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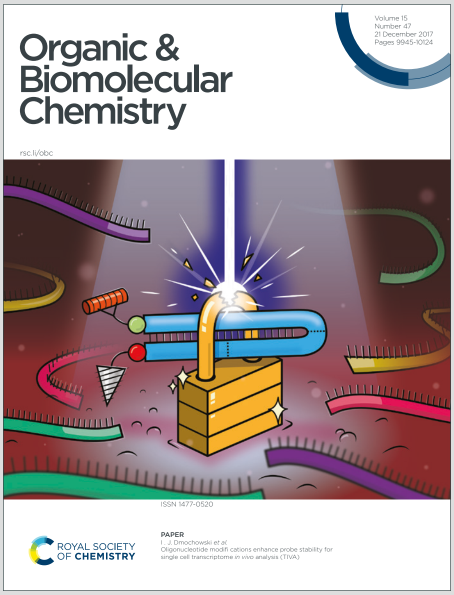

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REVIEW

The Sulfo-Click Reaction: Expanding the Toolbox for Bioconjugation and Medicinal Chemistry

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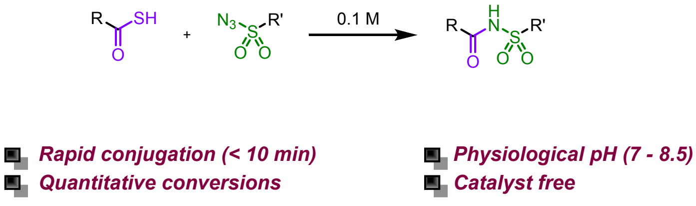
The sulfo-click reaction, which relies on the chemoselective coupling of sulfonyl azides with thioacids to yield *N*-acylsulfonamides, has emerged as a powerful and versatile transformation in synthetic chemistry and bioconjugation. Its high efficiency, operational simplicity, and compatibility with mild conditions and diverse functional groups have enabled broad applications in both chemical biology and medicinal chemistry. Notably, this reaction supports site-selective bioconjugation in aqueous media and is well suited to complex biomolecular systems, facilitating the preparation of functionalized bioconjugates. In addition, the resulting *N*-acylsulfonamide motif has attracted significant interest in drug design as a metabolically stable bioisostere of carboxylic acids and phosphates. In this review, we summarize the development of the sulfo-click reaction, discuss its mechanistic features and reaction scope, and highlight recent advances and emerging applications, underscoring its value as a modular and reliable platform for both fundamental and applied research.

1. Introduction

The concept of "click" chemistry was introduced by K. Barry Sharpless in 2001 as a guiding framework for the development of efficient, high-yielding, and selective transformations suited to modular synthesis.[1] Such reactions are defined by their operational simplicity, robustness, and ability to proceed under mild conditions while producing minimal and benign by-products. These features have driven their widespread adoption across diverse fields including chemical biology, medicinal chemistry and biomolecular engineering.

Among the most popular click reactions, the copper-catalyzed azide-alkyne cycloaddition (CuAAC) represents a cornerstone transformation, enabling the efficient and high selectivity synthesis of 1,2,3-triazoles with high selectivity and efficiency.[2-5] Similarly, the strain-promoted azide-alkyne cycloaddition (SPAAC) was developed to eliminate the need for metal catalysts,[6] thereby broadening its applicability in bioconjugation. Beyond these examples, a diverse range of click-type reactions has emerged, all sharing key features such as fast reaction rates, high chemoselectivity, and compatibility with complex molecular environments. Collectively, these transformations have driven major advances in chemical biology, drug discovery, and materials science by offering robust and modular strategies for molecular assembly, labelling, and functionalization.[7-13]

Over the past decade, the sulfo-click reaction has emerged as a valuable addition to the click chemistry toolbox. This transformation relies on the highly chemoselective coupling of thiocarboxylic acid (thioacid) and sulfonyl azides, affording *N*-acylsulfonamide under mild, catalyst-free conditions (Scheme 1). These motifs are of significant interest due to their presence in pharmaceuticals,[14] or polymeric materials,[15, 16] making the sulfo-click reaction a promising strategy for both synthetic and industrial applications.



Scheme 1. The sulfo-click reaction between a thioacid and a sulfonyl azide. Adapted with permission from ref 78. Copyright 2020, American Chemical Society.

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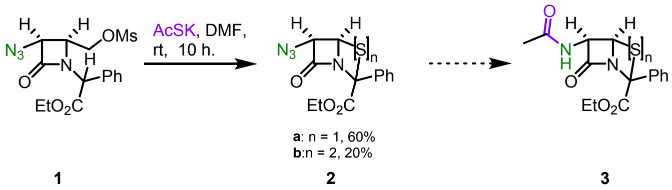
In response to the growing demand for efficient and selective methods to couple molecular partners, particularly in aqueous and biologically relevant environments, the sulfo-click reaction represents a powerful approach with broad synthetic potential. In this review, we outline its historical development, examine its mechanistic features, and discuss its scope and applications in bioconjugation and medicinal chemistry.

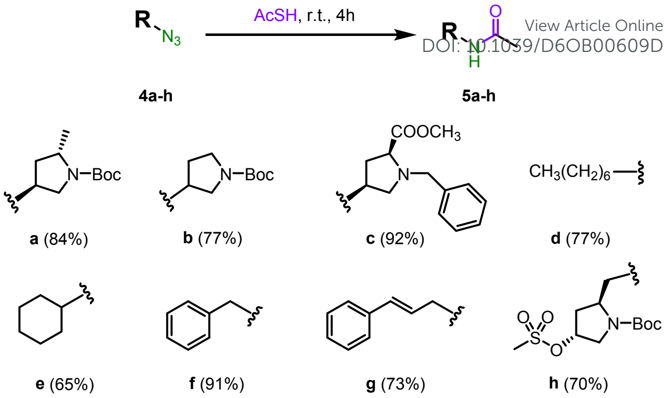
2. The emergence of the Thioacid/Azide Ligation

The earliest report of a reaction between a thioacid and an azide dates back to 1980, when Hakimelahi and Just described it in the context of the synthesis of 2,2-disubstituted tetrahydrothiophenes **2**.[17] In their study, potassium thioacetate was reacted with an azide-bearing β-lactam derivative **1** in DMF at room temperature to form the targeted 2,2-disubstituted tetrahydrothiophenes **2** Following the anticipated S_N2 displacement of the mesylate by potassium thioacetate, smooth cyclization takes place. This transformation is probably favored thermodynamically and facilitated by the heightened acidity of the C–H bond adjacent to both the ester and phenyl groups. Interestingly, prolonged reaction times led to the unexpected formation of an amide side product **3** (Scheme 2). This observation constitutes the first reported example of an amide bond synthesized from a thioacid and an azide, thereby paving the way for subsequent developments.



Scheme 2. Synthesis of 2,2-disubstituted tetrahydrothiophenes leading to amide bond formation.[17]

A few years later, in 1988, Rosen *et al.* reported a chemoselective approach for the reductive acetylation of azides, using thioacetic acid either as a solvent or a co-solvent. This approach enabled the efficient conversion of azides **4a-h** into the corresponding acetamides **5a-h** under mild reaction conditions. The scope of this methodology was demonstrated on a series of aliphatic azides, which were smoothly transformed into the desired products in good yields through a simple and practical procedure (scheme 3).[18]



Scheme 3. Reductive acylation of azides with thioacetic acid.[18]

Two years later, this transformation was extended to carbohydrates using a combination of thioacetic acid and potassium thioacetate, further expanding its synthetic scope.[19] These studies highlighted the potential of thioacids and azides as partners for chemoselective amide bond formation.

A decade later, Williams *et al.* revisited this transformation and coined the term Thioacid/Azide Ligation (TAL).[20] In their seminal study, the authors reassessed the mechanistic hypotheses previously proposed. Earlier reports had suggested a pathway involving the initial reduction of the azide to an amine, followed by an atypical acylation step (scheme 4), possibly mediated by trace amounts of H_2S as suggested by Rosen *et al.*[18] However, experimental investigations challenged this hypothesis. When benzylamine **6** was treated with trifluoroacetic acid (1.0 eq.) in dichloromethane (0.5 M), followed by a slight excess of thioacetic acid (1.3 eq.), only traces of the expected amide **7** were observed (<4%) after 15 hours at room temperature. In contrast, under identical conditions, benzyl azide **8** was converted into *N*-benzyl acetamide **9** in 42% yield, thereby supporting an alternative mechanistic pathway (scheme 4).

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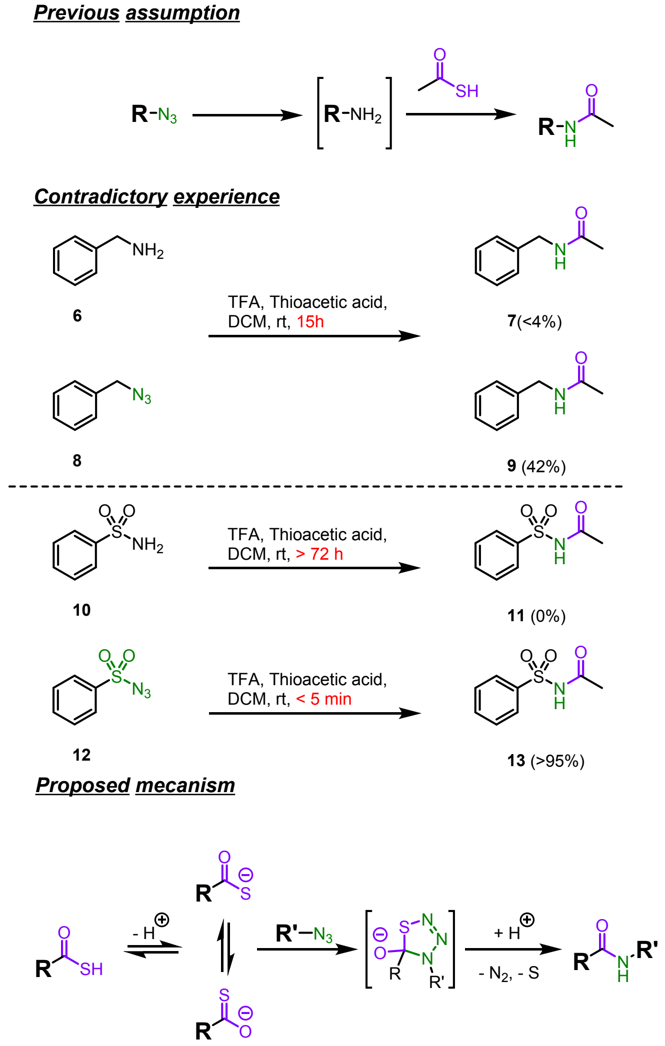
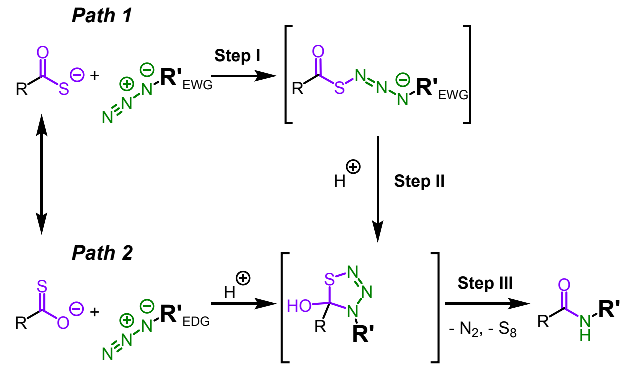


Scheme 4. Mechanistic study regarding the TAL.[18, 21]

Furthermore, benzenesulfonyl azide **12** reacted rapidly with thioacids, producing *N*-acylsulfonamides **13** in excellent yields (>95%), whereas benzenesulfonamide **10** remained unreactive even after prolonged reaction times (scheme 4).

These findings suggested that direct reduction of the azide is not a prerequisite for amide bond formation in this transformation. On this basis, the authors proposed a mechanism involving the formation of a thiatriazoline intermediate which could arise either through a [3+2] cycloaddition or through a diazo-transfer-like pathway. Subsequently, a [3+2] retrocycloaddition would furnish the amide product, with concomitant release of nitrogen gas and elemental sulfur (scheme 4). In the same study, a comprehensive evaluation of azide reactivity, revealed a strong dependence on their electronic properties (scheme 5). In particular, electron-deficient azides, such as sulfonyl azides, reacted rapidly at room temperature, with reactions reaching completion within minutes (k_{obs} = 5.7 × 10^{-3} $M^{-1}{\cdot}s^{-1}$ for EWG = SO_2Ph at 21°C). In contrast, electron-rich azides exhibited markedly lower reactivity, requiring elevated temperatures (≥ 60 °C) and prolonged reaction times (up to 36 h; k_{obs} = 4.5 × 10^{-6} $M^{-1}{\cdot}s^{-1}$ for EDG = CH_2Ph at 21 °C) to reach full conversion. The addition of a base, particularly 2,6-lutidine, was found to significantly accelerate the reaction, whereas acidic aqueous conditions favored the formation of thioamides.[22] To overcome the limited reactivity of electron-rich or sterically hindered azides, Fazio and Wong later developed a $RuCl_3$-promoted variant, significantly reducing reaction times to 18 hours in either H_2O or MeOH at room temperature with yields ranging from 36 to 91%.[23] Notably, this ligation proved compatible with a broad range of solvents, including aqueous media, and was successfully applied to biologically relevant substrates such as amino acids, β-glucosyl azide, and azidothymidine.[21]

These initial studies paved the way for more detailed mechanistic investigations, particularly focusing on the electronic density of the azido moiety, with the objective of improving the methodology and identifying related transformations.[20, 24, 25] Two distinct mechanisms, both involving a thiatriazoline intermediate, were proposed and validated through experimental evidence and density functional theory (DFT) calculations. These calculations examined the potential energy surfaces of reactions between thioacetate ion or thioacetic acid with methanesulfonazide and methyl azide. The key difference between the two mechanisms lies in the nature of the initial step. Electron-rich azides undergo a concerted process with thiocarboxylates or thiocarboxylic acids, directly forming the thiatriazoline in a single step. In contrast, electron-deficient azides initially form a nitrogen-sulfur bond to generate a linear intermediate, followed by subsequent step involving nitrogen-carbon bond formation and protonation to yield the thiatriazoline species. In both pathways, a retro-[3+2] cycloaddition then furnishes the amide product with the concomitant release of nitrogen gas and elemental sulfur (scheme 5).



Scheme 5. Proposed mechanisms of the TAL involving electron-deficient (Path 1) or electron-rich (Path 2) azide. EDG = electron-donating group; EWG = electron-withdrawing group.

Among electron-deficient azides, sulfonyl azides are particularly significant owing to their strong electrophilic character and straightforward synthesis, typically achieved *via* nucleophilic substitution of sulfonyl chlorides with azide salts. This highly reactive class of azides is at the core of the "sulfo-click" reaction. Although a few studies have explored applications of other electron-deficient azides, such as acyl azides[26-29] or perfluoroaryl azides,[30] this review focuses exclusively on sulfonyl azides.

3. The sulfo-click reaction: a powerful approach for bioconjugation

Selective and orthogonal bioconjugation reactions continue to expand the toolbox of chemical biology, enabling the covalent attachment of biomolecules to synthetic probes or functional moieties. Despite the broad utility of established ligation chemistries, there remains a need for reactions that combine mild, aqueous-compatible conditions with high chemoselectivity and tunable linkage stability. In this context, the sulfo-click reaction has emerged as a particularly promising approach. It enables the efficient coupling of thioacids and azides to form robust *N*-acylsulfonamide linkages, which can be further modulated for cleavability or specific functional outputs. Its orthogonality and reliability have been demonstrated across a range of bioconjugation applications, including the modification of peptides, proteins, carbohydrates, and nucleic acids, underscoring its potential as a versatile biocompatible click reaction.

3.1. Amino acid, peptide and protein

Given the structural similarity between native amide bonds and the *N*-acylsulfonamide moiety formed during the sulfo-click reaction, early applications focused on peptide chemistry. Indeed, the sulfo-click reaction offers several advantages over conventional amidation reactions between amines and carboxylic acids,[31, 32] which often require large amounts of expensive coupling reagents and generate stoichiometric quantities of waste.[33] Moreover, amide bonds are fundamental structural motifs in organic molecules, serving as building blocks of natural peptides and being widely present in polymers, natural products, and approximately 25% of all marketed drugs.[34] This central importance has driven the development of numerous catalytic, efficient, and environmentally sustainable methodologies for amide bond formation.[35-37] For electron-deficient amines such as sulfonamides, whose nucleophilicity is strongly diminished by electron-withdrawing substituents and which therefore remain unreactive under mild acylation conditions, the sulfo-click reaction provides a particularly suitable alternative to conventional amidation approaches.[38-44] In contrast, electron-rich amines are prone to undesired side reactions, which can complicate their use in amide bond formation.[21]

The first application of the sulfo-click reaction in amino acid and peptide chemistry was reported by the Liskamp group in 2005.[45] They demonstrated that protected β-substituted aminoethane sulfonyl azides could be efficiently coupled with amino thioacids to form α-amino acyl sulphonamides. Building on their previous work on the efficient five-step synthesis of *N*-protected β-aminoethanesulfonyl chlorides **17**,[46] they developed a strategy to access these derivatives from a wide range of Fmoc- and Cbz-*N*-protected amino acids **14**, including those bearing functionalized side chains. The chlorine atom was then efficiently replaced with an azide group, leading to the desired β-substituted aminoethane sulfonyl azides **18** (scheme 6).

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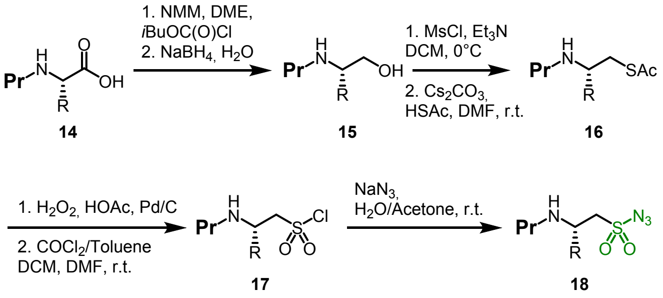

Scheme 6. General synthetic route to *N*-protected β-aminoethanesulfonyl azides. Pr = Fmoc or Cbz; R = side chain protecting groups corresponding to various amino acids (Ala, Val, Leu, Phe, or Ser). Overall yields: 32-60%.

Thereafter, the authors carried out the sulfo-click reaction using commercially available thioacids (*i.e.* thioacetic acid or thiobenzoic acid) in $CHCl_3$ or DMF in the presence of 2,6-lutidine at room temperature. This approach resulted in quantitative conversions and high isolated yields (87% to quantitative). To extend this methodology to dipeptide synthesis, Boc-Leu-SH **20** was first prepared from Boc-protected leucine hydroxysuccinimide ester (Boc-Leu-ONSu) by treatment with sodium hydrogen sulfide (NaHS).[47] Subsequently, the sulfo-click reaction was carried out under the same condition affording the desired dipeptide **21** in 81% isolated yield (Scheme 7).

Afterwards, they investigated the reaction between a dipeptide thioacid **23** and Cbz-Phe-$\Psi[CH_2SO_2]$-N_3 **22** to synthesize a tripeptide mimic **24** (scheme 7). Under the same conditions, the sulfonyl-azide **22** reacted efficiently with dipeptide thioacid **23**, yielding the orthogonally protected tripeptide mimic **24**, containing an *N*-acylsulfonamide moiety, in 99% yield (scheme 7). Both amino groups could be selectively deprotected, allowing controlled elongation of the peptide chain in either direction. The authors then confirmed the complete compatibility of the reaction with aqueous media,[21] by successfully conducting the sulfo-click reaction in a THF:H_2O mixture. This pioneering work led to the development of the first synthesis of peptidyl *N*-acylsulfonamides using sulfonyl azides and (amino)thio or peptide thioacids under mild conditions.

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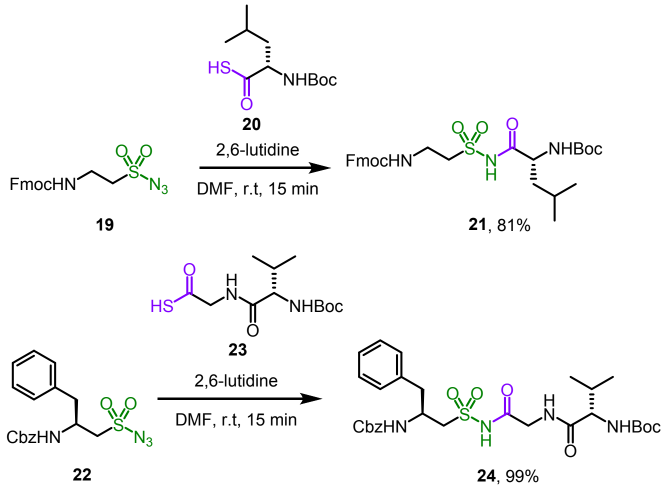
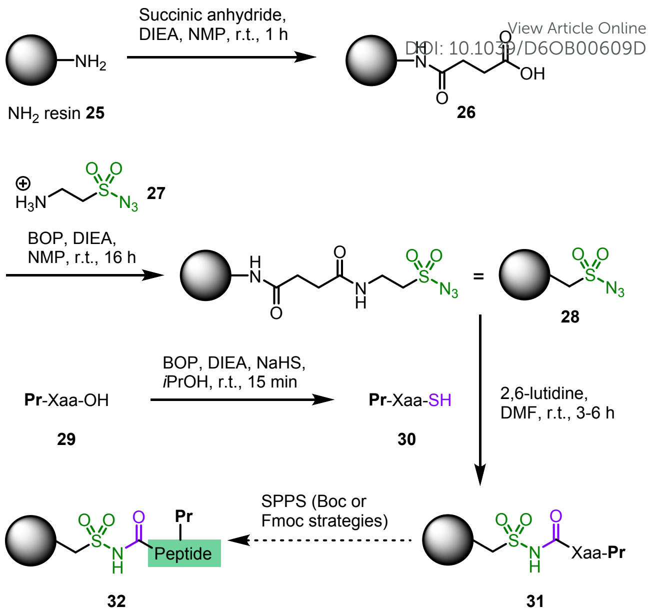


Scheme 7. Synthesis of peptidyl sulfonamide **24** using the sulfo-click reaction.

In a follow-up study, Liskamp and co-workers reported a robust and chemoselective strategy for the on-resin construction of *N*-acylsulfonamide linkers, offering a valuable platform for the *C*-terminal functionalization of peptides.[48] This approach relies on resin-bound sulfonyl azides, which undergo efficient coupling with Boc- or Fmoc-protected amino thioacids under mild conditions forming stable solid-supported *N*-acylsulfonamide linkages. Subsequent activation of the *N*-acylsulfonamide through microwave-assisted alkylation, followed by reaction with a variety of functionalized nucleophiles, enables the synthesis of *C*-terminal modified peptides (Scheme 8). The synthetic route began with the multigram-scale preparation of 2-aminoethanesulfonyl azide **27** from taurine,[49] which was thereafter coupled to a carboxylic acid-functionalized resin **26** (generated by reacting a commercially available amine resin **25** with succinic anhydride) *via* BOP-mediated amide bond formation. In parallel, *N*-protected amino thioacids **30** were prepared from their corresponding Boc- or Fmoc-protected precursors **29** using BOP and DIEA as activating agents, followed by treatment with sodium hydrosulfide (NaHS) in isopropanol to afford the thioacids **30**.[50] The later were directly coupled to the resin-bound sulfonyl azide **28** *via* the sulfo-click reaction performed in DMF at room temperature in the presence of 2,6-lutidine leading to the functionalized resin **31**. This chemoselective and mild protocol ensured high efficiency while preserving the stereochemical integrity of the amino acids and was compatible with both Boc and Fmoc protective groups for SPPS applications (**32**).



Scheme 8. Preparation of the resin-bound sulfonyl azide was followed by the application of the sulfo-click reaction using protected amino thioacids (Pr-Xaa = Boc-Phe, Fmoc-Ser(tBu), or Boc-Phe), enabling subsequent peptide chain elongation through either Boc- or Fmoc-based SPPS protocols.

Microwave-assisted activation of the *N*-acylsulfonamide linker in **32**, followed by reaction with various nucleophiles, enabled the synthesis of C-terminally modified peptides **34**. To demonstrate the interest of this strategy, the authors prepared an RGD-containing octapeptide (VGRGDSLA, Scheme 9) assembled on the *N*-acylsulfonamide linker. Peptide elongation was performed using standard Fmoc-based SPPS, with the final valine residue introduced in its Boc-protected form.

Activation of the *N*-acylsulfonamide linker was achieved *via* microwave-assisted alkylation using alkyl bromides. Among the reagents tested, 4-nitrobenzyl bromide proved the most effective, likely due to its strong electron-withdrawing character. The activation step was carried out by treating the resin with the alkyl bromide and DIEA in DMF at 150 °C for 6 minutes, affording the activated intermediate **33**. Subsequent reaction with a range of nucleophiles under mild conditions provided access to diverse C-terminally modified peptides **34**. The scope of the method was demonstrated using nucleophiles such as allylamine, propargylamine, and 2-aminoethanesulfonyl azide. These functionalities enable further transformations, including Diels-Alder, CuAAC, and sulfo-click reactions, thereby expanding the utility of SPPS toward the preparation of functionalized and reactive peptide probes.

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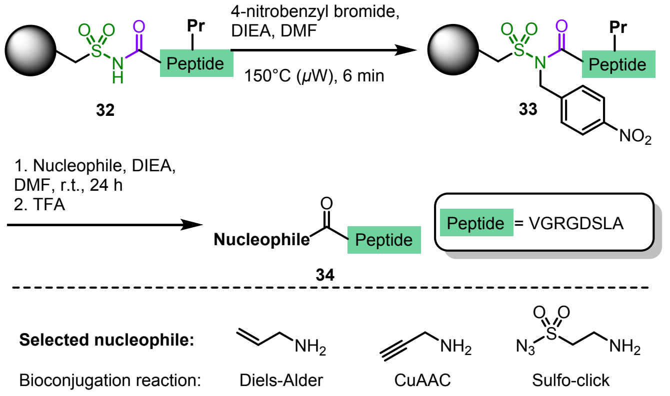
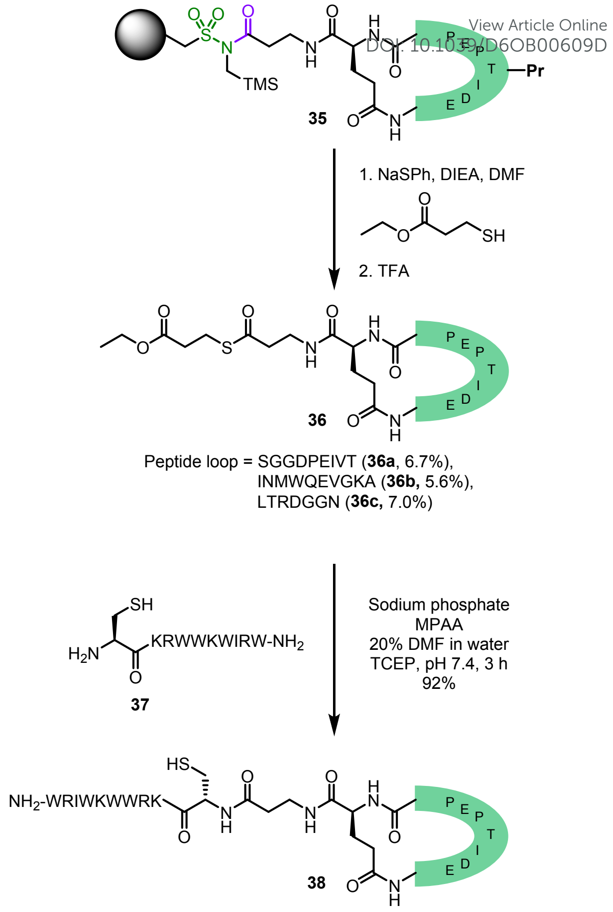


Scheme 9. Established optimized conditions for the functionalization and cleavage of resin-bound *N*-peptidyl sulfonamides, allowing the synthesis of *C*-terminal modified peptides; selected examples of nucleophiles used and associated chemical reactions.

The group of Liskamp later extended this strategy to the synthesis of cyclic peptide thioesters suitable for native chemical ligation (NCL).[51] Using the same *N*-acylsulfonamide linker introduced *via* the sulfo-click reaction at the resin-bound *C*-terminus, peptides were assembled by standard SPPS and subsequently cyclized on-resin through macrolactamization.[52] Following the approach outlined in Scheme 8, the anchored cyclic peptide bearing the *N*-acylsulfonamide linker was activated using trimethylsilyldiazomethane (**35**, Scheme 10). Subsequent nucleophilic cleavage, performed under forcing conditions to overcome steric hindrance, afforded the corresponding cyclic peptide thioesters **36**. This strategy was applied to several model sequences derived from the HIV gp120-CD4 interaction, demonstrating the feasibility of accessing structurally constrained peptides suited for NCL.



Scheme 10. Synthesis of a resin functionalized with a cleavable *N*-acylsulfonamide linker. General SPPS procedure for the preparation of cyclic peptides containing a thioester handle and NCL between LTRDGGN Loop **36c** and H-CKRWWKWIRW-NH_2 Peptide **37**.

To demonstrate the synthetic utility of these cyclic peptide thioesters, **36c** was engaged in NCL with an *N*-terminal cysteine-containing antimicrobial peptide **37** (HHC-10). Ligation was conducted in a mixture of aqueous buffer containing 20% DMF to improve solubility and resulted in complete conversion to the ligation product **38** in 3 hours, with a final isolated yield of 92% after HPLC purification (scheme 10). This study illustrates the versatility of the sulfo-click reaction for installing the *N*-acylsulfonamide moiety prior to SPPS elongation and highlights its potential as a modular tool for assembling complex, functionalized cyclic peptide architectures suitable for further ligation chemistry.[52]

In 2008, Liu *et al.* reported a mild and efficient approach to synthesize peptide thioacids directly on solid support.[53] Their strategy began with the preparation of peptide thioesters on ChemMatrix® resin, employing both Fmoc- and Boc-based solid-phase peptide synthesis (SPPS) protocols. The resin-bound peptide thioesters **40** were then transformed into peptide thioacids **41** through a hydrothiolysis reaction, using various hydrosulfide ion sources. This reaction enabled the simultaneous cleavage from the resin and conversion of the *C*-terminal thioester into a thioacid in a single, aqueous-compatible step (scheme 11).

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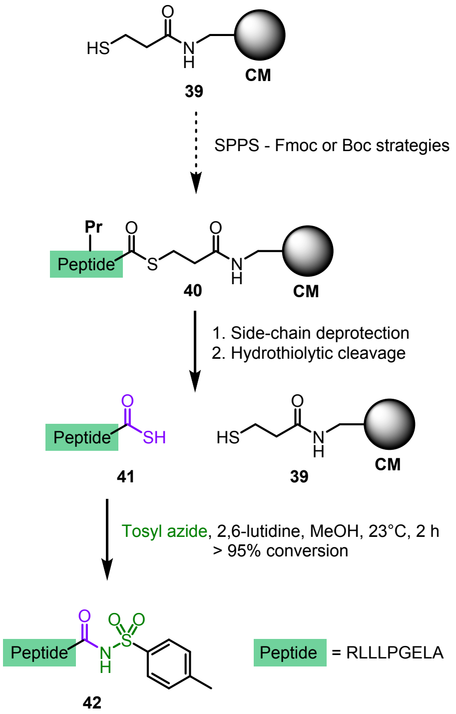
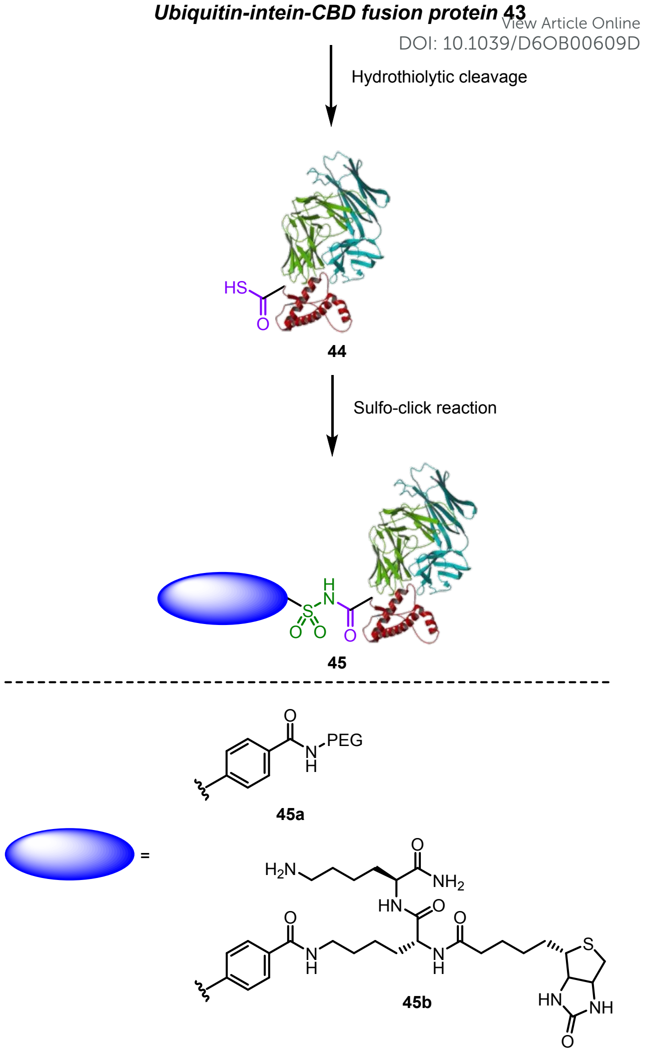


Scheme 11. Selected example of synthesis of peptide thioacids by solid-phase hydrothiolysis followed by a sulfo-click reaction.[53] CM = ChemMatrix resin. Hydrothiolytic conditions: 0.2 M $(NH_4)_2S$, 0.3M HEPES buffer, 6M guanidine-HCl, pH 8.6, r.t.

Additionally, the peptide thioacid H-RLLLPGELA-SH **41** was subjected to a sulfo-click reaction with tosyl azide in wet methanol, using 2,6-lutidine as a base. The reaction proceeded at room temperature affording the corresponding *N*-acylsulfonamide products **42** in excellent yield (scheme 11). Here, the sulfo-click reaction proved particularly valuable by directly transforming peptide thioacids, generated in a one-pot hydrothiolysis step, into *N*-acylsulfonamide conjugates under mild, aqueous conditions. The simplicity of this tandem process contrasts with lengthier protection-deprotection manipulations usually required for peptide *C*-terminal modifications, thus offering an efficient route for biocompatible peptide ligation and conjugation.[53]

The following year, the group or Liu reported a novel chemoselective approach for *C*-terminal modification of recombinant proteins[54] leveraging the sulfo-click reaction to achieve site-specific bioconjugation. The methodology began with the cloning of the ubiquitin gene into the pTYB1 vector, which encodes the *S. cerevisiae* VMA intein. Upon overexpression in *E. coli*, the resulting ubiquitin-intein-CBD fusion protein **43** was affinity-purified *via* binding to chitin resin. Hydrothiolytic cleavage was then carried out by incubating the bound fusion protein at 37 °C overnight in an aqueous buffer, generating the ubiquitin thioacid **44** at its *C*-terminus as originally described by Kinsland *et al.*[55]



Scheme 12. *C*-terminal thioacid ubiquitin elaboration followed by *C*-terminal PEGylation and biotinylation using the sulfo-click reaction. Hydrothiolytic conditions: 0.1 M Na_2S, 0.25 M HEPES, 0.5 M NaCl, 1 mM EDTA, pH 8.0, 37°C, O/N. Sulfo-click conditions: 0.3 mM thioacid ubiquitin in 6 M Gdn-HCl containing 3 mM 2,6-lutidine with 10 eq. of biotin or PEG sulfonyl azide derivatives at r.t.

This unique thioacid moiety was subsequently reacted with electron-deficient sulfonyl azides bearing biofunctional tags [polyethylene glycol (PEG) or biotin] resulting in *N*-acylsulfonamide linkages formation (scheme 12). These reactions were performed under mild aqueous conditions that maintained the structural integrity of the protein. Using ubiquitin as a model, this work demonstrated efficient protein *C*-terminal PEGylation (**45a**) and biotinylation (**45b**), yielding homogeneous conjugates in high yield while preserving native protein structure. The ability to directly couple thioacid-derivatized proteins with tagged sulfonyl azides distinguishes this approach from non-selective ligations (*e.g.* traditional amide chemistry) and provides a reliable route to protein conjugates.

In 2009, Crich et al. reported a versatile method for synthesizing peptide thioacids using a 9-Fluorenylmethyl thioester (Fm-SR) linker compatible with Boc-based SPPS.[56] This

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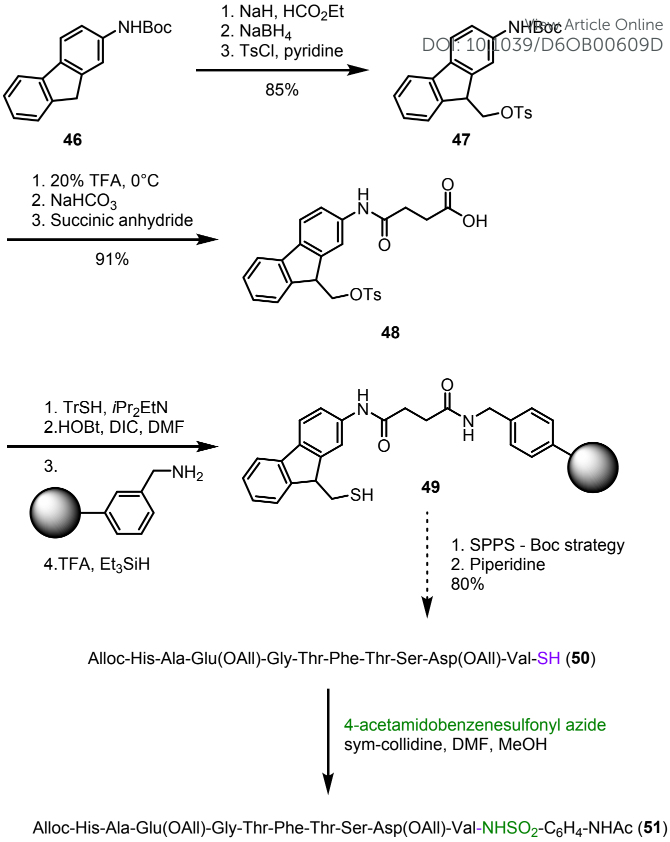
approach relies on anchoring the growing peptide chain to the resin via a 9-Fluorenylmethyl thioester-based linker, which remains stable throughout the repeated acidic treatments typical of Boc-SPPS protocols. Upon completion of peptide elongation, the linker is cleaved under mild basic conditions (*e.g.*, piperidine), releasing the *C*-terminal thioacid **50** in high yield and purity. The mercapto-functionalized linker *N*-[9-(OTs)-9H-fluoren-2-yl]succinamic acid **48** was synthesized from 9H-fluoren-2-(Boc)amine **46** *via* a multi-step sequence. Following -OTs substitution with tritylmercaptan and carboxylic acid activation, the linker was coupled to aminomethylpolystyrene resin and deprotected to yield the resin-bound fluorenylmethylthiol **49**. Standard Boc-based SPPS, using DIC/HOBt or HBTU as coupling agents, allowed assembly of peptidyl thioacids (scheme 13). Cleavage with piperidine released C-terminal thioacids, typically as N-Boc-protected peptides of high purity, confirmed by ESI-TOF MS and RP-HPLC. A variety of peptide thioacids were synthesized, including model and bioactive sequences from GLP-1[57], hsPLAA2,[58] and hPTH.[59] Importantly, Asn and Gln residues were incorporated without side-chain amide protection, with no significant cyclization observed. The strategy relied on Fmoc-based side-chain protection for amines and hydroxyls, and 9-Fluorenylmethyl esters or thioethers for acids and thiols, avoiding additional deprotection steps. When required, allyl- or alloc-protected building blocks enabled side-chain protection retention. A selected thioacid peptide (Alloc-His-Ala-Glu(OAll)-Gly-Thr-Phe-Thr-Ser-Asp(OAll)-Val-SH) was further converted into *C*-terminal *N*-acylsulfonamide **51** through the sulfo-click reaction, implemented with commercially available 4-acetamidobenzenesulfonyl azide, demonstrating the utility of this approach for peptide *C*-terminal modification (scheme 13).



Scheme 13. Preparation of the thiol-functionalized resin **49**, followed by SPPS, peptide cleavage, and a subsequent sulfo-click reaction.

In 2012, Rademann *et al.* introduced a novel and efficient Fmoc-compatible strategy for the SPPS of peptide thioacids,[60] offering a valuable alternative to previously established methods.[56, 61] This approach relies on the installation of a 2-cyanoethyl thioester moiety at the peptide *C*-terminus following standard Fmoc-based SPPS on a thiol functionalized 2-chlorotrityl chloride resin **52** previously reported by the authors.[62] Following peptide chain elongation and *N*-terminal deprotection, the resin-bound peptide **53** is treated with 3-mercaptopropionitrile to generate the corresponding *S*-(2-cyanoethyl) thioester **54** (scheme 14). This intermediate is then efficiently converted into the free thioacid **55** under mild basic conditions using $(NH_4)_2S$. The procedure is straightforward, high yielding, and applicable to a wide range of peptide sequences and lengths. The authors further demonstrated the synthetic utility of the resulting peptide thioacids by implementing the sulfo-click reaction. Using the pentapeptide thioacid Ac-SYRGF-SH **55** as model they explored two strategies : first peptide **55** was ligated with a sulfonyl azide-functionalized peptide in aqueous buffer (4-$N_3SO_2C_6H_4$-LYRAG-NH_2 **56**) using 2,6-lutidine as a base, affording the ligation product **57** in 84% yield within 15 minutes; second, a one-pot approach enabled the direct conversion of the 2-cyanoethyl thioester **54** into the ligated product **58** by *in situ* β-elimination using DBU in DMF followed by the sulfo-click reaction with tosyl azide and 2,6-lutidine (scheme 14).

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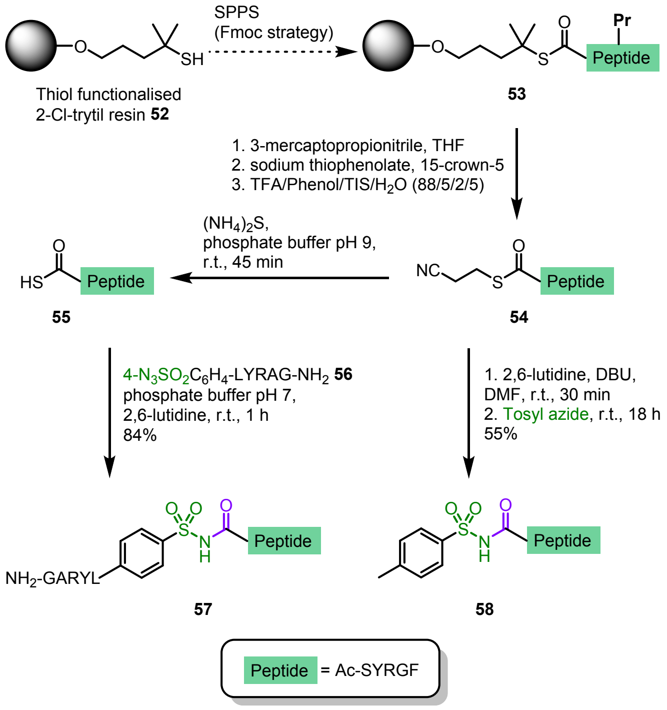
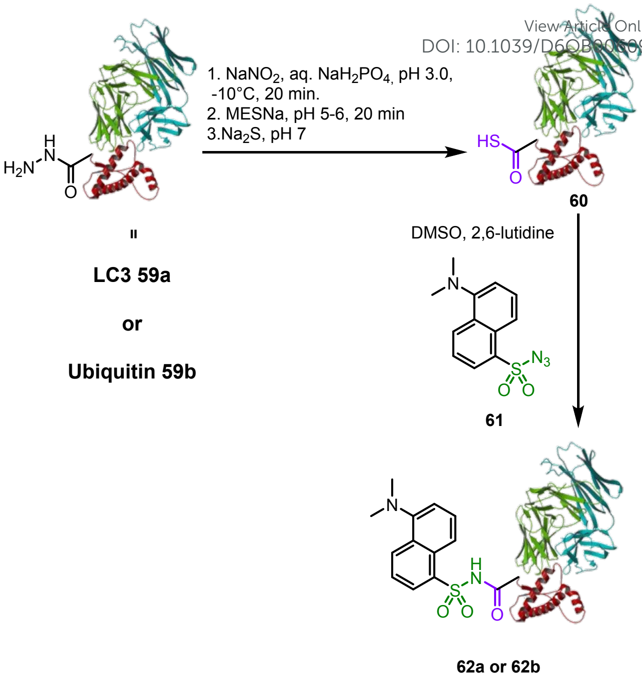


Scheme 14. Synthesis of peptide thioacids and implementation of the sulfo-click reaction.



Scheme 15. Synthesis of protein thioacids from protein acyl hydrazines and implementation of the sulfo-click reaction with Dansyl-N$_3$.

In 2014, Yang and co-workers reported an efficient one-pot strategy to convert peptide and protein hydrazides into *C*-terminal thioacids under mild aqueous conditions, enabling both peptide ligation and site-specific protein labelling.[63] The method proceeds *via* three steps: NaNO$_2$-mediated activation of peptide hydrazides under acidic conditions (pH 3.0) to form acyl azides, conversion of these intermediates into C-terminal thioesters using 2-mercaptoethanesulfonic acid sodium salt (MESNa) at slightly acidic pH (5.0-6.0), and *in situ* hydrothiolysis with Na$_2$S at pH 7.0 and room temperature to yield thioacids in high purity and yield using the model peptide Leu-Tyr-Arg-Ala-Gly-NHNH$_2$ (LYRAG-NHNH$_2$), the corresponding thioacid LYRAG-SH was obtained in 48% isolated yield after 1 hour at room temperature. To evaluate the scope of the method, a series of pentapeptides Leu-Tyr-Arg-Ala-**X**-NHNH$_2$, with various *C*-terminal residues were synthesized. Peptides ending with Ser, Phe, Ala, and Leu were quantitatively converted to thioacids within 3 hours under standard conditions (pH 7.0, 100 mM Na$_2$S, room temperature), while bearing bulkier residues such as Pro and Val required longer reaction times but still gave excellent conversions, indicating good substrate tolerance. The method was successfully applied to the recombinant LC3 protein (LC3-NHNH$_2$ **59a**), produced *via* intein-CBD fusion expression (scheme 15).[55]

The latter was efficiently converted into its *C*-terminal thioacid form **60** (LC3-SH, microtubule-associated protein 1A/1B-light chain 3)[64] under the same conditions previously applied to peptides. This transformation enabled site-specific conjugation with dansyl azide **61** *via* the sulfo-click reaction in DMSO using 2,6-lutidine as a base, yielding the fluorescently labeled LC3 protein **62a** (Scheme 15). Noteworthy, because dansyl azide **61** itself is non-fluorescent, this strategy provides a highly valuable fluorogenic platform.[65] Similar results were obtained with ubiquitin hydrazide **59b** (Scheme 15),[54] further demonstrating the broad applicability of this method for site-selective protein labelling.

In 2019, Hayashi and co-workers developed a multifunctional photocrosslinking strategy based on a diazirine-containing probe, featuring a cleavable *N*-acylsulfonamide linkage and a cinnamate-derived photo-convertible tag to improve protein target identification in photoaffinity labelling (PAL).[66] The diazirine moiety, known for generating highly reactive carbenes upon UV irradiation, was chosen for its fast crosslinking kinetics and minimal background reactivity.[67] The key innovation is the *N*-acylsulfonamide group serving as a "click-on/off" cleavable linker between the ligand and the photoreactive core. This linker is hydrolytically stable under physiological conditions, but can be easily cleaved after *N*-alkylation (*e.g.* with iodoacetamide) as previously demonstrated by Liskamp and co-workers,[48] enabling mild and selective release of labelled proteins from avidin-functionalized beads. To construct the probe **70**, the authors employed a sulfo-click reaction between a diazirine thioester **64**, deprotected *in situ* with DBU, and the biotin-sulfonyl azide functionalised peptide **69** obtained via the incorporation of the sulfonyl azide-modified amino acid **66** (Fmoc-Ala(SO$_2$-N$_3$)-OH) during SPPS (Scheme 16).

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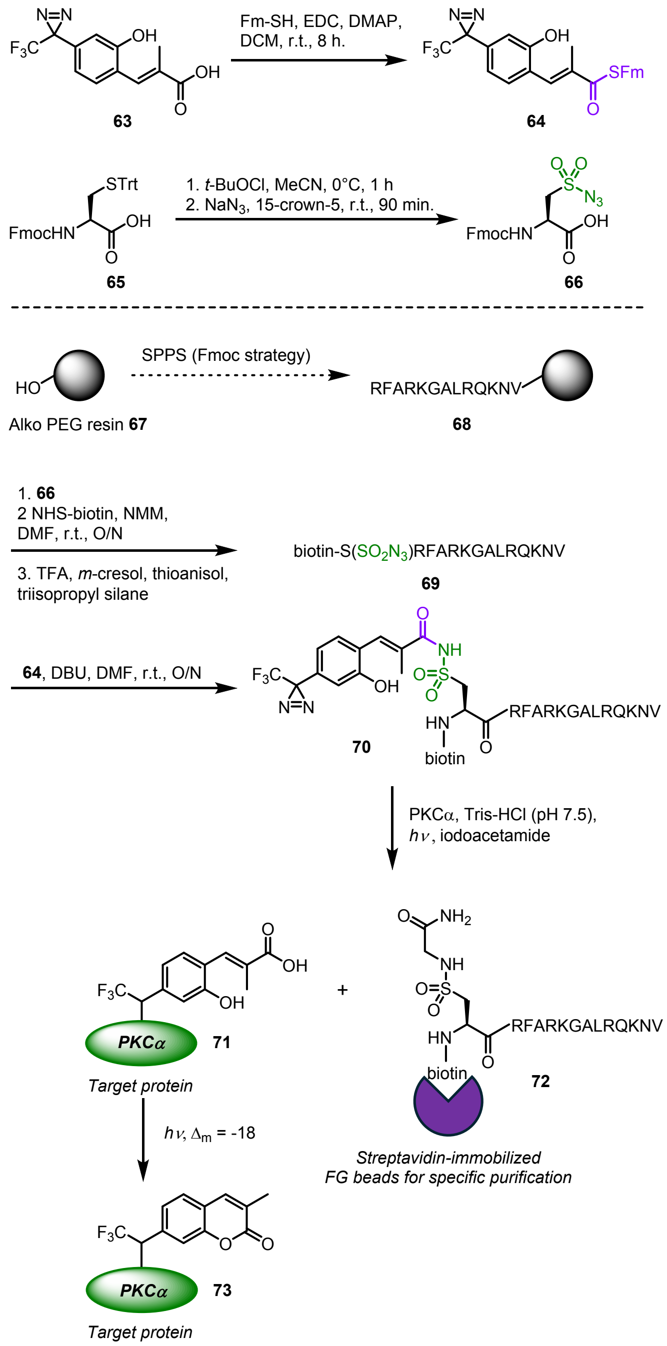

Scheme 16. Synthesis of diazirine thioester derivative **64**, Fmoc-Ala(SO_2-N_3)-OH **66**, and photoprobe **70**. PKCα protein tagging using probe **70**, followed by iodoacetamide-mediated *N*-acylsulfonamide cleavage for streamlined purification. Identification *via* UV-induced coumarin formation.

Two versions of probe **70** were synthesized, differing in the position of the diazirine-sulfonamide moiety along a peptide sequence derived from a known protein kinase Cα (PKCα) inhibitor.[68] Both probes retained inhibitory activity against PKCα, as confirmed by ATPase assays. Upon UV irradiation, the diazirine moiety was covalently crosslinked to adjacent residues on recombinant PKCα, and successful labelling was detected *via* avidin-HRP chemiluminescence. A distinctive advantage of this system relies on the cinnamate scaffold, which undergoes a photoinduced intramolecular cyclization upon subsequent UV irradiation, successfully yielding a coumarin fluorophore **73** (Scheme 16). This transformation facilitates peptide identification through a characteristic mass shift (Δm = -18 u) and confirms the origin of low-abundance signals, thereby reducing false positives in MS analysis. LC-MS/MS of tryptic digests revealed multiple labelled residues, including Asp463, Gly348, and Lys352, corresponding to specific regions of the kinase binding pocket. Structural mapping using PDB data (4RA4) showed that each probe variant captured distinct binding interface regions, providing detailed insights into ligand orientation and interaction surfaces. The *N*-acylsulfonamide linkage exhibited exceptional stability under standard PAL conditions (neutral/acidic pH, light exposure, SDS buffer), but could be selectively cleaved by iodoacetamide treatment leading to compound **72** and allowing high-yield elution (>80%) of labelled proteins while preserving protein integrity. This selective cleavage also prevents co-elution of endogenously biotinylated proteins, a common contaminant after affinity purification procedures (Scheme 16). To validate the approach in complex systems, the authors applied it to HeLa lysates and successfully identified endogenous targets, including phosphoglycerate kinase 1 (PGK1), with Lys11 identified as a labelled residue. The photoconversion of cinnamate to coumarin further allowed differentiation between labelled peptides and background based on retention time and mass signature, a key advantage when signal intensities are near detection limits. Overall, this innovative photoaffinity labelling strategy, combined with the sulfo-click reaction enables modular incorporation of a cleavable *N*-acylsulfonamide linker. Compared with conventional cleavable systems such as dithionite-sensitive azobenzene,[69] photosensitive nitrophenyl,[70] or periodate-cleavable vicinal diol linkers,[71] the *N*-acylsulfonamide motif offers superior chemical stability under physiological conditions and selective cleavage under mild alkylation conditions, minimizing sample degradation and preserving labelling specificity.

3.2. Carbohydrates

In 2015, Rohmer *et al.* reported a novel approach for functionalizing carbohydrates with sulfonyl azide groups, enabling their use for the sulfo-click reaction.[72] The authors developed an efficient strategy to modify both *β*-glucose pentaacetate **74** and *β*-galactose pentaacetate **75**, employing 2-hydroxyethane-sulfonyl azide **76** synthesized from mercaptoethanol as previously described by King *et al.*[73] Initial attempts using the Koenigs-Knorr and trichloroacetimidate glycosylation methods resulted in poor yields. However, the use of a BF_3·etherate-catalysed glycosylation reaction significantly improved the outcome, affording the desired sulfonyl azide-functionalized carbohydrate derivatives **77** and **78** in 65% and 70% yields respectively (scheme 17). The thioacid partners employed in this study were synthesized using two distinct strategies based on amino acid derivatives. The first approach involved the preparation of activated NHS esters, which react

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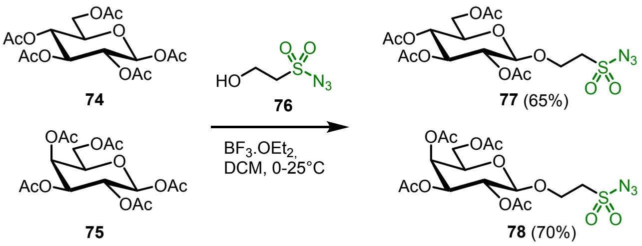
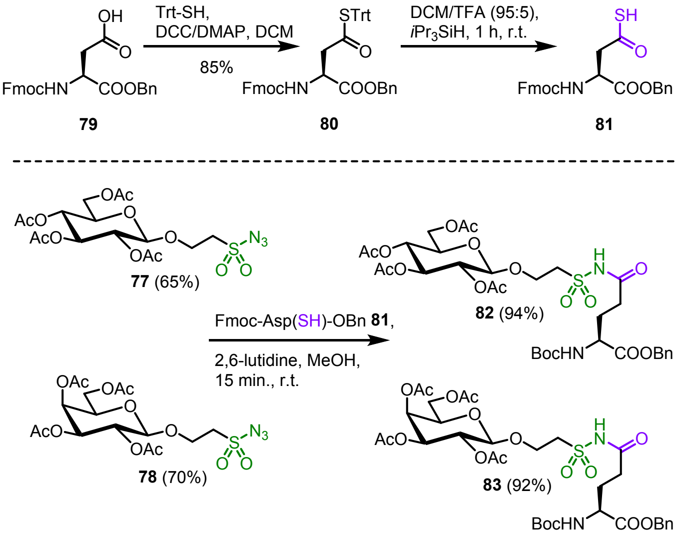
with NaHS to afford the corresponding thioacids.[47] However, this method presented practical limitations, including undesired transesterification in methanol and oxidative dimerization of thioacids into diacyl disulfides under basic conditions.



Scheme 17. Synthesis of sulfonyl azide-modified glycosides **77** and **78**.

To address these challenges, an alternative strategy was investigated to generate the thioacid under acidic conditions. Trityl thioesters were selected as precursors, as they can be quantitatively converted into thioacids upon treatment with diluted trifluoroacetic acid. Accordingly, the protected aspartic acid derivative **79** was first converted into the corresponding trityl thioesters **80** *via* DCC/DMAP-mediated activation. Subsequent treatment with 5% TFA in DCM yielded the thioacid Fmoc-Asp(SH)-OBn **81** (Scheme 18). After solvent removal, the crude thioacid was directly engaged in a sulfo-click reaction with sulfonyl azides **77** and **78**, affording the corresponding products **82** and **83** in overall yields of 94% and 92% over the two steps, respectively (Scheme 18) thus demonstrating the value of the sulfo-click reaction for carbohydrate bioconjugation, overcoming limitations often associated with conventional methods that typically rely on metal catalysis or harsh activation conditions.[74]



Scheme 18. Synthesis of Fmoc-Asp(SH)-OBn **81** ; selected examples of carbohydrate bioconjugation mediated by the sulfo-click reaction.

3.3. Nucleic acids

In 2003, Williams *et al.* reported the implementation of the TAL to the unactivated 3′-azido-3′-deoxythymidine using commercially available thioacetic and thiobenzoic acids under aqueous conditions at 60 °C for 36 hour, affording the corresponding products in 77% and 68% isolated yields, respectively.[21] In light of our ongoing efforts toward the development of 5′-modified nucleoside derivatives for constructing novel internucleosidic linkages,[75-77] and the growing interest in the sulfo-click reaction as a tool for functional bioconjugation, we decided to explore the applicability of this reaction in the context of nucleic acid chemistry.[78] We first decided to introduce the thioacid functionality at the 4′-position of nucleosides, employing a strategy based on the deprotection of a 9-Fluorenylmethyl (Fm)-protected nucleoside 4′-thioester. An efficient three-step synthetic route was developed starting from 3′-*O*-TBDMS-protected nucleosides **86a-d** (Scheme 19). The 5′-hydroxyl of nucleosides **86a-d** were oxidized to the corresponding carboxylic acids using TEMPO in the presence of BAIB as co-oxidant.[79] These intermediates were then coupled with 9-Fluorenylmethylthiol (Fm-SH) to afford the protected thioesters in high yields. Final deprotection of both the thioester and TBDMS groups using TBAF in THF led to the 4′-thiocarboxylate derivatives as tetrabutylammonium (TBA) salts **87a-d**. In parallel, a series of sulfonyl azide partners were synthesized in a single step under classical peptide coupling conditions, using 2-aminoethanesulfonyl azide as a short linker.[49] To demonstrate the broad applicability of the sulfo-click reaction to nucleoside derivatives, a diverse set of functional labels was selected, including biotin **88 a-d**, an amino acid **89a-d**, a modified nucleoside **90a-d**, the fluorophore Cy5.0 **91**, and an hexapeptide **92** (Scheme 19).

Kinetic studies demonstrated that the reaction proceeded to full conversion within 10 minutes at room temperature under aqueous conditions (pH 8.5). The reaction followed second-order kinetics, with rate constants of $k = 2.0 \times 10^{-1}$ $M^{-1}.s^{-1}$ at 25°C and $k = 2.4 \times 10^{-1}$ $M^{-1}.s^{-1}$ at 37°C, significantly surpassing the rate reported under organic conditions ($k = 5.7 \times 10^{-3}$ $M^{-1}.s^{-1}$ at 21°C).[24] These rate constants are comparable to, or even exceed, those reported for other bioorthogonal ligation methods, such as the Staudinger ligation ($k \approx 10^{-4}$-10^{-3} $M^{-1}.s^{-1}$) and the SPAAC ($k \approx 10^{-2}$ - 1 $M^{-1}.s^{-1}$).[80]

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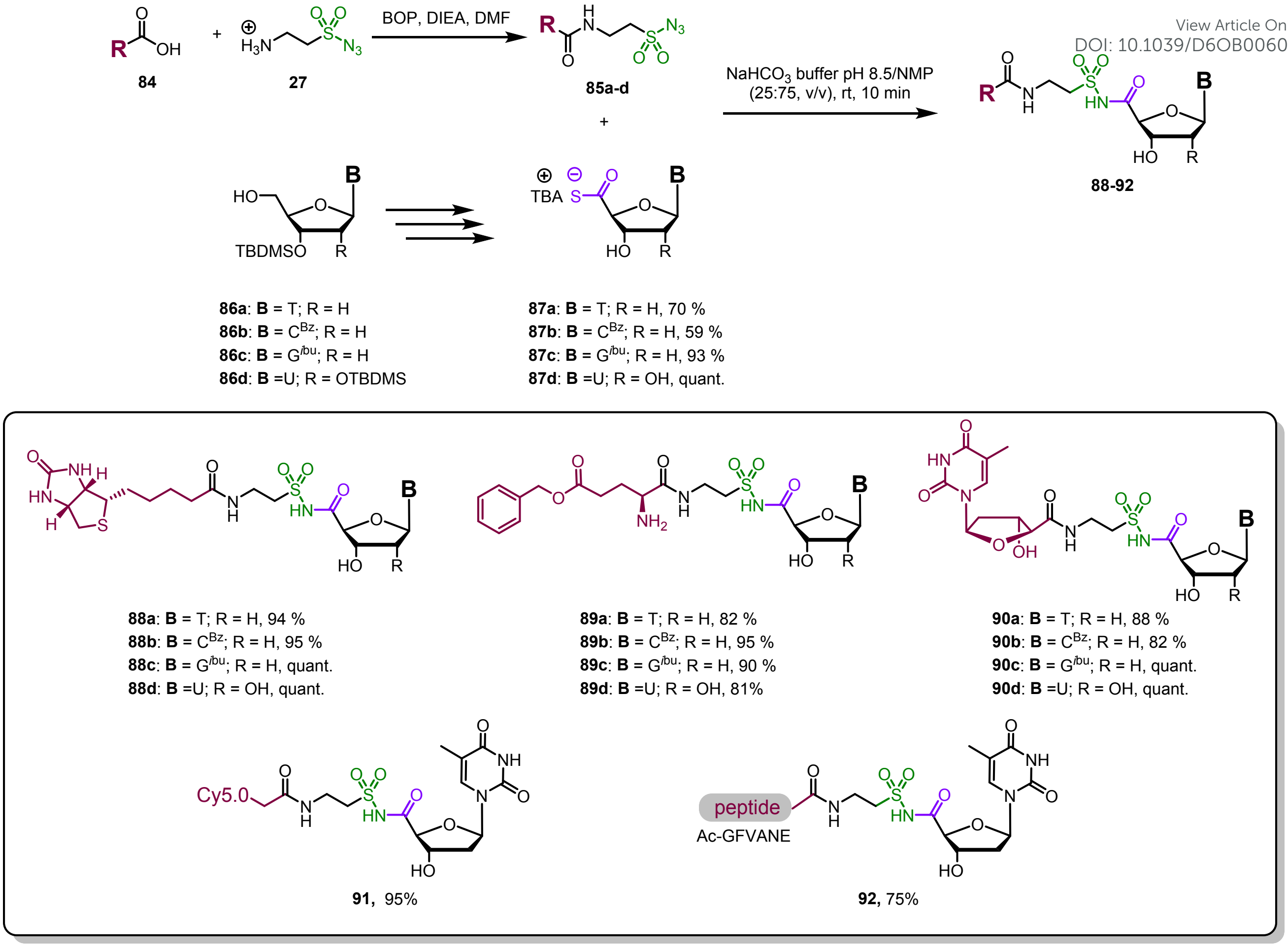

Scheme 19. Synthesis of 4′-thioacid nucleosides and sulfonyl azide derivatives, followed by their coupling *via* the sulfo-click reaction under biocompatible aqueous conditions. Adapted with permission from ref 78. Copyright 2020, American Chemical Society.

Following this initial work, we subsequently investigated the application of the sulfo-click reaction for medicinal chemistry-oriented modifications notably by exploiting the *N*-acylsulfonamide moiety as a phosphate bioisostere.[14]

In particular, we developed the synthesis of a new class of 4′-(*N*-acylsulfonamide) modified adenosine nucleosides as inhibitors of the SARS-CoV-2 nsp14 N7-guanine-methyltransferase, an enzyme essential for viral RNA cap formation and evasion of host immune responses (Scheme 20).[81] The sulfo-click reaction was exploited to install *N*-acylsulfonamide groups at the 4′-position of adenosine nucleosides in aqueous media, with sodium bicarbonate as the sole additive. The synthesis of sulfonyl azides **(93a,b,e-g)** was achieved in a one-step reaction using commercially available sulfonyl chlorides, which were treated with sodium azide in acetonitrile at 60°C for 16 hours.[82] Compound **93d** was synthesized by deprotecting 4-acetamidobenzenesulfonyl azide **93c** with concentrated aqueous HCl.[83] Thereafter, we extended our previous methodology to synthesize 4′-thioacid adenosine derivatives (**94** and **95**) through the three-step process previously developed.[78] These derivatives were then engaged in sulfo-click reactions with the sulfonyl azides prepared using our optimized conditions previously described affording the corresponding 4′-(*N*-acylsulfonamide) adenosine derivatives (**96a-d, 97a-g,** 84%-quant.).[78] After final deprotection, a library of 20 unprotected adenosine derivatives **98a-c and 99a-f,** were obtained with high yields (81-88% over two steps, Scheme 20).

These compounds were evaluated for their ability to inhibit SARS-CoV-2 N7-methyltransferase (nsp14) using a radioactive MTase assay.[84] The results showed only moderate inhibitory activity (11-40%) for six compounds at 5 µM, with none exhibiting inhibition comparable to the sulfonamide derivatives (IC50 < 0.5 µM).[85]

These findings demonstrate the sulfo-click reaction as an efficient and versatile method for installing 4′-*N*-acylsulfonamide groups on adenosine nucleosides. In contrast to earlier approaches, which typically required strictly anhydrous conditions and extended reaction times,[112, 113] this method proceeds rapidly in aqueous media with only minimal additives, affording products in excellent yields and purity.

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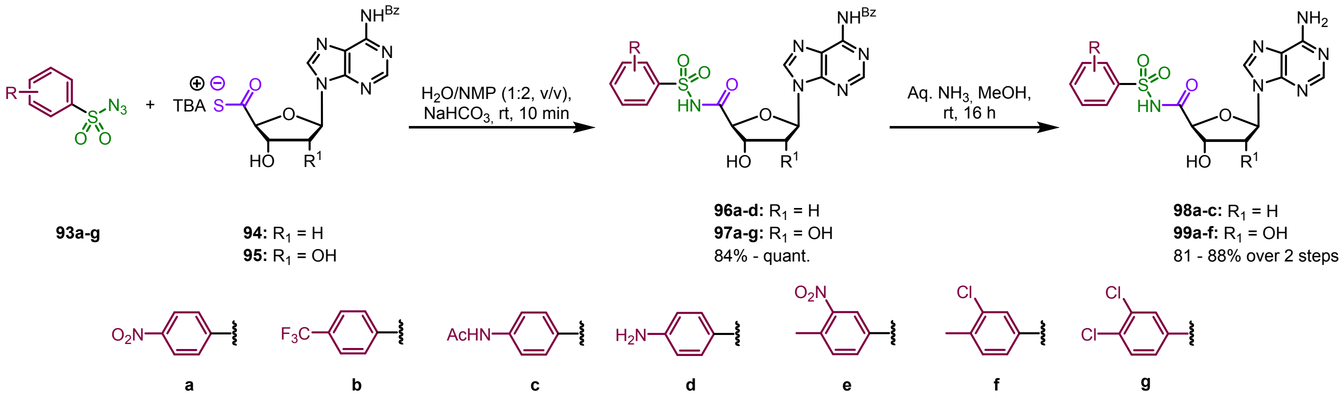

Scheme 20. Synthesis of the 20 adenosine derivatives synthesized. Adapted with permission from ref 81. Copyright 2022, Royal Society of Chemistry.

Subsequently, in 2024 we reported an innovative strategy for the synthesis of modified cyclic dinucleotides (CDNs) using the sulfo-click reaction.[86] CDNs are key second messengers that activate the STING (Stimulator of Interferon Genes) protein, which plays a central role in innate immunity and has garnered significant interest for therapeutic applications.[87-95] In this work, we synthesized four new CDN analogues in which one phosphate diester linkages was replaced with an *N*-acylsulfonamide moiety, offering a bioisosteric replacement with distinct electronic and hydrogen bonding properties. The design aimed to enhance chemical stability while retaining or modulating biological activity. A convergent synthetic strategy was developed to access novel CDN analogues incorporating the *N*-acylsulfonamide linkage *via* the sulfo-click reaction (Scheme 21). The 3′-hydroxyl group of commercially available 5′-*O*-DMTr nucleosides was activated using 4-nitrophenyl chloroformate and coupled with 2-aminoethanesulfonyl azide[49] to afford sulfonyl azide-modified building blocks **100a,b**. The synthesis of 4′-thioacid nucleoside counterparts was performed according to previously reported methods,[78, 81, 96] enabling their coupling under previously optimized aqueous sulfo-click conditions to generate nucleoside dimers **101a-c** in high yields (87-96%),.[78, 96] These dimers were subsequently converted into phosphoramidite precursors, and following a series of deprotection steps, H-phosphonate intermediates **102a-c** were obtained with good yields over three steps (65-69%). Cyclization using pivaloyl chloride proceeded efficiently (~85% yield), and final oxidation steps enabled the introduction of phosphodiester or phosphorothioate linkages. Final removal of nucleobase-protecting groups delivered the four target CDNs **103a-c** in overall yields of about 15% (Scheme 21).

The CDN analogues were evaluated across four different STING protein haplotypes but showed no significant activity in this assessment. Nevertheless, the study demonstrated the synthetic value of the approach, the sulfo-click reaction enabled the efficient, bioisosteric replacement of phosphates with *N*-acylsulfonamides moiety, providing straightforward and convergent access to CDN analogues under mild conditions paving the way for future development of structurally diverse CDN-based therapeutics.

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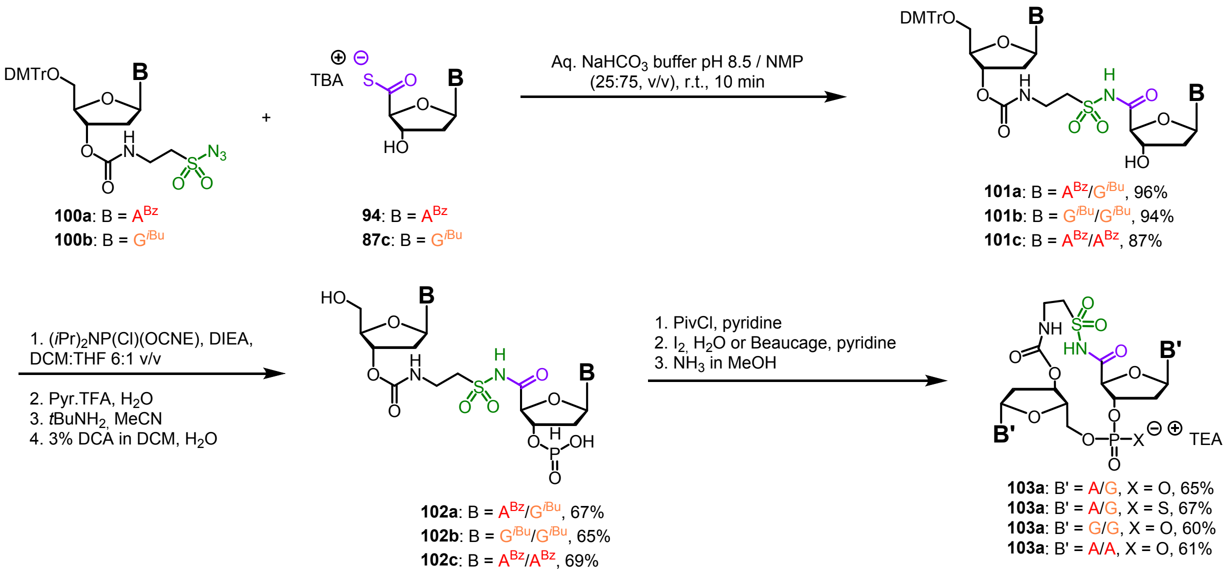

Scheme 21. Sulfo-click assembly of nucleoside precursors **100a,b** and **94/87c** for the synthesis of CDNs **103a-d**. Adapted with permission from ref 86. Copyright 2024, American Chemical Society.

Building on the internucleoside linkage originally developed for CDN (Scheme 21),[86] we recently demonstrated its incorporation into oligonucleotide (ODN) sequences, thereby extending its potential application to antisense oligonucleotides (ASOs).[97] Specifically, we synthesized a thymidine dimer connected through a (2-(*N*-acetylsulfamoyl)ethyl)carbamate (NAC) linkage *via* the sulfo-click reaction, and its subsequent integration into several ODNs to evaluate their biophysical properties.[98] This nine-bond internucleoside linkage merges carbamate and *N*-acylsulfonamide functionalities to yield a negatively charged and enzymatically stable backbone.

The synthesis of the NAC linkage began with the preparation of sulfonyl azide **100c** from 5′-*O*-DMTr-thymidine **104** in two steps, involving carbonate activation followed by reaction with 2-aminoethanesulfonyl azide, following protocols previously described on A and G.[86] The key sulfo-click reaction between this sulfonyl azide and the 4′-thioacid thymidine derivative **87a**[78] was performed under aqueous conditions, yielding dimer **101d** in 88% yield. Subsequent phosphitylation gave the phosphoramidite building block **106** in an overall 42% yield over four steps (Scheme 22). After optimization of coupling conditions, this building block was then incorporated at various positions within a 12-mer ODN using solid-phase synthesis (Table 1).

Table 1 T_m values of the duplexes formed between **ODN1–6** and their complementary ssDNA or ssRNA[a]

	Sequences[a]	Complementary strand Tm (ΔTm)/°C	
		DNA	RNA
ODN 1	5′-d(TTCTGTTACATT)-3′	36.7	38.2
ODN 2	5′-d(T_{NAC}TCTGTTACATT)-3′	35.9 (-0.8)	36.2 (-2)
ODN 3	5′-d(TTCTGTTACATT_{NAC}T)-3′	35.7 (-1)	36.1 (-2.1)
ODN 4	5′-d(TTCTGTT_{NAC}TACATT)-3′	29.4 (-7.3)	30 (-8.1)
ODN 5	5′-d(T_{NAC}TCTGTTACATT_{NAC}T)-3′	34.9 (-1.8)	33.9 (-4.3)
ODN 6	5′-d(T_{NAC}TCTGTT_{NAC}TACATT_{NAC}T)-3′	27.3 (-9.4)	-

[a] Conditions: 10 mM sodium cacodylate buffer (pH 7), 100 mM NaCl, and 3 μM of each ODN. The Tm values reflect the average of three measurements. Target DNA sequence: 5′-AATGTAACAGAA-3′. Target RNA sequence: 5′-AAUGUAACAGAA-3′. All linkages except for NAC are phosphodiesters.

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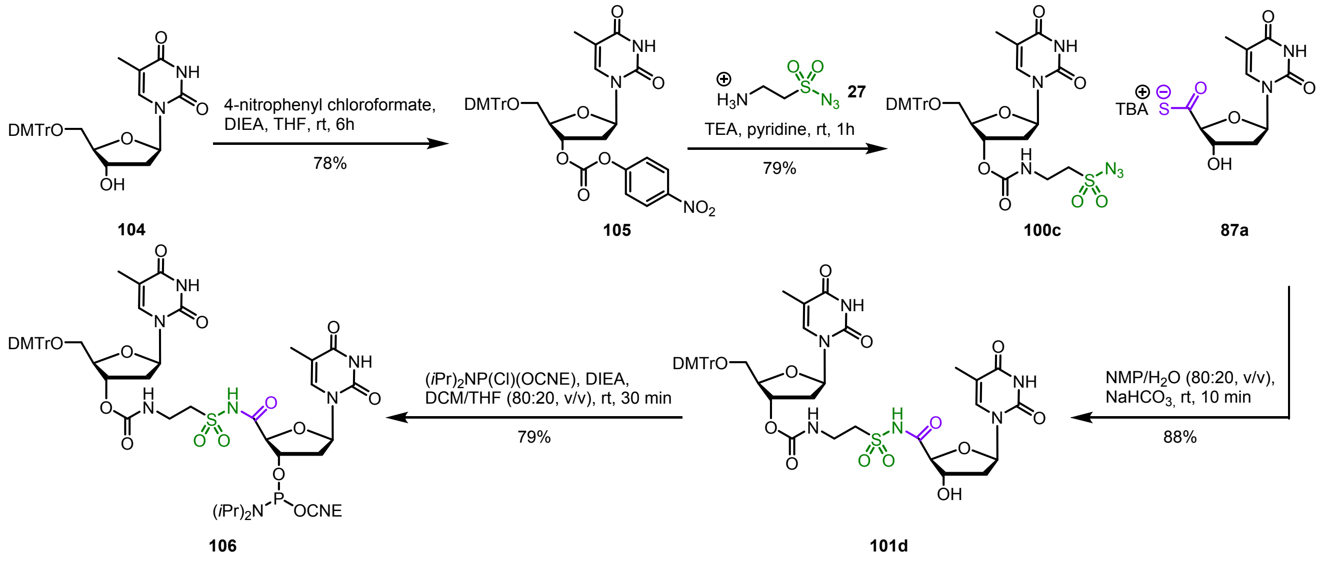

Scheme 22 Synthesis of NAC-linked phosphoramidite dimer **106**. Adapted with permission from ref 98. Copyright 2025, Royal Society of Chemistry.

Thermal denaturation studies revealed that the position of the NAC linkage strongly influences duplex stability with complementary DNA or RNA strands (Table 1). Central placement of the NAC linkage caused significant destabilization (up to -8.1 °C in T_m), likely due to perturbations in base stacking and backbone flexibility, whereas terminal modifications exhibited minimal effects on duplex stability (ΔT_m ~ -1 °C). Evaluation of the nuclease resistance of the NAC-modified oligonucleotides revealed a complete resistance to 3′-exonuclease (calf spleen phosphodiesterase) and partial resistance to 5′-exonuclease (snake venom phosphodiesterase). These findings suggest that the NAC linkage could be strategically employed in gapmer architectures where the modified segment serves to protect the ends of the molecule while preserving central RNase H-compatible regions.[99] Earlier approaches to *N*-acylsulfonamide internucleoside linkages have been reported by Matteucci[100] and Widlanski.[101] They both relied on the preparation of 3′-sulfonamide nucleosides followed by DCC-mediated coupling to 4′-carboxylic acid derivatives. While these studies provided the desired dinucleotides, the synthetic route was long and low-yielding, with overall yields below 8% for the key building blocks and only moderate yields (~65%) for the final coupling. In contrast, the sulfo-click strategy enables direct and high-yielding access to NAC-linked thymidine dimers (42% overall yield) despite the structural complexity of the linkage. This approach is fully compatible with standard solid-phase ODN synthesis, allowing site-specific incorporation into ODNs.

4. Medicinal chemistry and drug development

The *N*-acylsulfonamide moiety has attracted growing attention in medicinal chemistry owing to its peculiar physico-chemical properties. Recognized as a bioisostere of carboxylic acids and phosphates,[14] this functional group exhibits comparable acidity (pKa ≈ 3.5-4.5) and hydrogen-bonding capabilities, while offering enhanced metabolic and chemical stability. Therefore, *N*-acylsulfonamides are increasingly incorporated into pharmacologically active compounds, including antivirals, antibacterial and anticancer agents. [102-112] Their ability to form stable interactions with biological targets, combined with improved resistance to enzymatic hydrolysis, contributes to their favourable drug-like profile. In addition, the *N*-acylsulfonamide scaffold has been explored in the design of nucleotide and peptide mimetics, where it enhances binding affinity and target selectivity. Altogether, the incorporation of this motif provides medicinal chemists with a powerful tool for fine-tuning molecular interactions and modulating physico-chemical properties of lead compounds.

Notable examples include inhibitors of the hepatitis C virus (HCV) NS3-4A protease,[105] asparagine synthetase (ASNase),[102] carbonic anhydrase,[107] and various bacterial enzyme,[109, 113] as well as compounds with antiproliferative activity against tumors by targeting proteins such as Bcl-2[110] or MMP2 (Fig. 1).[112]

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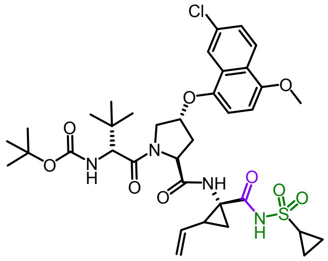
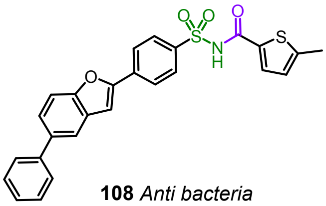
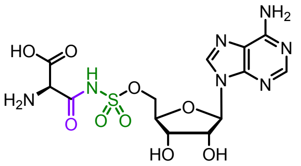


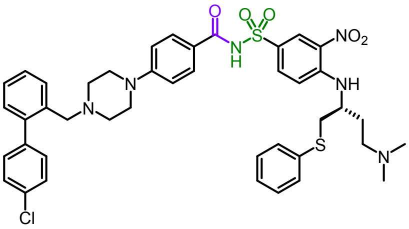
107 *Anti virus*
Asunaprevir (BMS-650032), hepatitis C virus (HCV)
NS3 protease inhibitor, IC_{50} 0.2 nM-3.5 nM



108 *Anti bacteria*
E. coli, MurC inhibitor,
IC_{50} 2.3 μm



109 *Anti leukemia*
Human asparagine synthetase inhibitor
K_i* of 728 nM



110 *Anti cancer*
Navitoclax (ABT-737), Bcl-2, Bcl-xL
and Bcl-w inhibitor with EC_{50} of 30.3 nM,
78.7 nM, and 197.8 nM, respectively.

Fig. 1. Selected examples of bioactive *N*-acylsulfonamide derivatives.[102, 103, 106, 111]

In this context, the advent of the sulfo-click reaction offers medicinal chemists a direct, modular, and high-yielding access to this pharmacophore under aqueous and mild conditions. Compared with conventional coupling strategies, the sulfo-click reaction minimizes the need of protecting groups and avoids harsh reagents, thereby enabling the rapid preparation of pharmacologically relevant libraries.

4.1. Small-molecule drug design

Kinetic target-guided synthesis (KTGS) is an innovative strategy to identify biologically active compounds, allowing the simultaneous exploration of multiple molecular scaffolds.[114-116] Unlike conventional fragment-based drug discovery approaches, KTGS places the biological target at the heart of the ligand assembly process. In this unconventional method, the target itself facilitates the formation of its optimal multidentate ligand by accelerating the reaction between complementary reactive fragments. This acceleration occurs through proximity effects and precise molecular orientation within the binding site, leading to selective product formation that favors biologically relevant interactions. KTGS has demonstrated its broad applicability across various biological systems, including enzymes, RNA molecules, protein-protein interaction interfaces, and phosphate-binding sites, under *in vitro* conditions.[114-116] In this context, bioorthogonal and biocompatible reactions are particularly attractive as recently highlighted in a review.[115] The sulfo-click reaction, fulfilling the requirements of a bioorthogonal and biocompatible transformation, is particularly well-suited for KTGS applications. As this topic has been comprehensively reviewed by Manetsch it will not be discussed in detail herein.[117] To illustrate the versatility of the sulfo-click reaction in KTGS strategies one representative example is presented. The Manetsch group employed this reaction for the *in situ* assembly of Bcl-xL inhibitors (Scheme 23),[118, 119] targeting a protein involved in apoptosis regulation. The strategy consisted in incubating sulfonyl azide and thioacid fragments (also evaluated as 9-Fluorenylmethyl thioester precursors, deprotected *in situ* with 5% piperidine in DMF) in the presence of Bcl-xL under physiological conditions).[120] HPLC-MS analyses of the reaction mixture confirmed the formation of **SZ4TA2 111**, containing the *N*-acylsulfonamide moiety and exhibiting high affinity for the target (IC_{50} = 78.8 nM). Noteworthy, this active compound had previously been identified by Abbott Laboratories,[121] highlighting the value of the sulfo-click reaction as a valuable tool for KTGS -based discovery and optimizing protein-protein interaction modulators.

More recently, Manetsch and co-workers described a variant of the sulfo-click reaction using selenoacids instead of thioacids, which they coined as “seleno-click amidation” in the context of KTGS applications.[122] This modification takes advantage of the higher reactivity of selenoacids, enabling the reaction to proceed with excellent efficiency even at low temperatures (4 °C). Such conditions are particularly beneficial for thermally sensitive biological systems, where very mild reaction environments are required to preserve the integrity of targets or ligands.

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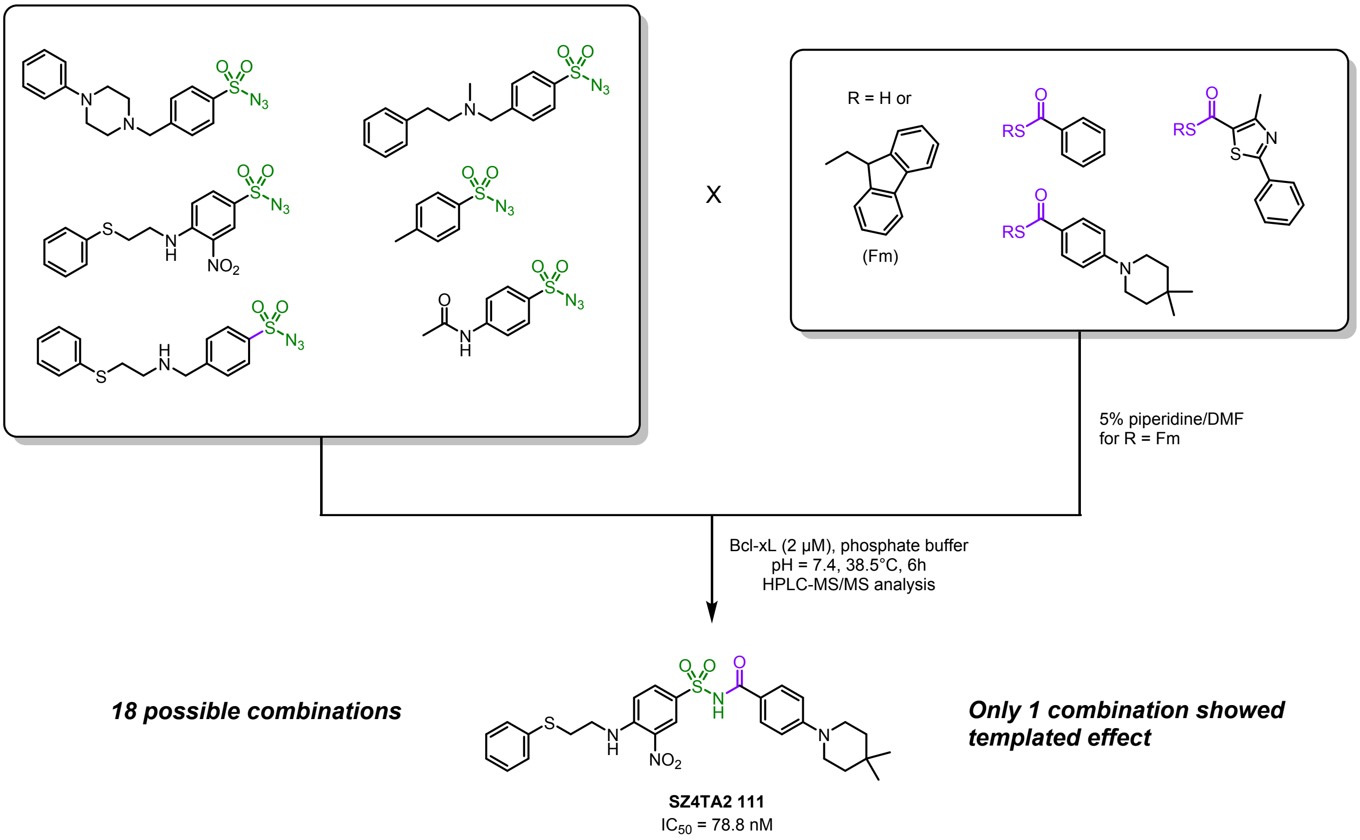

Scheme 23. KTGS was applied using a variety of sulfonyl azides and thioacid fragments to facilitate the discovery of novel Bcl-xL inhibitors.

Apart from KTGS-based strategy, we recently investigated the potential of the *N*-acylsulfonamide moiety as an alternative pharmacophore to classical sulfonamides in the development of antibacterial agents.[123] Traditional sulfa drugs, which inhibit bacterial dihydropteroate synthase (DHPS) in the folate biosynthesis pathway,[124, 125] have seen declining efficacy due to widespread resistance.[126] In this work, the sulfo-click reaction was employed to synthesize and assess the biological activity of 17 novel *N*-acylsulfonamide derivatives along with the known antibiotic sulfacetamide.[127, 128] First, 4-acetamidobenzenesulfonyl azide **93c** was hydrolyzed in concentrated HCl to yield 4-aminobenzenesulfonyl azide **93d**.[83] This choice was motivated by the desire to generate analogues structurally related to the natural *p*-aminobenzene scaffold, as well as hydrophobic counterparts, to enable both synthetic and biological comparisons. The corresponding thioacids **112a-i** were either purchased or synthesized in one step from carboxylic acids using a modified version of the procedure described by Kanai using AcSK and catalytic Ac_2S in DMF.[129] Then, the sulfo-click reactions were performed under standard optimized conditions, yielding *N*-acylsulfonamide products **113a-i** and **114a-i** within 10 minutes (Scheme 24). The method proved robust with both electron-donating and electron - withdrawing sulfonyl azides and tolerated various aryl thioacid substituents, consistently affording high isolated yields.

This efficiency, combined with the simplicity and rapidity of the one-step process, highlights the superiority of this approach over traditional multistep protocols, which typically suffer from lower overall yields and more demanding conditions.[130-133] The antibacterial activity of these compounds was evaluated against four relevant bacterial strains: *E. coli* and *P. aeruginosa* (Gram-negative), and *B. subtilis* and *S. aureus* (Gram-positive). Among the synthesized molecules, three derivatives (**114b, 114h, and 114i**) exhibited interesting antibacterial activity, with minimum inhibitory concentrations (MICs) comparable to sulfacetamide, particularly against *E. coli* and *B. subtilis*.

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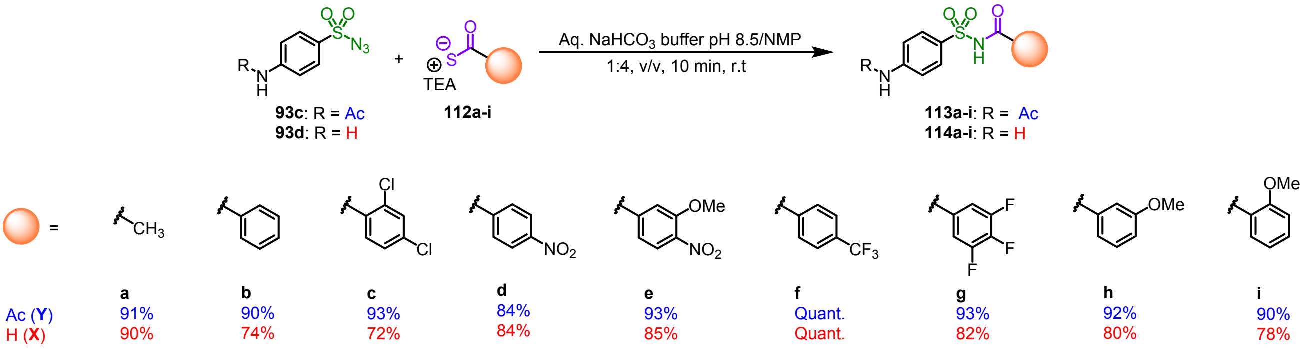
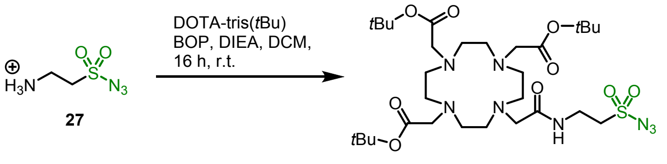


Scheme 24. Scope of the new sulfa drugs synthesized. Adapted with permission from ref 123. Copyright 2023, Royal Society of Chemistry.

Structure-activity relationship analysis revealed that electron-donating methoxy substituents on the aromatic ring enhanced activity, while electron-withdrawing groups had a detrimental effect. Interestingly, positional effects were observed: compound **114i** (ortho-methoxy) was active against *E. coli*, while **114h** (meta-methoxy) was active against *B. subtilis*, demonstrating structural differences in DHPS active sites across strains. Additionally, compounds **114b**, **114h**, and **114i** were assessed for their ability to inhibit bacterial biofilm formation (a key factor in antibiotic resistance) at 0.5 MIC concentration.[134, 135] Compound **114h** exhibited the strongest antibiofilm effect, reducing biofilm growth by up to 42% in *E. coli* and 23% in *P. aeruginosa*, surpassing sulfacetamide. None of the active compounds showed cytotoxicity in human keratinocyte (HaCaT) or intestinal epithelial (Caco-2) cell lines. These results highlight not only the synthetic efficiency of the sulfo-click reaction over traditional protocols that are often low-yielding and operationally demanding but also its potential to accelerate the discovery of novel bioactive compounds.

4.2. Radiolabelling and theranostics

In 2010, Liskamp and coworkers reported the design, synthesis, and biological evaluation of a series of DOTA-conjugated mono-, di-, and tetrameric analogues of [Tyr³]octreotide, developed as potential agents for tumor imaging and radionuclide therapy.[136, 137] Their synthetic strategy combined CuAAC-mediated assembly of multivalent peptide scaffolds with subsequent metal-free attachment of the DOTA chelator *via* the sulfo-click reaction. In order to implement this reaction, they first synthesized the *tert*-butyl protected DOTA sulfonyl azide derivative **115** *via* BOP-mediated coupling of DOTA-Tris(*t*Bu) with 2-aminoethanesulfonyl azide **27**,[49] affording **115** in 20% isolated yield after purification (Scheme 25). Mono-, di-, and tetrameric dendrimeric alkynes bearing TMOB-protected thioacid groups **119a-c** were prepared by EDCI-mediated esterification of alkyne-derived benzoic acids **117a-c**[138-140] with TMOB-thiol **118** (Scheme 26). Azide **116** and terminal alkynes **119a-c** were coupled *via* a CuAAC click reaction (Scheme 26), affording the corresponding triazole derivatives **120a** (47%), **120b** (63%), and **120c** (40%).



115 (20%)

Scheme 25. Synthesis of *t*Bu-protected DOTA-derived sulfonyl azide **115**. Adapted with permission from ref 137. Copyright 2010, American Chemical Society.

Radiolabeled [Tyr³]octreotide *N*-acylsulfonamides **121a-c** were synthesized from thioesters **120a-c** via a four-step sequence. Thioesters were first deprotected to generate thioacid intermediates, which then underwent the sulfo-click reaction with DOTA-sulfonyl azide to form *t*Bu-protected *N*-acylsulfonamide DOTA conjugates. Acid treatment removed the protecting groups, and the resulting conjugates were efficiently radiolabeled with ^{111}In, yielding the final radiolabeled compounds (Scheme 27).

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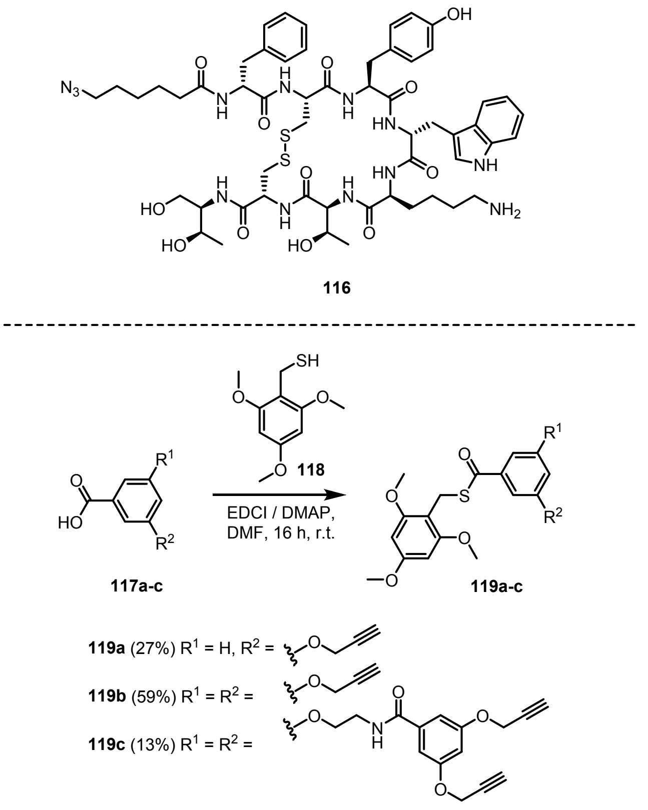
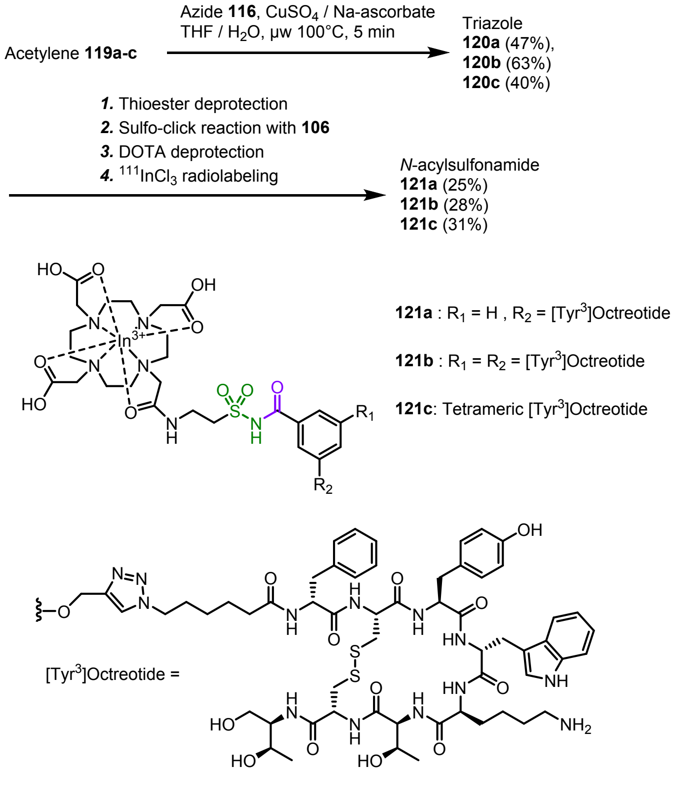


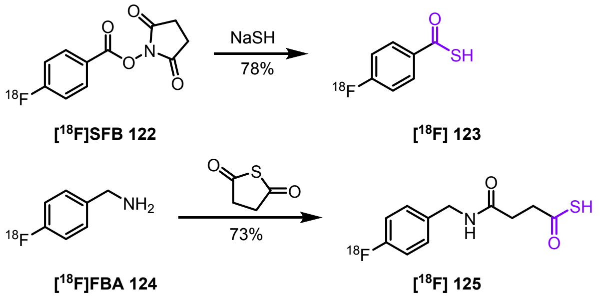
Scheme 26. Chemical structure of N_3-Ahx-D-Phe-cyclo(Cys-TyrD-Trp-Lys-Thr-Cys)-Threol **116** ; synthesis of TMOB-protected mono-, di-, and tetrameric alkyne-derived thio Esters **119a-c**, respectively. Adapted with permission from ref 137. Copyright 2010, American Chemical Society.

Radio-HPLC analysis indicated radiochemical yields >98% for ^{111}In-labeled **121a** (specific activity: 13.6 GBq/µmol) and **121b** (22.7 GBq/µmol), and 80% for ^{111}In-labeled **121c** (43.3 GBq/µmol). Competitive binding assays using AR42J rat pancreatic tumor cells revealed that the monomeric [Tyr³]octreotide conjugate **121a** displayed the highest affinity (IC_{50} = 1.32 nM), followed by the dimeric analogue **121b** (IC_{50} = 2.45 nM) and the tetrameric variant **121c** (IC_{50} = 14.0 nM). In biodistribution studies conducted in BALB/c nude mice bearing subcutaneous AR42J tumors, the [^{111}In]-labeled monomeric [Tyr³]octreotide conjugate **121a** exhibited the highest tumor uptake, reaching 42.3 ± 2.8 % ID/g at 2 h post-injection, significantly surpassing that of the dimeric conjugate **121b**, with sustained tumor retention of 25.3 ± 5.9 % ID/g at 2 h and 12.1 ± 1.3 % ID/g at 24 h post-injection. Biodistribution studies of the [^{111}In]-labeled tetramer **121c** revealed that excessive multivalency compromised its pharmacological profile. Unlike its monomeric **121a** and dimeric **121b** counterparts, the tetramer exhibited negligible tumor uptake and high sequestration in the liver and spleen. Collectively, these data underscore the efficacy of [Tyr³]octreotide constructs as effective vehicles for targeted radionuclide delivery in cancer theranostics. This approach circumvented the persistent issue of residual copper contamination encountered in earlier CuAAC-based strategies, which can compromise chelator integrity and radionuclide complexation.[140, 141]



Scheme 27. Synthesis of mono-, di-, and tetrameric DOTA-conjugated [Tyr³]octreotide sulfonamides **121a-c**. Adapted with permission from ref 137. Copyright 2010, American Chemical Society.

In 2019, Wuest and co-workers reported the first application of the sulfo-click reaction for the synthesis of ^{18}F-labeled *N*-acylsulfonamide derivatives using ^{18}F-labeled thioacids for positron emission tomography (PET) imaging applications.[142] They implemented a one-pot three-component reaction that proceeded efficiently under mild conditions in less than 30 minutes. A broad range of ^{18}F-labeled *N*-acylsulfonamide derivatives with radiochemical conversions ranging from 38% to 99% were obtained. Additionally, the method enabled the radiolabelling of peptides, yielding isolated and decay-corrected radiochemical conversions (RCCs) of 20-25%.



Scheme 28. Synthesis of ^{18}F-labeled thio acids **[^{18}F] 123** and **[^{18}F] 125**. Adapted with permission from ref 142. Copyright 2019, Royal Society of Chemistry.

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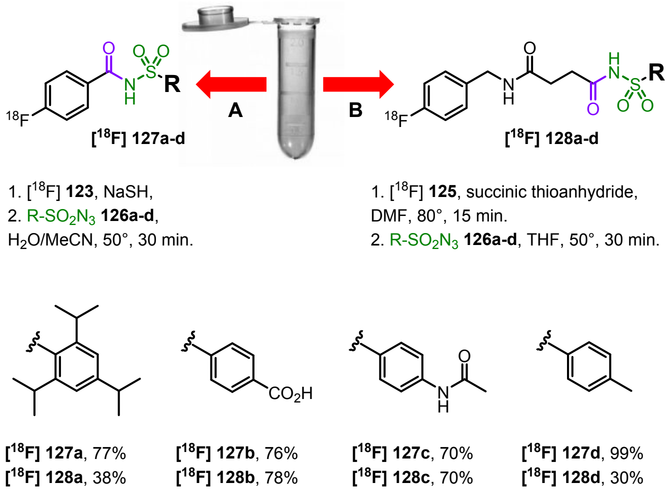
The authors investigated first the *in situ* generation of ^{18}F-labeled thioacids **[^{18}F] 123** and **[^{18}F] 125** by rapid reaction of commercially available ^{18}F-labeled precursors, *N*-succinimidyl 4-[^{18}F]fluorobenzoate (**[^{18}F]SFB 122**, 78%) and 4-[^{18}F]fluorobenzylamine (**[^{18}F]FBA 124**, 73%), respectively, with either NaSH or succinic thioanhydride (Scheme 28). These intermediates were not isolated and directly subjected to one-pot, three-component coupling reactions with various sulfonyl azides. (Scheme 29). Thioacid **[^{18}F] 123** afforded high RCCs (70-99%) across all four model sulfonyl azides **126a-d** leading to radiolabelled **[^{18}F] 127a-d** derivatives. **[^{18}F] 125** also performed well leading to **[^{18}F] 128a-d**, but the yields of **[^{18}F]128a** and **[^{18}F]128d** were lower (38% and 30%, respectively). These reduced RCCs may be attributed to the lower acidity of **[^{18}F] 125** compared to **[^{18}F] 123** and the steric and electronic properties of the coupling sulfonyl azide partners (Scheme 29).



Scheme 29. Two one pot/three-component reaction schemes were explored: (A) [^{18}F]SFB/NaSH/R–SO_2N_3 **126a-d** and (B) [^{18}F]FBA/succinic thioanhydride/R–SO_2N_3 **126a-d** in DMF/THF. Yields indicated are RCCs. Adapted with permission from ref 142. Copyright 2019, Royal Society of Chemistry.

Thereafter, compounds of interest were used to demonstrate the utility of the sulfo-click reaction for the synthesis of drug-like PET radiotracers. A celecoxib-derived sulfonyl azide was prepared as a labelling precursor by replacing the sulfonamide pharmacophore of celecoxib with a sulfonyl azide group. Sulfo-click reactions of thioacids **[^{18}F] 123** and **[^{18}F] 125** with the celecoxib-derived sulfonyl azide precursor afforded the corresponding ^{18}F-labeled *N*-acylsulfonamides **[^{18}F] 129** and **[^{18}F] 130** in comparable radiochemical conversions of 67% and 55%, respectively (Fig. 2). Additionally, peptide radiolabelling was studied using a sulfonyl azide-modified model tetrapeptide, Gly-Leu-Ser-Phe, synthesized *via* standard Fmoc-based SPPS. The sulfonyl azide was introduced at the *N*-terminal using 4-(azidosulfonyl)benzoic acid. Radiolabelling using the sulfo-click reaction with **[^{18}F] 123** and **[^{18}F] 125** provided the corresponding labelled peptides **[^{18}F] 131** and **[^{18}F] 132** in decay-corrected radiochemical yields of 25% and 20%, respectively, (Fig. 2). These features establish the sulfo-click reaction as an alternative practical and generalizable route to radiotracers and theranostic agents.

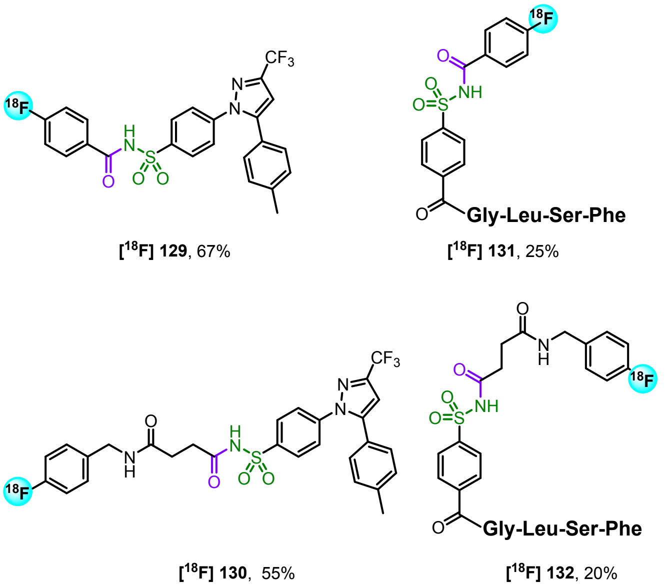


Fig. 2. Radiosynthesis of *N*-acylsulfonamides **[^{18}F] 129** and **[^{18}F] 130** based on anti-inflammatory drug celecoxib and ^{18}F-labeled peptides **[^{18}F] 131** and **[^{18}F] 132** using the sulfo-click reaction as bioconjugation method. Yields indicated are RCCs. Adapted with permission from ref 142. Copyright 2019, Royal Society of Chemistry.

Summary and outlook

Since its initial development, the sulfo-click reaction has become a cornerstone of the click chemistry repertoire. It is operationally simple, proceeds under mild, transition-metal-free conditions, and is compatible with biomolecules in aqueous media. This reaction efficiently generates *N*-acylsulfonamide moieties, which are structurally analogous to native amide and peptide bonds, offering clear advantages over many traditional click strategies. As highlighted in this review, its applications now span site-specific biomolecule labeling, the synthesis of pharmacologically relevant compounds, and the development of enzyme inhibitors. pharmacologically relevant compounds and enzyme inhibitors.

Kinetically, the sulfo-click reaction is slower than the fastest bioorthogonal reactions, such as strain-promoted azide-alkyne cycloaddition or inverse-electron-demand Diels-Alder reactions. Nevertheless, its moderate reaction rates are sufficient for *in vitro* applications, particularly when considering its high chemoselectivity, its close bioisosteric relationship to native amide and phosphate functionalities, and the robustness of the *N*-acylsulfonamide linkage. In this context, the sulfo-click reaction fills a complementary position within the click chemistry landscape.

Despite these advantages, current limitations must be acknowledged when considering *in cellulo* or *in vivo*

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applications. These include the need for relatively high reactant concentrations and potential competition with cellular nucleophiles. Furthermore, hydrolytic or oxidative instability of some thioacid derivatives can limit their effective lifetime in biological media. Addressing these challenges will likely require a combination of proximity-driven or templated reaction strategies. Fine-tuning the electronic properties of both coupling partners to increase the reaction kinetics while preserving chemoselectivity, as well as the design of masked or activatable sulfo-click reagents, could further expand the applicability of the reaction in living systems.

Taken together, these considerations suggest that the future impact of the sulfo-click reaction will not rely solely on further acceleration of its kinetics, but rather on its thoughtful integration into biological systems where structural relevance, stability and chemoselectivity are paramount. With continued methodological refinement, the sulfo-click reaction is well positioned to evolve from a robust *in vitro* ligation strategy into a versatile tool for chemical biology and medicinal chemistry applications.

Author contributions

We strongly encourage authors to include author contributions and recommend using CRediT for standardised contribution descriptions. Please refer to our general author guidelines for more information about authorship.

Conflicts of interest

There are no conflicts to declare.

Data availability

A data availability statement (DAS) is required to be submitted alongside all articles. Please read our full guidance on data availability statements for more details and examples of suitable statements you can use.

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No primary research results, software or code have been included and no new data were generated or analysed as part of this review.