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Chemoselective Preparation of "Clickable" Aryl Sulfonyl Fluoride Monomers. A Toolbox of Highly Functionalized Intermediates for Chemical Biology Probe Synthesis

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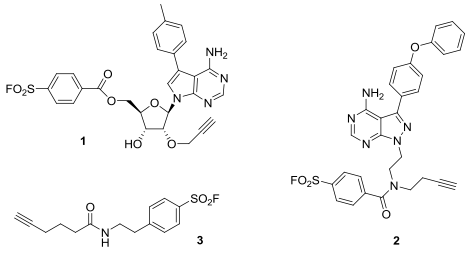
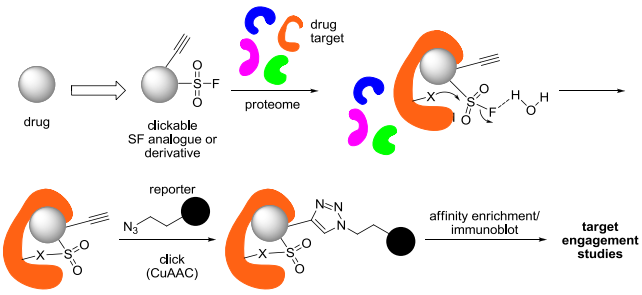
In memory of Ralph P. Robinson (senior) 1939-1976.

Abstract: Sulfonyl fluoride (SF)-based activity probes have become important tools in chemical biology. Herein, exploiting the relative chemical stability of SF to carry out a number of unprecedented SF-sparing functional group manipulations, we report the chemoselective synthesis of a toolbox of highly functionalized aryl SF monomers that we have used to quickly prepare SF chemical biology probes. In addition to SF, the monomers bear an embedded click handle (a terminal alkyne that can perform copper(I)-mediated azide alkyne cycloaddition). The monomers can be used either as fragments to prepare clickable SF analogues of drugs (biologically active compounds) bearing an aryl ring or, alternatively, attached to drugs as "minimalist" clickable aryl SF substituents.

Sulfonyl fluoride (SF)-based activity probes have become important tools in chemical biology and molecular pharmacology.[1,2] SFs are capable of modifying most amino acid side chains but, in general, they react with proteins only in a context-specific manner, i.e., only when bound in an active site or binding pocket. Aryl SFs are typically slow to hydrolyze under physiologic conditions and therefore are more commonly incorporated into chemical biology probes than are aliphatic SFs, which are typically more prone to hydrolysis.[3] Attachment of SF to a compound of biological interest results only in modest changes to overall lipophilicity and thus minimal perturbations to cell permeability and distribution can be anticipated. SF probes (e.g., **1**-**3**, Scheme 1)[4,5,6] that contain an embedded click handle (a terminal alkyne that can perform copper(I)-mediated azide alkyne cycloaddition - CuAAC[7,8]) are of particular interest in developing technologies for measuring target engagement in intact cells. Subsequent to probe binding to target, a reporter group can be attached to the click handle using CuAAC thereby allowing measurement of protein activity and target occupancy (Scheme 2).[9] In view of the fact that target engagement studies and related chemical biology technologies play increasingly important roles in contemporary drug discovery, quick access chemical biology probes can be critical for rapid project progression and decision making.[10,11]



Scheme 1. Sulfonyl fluoride chemical biology probes containing an imbedded click handle.



Scheme 2. Employing clickable SF probes in target engagement studies; X = O (tyrosine, serine, threonine), S (cysteine) or NH (lysine, arginine).

Herein, exploiting the relative chemical stability of SF to carry out a number of unprecedented SF-sparing functional group manipulations, we report the chemoselective synthesis of a toolbox of trifunctional aryl SF monomers (**4**-**8**), that we have used to quickly prepare clickable SF probes in support of drug discovery projects (Scheme 3). Based on our experience, we anticipate these compounds will find general utility in chemical biology research. For ease of synthesis, the click handle and SF can be simultaneously introduced. As shown in Scheme 4, the monomers can be used as fragments to prepare clickable SF analogues of drugs (biologically active compounds) bearing an aryl ring. Alternatively, the monomers can be attached to drugs as "minimalist" (irreducibly small) clickable aryl SF substituents, complementing, for example, the set of minimalist diazirine photo-crosslinkers described by Yao *et al.*[11] The range of functionalities (FG^1) in the toolbox allows a number of options for coupling depending on the functionality (FG^2) present on the drug or fragment of interest. Of course the utility of the clickable SF analogues prepared using the monomer set will be

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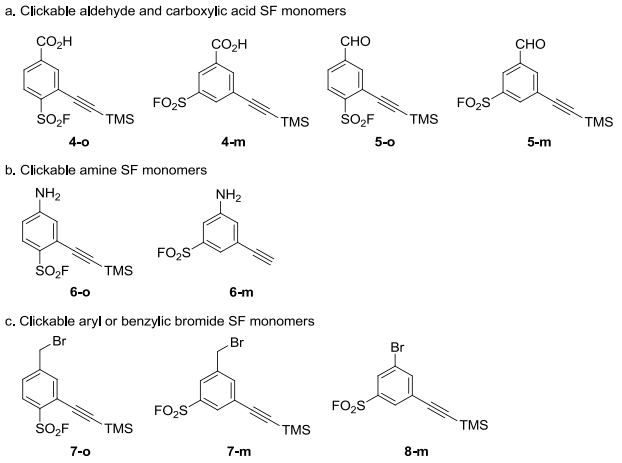
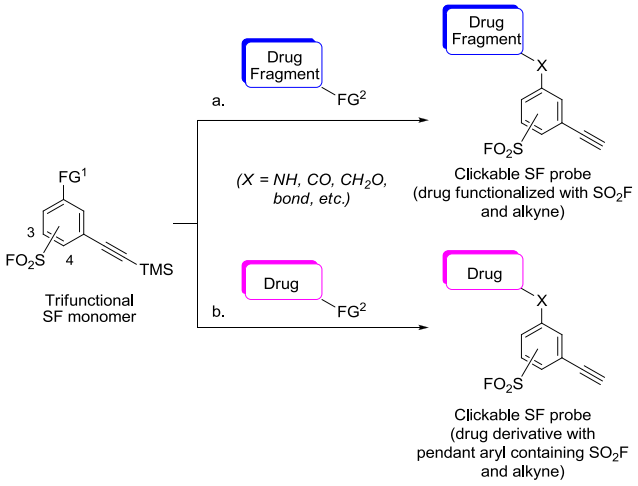
dependent on structure-activity relationships for the target protein in question.



Scheme 3. Clickable SF monomer toolbox (**4**-**8**).



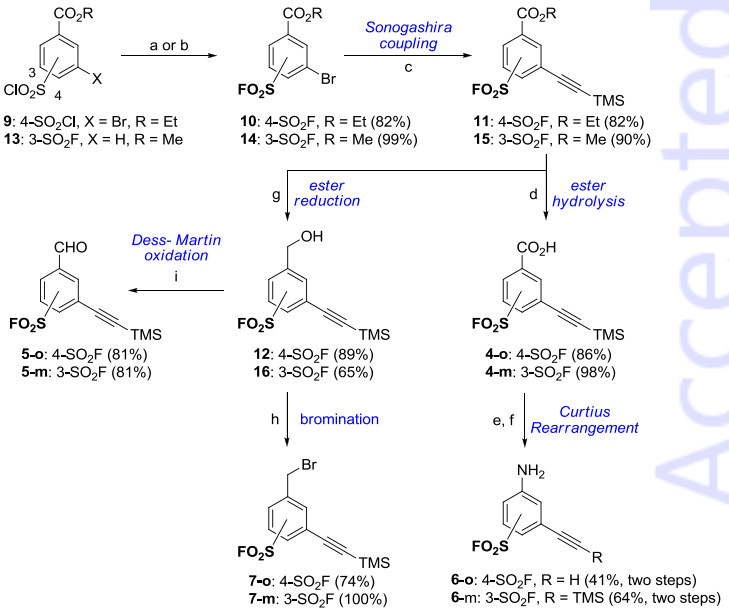
Scheme 4. a: clickable SF monomer is used as an aryl fragment for combination with another functionalized drug fragment; the product is a close analogue of the parent drug bearing an aryl side chain; b: clickable SF monomer is attached to a drug bearing functionality suitable for derivatization.

In planning the synthesis of the monomers, we needed to consider potential challenges associated with chemoselectively introducing three potentially reactive functionalities onto the aromatic ring, for example introducing FG^1 in the presence of SF and alkyne or attaching alkyne in the presence of FG^1 and SF. Fortunately, as recently reviewed by Sharpless *et al.*,[2] the relative chemical stability of SF in comparison to other sulfonyl halides can be exploited in synthesis. For example, SFs are particularly stable to reduction, which allows chemoselective reduction of other functional groups in the same molecule. As described herein, the routes to **4**-**8** significantly expand the scope of known functional group manipulations that can be carried out in the presence of SF.

We first established routes to the 1,3,4-trisubstituted ("ortho") monomers **4-o, 5-o, 6-o and 7-o** (Scheme 5). We were concerned about the stability of the alkyne under conditions typically employed for introduction of SF, e.g., thiobenzyl ether chlorination/dealkylation. Thus, hoping to capitalize on the relative stability of SF, we elected to introduce SF at an early stage and investigate chemoselective functional group manipulations in its presence.

Treatment of commercially available ethyl 3-bromo-4-(chlorosulfonyl)benzoate (**9**) with KF in acetonitrile gave SF **10** in good yield (82%). Although subsequent Sonogashira coupling[12] with ethynyltrimethylsilane cleanly afforded **11** (82%), exposure of **11** to standard aqueous hydrolysis conditions (aq. LiOH/MeOH/THF, 0°C) led to rapid and selective hydrolysis of the SF group. This result was consistent with observations suggesting that SF reactivity is increased when a hydrogen bond donor (in this case H_2O) is available to assist fluoride as a leaving group. Therefore, switching to an aprotic system was expected to reverse the chemoselectivity in favor of ester hydrolysis. Indeed this was the case, carboxylic acid monomer **4-o** being isolated in good yield (86%) upon exposure of **11** to trimethyltin hydroxide in 1,2-dichloroethane at 80°C.[13] Also to our satisfaction, the SF function remained intact after Curtius rearrangement (diphenylphosphoryl azide, Et_3N, 80°C) and cleavage of the resulting *tert*-butyl carbamate to give amino monomer **6-o** (41% over 2 steps).

Reflecting the high degree of stability of SF under reductive conditions, **11** underwent smooth reduction with diisobutylaluminum hydride (DIBAL) to yield intermediate alcohol **12** (89%). This was then converted to bromomethyl monomer **7-o** (74%) and aldehyde monomer **5-o** (81%).

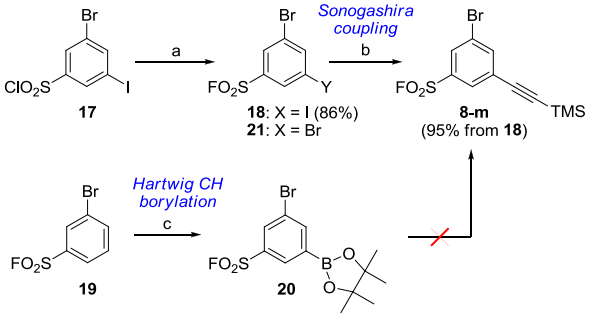


Scheme 5. Synthesis of monomers **4**-**7**, highlighting key orthogonal reactions leaving SF intact (**blue**): a) KF, MeCN; b) DBI, H_2SO_4; c) ethynyltrimethylsilane, Et_3N, CuI (cat.), $PdCl_2(PPh_3)_2$ (cat.); d) Me_3SnOH, DCE,

80°C; e) DPPA, Et_3N, *t*-BuOH, 90°; f) HCl, 1,4-dioxane or EtOAc; g) DIBAL, DCM, -40°C; h) CBr_4, PPh_3, DCM; i) Dess-Martin periodinane, DCM.

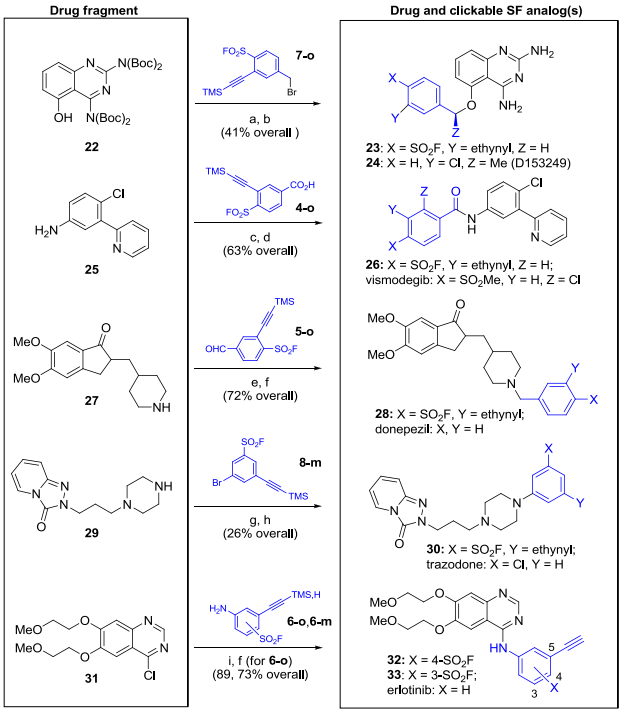
We also prepared the corresponding 1,3,5-trisubstituted ("meta") monomers **4-m**, **5-m**, **6-m**,[14] and **7-m** in to order minimize the potential for interference between the SF and alkyne groups, i.e., steric hindrance of covalent protein labelling by the SF or subsequent reporter attachment via 1,3-dipolar cycloaddition to the alkyne. These monomers were prepared in a similar way to the synthesis of the "ortho" monomers, except that bromide **14** was obtained from methyl 3-(fluorosulfonyl)benzoate (**13**) by selective bromination at the 5-position using dibromoisocyanuric acid (DBI) in H_2SO_4 (99%) (Scheme 5).

Aryl bromide monomer **8-m** was accessed from commercially available 3-bromo-5-iodobenzenesulfonyl chloride (**17**) via reaction with KF (to yield **18**) followed by selective Sonogashira coupling (Scheme 6). However, because of the high cost of commercial **17** (~$1000/g), we explored alternative approaches including Hartwig-Miyaura CH borylation[15] of 3-bromobenzenesulfonyl fluoride (**19**). Although we were pleased that borylation of **19** took place efficiently to provide **20**, attempts to obtain **8-m** by subsequent Sonogashira coupling were not successful. 3,5-Dibromobenzenesulfonyl fluoride (**21**) could be used as the Sonogashira coupling partner, but, due to competing formation of the bis-coupling product, isolated yields of **8-m** were quite low.



Scheme 6. Synthesis monomer **8-m**, highlighting key orthogonal reactions leaving SF intact (**blue**): a) KF, MeCN; b) ethynyltrimethylsilane, Et_3N, CuI (cat.), $PdCl_2(PPh_3)_2$ (cat.); c) B_2pin_2, 2 mol% $[Ir(cod)(OMe)]_2$, 4 mol% dtpby, heptane, CPME, 140°C.

Our first application of one of the monomers to an ongoing project was for an improved synthesis of **23**, a chemical biology probe based on D153249 (**24**), an inhibitor of the mRNA decapping scavenger enzyme DcpS (Scheme 7).[16] As previously disclosed, we used **23** to quantify in-cell target occupancy by DcpS inhibitors. In the original route to **23**, the alkyne was installed late in the synthesis by way of a low-yielding (~5%) Stille coupling on a fully elaborated diaminoquinazoline template. In the new convergent route using monomer **7-o**, chemoselective alkylation of phenol **22** with **7-o** took place to provide **23** after Boc deprotection (41% overall). Cleavage of the TMS group conveniently took place under the alkylation conditions. Notably, we did not see evidence of competing reaction of the phenolate with the SF group under the reaction conditions.



Scheme 7. Coupling of monomers to drug fragments. a) **7-o**, K_2CO_3, acetone; b) HCl, dioxane; c) **4-o**, $(COCl)_2$, DCM, then **25**, Et_3N, DCM; d) KF, acetone, MeCN, H_2O; e) **5-o**, $NaHB(OAc)_3$, AcOH, DCM; f) TBAF, THF; g) **8-m**, 10 mol% RuPhos-G3, Cs_2CO_3, 1,4-dioxane, 70°C; h) KF, acetone, H_2O; i) **8-m**, MeCN, 82°C.

To demonstrate an ability to use other coupling chemistries for monomer attachment without the occurrence of significant side-reactions involving the SF or alkyne, drug analogues **26**, **28**, **30**, **32** and **33** representing different target classes, were prepared from drug fragment precursors **25**, **27**, **29** and **31** (Scheme 7). Based on literature pertaining to the relative stability of aryl SF in the presence of amines and considering the mild (room temperature) conditions employed, we did not anticipate problems with SF reactivity during amide bond formation and reductive amination. Indeed this was the case as exemplified by smooth formation of vismodegib analogue **26** via acid **4-o** (63%, 2 steps including TMS removal) and donepezil analogue **28** from aldehyde **5-o** (72%, 2 steps). On the other hand, given the elevated temperatures often required, we were concerned whether conditions for S_NAr and Buchwald-Hartwig[17] couplings would be compatible with SF monomers. However, as shown by the preparation of trazodone analogue **30** from aryl bromide monomer **8-m** (26%, 2 steps) and erlotinib analogues **32** (89%, 2 steps) and **33** (73%) from amine monomers **6-o** and **6-m** respectively, useful yields of the drug analogues could be obtained.

As a second demonstration of the utility of the monomer set and derived drug analogues in a chemical biology setting we

applied erlotinib SF analogue **32** to assessing drug-target engagement in live cells (Figure 1). A crystal structure of erlotinib with its primary target, epidermal growth factor receptor (EGFR, PDB 1M17),[18] highlighted the proximity of the conserved lysine to the 4-position of the erlotinib aniline ring (Figure 1a). We thus anticipated that, of the two isomeric SF erlotinib analogues prepared, the 1,3,4-substituted isomer **32** would best position the SF for chemical labelling of the conserved lysine residue in the ATP-binding site of the EGFR protein, in a manner similar to other SF-containing kinase inhibitors (e.g., **1**).[4,19] As expected, intact mass spectrometry studies showed that **32** labelled EGFR (Figure 1b), and the extent of labelling was significantly higher than that seen for the 1,3,5-substituted isomer **33**. Peptide mapping experiments confirmed the site of labelling as the conserved Lys745 (Supporting Information). Also, as determined by ELISA in non-small cell lung cancer H3255 cells, both **32** and **33** exhibited significant inhibition of EGFR cellular autophosphorylation at Tyr1068 (IC_{50} = 116 and 74 nM respectively). With these encouraging results, we next explored the ability of **32** to report on live cell target engagement. Treatment of H3255 cells with **32**, followed by cell lysis, subsequent attachment of biotin azide, and streptavidin-mediated enrichment followed by Western blot, showed that the probe labelled EGFR in live cells (Figure 1c). Specificity was confirmed by competing the labelling with parent erlotinib in a concentration-dependent manner, thus confirming the utility of **32** as an in-cell target occupancy probe.

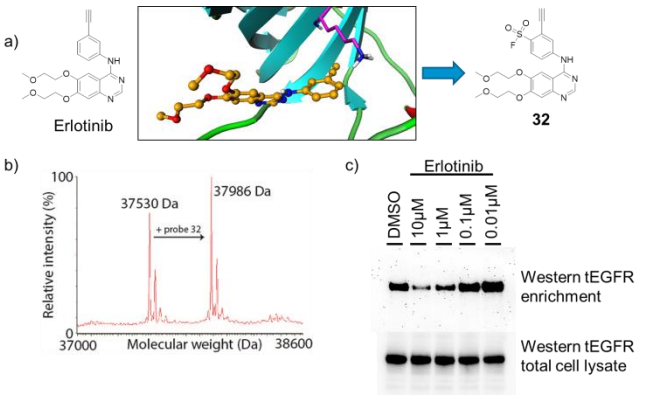


Figure 1. a) Design of sulfonyl fluoride, clickable EGFR probe **32** based on the proximity of the SF warhead to the conserved Lys (erlotinib-EGFR crystal structure shown, PDB 1M17). b) Intact mass spectrum of EGFR following addition of **32**, illustrating a commensurate mass shift for the adduct. c) *Top: in situ* probe **32** labelling of single mutant EGFR in H3255 cells is specifically competed with erlotinib in a concentration-dependent manner; *Bottom:* equal levels of EGFR in erlotinib-treated H3255 cells.

In summary, we have developed chemistry allowing the preparation of a toolbox of "clickable" aryl sulfonyl fluoride (SF) monomers that may be used as fragments or minimalist substituents in the rapid synthesis of chemical biology probes. As exemplified by chemoselective hydrolysis of esters **11** and **15**, Curtius rearrangement of acids **4**-**o** and **4**-**m**, reductive amination of amine **27,** Buchwald amination of **29**, our work significantly expands the scope of known orthogonal chemical transformations that can be carried out in the presence of SF.[18] We used representative compounds from the monomer set to prepare clickable SF analogues of known drugs or drug candidates, thus illustrating their potential for application in "real world" situations. Further showing utility, as previously disclosed, D153259 analogue **23** (prepared in improved overall yield from monomer **7**-**o**), and, as presented here, erlotinib analogue **32** (prepared from amine monomer **6**-**o**) have been employed as chemical probes for measuring target occupancy in live cells.

We continue to define the scope of sulfonyl fluoride-sparing chemical transformations as applied to a range of substrates and to develop new monomers and chemistries for SF chemical biology probe synthesis. Assessment of the broader proteome reactivity of **32** is a subject of ongoing work in our group.

Experimental Section

See Supporting Information.

Acknowledgements

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Keywords: chemical biology • click chemistry • medicinal chemistry • proteomics • sulfonyl fluoride

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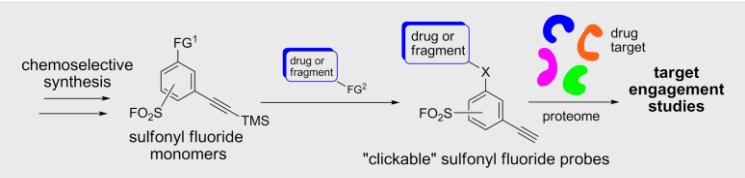

Expanding the scope of known synthetic transformations that can be carried out in the presence of sulfonyl fluoride (SF), a toolbox of trifunctional aryl SF monomers is now available that can be used to quickly prepare "clickable" SF chemical biology probes. The monomers can be used either as fragments to prepare clickable SF analogues of drugs bearing an aryl ring or, alternatively, attached to drugs as "minimalist" clickable aryl SF substituents.

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