusions

We have designed and synthesized two multifunctional photolabels **1** and **2**. Photoactivation studies with labeling agents **1** and **2** revealed that photolabel **1**, in which the diazirine ring is directly incorporated in the fluorous tag, mainly undergoes a self-deactivating elimination to yield a polyfluorinated alkene. This potential self-deactivation of the transient carbene suggests that photoprobe **1** is not ideal for specific photolabeling of protein or proteome samples. Conversely, photolabel **2** was found to be a reliable photolabel for derivatization with target specific ligands. As a proof of feasibility to derivatize photolabel **2**, mannose-containing photoaffinity probe **3** was synthesized and its efficacy in fluorous enrichment was evaluated. On the route to synthesize photolabel **2**, we have optimized an efficient and low hydrogen pressure method to hydrogenate a carbon-carbon double bond on a perfluorinated alkenyl-aryl bromide.

Experimental section

General information

All NMR experiments were performed on a Varian Inova 400 MHz spectrometer (^{1}H at 400, ^{13}C at 100 and ^{19}F at 376 MHz). Chemical shifts (in ppm, δ scale) are reported using solvent peak as internal standard. The ESI high resolution mass spectra were recorded using Agilent G1969A mass detector with time-of-flight (TOF) analyzer. Thin layer chromatography was performed on EMD silica gel 60-F plates and the spots were visualized with UV light or iodine stain. Purification of reaction crude was done by flash chromatography using EMD silica gel (230–400 mesh). The elemental analysis were carried out at Atlantic Microlab, Inc., Georgia. The melting points of compounds were measured on Electrothermal Mel-Temp 3.0. The photoactivation studies were done using Orel Instruments housing with Osram 150 W XBO xenon short-arc lamp. The light source was fitted with a Schott filter WG-320 to eliminate UV lights below 320 nm. The photoactivation studies were done as reported by Brunner and Richards.[19]

(5-Bromo-1,3-phenylene)bis(methylene)bis(oxy)bis(*tert*-butyldimethylsilane) (5). To a solution of (5-bromo-1,3-phenylene)-dimethanol[25] **4** (1.21 g, 5.57 mmol) in dry dichloromethane (35 mL), TBSCl (2.10 g, 13.9 mmol) and imidazole (1.13 g, 16.6 mmol) were added and stirred at room temperature for 16 h. The reaction mixture was quenched with sat. ammonium chloride solution and extracted with dichloromethane. The organic layer was dried over anhydrous sodium sulfate and concentrated. The resulting crude was purified by silica column chromatography (19 : 1, hexanes and ethyl acetate) to give **5** (2.33 g, 94%) as a colorless oil. R_f 0.72 (19 : 1 hexanes and ethyl acetate). δ_H (400 MHz; $CDCl_3$) 7.32 (2H, s), 7.19 (1H, s), 4.70 (4H, s), 0.93 (18H, s), 0.09 (12H, s). δ_C (100 MHz; $CDCl_3$) 143.8, 127.7, 122.5, 122.3, 64.5, 26.1, 18.6, −5.0. Elemental analysis (CHN) calculated for $C_{20}H_{37}BrO_2Si_2$ (%) C, 53.9; H, 8.4; N, 0.0; found C, 54.15; H, 8.4; N, 0.0.

1-(3,5-Bis((*tert*-butyldimethylsilyloxy)methyl)phenyl)-2,2,3,3,4,4,5,5,6,6,7,7,7-tridecafluoroheptan-1-one (6). To a solution of **5** (1.94 g, 4.35 mmol) in dry diethyl ether (30 mL) under argon, *n*-butyllithium (4.35 mL of 2.5 M soln in hexane) was added dropwise at −40 °C. The reaction mixture was warmed to 10 °C and stirred for 2 h, then cooled back to −50 °C and methyl perfluoroheptanoate (6.57 g, 17.4 mmol) was added dropwise. The solution was warmed to room temperature and

stirred for 4 h. Later the reaction mixture was quenched with saturated ammonium chloride solution and extracted with ether. The organic layer was dried over anhydrous sodium sulfate and concentrated. The resulting crude was purified by silica column chromatography (100% hexanes) to yield **6** (1.76 g, 57%) as a colorless oil. δ_H (400 MHz; $CDCl_3$) 7.91 (2H, s), 7.62 (1H, s), 4.79 (4H, s), 0.94 (18H, s), 0.11 (12H, s). δ_C (100 MHz; $CDCl_3$) 183.6 (t, J = 25.8 Hz), 142.9, 130.6, 128.3, 126.5, 120.9–104.9 (m), 64.4, 26.1, 18.6, −5.2. δ_F (376 MHz; $CDCl_3$) −81.3 (3F), −113.1 (2F), −121.3 (2F), −121.6 (2F), −123.2 (2F), −126.6 (2F).

1-(3,5-Bis((*tert*-butyldimethylsilyloxy)methyl)phenyl)-2,2,3,3,4,4,5,5,6,6,7,7,7-tridecafluoroheptan-1-one oxime (7). A suspension of hydroxylamine HCl (0.687 g, 9.88 mmol) and sodium acetate trihydrate (2.02 g, 14.8 mmol) in ethanol (7.5 mL) was stirred for 15 min and allowed to settle. The supernatant from this mixture was added dropwise to a solution of **6** (1.76 g, 2.47 mmol) in ethanol (7.5 mL) and refluxed for 16 h. The reaction was cooled and solvent was evaporated under vacuum. The resulting residue was extracted with ether, washed with water and concentrated after drying the organic layer over anhydrous sodium sulfate. The crude was purified by silica column chromatography (19 : 1, hexanes and ethyl acetate) to give **7** (0.788 g, 44%) as a colorless oil. R_f 0.36 (9 : 1, hexanes and ethyl acetate). δ_H (400 MHz; $CDCl_3$) 9.53 (1H, s), 7.38 (1H, s), 7.22 (2H, s), 4.77 (4H, s), 0.93 (18H, s), 0.09 (12H, s). δ_C (100 MHz; $CDCl_3$) 149.0 (t, J = 24.7 Hz), 141.9, 126.8, 125.9, 125.4, 118.8–108.1 (m), 64.9, 26.0, 18.6, −5.1.

1-(3,5-Bis((*tert*-butyldimethylsilyloxy)methyl)phenyl)-2,2,3,3,4,4,5,5,6,6,7,7,7-tridecafluoroheptan-1-one *O*-tosyl oxime (8). To a solution of **7** (0.207 g, 0.28 mmol) in dichloromethane (0.5 mL), diisopropylethylamine (44.1 mg, 0.34 mmol) and 4-dimethylaminopyridine (2.6 mg, 21 μmol) were added under argon. The reaction mixture was cooled to 0 °C and tosyl chloride (65 mg, 0.34 mmol) was added in small portions. The solution was warmed to room temperature and stirred for 40 min and the reaction mixture was quenched with water and extracted with dichloromethane. After being dried over anhydrous sodium sulfate, the organic layer was concentrated. The resulting crude was purified using silica column chromatography (19 : 1, hexanes and ethyl acetate) to give **8** (0.210 g, 84%) as a colorless oil. R_f 0.65 (9 : 1, hexanes and ethyl acetate). δ_H (400 MHz; $CDCl_3$) 7.84 (2H, d, J = 8.3 Hz), 7.40 (1H, s), 7.35 (2H, d, J = 8.3 Hz), 7.10 (2H, s), 4.75 (4H, s), 2.45 (3H, s), 0.92 (18H, s), 0.09 (12H, s). δ_C (100 MHz; $CDCl_3$) 155.9 (t, J = 26.2 Hz), 146.2, 142.6, 131.6, 129.9, 129.5, 126.5, 125.0, 124.5, 118.8–105.7 (m), 64.5, 26.1, 21.8, 18.5, −5.1.

(5-(3-(Perfluorohexyl)-3*H*-diazirin-3-yl)-1,3-phenylene)dimethanol (1). To a solution of **8** (68 mg, 77.1 μmol) in acetonitrile, 50% aqueous solution of HF (0.3 mL) was added slowly and stirred for 4 h. The reaction was quenched with saturated sodium bicarbonate solution, extracted with ethyl acetate and concentrated after drying over anhydrous sodium sulfate. The crude was dissolved in dry ether (1 mL) under argon and cooled to −50 °C. Ammonia (around 2 mL) was condensed in the cooled flask and left to stir at this temperature for 8 h. Ammonia was evaporated by warming the reaction mixture to room temperature followed by the addition of water and extraction with ethyl acetate. The organic layer was dried with anhydrous sodium sulfate and concentrated under vacuum. This crude was dissolved in methanol (1.5 mL) and triethylamine (60 mg) was added to it, followed by the dropwise addition of iodine solution in methanol (30 mg mL^{-1}) until the color of iodine persisted. The reaction proceeded for another 30 min and the solvent was evaporated, followed by the addition of water and extraction with ethyl acetate. The organic layer was dried over anhydrous sodium sulfate, concentrated and the resulting crude was purified using silica column chromatography (97 : 3, dichloromethane and methanol) to give **1** (18.9 mg, 49%) as a white solid. R_f 0.51 (23 : 2, dichloromethane and methanol). Mp: 69–70 °C. δ_H (400 MHz; CD_3OD) 7.43 (1H, s), 7.33 (2H, s), 4.85 (2H, s), 4.61 (4H, s). δ_C (100 MHz; CD_3OD) 144.9, 130.4, 128.3, 126.3, 119.6–109.0 (m), 64.5, 29.1 (t, J = 28.6 Hz). δ_F (376 MHz; CD_3OD) −82.85 (3F), −110.95 (2F), −121.50 (2F), −123.39 (2F), −124.28 (2F), −127.77 (2F). HRMS (ESI^+) for $[M + Na]^+$; calculated: 519.03487, found: 519.03391 (error = −1.84 ppm).

***tert*-Butyl 3-bromo-5-iodophenylcarbamate (11).** To a solution of 3-bromo-5-iodobenzoic acid (5.0 g, 15.3 mmol) in *t*-butanol (25 mL), triethylamine (2.01 g, 19.9 mmol) and diphenylphosphorylazide (4.63 g, 16.8 mmol) were added and refluxed for 24 h under argon. The reaction mixture was concentrated under vacuum, extracted with diethyl ether, and washed with 10% NaOH solution, water (2 times) and then brine solution. After drying over anhydrous sodium sulfate, the organic layer was concentrated and the crude was purified using silica column chromatography (9 : 1, hexanes and ethyl acetate) to give **11** (4.57 g, 75%) as a white solid. R_f 0.72 (4 : 1, hexanes and ethyl acetate). Mp: 97–98 °C. δ_H (400 MHz; $CDCl_3$) 7.65 (1H, s), 7.54 (1H, s), 7.48 (1H, s), 6.44 (1H, s), 1.49 (9H, s). δ_C (100 MHz; $CDCl_3$) 152.2, 140.7, 134.2, 125.8, 123.2, 120.8, 94.3, 81.7, 28.5. HRMS (ESI^+) for $[M + H]^+$; calculated: 397.92471, found: 397.92424 (error = −1.17 ppm).

Dimethyl 3′-amino-5′-bromobiphenyl-3,5-dicarboxylate (12). To an argon back-flushed flask, tetrahydrofuran (150 mL), 2 M sodium carbonate solution (45 mL), **10** (10.95 g, 46.0 mmol), **11** (11.5 g, 28.8 mmol) and bis(triphenylphosphine)palladium(II) dichloride (1.01 g, 1.44 mmol) were added and refluxed for 24 h. The reaction mixture was cooled, extracted with ethyl acetate and washed with water. The organic layer was dried with anhydrous sodium sulfate and concentrated. The resulting crude was dissolved in dichloromethane (60 mL) followed by the addition of trifluoroacetic acid (20 mL) and stirred for an hour. The reaction mixture was concentrated and purified by silica column chromatography (7 : 3, hexanes and ethyl acetate) to give the product **12** (5.97 g, 57%). R_f 0.37 (4 : 1, hexanes and ethyl acetate). Mp: 195–197 °C δ_H (400 MHz; CD_3OD) 8.59 (1H, t, J = 1.4 Hz), 8.38 (2H, d, J = 1.5 Hz), 7.03 (1H, t, J = 1.5 Hz), 6.93 (1H, t, J = 1.6 Hz), 6.90 (1H, t, J = 1.7 Hz), 3.98 (6H, s). δ_C (100 MHz; CD_3OD) 167.55, 151.93, 142.95, 142.84, 133.03, 132.74, 130.46, 124.72, 119.49, 118.35, 113.23, 53.18. HRMS (ESI^+) for $[M + Na]^+$; calculated: 385.99984, found: 385.99896 (error = −2.29 ppm).

Dimethyl 3′-bromo-5′-(3,3,4,4,5,5,6,6,7,7,8,8,8-tridecafluorooct-1-enyl)biphenyl-3,5-dicarboxylate (13). To a 50% aqueous solution of tetrafluoroboric acid (70 mL) and water (70 mL) at 0 °C, **12** (5.97 g, 16.4 mmol) was added. A solution of sodium nitrite (1.69 g, 24.6 mmol) in water (10 mL) was added dropwise to the reaction mixture over 10 min and warmed to room temperature.

After 16 h the reaction mixture was filtered and washed with 5% tetrafluoroboric acid followed by diethyl ether. Upon drying the residue under argon, it was suspended in degassed methanol (160 mL) followed by the addition of palladium(II) acetate (55 mg, 245 μmol) and perfluorohexylethylene (7.94 g, 22.9 mmol). The suspension was warmed to 40 °C and stirred until gas evolution ceased and then stirred for an additional 30 min. The reaction mixture was concentrated, extracted with ether and washed with water followed by drying the organic layer over anhydrous sodium sulfate and concentration under vacuum. The crude was purified using silica column chromatography (17 : 3, hexanes and ethyl acetate) to give the product **13** as a white solid (7.7 g, 68%). R_f 0.53 (4 : 1, hexanes and ethyl acetate). mp: 156–157 °C. δ_H (400 MHz; $CDCl_3$) 8.68 (1H, s), 8.39 (2H, s), 7.77 (1H, s), 7.65 (1H, s), 7.60 (1H, s), 7.17 (1H, d, J = 16.0 Hz), 6.36–6.22 (1H, m), 3.97 (6H, s). δ_C (100 MHz; $CDCl_3$) 166.1, 142.0, 139.9, 138.20 (t, J = 9.3 Hz), 136.3, 132.4, 131.9, 131.8, 130.5, 130.0, 125.6, 124.0, 123.0–107.3 (m), 117.0 (t, J = 23.3 Hz), 52.8. δ_F (376 MHz; $CDCl_3$) −81.24 (3F), −111.85 (2F), −121.99 (2F), −123.27 (2F), −123.44 (2F), −126.56 (2F). Elemental analysis (CHN, F) calculated for $C_{24}H_{14}BrF_{13}O_4$ (%) C, 41.6; H, 2.0; N, 0.0; F, 35.6; found C, 41.9; H, 1.9; N, 0.0; F, 35.9.

Dimethyl 3′-bromo-5′-(3,3,4,4,5,5,6,6,7,7,8,8,8-tridecafluorooctyl)biphenyl-3,5-dicarboxylate (14). To a solution of **13** (1.59 g, 2.30 mmol) in ethyl acetate (50 mL), platinum oxide (26 mg, 115 μmol) was added and the reaction mixture was shaken in a hydrogenator under 40 psi of hydrogen gas. After 3 days, another portion of platinum oxide (26 mg, 115 μmol) was added and the reaction was shaken under 40 psi for another 3 days and the reaction mixture was filtered and concentrated. The crude was purified using silica column chromatography (4 : 1, hexanes and ethyl acetate) to give the product **14** (1.32 g, 83%) as a white solid. R_f 0.25 (4 : 1 hexanes and ethyl acetate). mp: 77–80 °C. δ_H (400 MHz; $CDCl_3$) 8.66 (1H, s), 8.38 (2H, s), 7.66 (1H, s), 7.40 (2H, s), 3.97 (6H, s), 2.96 (2H, dd, J = 10.0, 6.7 Hz), 2.49–2.33 (2H, m). δ_C (100 MHz; $CDCl_3$) 166.2, 142.2, 141.8, 140.4, 132.4, 131.6, 131.3, 130.2, 128.9, 126.2, 123.7, 121.6–106.5 (m), 52.8, 33.0 (t, J = 22.0 Hz), 26.5. δ_F (376 MHz; $CDCl_3$) −81.24 (3F), −114.98 (2F), −122.29 (2F), −123.29 (2F), −123.83 (2F), −126.58 (2F). Elemental analysis: calculated for $C_{24}H_{16}BrF_{13}O_4$ (%) C, 41.5; H, 2.3; Br, 11.5; F, 35.5; O, 9.2; found C, 41.75; H, 2.2; Br, 11.6; F, 35.7; O, 9.5.

(3′-Bromo-5′-(3,3,4,4,5,5,6,6,7,7,8,8,8-tridecafluorooctyl)biphenyl-3,5-diyl)bis(methylene)-bis(oxy)bis(*tert*-butyldimethylsilane) (15). To a suspension of lithium aluminium hydride (211 mg, 5.56 mmol) in dry tetrahydrofuran (9 mL), a solution of **14** (1.288 g, 1.85 mmol) in dry tetrahydrofuran (11 mL) was added dropwise at 0 °C under argon. Another 4 mL of tetrahydrofuran was added through the dropping funnel to rinse down the dropping funnel and warmed to room temperature. After 45 min the reaction was quenched by slow addition of saturated sodium sulfate solution and extracted with ethyl acetate followed by drying the organic layer with anhydrous sodium sulfate and concentrated under vacuum. To the concentrated residue, dichloromethane (12 mL), TBDMS-Cl (838 mg, 5.56 mmol) and imidazole (757 mg, 11.1 mmol) were added at room temperature and stirred overnight. The reaction was quenched with saturated ammonium chloride solution, extracted with dichloromethane and the organic layer was dried over anhydrous sodium sulfate and concentrated under vacuum. The resulting crude was purified using silica column chromatography (97 : 3, hexanes and ethyl acetate) to yield **15** (1.38 g, 86%) as a colorless oil. R_f 0.68 (19 : 1, hexanes and ethyl acetate). δ_H (400 MHz; $CDCl_3$) 7.61 (1H, s), 7.37 (2H, s), 7.34 (1H, s), 7.33 (2H, s), 4.80 (4H, s), 3.06–2.84 (2H, m), 2.55–2.27 (2H, m), 0.96 (18H, s), 0.12 (12H, s). δ_C (100 MHz; $CDCl_3$) 144.2, 142.5, 141.8, 139.4, 130.2, 128.9, 126.1, 123.7, 123.6, 123.3, 121.6–107.0 (m), 65.1, 33.1 (t, J = 22.2 Hz), 25.3–27.5 (m), 18.7, −5.1. δ_F (376 MHz; $CDCl_3$) −81.24 (3F), −115.03 (2F), −122.29 (2F), −123.29 (2F), −123.89 (2F), −126.58 (2F).

1-(3′,5′-Bis((*tert*-butyldimethylsilyloxy)methyl)-5-(3,3,4,4,5,5,6,6,7,7,8,8,8-tridecafluorooctyl)biphenyl-3-yl)-2,2,2-trifluoroethanone (16). To a solution of **15** (2.64 g, 3.05 mmol) in dry diethyl ether (13 mL), *n*-butyllithium (3.0 mL of 2.5 M solution in hexane, 7.5 mmol) was added dropwise at −50 °C under argon and warmed to 0 °C. After 2 h the reaction was cooled back to −50 °C and a solution of methyl trifluoroacetate (975 mg, 7.62 mmol) in dry diethyl ether (1.5 mL) was added dropwise and stirred for another 3 h. The reaction was quenched with saturated ammonium chloride solution and extracted with diethyl ether. The organic layer was dried over anhydrous sodium sulfate and concentrated under vacuum. The resulting crude was purified using silica column chromatography (9 : 1, hexanes and ethyl acetate) to yield **16** (1.69 g, 63%) as a pale yellow oil. R_f 0.57 (17 : 3, hexanes and ethyl acetate). δ_H (400 MHz; $CDCl_3$) 8.21 (1H, s), 7.92 (1H, s), 7.82 (1H, s), 7.50 (2H, s), 7.37 (1H, s), 4.86 (4H, s), 3.10 (2H, dd, J = 8.3, 6.0 Hz), 2.59–2.40 (2H, m), 0.99 (18H, s), 0.16 (12H, s). δ_C (100 MHz; $CDCl_3$) 180.63 (q, J = 35.1 Hz), 143.52, 142.88, 141.17, 139.18, 134.35, 131.27, 128.58, 127.56, 123.85, 123.56, 122.31–106.79 (m), 64.99, 32.99 (t, J = 22.1 Hz), 24.5–27.5 (m), 18.65, −5.20. δ_F (376 MHz; $CDCl_3$) −71.78 (3F), −81.40 (3F), −114.96 (2F), −122.35 (2F), −123.37 (2F), −123.91 (2F), −126.68 (2F). Elemental analysis (CHN, F) calculated for $C_{36}H_{44}F_{16}O_3Si_2$ (%)C, 48.9; H, 5.0; N, 0.0; F, 34.3; found C, 49.3; H, 5.1; N, 0.0; F, 34.4.

1-(3′,5′-Bis((*tert*-butyldimethylsilyloxy)methyl)-5-(3,3,4,4,5,5,6,6,7,7,8,8,8-tridecafluorooctyl)biphenyl-3-yl)-2,2,2-trifluoroethanone *O*-tosyl oxime (17). A suspension of hydroxylamine HCl (276 mg, 3.96 mmol) and sodium acetate trihydrate (809 mg, 5.95 mmol) in ethanol (5 mL) was stirred for 15 mins and allowed to settle. The supernatant from this mixture was added dropwise to a solution of **16** (1.755 g, 1.98 mmol) in ethanol (6 mL) and refluxed for 16 h. The solvent was evaporated from the reaction mixture under vacuum and the residue was extracted with ether and washed with water. After drying the organic layer over anhydrous sodium sulfate it was concentrated under vacuum. The resulting residue was dissolved in dry dichloromethane (6 mL) followed by the addition of DIEA (307 mg, 2.4 mmol), and DMAP (18 mg, 7.5 mol%) under argon at room temperature. The reaction mixture was cooled to 0 °C and tosyl chloride (457 mg, 2.4 mmol) was added in small portions, warmed to room temperature and stirred for 40 min. The reaction was quenched with saturated ammonium chloride solution and extracted with dichloromethane followed by drying the organic layer over anhydrous sodium sulfate and concentration under vacuum. The resulting crude was purified using silica column chromatography (49 : 1, hexanes and ethyl acetate) to yield **17** (1.14 g, 55%). R_f

0.46 (19 : 1 hexanes and ethyl acetate). δ_H (400 MHz; $CDCl_3$) 7.89 (2H, d, J = 8.0 Hz), 7.58 (1H, s), 7.45–7.32 (6H, m), 7.16 (1H, s), 4.82 (4H, s), 3.16–2.94 (2H, m), 2.62–2.28 (5H, m), 0.97 (18H, s), 0.13 (12H, s). δ_C (100 MHz; $CDCl_3$) 154.3 (q, J = 33.7 Hz), 146.4, 143.2, 142.7, 140.7, 139.4, 131.4, 130.5, 130.1, 129.5, 127.0, 126.0, 125.4, 123.7, 123.6, 121.7–107.7 (m), 65.0, 32.9 (t, J = 22.3 Hz), 28.72–23.11 (m), 21.9, 18.6, –5.2. δ_F (376 MHz; $CDCl_3$) –61.93, –67.41 (3F), –71.71, –81.28 (3F), –114.95 (2F), –122.28 (2F), –123.29 (2F), –123.84 (2F), –126.59 (2F).

3-(3′,5′-Bis((*tert*-butyldimethylsilyloxy)methyl)-5-(3,3,4,4,5,5,6,6,7,7,8,8,8-tridecafluorooctyl)biphenyl-3-yl)-3-(trifluoromethyl)-diaziridine (18). To a solution of **17** (1.0 g, 0.948 mmol) in dry diethyl ether (6 mL), ammonia (10 mL) was condensed at –60 °C and stirred for 8 h. Ammonia was evaporated by warming the reaction to room temperature and extracted with ether followed by washing the organic layer with water. The ether layer was dried over anhydrous sodium sulfate and concentrated under vacuum followed by purification with silica column chromatography (9 : 1, hexanes and ethyl acetate) to yield **18** (677 mg, 79%) as a white solid. R_f 0.32 (9 : 1, hexanes and ethyl acetate). mp: 65–66 °C. δ_H (400 MHz; $CDCl_3$) 7.75 (1H, s), 7.53 (1H, s), 7.44 (3H, s), 7.33 (1H, s), 4.83 (4H, s), 3.02 (2H, dd, J = 10.0, 6.7 Hz), 2.85 (1H, d, J = 8.6 Hz), 2.57–2.35 (2H, m), 2.30 (1H, d, J = 8.7 Hz), 0.98 (18H, s), 0.14 (12H, s). δ_C (100 MHz; $CDCl_3$) 143.0, 142.6, 140.6, 139.8, 133.1, 129.1, 126.9, 125.7, 123.6, 123.5, 120.0–107.6 (m), 65.0, 58.2 (q, J = 36.0 Hz), 33.1 (t, J = 21.8 Hz), 29.2–23.2 (m), 18.6, –5.1. δ_F (376 MHz; $CDCl_3$) –75.88 (3F), –81.35 (3F), –115.05 (2F), –122.34 (2F), –123.33 (2F), –123.90 (2F), –126.65 (2F). Elemental analysis (CHN, F) calculated for $C_{36}H_{46}F_{16}N_2O_2Si_2$ (%)C, 48.1; H, 5.2; N, 3.1; F, 33.8; found C, 48.7; H, 5.2; N, 3.0; F, 33.3.

(3′-(3,3,4,4,5,5,6,6,7,7,8,8,8-Tridecafluorooctyl)-5′-(3-(trifluoromethyl)-3*H*-diazirin-3-yl)biphenyl-3,5-diyl)dimethanol (2). To a solution of **18** (0.317 g, 0.353 mmol) in dry diethyl ether (4.5 mL), AgO (0.655 g, 5.29 mmol) was added and stirred for 2 h. The reaction mixture was filtered and the residue was washed with diethyl ether. The filtrate was concentrated and dissolved in ethanol (6 mL) and pyridine *p*-toluenesulfonate (177 mg, 0.706 mmol) was added to it and stirred overnight at room temperature. After concentrating the reaction mixture under vacuum it was extracted with ethyl acetate and washed with water. The organic layer was dried over anhydrous sodium sulfate and concentrated, followed by silica column chromatography (97 : 3, dichloromethane and methanol) to give **2** (0.171 g, 72%) as a white solid. R_f 0.38 (23 : 2, dichloromethane and methanol). Mp: 90–91 °C. δ_H (400 MHz; $CDCl_3$) 7.40 (1H, s), 7.34 (3H, s), 7.17 (1H, s), 7.02 (1H, s), 4.65 (4H, s), 3.39 (2H, s), 2.92 (2H, dd, J = 9.8, 6.7 Hz), 2.44–2.28 (2H, m). δ_C (100 MHz; $CDCl_3$) 142.5, 142.3, 141.0, 140.3, 130.6, 128.8, 125.6, 125.2, 125.1, 123.9, 121.7–107.9 (m) 64.9, 32.9 (t, J = 22.1 Hz), 28.6 (q, J = 40.5 Hz), 26.7. δ_F (376 MHz; $CDCl_3$) –65.70 (3F), –81.45 (3F), –115.09 (2F), –122.43 (2F), –123.42 (2F), –123.93 (2F), –126.73 (2F). HRMS (ESI^+) for $[M + NH_4]^+$; calculated: 686.12945, found: 686.12865 (error = –1.18 ppm).

(2*S*,2′*S*,3*S*,3′*S*,4*S*,4′*S*,5*S*,5′*S*,6*R*,6′*R*)-6,6′-(3′-(3,3,4,4,5,5,6,6,7,7,8,8,8-Tridecafluorooctyl)-5′-(3-(trifluoromethyl)-3*H*-diazirin-3-yl)biphenyl-3,5-diyl)bis(methylene)bis(oxy)bis(methylene)bis-(tetrahydro-2*H*-pyran-2,3,4,5-tetraol) (3). To a solution of **2** (40 mg, 59.8 μmol) and mannose pentaacetate (70.4 mg, 180 μmol) in anhydrous dichloromethane (0.55 mL), $BF_3{\cdot}Et_2O$ (59 mg, 415 μmol) was added at 0 °C under argon and stirred for 30 min. The reaction mixture was then warmed to room temperature and stirred for additional 18 h followed by quenching with water. The reaction mixture was extracted with dichloromethane and the organic layer was dried over anhydrous sodium sulfate and concentrated. The residue was dissolved in anhydrous methanol (0.4 mL) followed by the addition of 5 M sodium methoxide solution (40 μL) and stirred at room temperature, under argon, for 3 h. The reaction was quenched with the addition of Amberlyst-15 and filtered. The filtrate was concentrated and the resultant residue was purified by reverse phase semi-prep HPLC.[26] The collected peaks with the desired compound, as identified by LC-MS, were concentrated and lyophilized to give **3** (25.6 mg, 43%) as a white solid. δ_H (400 MHz; CD_3OD) 7.69 (1H, s), 7.53 (2H, s), 7.48 (1H, s), 7.32 (1H, s), 7.19 (1H, s), 4.88 (2H, d, J = 1.7 Hz), 4.84 (13H, m, including solvent peak), 4.64 (2H, d, J = 12.0 Hz), 3.91–3.81 (4H, m), 3.79–3.69 (4H, m), 3.67–3.58 (4H, m), 3.10–3.00 (2H, m), 2.65–2.46 (2H, m). δ_C (100 MHz; CD_3OD) 143.9, 143.1, 141.5, 140.5, 131.2, 130.5, 128.7, 127.5, 126.9, 124.8, 124.2–108.2 (m), 100.9, 75.2, 72.8, 72.3, 69.8, 68.8, 63.1, 33.4 (t, J = 19.2), 29.7 (q, J = 40.4), 27.5. δ_F (376 MHz; CD_3OD) –67.41 (3F), –82.84 (3F), –115.74 (2F), –123.33 (2F), –124.31 (2F), –124.74 (2F), –127.75 (2F). HRMS (ESI^+) for $[M+Na]^+$; calculated: 1015.1905, found: 1015.1906 (error = 0.1 ppm). Please refer to ESI† for ^{1}H-COSY and HMQC.

(3′-(3,3,4,4,5,5,6,6,7,7,8,8,8-Tridecafluorooctyl)-5′-(2,2,2-trifluoro-1-[$^2H_3$]methoxy[1-^{2}H]ethyl)biphenyl-3,5-diyl)dimethanol (19). Photolabel **2** (3.6 mg, 5.3 μmol) was dissolved in methanol-D4 (0.6 mL) in a 5 mm NMR tube. This solution was subjected to photoirradiation with UV light (>320 nm) and the photoactivation was followed at various time intervals using ^{19}F NMR. After one hour of UV irradiation the sample was transferred to a 2 mL vial and subjected to HPLC purification[27] and the peak at 20.2 min was collected and concentrated, followed by lyophilization to give **19**. δ_H (400 MHz; CD_3OD) 7.63 (1H, s), 7.61 (1H, s), 7.55 (2H, s), 7.36 (2H, s), 4.69 (4H, s), 3.08–3.01 (2H, m), 2.71–2.42 (2H, m). δ_F (376 MHz; CD_3OD) –78.59 (3F, s), –82.85 (3F, t), –115.79 (2F, m), –123.34 (2F, m), –124.32 (2F, m), –124.79 (2F, m), –127.76 (2F, m). HRMS (ESI^+) for $[M+NH_4]^+$; calculated: 694.17463, found: 694.17198 (error = –3.82 ppm).

(5-(2,2,3,3,4,4,5,5,6,6,7,7,7-Tridecafluoro-1-[$^2H_3$]methoxy[1-^{2}H]heptyl)-1,3-phenylene)dimethanol (21). Photolabel **1** (50 mg, 101 μmol) was dissolved in methanol-D4 (5 mL) in a test tube. This solution was subjected to photoirradiation with UV light (>320 nm) and the photoactivation was followed at various time intervals using ^{19}F NMR. After 2 h of UV irradiation the sample was subjected to HPLC purification[27] and the major peak at 14.1 mins was collected and concentrated, followed by lyophilization to give **21**. δ_H (400 MHz, CD_3OD) 7.44 (1H, s), 7.38 (2H, s), 4.85 (4H, s, including solvent peak), 4.66 (4H, s). δ_C (101 MHz, CD_3OD) 143.7, 133.4, 127.9, 127.5, 64.9. HRMS (ESI^+) for $[M+NH4]^+$; calculated: 522.12464, found: 522.12456 (error = –0.16 ppm).

(5-(2,3,3,4,4,5,5,6,6,7,7,7-Dodecafluoro-1-[$^2H_3$]methoxyhept-1-enyl)-1,3-phenylene)dimethanol (22). Photolabel **1** (50 mg,

101 μmol) was dissolved in methanol-D4 (5 mL) in a test tube. This solution was subjected to photo irradiation with UV light (>320 nm) and the photoactivation was followed at various time intervals using ^{19}F NMR. After 2 h of UV irradiation the sample was subjected to HPLC purification[27] and the major peak at 12.9 min was collected and concentrated, followed by lyophilization to give **22**. δ_H (400 MHz; CD_3OD) 7.50 (1H, s), 7.25 (2H, s), 4.66 (4H, s). δ_F (376 MHz; CD_3OD) –82.90 (3F, t), –112.41 (2F, m), –123.81 (2F, m), –124.46 (2F, m), –127.86 (2F, m), –157.96 (1F, m). HRMS (ESI$^+$) for $[M+NH4]^+$; calculated: 501.1121, found: 501.1117 (error = –0.97 ppm). Please refer to ESI† for ^{19}F-COSY and ^{19}F-NOESY.

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27 Column: Agilent Eclipse XDB-C18 PN 990967-202. Gradient: 40% to 70% of acetonitrile in water with 0.05% TFA over 20 min followed by 100% acetonitrile for 5 min (5 ml min^{-1}).

