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





# A modular flow platform for sulfur(VI) fluoride exchange ligation of small molecules, peptides and proteins

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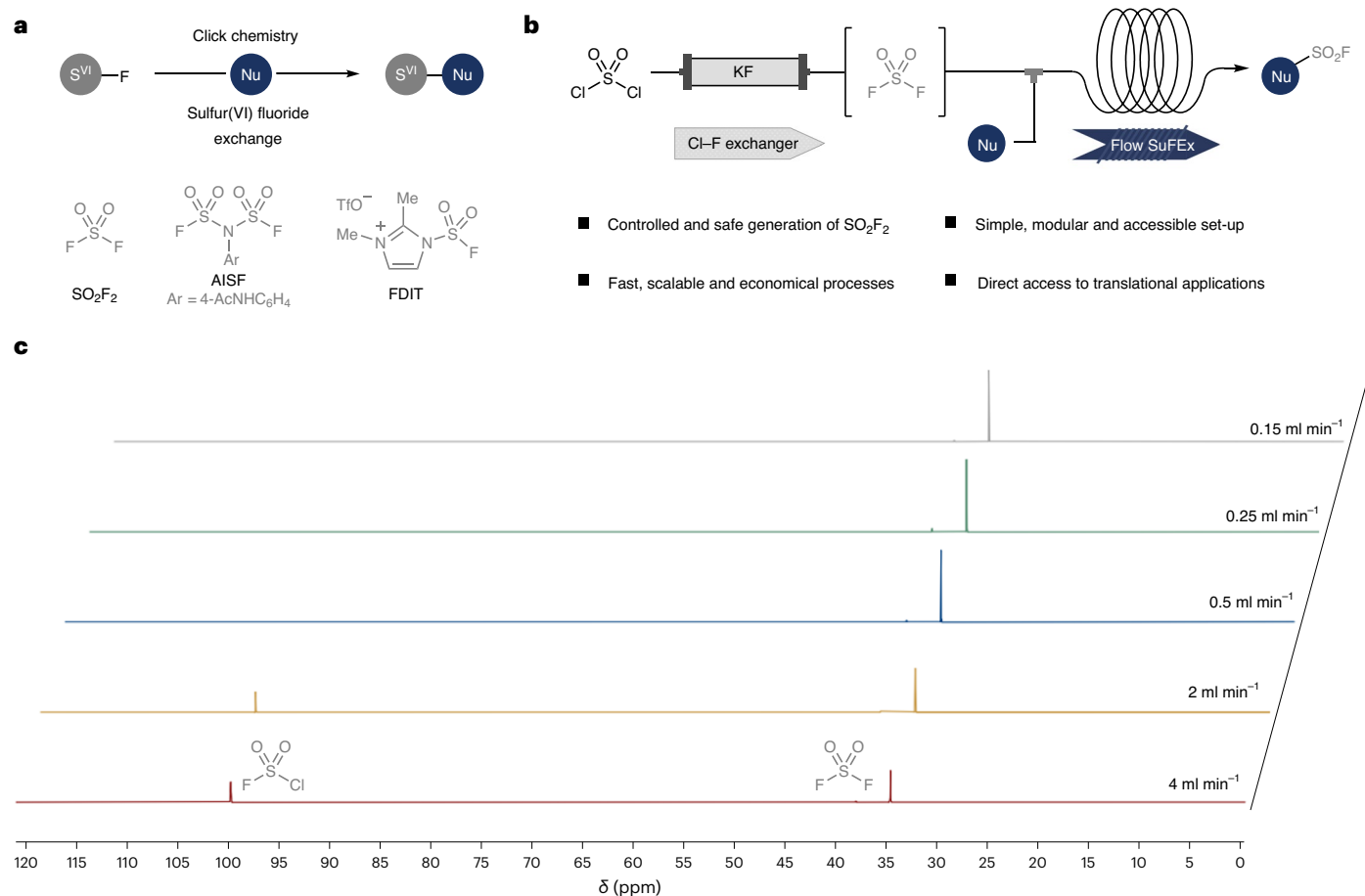
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Sulfur(VI) fluoride exchange click chemistry is a formidable tool to rapidly and effectively link chemical structures. Despite advances in the field in recent years, the installation of the sulfonyl fluoride handle still requires the use of purpose-designed, expensive and non-atom-economic reagents. The use of the  $\text{SO}_2\text{F}_2$  for sulfonyl fluoride synthesis has been thwarted by the difficulties associated with the manipulation and dosage of this toxic gas, and by its apparent low reactivity with amino functionalities. Here we report a modular flow platform that can generate on demand, and efficiently dose, gaseous  $\text{SO}_2\text{F}_2$ . The use of flow technologies allows many lingering limitations of this transformation to be overcome, resulting in reduced reaction times, efficient reactivity and broad substrate scope. The effectiveness of the process was demonstrated by the successful synthesis of a diverse set of fluorosulfates and sulfamoyl fluorides, including those derived from biorelevant compounds, peptides and proteins.

Click chemistry is a powerful and efficient method for rapidly connecting chemical fragments, enabling the modification of biologically active molecules<sup>1,2</sup>. Among the various types of click chemistry, sulfur(VI) fluoride exchange (SuFEx) reactions have emerged as a reliable resource for drug discovery<sup>3–5</sup>, chemical biology<sup>6–8</sup>, polymer chemistry<sup>9,10</sup> and surface modifications<sup>11,12</sup>. In particular, the unique properties of the sulfur(VI)–fluorine bond make SuFEx reactions highly versatile, enabling the formation of covalent bonds under mild conditions<sup>13</sup>. The S(VI)–F bond is highly stable, allowing it to withstand harsh conditions, yet is readily cleavable in the presence of a suitable activator or reaction partner<sup>14,15</sup>. Consequently, the versatility and efficiency of SuFEx reactions make them a valuable tool for researchers in a wide range of fields, including synthetic chemistry, drug discovery and materials science<sup>16</sup>.

Among the various SuFEx hubs, the  $-\text{SO}_2\text{F}$  moiety has received substantial attention due to its unique biophysical properties<sup>17</sup> and its potential as a versatile connector between nucleophilic entities<sup>18</sup>. One method for installing this moiety is the use of gaseous sulfonyl fluoride ( $\text{SO}_2\text{F}_2$ ), which has been demonstrated for various organic molecules by Sharpless and co-workers<sup>13</sup>. However, despite its potential as an economic and traceless compound, its mild toxicity<sup>19</sup> and the handling difficulties it presents have motivated researchers to seek more practical alternatives. While in situ generation of  $\text{SO}_2\text{F}_2$  from 1,1'-sulfonyldiimidazole (SDI, Fig. 1a)<sup>20</sup> or the use of solid reagents such as [4-(acetylamino)phenyl]imidodisulfonyl difluoride (AISF)<sup>21</sup> or 1-(fluorosulfonyl)-2,3-dimethyl-1*H*-imidazol-3-ium trifluoromethanesulfonate (FDIT)<sup>22</sup> has been explored, these alternatives require

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**Fig. 1 | SuFEx click chemistry.** **a**, Although stable under various conditions (hydrolysis, reduction/oxidation), S(VI) fluorides are susceptible to nucleophilic attack, enabling efficient click-type reactions. However, the synthesis of S(VI) fluorides can be cumbersome, requiring challenging reagents, such as gaseous  $SO_2F_2$  or atom-inefficient solid reagents. **b**, Flow-generated gaseous

$SO_2F_2$  enables a practical, fast and selective preparation of S(VI) fluoride reagents. **c**, Flow experiments showing the feasibility of the outlined strategy to produce the coveted  $SO_2F_2$  in high selectivity (results obtained by  $^{19}F$  NMR spectroscopy using 1,2-difluorobenzene as internal standard). Nu, nucleophile; Tf, trifluoromethanesulfonyl.

the use of  $SO_2F_2$  in their preparation and produce unnecessary by-products. Hence, these methods go against the principles of click chemistry and represent an ongoing challenge<sup>2</sup>. Addressing these challenges by developing a new strategy for producing and dosing  $SO_2F_2$  in a controlled manner from simple chemicals would represent a substantial advance in the field of SuFEx chemistry. Furthermore, such a process would notably reduce the number of synthetic steps needed for SuFEx handle installation and therefore streamline the overall process.

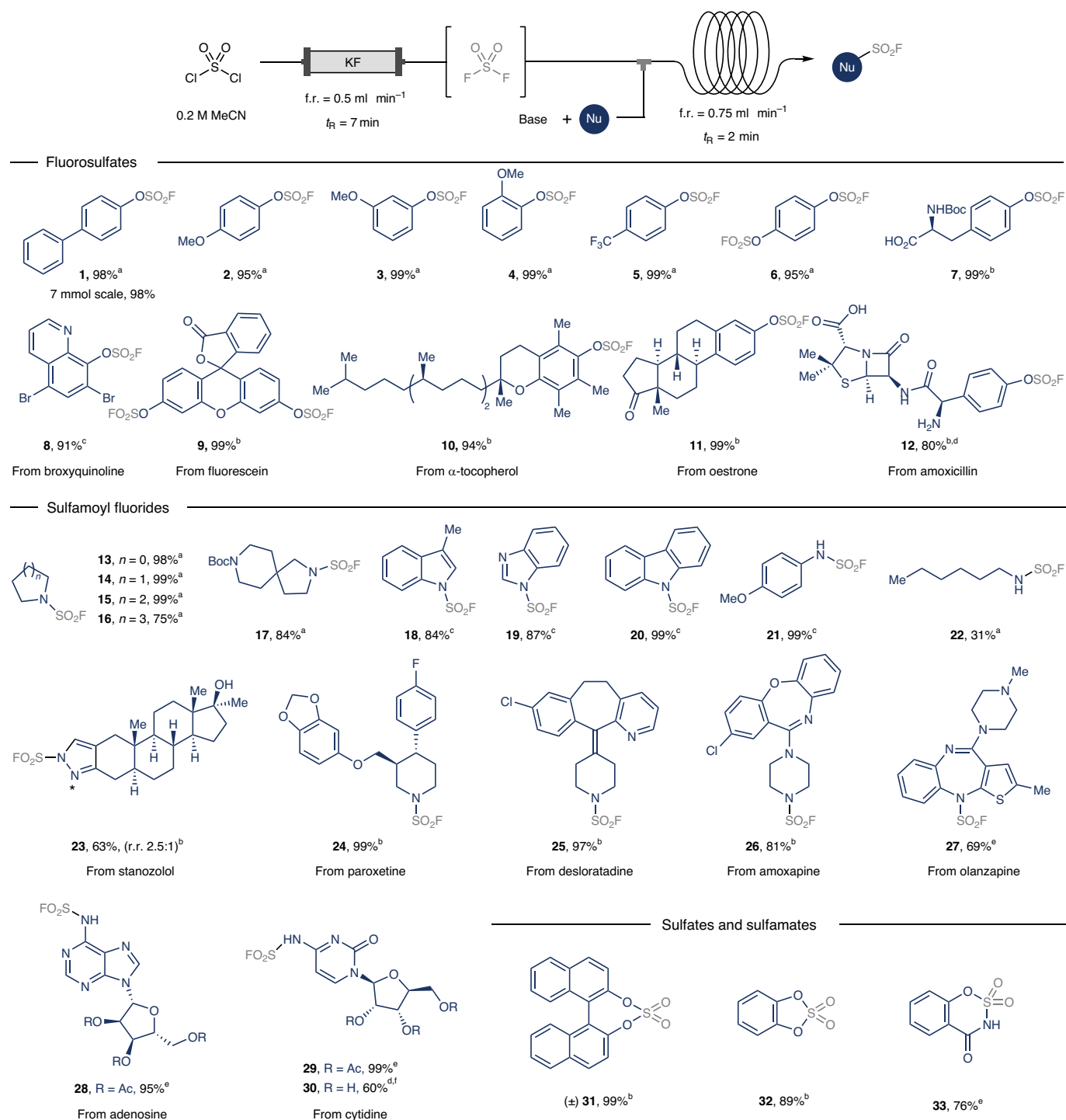
## Results

### Generation of $SO_2F_2$

To address the challenges associated with using  $SO_2F_2$  as a reagent, we considered using cheap commodity chemicals such as sulfuric chloride ( $SO_2Cl_2$ ) and potassium fluoride (KF) to generate  $SO_2F_2$  in situ. This approach was motivated by the greater thermodynamic stability of the S(VI)–F bond ( $-90 \text{ kcal mol}^{-1}$ ) compared with the S(VI)–Cl bond ( $-46 \text{ kcal mol}^{-1}$ ), which suggests that this exchange should be achievable<sup>13,23</sup>. Our initial batch experiments using  $SO_2Cl_2$  and KF in MeCN confirmed the feasibility of this approach, demonstrating that the successful conversion of first  $SO_2Cl_2$  to  $SO_2ClF$  and subsequently to  $SO_2F_2$  could be achieved within 2 h (Supplementary Information).

Due to the slow formation observed in batch reactions and the mixture of  $SO_2ClF$  and  $SO_2F_2$  obtained, we turned to flow technology

as a tool for more effectively generating and controlling the delivery of this reactive gas<sup>24,25</sup>. To this end, we designed a modular system (Fig. 1b) using microfluidic technology to greatly enhance the safety and scalability of the overall process<sup>26</sup>. Our modular system consists of two interconnected flow reactors. The first reactor is a packed-bed reactor filled with KF that generates  $SO_2F_2$  on demand via chlorine–fluorine exchange. The second reactor is where the generated gaseous  $SO_2F_2$  is mixed with the nucleophilic partner, ultimately yielding the desired SuFEx product. Because the first reactor generates  $SO_2F_2$  on demand, the reagent remains contained and is subsequently mixed with the nucleophilic partner in the second reactor. By immediately reacting away the toxic  $SO_2F_2$  in the SuFEx module, our modular system effectively eliminates the safety and practical concerns associated with the handling of this reagent, while generating only the required quantities. In addition, we anticipated that our use of flow technology would also reduce the time required for halogen substitution reactions due to enhanced liquid–solid contact in the first chlorine–fluorine exchange module and excellent gas–liquid mass transfer in the SuFEx module<sup>27</sup>. Our experiments confirmed the effectiveness of our modular system: when we directed a solution of  $SO_2F_2$  over the packed-bed reactor filled with a mixture of KF and glass beads (Fig. 1c; see Supplementary Information for further details), we observed a rapid and selective formation of  $SO_2F_2$ . We found that varying the flow rate was crucial for the selectivity of the transformation, and that  $0.5 \text{ ml min}^{-1}$  was the optimal flow rate in terms of conversion, selectivity and time

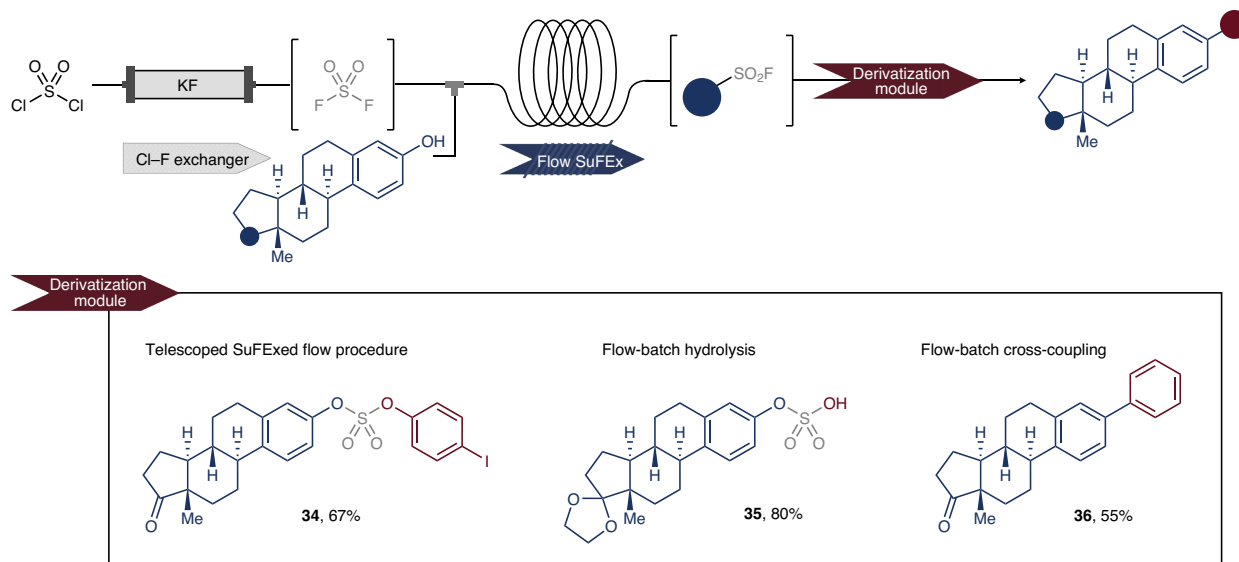
**Table 1 | Rapid SuFEx ligation of small molecules in flow, yielding fluorosulfates, sulfamoyl fluorides, sulfates and sulfamates**

All yields are those of isolated compounds. Standard conditions for  $\text{SO}_2\text{F}_2$  generation:  $\text{SO}_2\text{Cl}_2$  (2.0 equiv., 0.2 M in MeCN) passed through a 3.8 ml cartridge filled with a 1:1 mixture of KF and glass beads. Standard conditions for the second step are indicated in the footnotes. r.r., regiomer ratio; f.r., flow rate;  $t_{\text{R}}$ , residence time; DBU, 1,8-diazabicyclo[5.4.0]undec-7-ene; DMF, *N,N*-dimethylformamide; DMSO, dimethylsulfoxide; Boc, *tert*-butyloxycarbonyl. <sup>a</sup>Nucleophile (1.0 equiv., 0.2 M in MeCN),  $\text{Et}_3\text{N}$  (2.5 equiv.). <sup>b</sup>Nucleophile (1.0 equiv., 0.2 M in DMF),  $\text{Et}_3\text{N}$  (2.5 equiv.). <sup>c</sup>Nucleophile (1.0 equiv., 0.2 M in MeCN), DBU (4.0 equiv.); for **8**, 5.0 equiv. of DBU was used. <sup>d</sup>The compound has been isolated after an acetylation step. <sup>e</sup>Nucleophile (1.0 equiv., 0.2 M in DMF), DBU (4.0 equiv.). <sup>f</sup>Nucleophile (1.0 equiv., 0.1 M in DMSO),  $\text{K}_2\text{CO}_3$  (4.0 equiv.).

(7 min residence time), effectively avoiding the presence of undesired  $\text{SO}_2\text{Cl}$ . Under optimized conditions, the packed-bed reactor was able to produce -18 mmol of  $\text{SO}_2\text{F}_2$  starting from an -80 mmol KF bed (see Supplementary Information for further details).

### SuFEx ligation of small molecules

Having obtained promising results from our  $\text{SO}_2\text{F}_2$  generator, we proceeded to integrate it with the SuFEx module to enable the reaction with nucleophilic partners. By introducing the appropriate nucleophiles



**Fig. 2 | A three-step protocol that combines the  $\text{SO}_2\text{F}_2$  generator module, the SuFEx module and the derivatization module.** All yields are those of isolated compounds. A full description of the experimental details can be found in the Supplementary Information.

with an excess of a base, we were able to obtain a diverse range of SuFExed products with excellent yields in just 2 min of residence time (Table 1). This short residence time can be attributed to the intimate contact between gas and liquid phase in the flow (that is, enhanced gas–liquid mass transfer)<sup>28,29</sup>, which should allow for the generation of large libraries of SuFExed compounds with minimal effort and time. Notably, a variety of phenols could be cleanly converted to their corresponding fluorosulfates regardless of the position or electronic nature of the substituents (Table 1, compounds 1–6). Although using large quantities of gaseous  $\text{SO}_2\text{F}_2$  in batch reactions can be challenging, our flow protocol overcomes this issue, allowing for a gram-scale synthesis of fluorosulfate **1** by simply increasing the amount of starting materials pumped through the reactor assembly<sup>30</sup>. Importantly, no reoptimization of reaction conditions was required, and no loss of chemical efficiency was observed during the scaled-up experiment.

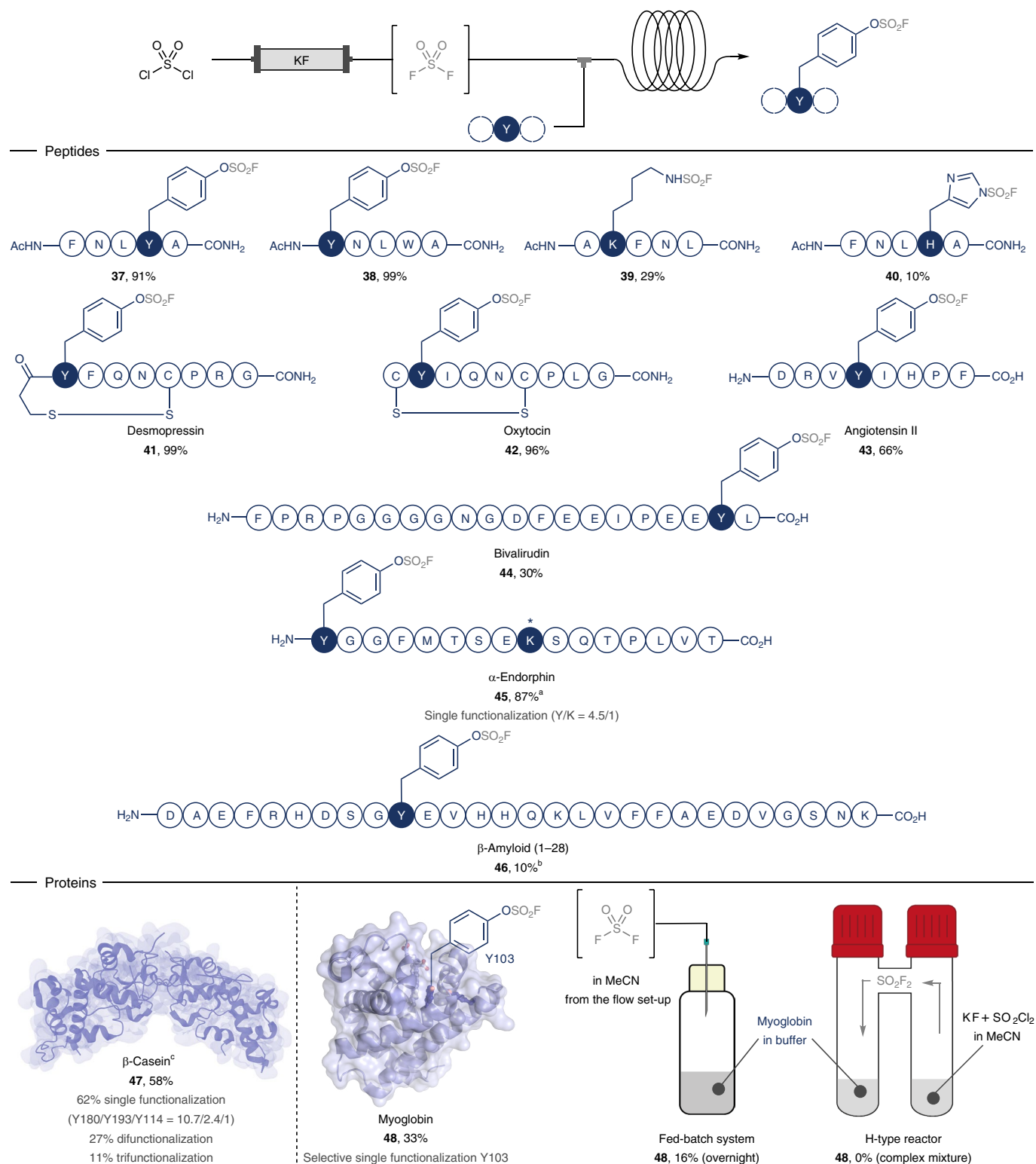
We further found that our flow protocol was not limited to the synthesis of simple phenol-based fluorosulfates. In fact, we observed that a wide variety of natural products, drugs and fluorescent tracers could be successfully reacted using this approach. For instance, *N*-Boc-protected tyrosine, broxyquinoline, fluorescein,  $\alpha$ -tocopherol, oestrone and unprotected amoxicillin were all cleanly converted in just 2 min of reaction time (Table 1, compounds 7–12), demonstrating the excellent functional group tolerance of our method. Similarly, the challenging class of nitrogen-based nucleophiles could also be subjected to our protocol, delivering the corresponding sulfamoyl fluorides in excellent yields. For example, four- to seven-membered ring secondary amines (Table 1, compounds 13–17), heterocyclic derivatives (Table 1, compounds 18–20), an aniline compound (Table 1, compound 21) and a primary amine (Table 1, compound 22) were effectively reacted with  $\text{SO}_2\text{F}_2$ . Our flow protocol was also effective in synthesizing analogues of several pharmaceutically relevant molecules, such as stanozolol, paroxetine, desloratadine, amoxapine and olanzapine, which rapidly yielded the desired SuFExed products (Table 1, compounds 23–27). Additionally, nucleosides such as adenosine and cytidine derivatives (Table 1, compounds 28–30) could be used as competent reaction partners. Finally, we were able to use bidentate nucleophiles, such as 1,1'-bi-2-naphthol, catechol and salicylamide (Table 1, compounds 31–33), to produce the corresponding sulfates and sulfamates.

Next, we capitalized on the modular nature of our set-up and developed a multistep process that orchestrates several reactions in a sequential fashion (Fig. 2). For instance, we streamlined a three-module

flow set-up to synthesize sulfate derivative **34** (67%) uninterrupted. After generating sulfonyl fluoride in the packed-bed reactor and trapping it with oestrone in the SuFEx capillary reactor, the resulting fluorosulfate was reacted with (4-iodophenoxy)trimethylsilane in a second SuFEx capillary. Our flow approach carefully balances the stoichiometry of the gaseous reagent, preventing any remaining  $\text{SO}_2\text{F}_2$  from unproductively consuming the silyl ether in the second SuFEx step<sup>13</sup>. Furthermore, after a solvent switch, the reaction crude can also immediately be used to carry out base-promoted hydrolysis of ethyleneglycol-protected oestrone to obtain bisulfate derivative **35** (80% yield) or a palladium-catalysed Suzuki–Miyaura-type cross-coupling to yield product **36** in 55% yield.

### SuFEx ligation of peptides and proteins

Our flow approach has demonstrated excellent functional-group tolerance and selectivity in producing SuFExed products from various small molecules. This is due to the favourable reaction conditions, including high mass transfer and excellent dosing of gaseous reagents, provided by the flow protocol which allows for reduced reaction times of just 2 min, thereby avoiding the formation of deleterious by-products. We next sought to explore the potential of our microfluidic strategy for the late-stage modification of unprotected peptides<sup>31</sup>. Our aim was to directly install the sulfur-centred electrophilic handle within the peptide core, allowing for later SuFEx-enabled derivatization opportunities<sup>32</sup>. Based on our previous experience with small molecules, we anticipated that the reaction would be kinetically favoured for tyrosine residues, which should enable site-selective modification within the complex peptidic framework. After a minimal reoptimization of the reaction conditions (Supplementary Information), we investigated the reactivity of different nucleophilic amino acid residues within different pentapeptides. We found that tyrosine-containing peptides were successfully converted in a site-selective fashion in just 2 min (Table 2, compounds 37 and 38). Our evaluation also showed limited modification at lysine and histidine (Table 2, compounds 39 and 40), while other nucleophilic residues, such as tryptophan and cysteine, were not reactive under our reaction conditions (Supplementary Information). Encouraged by these results, we subjected various complex and therapeutically valuable peptides to our flow protocol. Cyclic peptides (Table 2, compounds 41 and 42) and therapeutic drugs such as angiotensin II and bivalirudin (Table 2, compounds 43 and 44) were selectively modified at the tyrosine residue with good

**Table 2 | Application of the flow SuFEx ligation protocol to the direct modification of peptides and proteins**

Conversions reported as ratios of areas under the peak of product and starting compound obtained by liquid chromatography–mass spectrometry analysis. Peptides: standard conditions for the SO<sub>2</sub>F<sub>2</sub> generation: SO<sub>2</sub>Cl<sub>2</sub> (40 equiv., 0.2M in MeCN) passed through a 3.8ml cartridge filled with a 1:1 mixture of KF and glass beads, f.r.=0.5mlmin<sup>-1</sup>. Peptide (1.0equiv., 10mM in MeCN:H<sub>2</sub>O 1:1), Et<sub>3</sub>N (6.0equiv.), f.r.=0.25mlmin<sup>-1</sup>, t<sub>R</sub>=2min at room temperature. The asterisk above the α-endorphin structure represents the second amino acid (K, lysine), which is also modified in the reaction (alternative modification site). The accompanying image was created using the three-dimensional visualizer available on the PDB platform. In addition, the picture of myoglobin was generated using PyMol software. Proteins: standard conditions for the SO<sub>2</sub>F<sub>2</sub> generation: SO<sub>2</sub>Cl<sub>2</sub> (0.1M in MeCN) passed through a 3.8ml cartridge filled with a 1:1 mixture of KF and glass beads, f.r.=0.1mlmin<sup>-1</sup>. β-Casein (1.0equiv., 5mM in Tris buffer, pH7.7), TMG (10equiv.), f.r.=0.9mlmin<sup>-1</sup>, t<sub>R</sub>=1.5min at room temperature, SO<sub>2</sub>F<sub>2</sub> (2.2equiv.). Myoglobin (1.0equiv., 1mM in 10mM acetate buffer, pH5), TMG (1.0equiv.), f.r.=0.9mlmin<sup>-1</sup>, t<sub>R</sub>=1.5min at room temperature, SO<sub>2</sub>F<sub>2</sub> (11equiv.). TMG, 1,1,3,3-tetramethylguanidine. f.r., flow rate; t<sub>R</sub>, residence time. <sup>a</sup>α-Endorphin (6mM), SO<sub>2</sub>F<sub>2</sub> (67equiv.). <sup>b</sup>β-Amyloid (3mM), SO<sub>2</sub>F<sub>2</sub> (133equiv.). <sup>c</sup>The generic structure chosen to represent β-casein, due to the absence of a reported crystal structure, is protein PDB1RQF from the Protein Data Bank.

to excellent results. Even natural peptides, such as  $\alpha$ -endorphin and  $\beta$ -amyloid (1–28), were effectively converted into the corresponding fluorosulfates with good to excellent conversions (Table 2, compounds 45 and 46).

As the ultimate test for our SuFEx ligation protocol, we focused on the direct modification of proteins in flow. By minimizing lysine competition (Supplementary Information), we managed to exclusively install the electrophilic  $\text{SO}_2\text{F}$  handle on tyrosine residues in just 1.5 min. This is one of the fastest methods for direct protein modifications reported to date<sup>33</sup>. For instance, we merged a solution of  $\beta$ -casein with the  $\text{SO}_2\text{F}_2$ -containing stream and observed predominantly single, chemoselective functionalization at different tyrosine residues with a Y180/Y193/Y114 = 10.7/2.4/1 regioselectivity ratio (Table 2, compound 47). Notably, myoglobin was obtained as a single Y103-modified adduct (Table 2, compound 48), without observing denaturation or loss of the haem group, demonstrating the mild nature of the protocol. Remarkably, when we attempted to perform the same experiments in fed-batch mode, only a 16% conversion was obtained, while using a batch H-type reactor only yielded a complex mixture of products (Table 2, bottom right). These results demonstrate that the enhanced mass transfer and confined access to the gaseous and hydrophobic  $\text{SO}_2\text{F}_2$  observed in capillary flow reactors are critical to enable efficient SuFEx hub installation in complex macromolecular systems. Overall, our modular flow system proves to be an invaluable tool, facilitating remarkably swift and high-yielding SuFEx reactions between  $\text{SO}_2\text{F}_2$  and an extensive array of structurally diverse substrates, including small molecules, biorelevant compounds, peptides and proteins.

## Conclusion

The practical flow protocol presented in this study enables the safe and efficient generation of the coveted gaseous  $\text{SO}_2\text{F}_2$  reagent, and produces high reaction rates of the subsequent SuFEx ligation, with wide applicability to various substrates, including therapeutically relevant small molecules, peptides and proteins. Based on these findings, we believe that this protocol opens up new opportunities in the field of SuFEx click chemistry. In particular, the use of this flow process makes  $\text{SO}_2\text{F}_2$  a viable reagent for installing the  $-\text{SO}_2\text{F}$  handle on a variety of phenol and amino functionalities.

## Method

### Flow generation and onward reaction of $\text{SO}_2\text{F}_2$

In a typical experiment,  $\text{SO}_2\text{F}_2$  (810  $\mu\text{l}$ , 10 mmol) was dissolved in dry acetonitrile (50 ml, 0.2 M) in an oven-dried, nitrogen-filled 100 ml round-bottom flask with a rubber septum. The 0.2 M solution of  $\text{SO}_2\text{F}_2$  was taken up with a 20 ml syringe and mounted on a syringe pump. The first time a packed bed was used, it was flushed with dry acetonitrile to fill the empty volume with solvent. Then 5 ml of the  $\text{SO}_2\text{Cl}_2$  solution was pumped at 0.5 ml  $\text{min}^{-1}$  to equilibrate the reactor. Once this procedure was done, the cartridge could be used continuously until its exhaustion. In parallel, in a 10 ml vial the nucleophile was charged. Next, solvent and an organic base were subsequently added. This mixture was then taken up with a 5 ml syringe and mounted on a second syringe pump. To start a reaction, the solution of  $\text{SO}_2\text{F}_2$  was constantly pumped through the equilibrated KF packed bed at 0.5 ml  $\text{min}^{-1}$  ( $t_{\text{R}} \approx 7$  min). The resulting  $\text{SO}_2\text{F}_2$  flow was then mixed with the nucleophile solution (pumped at 0.25 ml  $\text{min}^{-1}$ ) through a polyether ether ketone T-mixer. The combined feeds were connected to a 1.5 ml perfluoroalkoxy coil (inner diameter = 0.8 mm;  $t_{\text{R}} = 2$  min) which served as reactor for the SuFEx event. At the end of the coil, the organic crude was collected in a conical flask. Importantly, when the syringe pump of the nucleophile finished pushing the solution, the system was stopped (both syringe pumps), and the syringe of the nucleophile was quickly substituted for another one containing acetonitrile. Then the flow rate of the latter pump was set to 0.75 ml  $\text{min}^{-1}$  to push the remaining reaction crude of the perfluoroalkoxy coil, while the syringe pump of the  $\text{SO}_2\text{F}_2$  solution

remained stopped. The collected organic crude was diluted with ethyl acetate, and washed with 10% HCl (aq.) and then with brine. Next, the organic phase was dried over  $\text{Na}_2\text{SO}_4$ , filtered and concentrated under reduced pressure to obtain the SuFExed products.

## Data availability

The authors declare that the data supporting the findings of this study are available within the paper and its Supplementary Information.

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## Author contributions

M.B. and D.M. conceived the project. M.B., D.M. and J. S. performed and analysed the experiments. O.B. and T.N.G. supervised the ligation of peptides and proteins. Z.Z., A.Y.V., A.F.G.G. and T.N.G. performed the analyses of the ligation of peptides and proteins. T.N. directed the project. M.B., D.M. and T.N. have written the manuscript with contributions from all the authors.

## Competing interests

The authors declare no competing interests.

## Additional information

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