Approaches to the Sulfation of Small Molecules: Current Progress and Future Directions

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Abstract

Sulfation is one of the most important modifications that occur to a wide range of bioactive small molecules including polysaccharides, proteins, flavonoids, and steroids. In turn, these sulfated molecules have significant biological and pharmacological roles in diverse processes including cell signalling, modulation of immune and inflammation response, anti-coagulation, anti-atherosclerosis, and anti-adhesive properties. This *essay* summarises the most encountered chemical sulfation methods of small molecules. Sulfation reactions using sulfur trioxide amine/amide complexes are the most used method for alcoholic or phenolic groups in carbohydrates, steroids, proteins, and aliphatic or alicyclic scaffolds. Despite the effectiveness of these methods, they suffer from some issues such as multiple-purification steps, toxicity issues (eg pyridine contamination), purification challenges, stoichiometric excess of reagents which leads to increase of a reaction cost, and intrinsic stability issues of both the reagent and product. Recent advances including SuFEx, the Malins *in situ* reagent approach and TBSAB show the widespread appeal of novel sulfating approaches that will enable a larger exploration of the field in the years to come by simplifying the purification and isolation to access bespoke sulfated small molecules.

Summary points (3-5 bullet points)

- Sulfation of small molecules is implicated in critical biological signalling cascades and a key phase II drug metabolism step.
- Methods to prepare sulfated molecules to enable biological study are both limited and have practical disadvantages to isolate tractable quantities of the desired sulfate.
- Emerging methods that build upon the early work of amine-sulfur trioxide complexes (e.g. tributylsulfoammonium betaine, TBSAB), *in situ* preparation of reactive sulfating complexes, and SuFEx methodology are gaining traction in the sulfation community.

Introduction

Sulfur has been recognised as one of the earliest known elements for its therapeutic properties by the ancient Greeks.[1] It is one of the essential components for all living organisms, occurring in the amino acids, methionine and cysteine, and in the vitamins, thiamine and biotin, amongst others.[1][2] Approximately, 250 FDA drugs bearing sulfur-containing functional groups were approved, including for hypertension, diabetes mellitus, bacterial infections, migraine, cardiovascular diseases (CVD), neurological disorders, cancer, and human immunodeficiency virus (HIV) (**Figure 1**).[1][2][3][4]

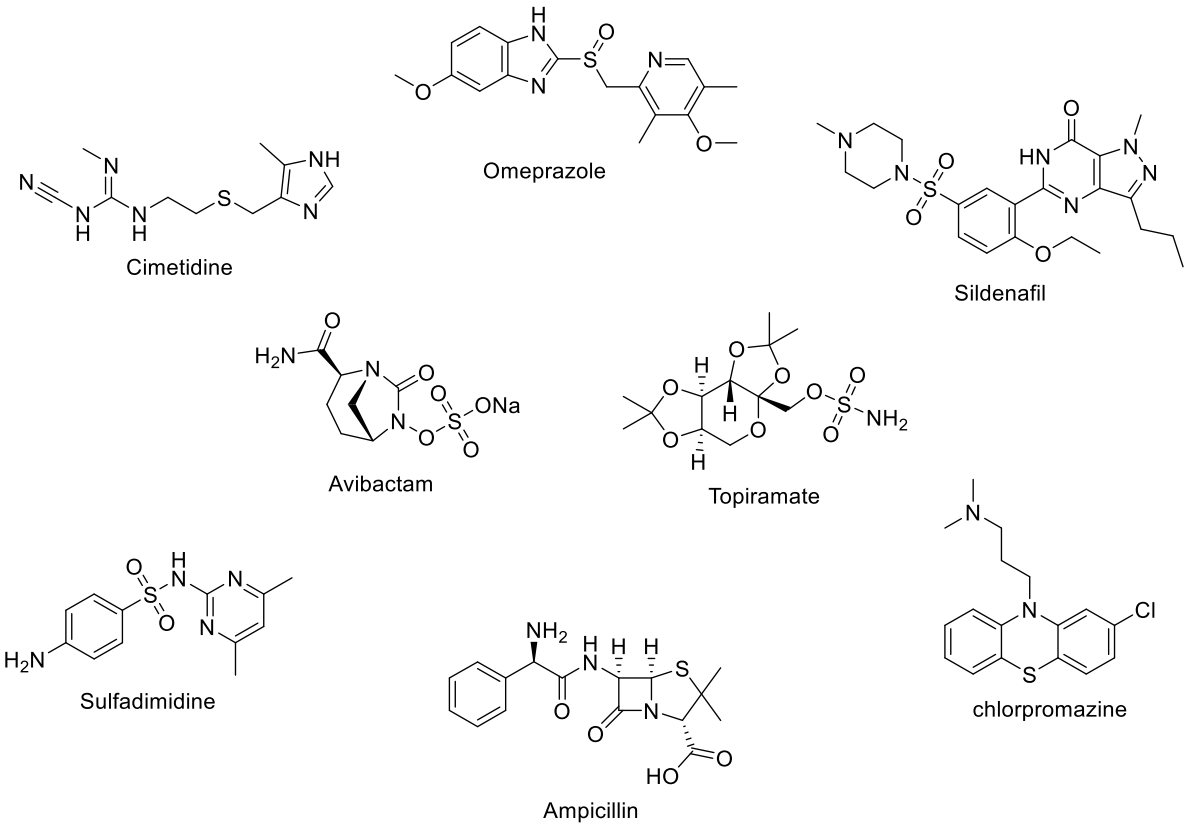


Figure 1. Selected examples of sulfur containing FDA-approved drugs.

Sulfur is the tenth most abundant element in nature, accounting about 0.03% to 0.06% of the earth's crust by weight.[1] The sulfur atom exists in multiple oxidation forms, ranging from -2 to +6. Sulfur is present predominantly in the +6-valance state as the sulfate form in the earth atmosphere.

Sulfur trioxide (SO_3) is a key precursor to sulfate and present in oleum, a solution of sulfur trioxide 25-65 % in sulfuric acid (H_2SO_4).[5] Moreover, sulfur trioxide is a strong Lewis acid, engages in reactions with Lewis bases such as trimethylamine (Me_3N), triethylamine (Et_3N), dioxane, and pyridine (Py). These reactions result in the formation of sulfur trioxide adducts, which are be used in the sulfation process of various organic substrates, leading to the formation of organosulfate esters (**Figure 2**).[6]

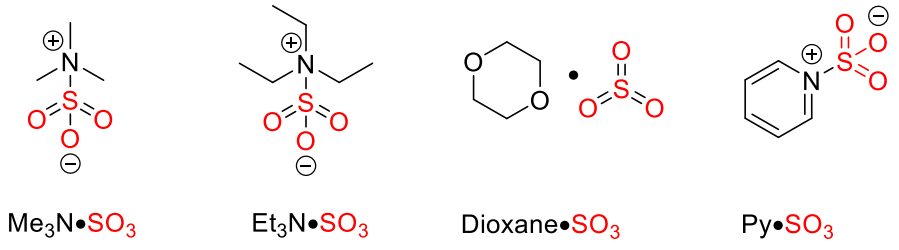
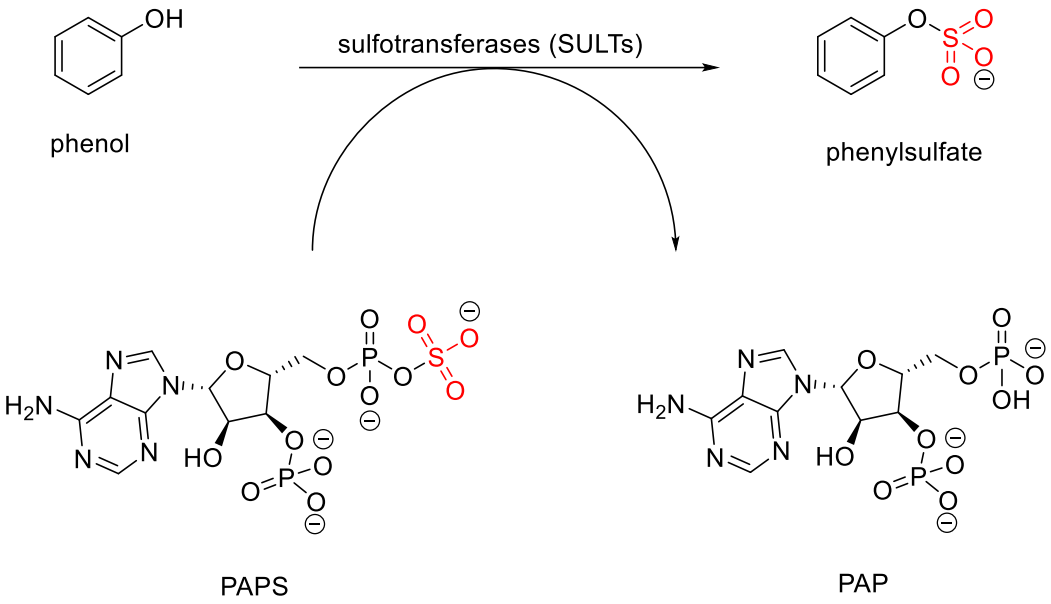


Figure 2: Sulfur trioxide complexes with trimethylamine, triethylamine, dioxane, and pyridine.

Sulfation is an important conjugation reaction during the phase II metabolism of xenobiotics.[7] The sulfation process which mainly occurs in the liver, increases the hydrophilicity of metabolites, facilitating their elimination from the body.[8] Sulfate conjugation usually results in reducing the biological activity of the metabolite,[9] but not always. Sulfation can increase the therapeutic activity of certain drugs including minoxidil sulfate, the active form of minoxidil used for

the treatment of hypertension and hair loss.[10-11] The sulfation reaction is catalysed by a group of enzymes called sulfotransferases (SULTs), which facilitate the transfer of the sulfate group from 3'-phosphoadenosine 5'-phosphosulfate (PAPS), the universal sulfate donor, to a nucleophilic site of an acceptor molecule such as phenol (**Scheme 1**).[12][13][14]



Scheme 1. Sulfation of an acceptor substrate (e.g. phenol) mediated by SULT enzyme and PAPS co-factor.

Since the sulfation of the antiseptic, phenol, was discovered in the urine of a patient in 1876 by Eugen Baumann [15] – the role of sulfated small molecules in man has evolved from a detoxification step to critical signalling mechanisms. Thus, methods to access these important sulfated biomolecules is critical to understanding the intricacies of the sulfate life cycle in man.

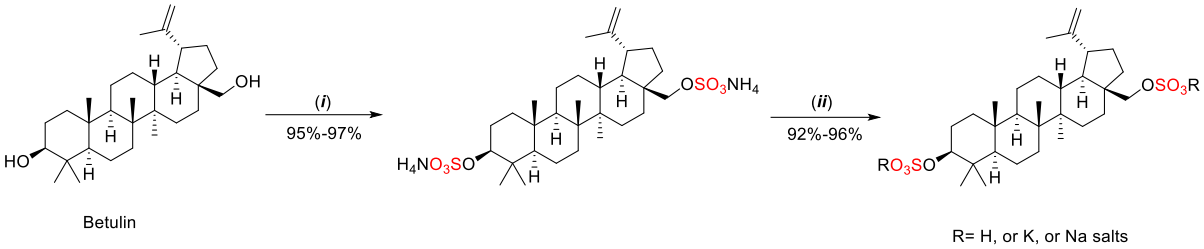
Small molecule chemical sulfation approaches

Organic *O*-sulfates and *N*-sulfamates have a number of crucial biological applications, ranging from the metabolism of xenobiotics to the downstream signalling of steroidal sulfates in pathological conditions.[16] Anti-coagulant, anti-viral, anti-inflammatory, immunomodulatory, and anti-tumour properties have been associated with sulfated polysaccharides, flavonoids, steroids, and proteins. Heparin and heparan sulfate are examples of glycosaminoglycans (GAGs) that contain sulfate groups which promote molecular interactions with protein ligands and binding at the cellular surface.[17] The incorporation of polar, hydrophilic sulfate groups onto drug-like molecules has facilitated the investigation of novel sulfated biomolecules as potential new therapies.[18] However, the chemical synthesis and purification of sulfated compounds are challenging when one or more sulfate groups are present, due to both anionic crowding, lack of regioselectivity, and poor solubility in organic solvents.[19][20] Successfully sulfated compounds remain sensitive to both acidic and high temperature reaction environments.[21]. As a result, the sulfation reaction is often the last step in a synthetic process, which restricts any potential chemical modifications.[22]

Given the growing interest in sulfation and the significant biological functions of sulfated molecules, several synthetic approaches to sulfate oxygen, nitrogen, oxime, and phosphate functionalities have been developed. This *essay* will explore the most commonly encountered and latest chemical sulfation approaches that have been applied to a wide range of small and biomolecules since the last major review in 2010.[23]

Sulfation using sulfuric acid and related reagents.

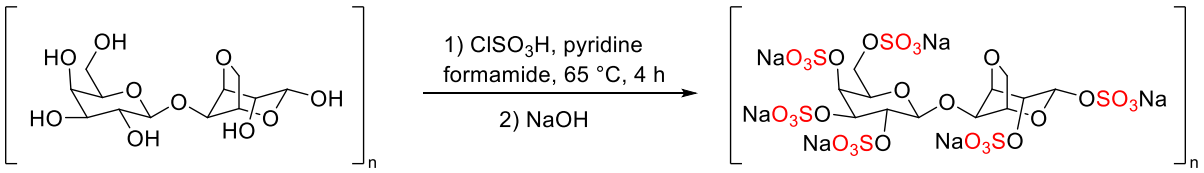
Sulfuric acid (H_2SO_4) has been employed for the sulfation of (cyclo)alkenes at modest temperature and pressure resulting in (cyclo)alkyl sulfates [6] and the sulfation of polysaccharides and flavonoids.[24][25] The high reactivity of sulfuric acid can be modified via the sulfamic acid (H_2NSO_3H) reagent, which has been used for the sulfation of saturated alcohols, carbohydrate, and flavonoids including the naturally occurring triterpenoid *betulin* which is found in the bark of birch trees which possesses anti-viral, anti-inflammatory, anti-oxidant, and anti-coagulant properties.[26] Betulin was sulfated using the sulfamic acid method in the presence of an urea $CO(NH_2)_2$ catalyst and alternatively DMF or 1,4-dioxane as solvent. Double sulfation of betulin was achieved and isolated as ammonium, sodium, and potassium salts (**Scheme 2**).



Scheme 2. Double sulfation of betulin with sulfamic acid (NH_2SO_3H). Conditions: (*i*) NH_2SO_3H, urea, DMF (60–70°C was 2.0–3.0 h) or 1,4-dioxane (70–75°C, 3.0–3.5 h); (*ii*) work up with 10% H_2SO_4, or 3–5% KOH, or 3–5% NaOH.

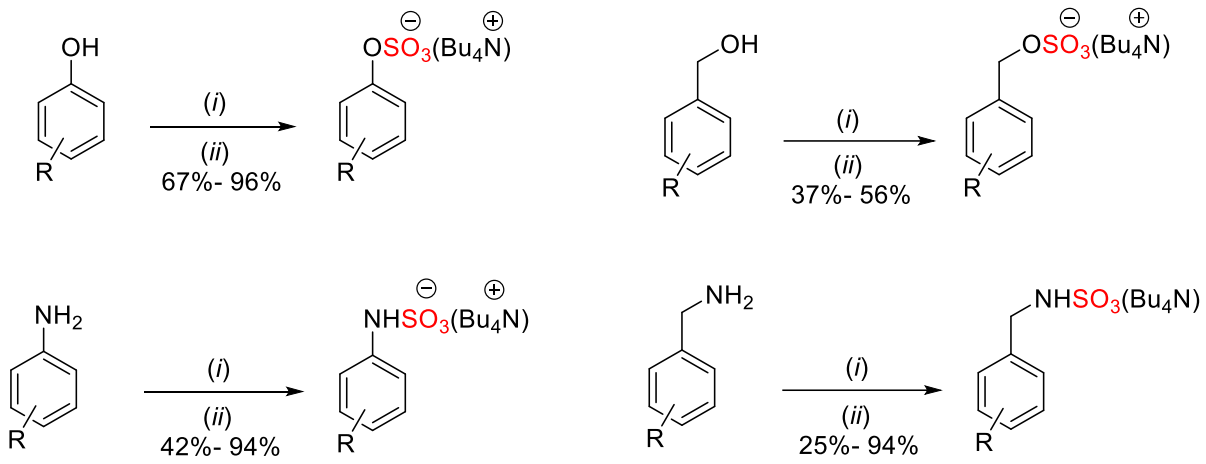
Sulfation Using Chlorosulfonic Acid ($ClSO_3H$) and its derivatives.

An alternative approach utilises chlorosulfonic acid, which has been applied for the sulfation of polysaccharides, phenolic acids, and flavonoids amongst others. Agarose sulfate, an example of sulfated seaweed polysaccharide which was proposed to have an anticoagulant activity comparable to heparin. Youping and co-workers have reported the formation of agarose sulfate using the chlorosulfonic acid protocol.[27] Agarose polysaccharide was dissolved in formamide solvent followed by the addition of chlorosulfonic acid/pyridine solution and stirred for 4 h at 65 °C affording agarose sulfate (**Scheme 3**).



Scheme 3: The sulfation reaction of agarose using $ClSO_3H$/pyridine method. N.B. chlorosulfonic acid was added dropwise to the cold pyridine (0 °C) due to the exothermic nature of chlorosulfonic acid.

A modified version of chlorosulfonic acid-pyridine procedure was implemented using chlorosulfonic acid followed by cation exchange with tetrabutylammonium hydrogensulfate (Bu_4NHSO_4).[28][29] The sulfation reaction of different organic molecules such as phenols, benzyl alcohols, anilines, and benzylamines using this modified protocol has been accomplished (**Scheme 4**).

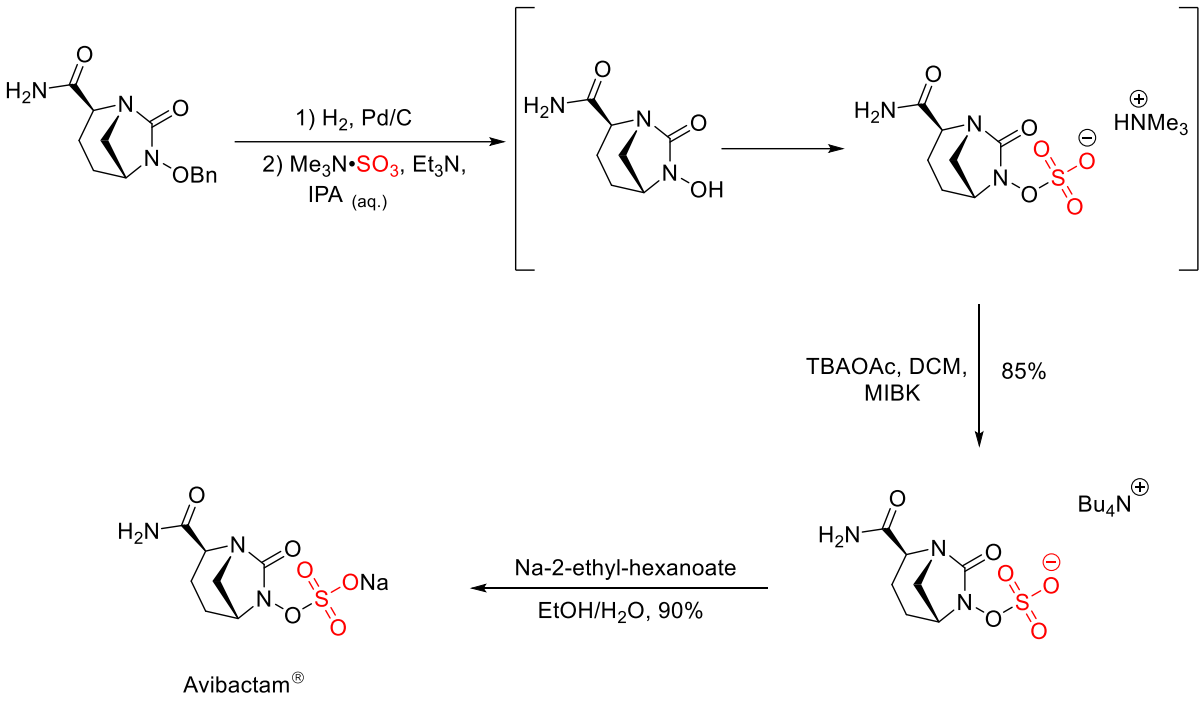


Scheme 4. Synthesis of sulfated phenols, benzyl alcohols, anilines, and benzylamine using $ClSO_3H$ as a source of sulfation, followed by the cation exchange with tetrabutylammonium cation. Conditions: (*i*) $ClSO_3H$ (5.0 mmol, 1 eq.), triethylamine and DCM, 0 °C to r.t., stirred for 1 h. (*ii*) tetrabutylammonium hydrogen sulfate (4.0 mmol), then extracted into DCM.

Sulfation Using formal Sulfur Trioxide Amine/Amide Complexes

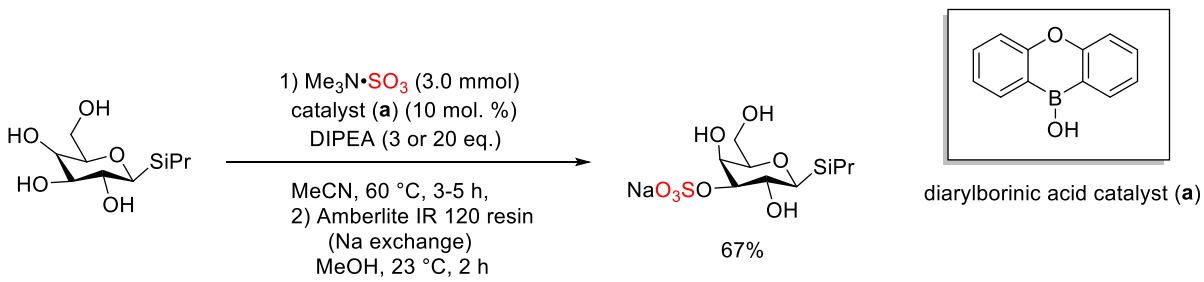
Trimethylamine-sulfur trioxide complex

The sulfation reaction using sulfur trioxide amine/amide complexes is the most commonly used method for alcoholic or phenolic groups in carbohydrates, flavonoids, steroids, proteins, and aliphatic or alicyclic scaffolds. Ball and co-workers have successfully used a sulfur trioxide trimethylamine complex ($Me_3N{\cdot}SO_3$) and a lipophilic cation-exchange to access Avibactam®, a sulfate containing β-lactamase inhibitor.[30] They reported a simultaneous one-pot deprotection and sulfation reaction of a hydroxylamine intermediate using the $Me_3N{\cdot}SO_3$ complex. The resulting intermediate was ion exchanged with tetrabutylammonium acetate (TBAOAc) making it more lipophilic and therefore facilitates the extraction of the organosulfate intermediate into DCM. Finally, a lipophilic sodium salt exchange reagent, sodium-2-ethyl-hexanoate (NEH) was added affording Avibactam® in 90% yield as the sodium salt (**Scheme 5**).



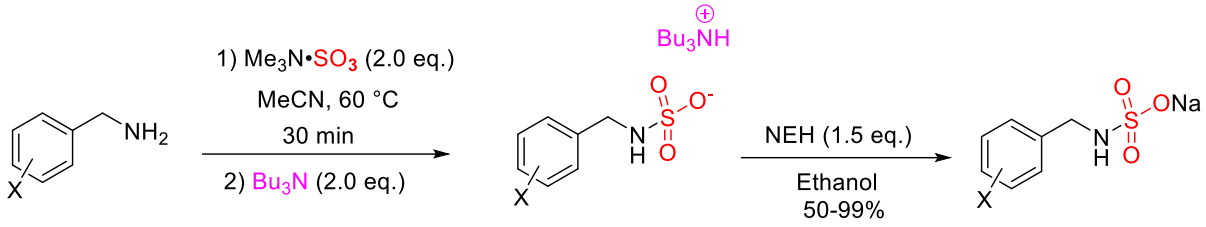
Scheme 5. The final sulfation steps of Avibactam® total synthesis.

The $Me_3N{\cdot}SO_3$ complex was reported for the site selective sulfation of polysaccharides including pyranoside scaffolds.[31] The site selective sulfation of pyranoside scaffolds was carried out under catalytic conditions using diarylborinic acids are highly active catalyst for several reactions including sulfation.[32][33] This method will lead to the sulfation of different OH group positions including *cis*-1,2-diol and 1,3-diol pyranoside derivatives. *β*-thioglycoside pyranoside was selected as a model for the site selective sulfation using $Me_3N{\cdot}SO_3$ and the addition of diarylborinic acid and DIPEA was critical to improve the isolated yield (**Scheme 6**).[31]



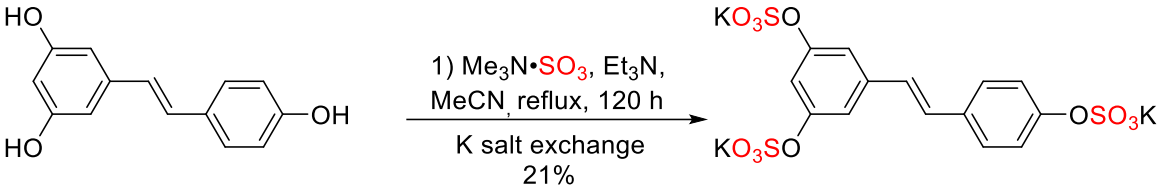
Scheme 6. Site selective sulfation of pyranoside scaffolds catalysed by diarylborinic acids (**a**).

The $Me_3N{\cdot}SO_3$ complex has also found use in the preparation of sulfamates which contain polar functional groups that are significant to a wide range of biological functions, including viral infection and protein- protein interaction. The sulfamation reaction of benzylamine derivatives were carried out using $Me_3N{\cdot}SO_3$ complex under a low reaction temperature (30-60 °C) affording the corresponding trimethylammonium cation.[34] This was subsequently exchanged with a more lipophilic counterion, tributylamine, then exchanged with sodium salt leading to the formation of sulfamates (**Scheme 7**) and employed in other examples.[35]



Scheme 7. The sulfamation reaction of benzylamine derivatives using $Me_3N{\cdot}SO_3$ in a combination with the lipophilic counterion (Bu_3N) and a sodium exchange strategy.

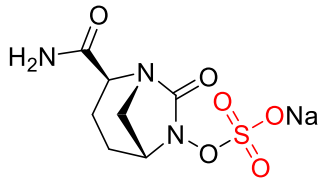
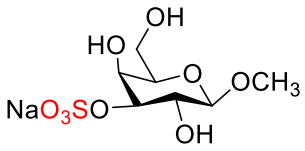
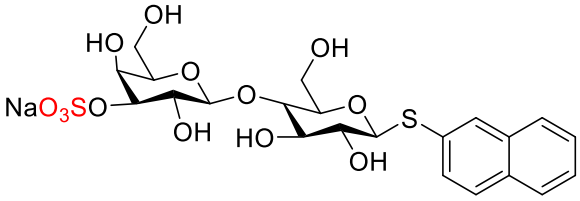
The use of $Me_3N{\cdot}SO_3$ complex was also reported in the sulfation of resveratrol, a naturally occurring polyphenolic that is found in many plants, such as peanuts, grapes, and berries.[36] It was reported that resveratrol has gained more interest due its important biological applications such as anti-inflammatory, anti-cancer, and antioxidant activity. The sulfation reaction of resveratrol was carried out using $Me_3N{\cdot}SO_3$ complex in the presence of base (Et_3N) at reflux affording the potassium salt of sulfated resveratrol (**Scheme 8**).

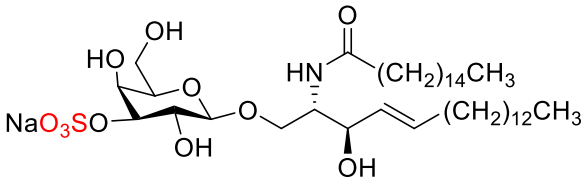
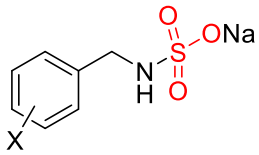
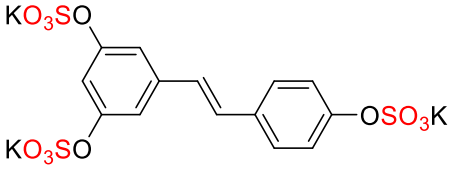


Scheme 8. Sulfation reaction of resveratrol using $Me_3N{\cdot}SO_3$ complex.

A summary of the scope and range of sulfated molecules achievable with Me_3NSO_3 complex are listed in **Table 1**.

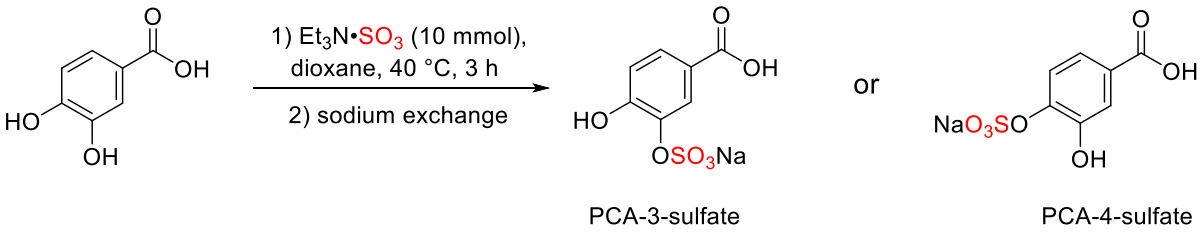
Table 1: Sulfation methods of organic molecules using $Me_3N{\cdot}SO_3$ complex

Entry	Sulfated substrate	Sulfating agent	Isolated yield	Ref.
1	 Avibactam	$Me_3N{\cdot}SO_3$	90%	[30]
2	 pyranoside derivatives	$Me_3N{\cdot}SO_3$	64-97%	[31]
3	 Sulfated Lactose-derived *β*-thioglycoside	$Me_3N{\cdot}SO_3$	66%	[31]

4	 Sulfated *β*-galactosylceramide	$Me_3N{\cdot}SO_3$	66%	[31]
5	 Sulfamate derivatives	$Me_3N{\cdot}SO_3$	50-99%	[34]
6	 Potassium resveratrol sulfate	$Me_3N{\cdot}SO_3$	21%	[36]

Triethylamine sulfur trioxide complex

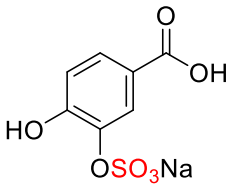
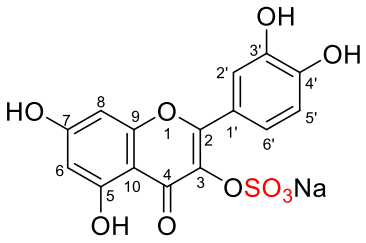
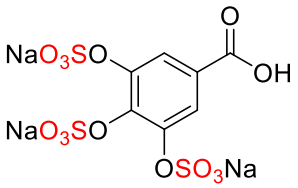
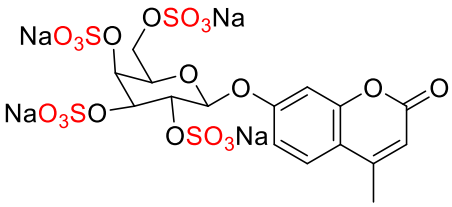
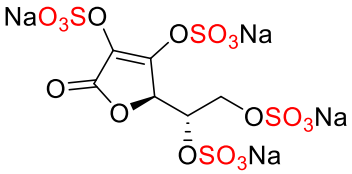
An alternative sulfur trioxide amine complex example is triethylamine SO_3 complex ($Et_3N{\cdot}SO_3$), which was also utilised for the sulfation of a wide range of small organic molecules such as flavonoids, polysaccharides, proteins, and steroids. For instance, the sulfation of polyphenolic flavonoids such as protocatechuic acid (PCA), quercetin, and catechin were reported using $Et_3N{\cdot}SO_3$ complex.[37] PCA sulfate lower the production of interleukin-6 (IL-6) and vascular cell adhesion molecule-1 (VCAM-1), pro-inflammatory cytokine genes that are linked to cardiovascular diseases.[38] Gutierrez-Zetina and co-workers have described the mono-sulfation reaction of PCA using superstoichiometric equivalents of $Et_3N{\cdot}SO_3$ (10-fold) at 40 °C for 3 h. As a result, two compounds were isolated as PCA-3-sulfate and PCA-4-sulfate, respectively (**Scheme 9**).[37]



Scheme 9. General procedure for sulfation of PCA using $Et_3N{\cdot}SO_3$ affording either PCA-3-sulfate or PCA-4-sulfate.

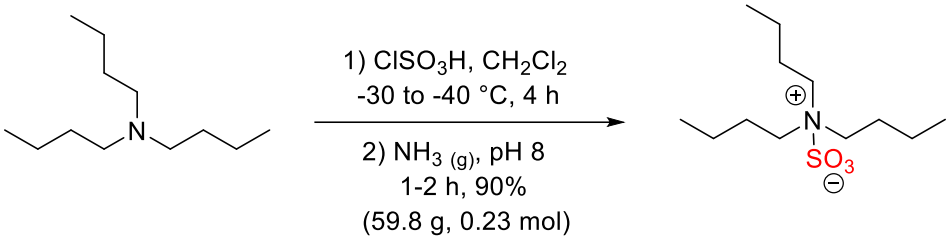
The mono-sulfate analogues of quercetin and catechin were also prepared following the mono-sulfation reaction procedure used with PCA employing $Et_3N{\cdot}SO_3$ as a source of sulfate.[39] Correia-da-Silva and co-workers have investigated the synthesis of persulfated compounds which can be used as an anticoagulant and antiplatelet agents for the treatment of thrombosis.[40] Sulfation of different polyphenolic molecules such as gallic acid, 4-methyl 7-hydroxycoumarin 7-ß-D-glucopyranoside, and ascorbic acid was afforded using $Et_3N{\cdot}SO_3$ (2-8 eq./OH group) in dimethylacetamide (DMA) at 65 °C for 24 h reaction duration. A summary of the scope and range of sulfated molecules achievable with Et_3NSO_3 complex are listed in **Table 2**.

Table 2: Sulfation methods of organic molecules using $Et_3N{\cdot}SO_3$ complex. N.R. = not reported.

Entry	Sulfated substrate	Sulfating agent	Isolated yield	Ref.
1	 PCA-3-sulfate	$Et_3N{\cdot}SO_3$	N.R.	[37]
2	 Quercetin 3-sulfate	$Et_3N{\cdot}SO_3$	N.R.	[39]
3	 Sulfated gallic acid	$Et_3N{\cdot}SO_3$	36%	[40]
4	 Sulfated 4-methyl 7-hydroxycoumarin	$Et_3N{\cdot}SO_3$	47%	[40]
5	 Sulfated ascorbic acid	$Et_3N{\cdot}SO_3$	7%	[40]

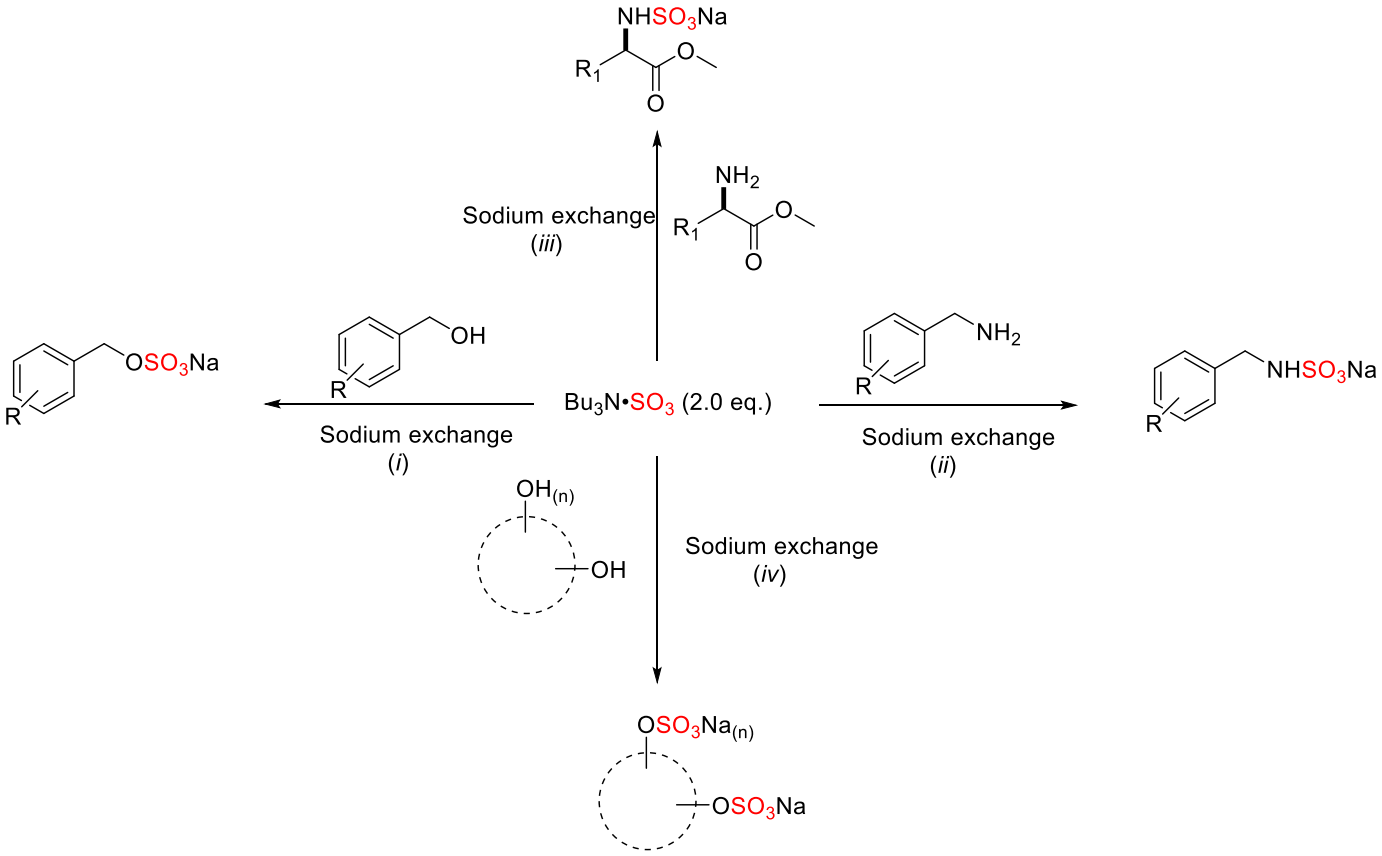
Tributylsulfoammonium betaine (TBSAB)

A novel sulfating reagent was recently developed by Gill and co-workers, tributylsulfoammonium betaine (TBSAB), which was applied to a wide range of small molecules including benzyl alcohols, benzylamines, steroids, carbohydrates, and proteins (**Scheme 10**).[41]



Scheme 10. Synthesis of sulfur trioxide tributylamine complex (TBSAB).

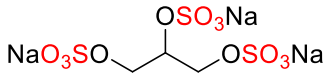
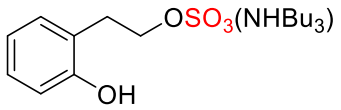
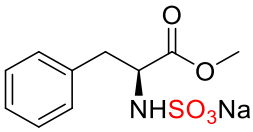
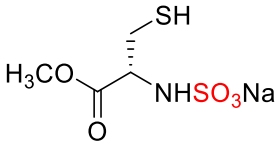
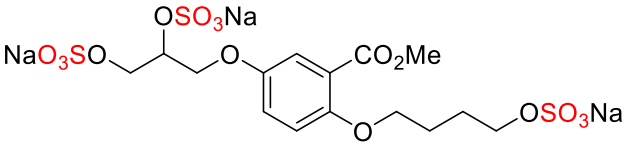
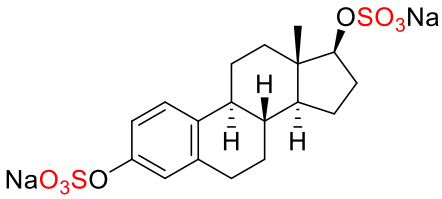
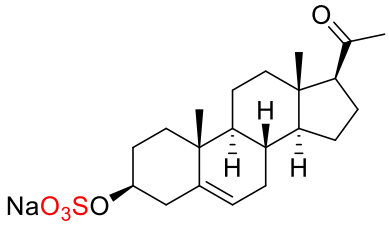
TBSAB provides a simplified purification and isolation of sulfated molecules due to the greater lipophilicity profile of the corresponding tributylammonium intermediate ($Log_{10}P$ = 4.01).[42] The scope of the TBSAB reagent is demonstrated in **Scheme 11**.



Scheme 11. General sulfation synthesis of some organic scaffolds using all-in-one reagent, $Bu_3N{\cdot}SO_3$. Conditions: (*i*) $Bu_3N{\cdot}SO_3$ (2.0 eq.), MeCN, 90 °C, up to 3 h, then NEH or NaI exchange. (*ii*) $Bu_3N{\cdot}SO_3$ (2.0 eq.), MeCN, 30 °C, up to 1 h, then NaI exchange. (*iii*) $Bu_3N{\cdot}SO_3$ (2.0 eq.), MeCN, r.t., up to 18 h, then NaI exchange. (*iv*) $Bu_3N{\cdot}SO_3$ (≥ 2.0 eq.), MeCN, 90 °C, up to 12 h, then NEH or NaI exchange. R_1= amino acid side chain.

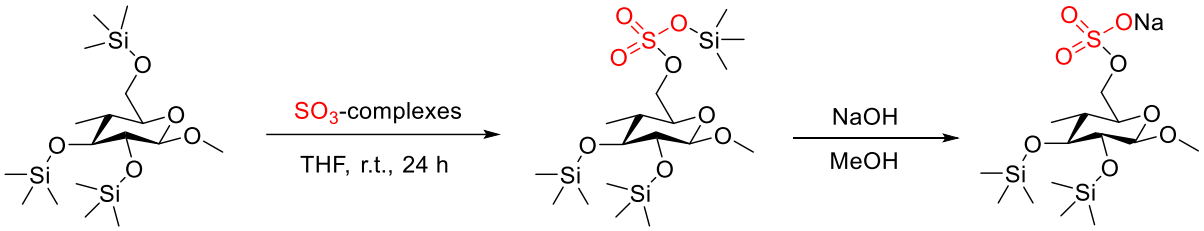
TBSAB was used for the sulfation and sulfamation of a wide range of benzyl alcohols, benzylamines, amino acids, and carbohydrates.[43][44] TBSAB has also been used for the chemoselective sulfation of steroids affording the corresponding sulfated steroid molecules such as, estrone sulfate, pregnenolone sulfate, and pregnanediol sulfate.[45] TBSAB has also found use as a reagent to install aniline *N*-sulfamates prior to their *inter*molecular rearrangement.[46] A summary of the scope and range of sulfated molecules achievable with Bu_3NSO_3 complex are listed in **Table 3**.

Table 3: Sulfation methods of selected biomolecules using $Bu_3N{\cdot}SO_3$ (TBSAB) complex.

Entry	Sulfated substrate	Sulfating agent	Isolated yield	Ref.
1	 Sulfated glycerol	$Bu_3N{\cdot}SO_3$	92%	[41]
2	 Sulfated 2-hydroxyphenyl ethanol	$Bu_3N{\cdot}SO_3$	74%	[41]
3	 *L*-phenylalanine methyl ester sulfamate	$Bu_3N{\cdot}SO_3$	60%	[43]
4	 *L*-cysteine methyl ester sulfamate	$Bu_3N{\cdot}SO_3$	50%	[43]
5	 Sulfated glycomimetics C3	$Bu_3N{\cdot}SO_3$	76%	[41][44]
6	 Estradiol sulfates	$Bu_3N{\cdot}SO_3$	84%	[41][45]
7	 pregnenolone sulfate	$Bu_3N{\cdot}SO_3$	98%	[45]

Pyridine-sulfur trioxide and dimethylformamide-sulfur trioxide complexes

Alternative sulfur trioxide complexes involving Py·SO_3 and DMF·SO_3 were used for the sulfation of polysaccharides and polyphenolic flavonoids.[47] For instance, Cellulose sulfate is an example of sulfated polysaccharide which has been studied for its potential biological and pharmaceutical applications such as anticoagulant, antimicrobial, antioxidant, and used in drug delivery system. Richter and co-workers have described the regioselective sulfation of the trimethylsilyl cellulose (TMSC) using sulfur trioxide complexes such as Py·SO_3, DMF·SO_3, and Et_3N·SO_3.[48] The synthesis of sodium cellulose sulfates were carried out by dissolving TMSC in tetrahydrofuran (THF) followed by the addition of sulfur trioxide complexes at room temperature for 24 h. The resulting intermediate was treated with sodium hydroxide yielding the final desired sodium cellulose sulfates (**Scheme 12**).



Scheme 12. The regioselective sulfation of trimethylsilyl cellulose (TMSC) using sulfur trioxide complexes.

Malins and co-workers have reported the use of Py·SO_3 and DMF·SO_3 complexes for the preparation of sulfated xylooligosaccharides that could be a promising therapeutic agent similar to the known exemplar, pentosan polysulfate (PPS).[49] Pentosan polysulfate is a semi-synthetic polysulfated xylan that is related to glycosaminoglycans (GAGs) containing β-1→4-linked xylooligosaccharides and was approved for the treatment of interstitial cystitis (inflammation of bladder) (**Figure 3**).[50]

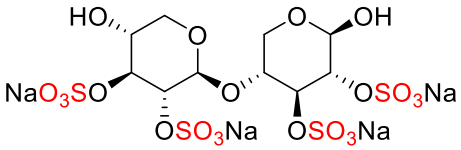
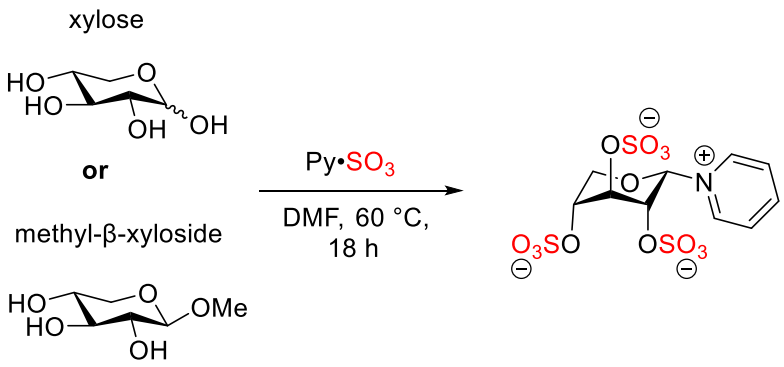


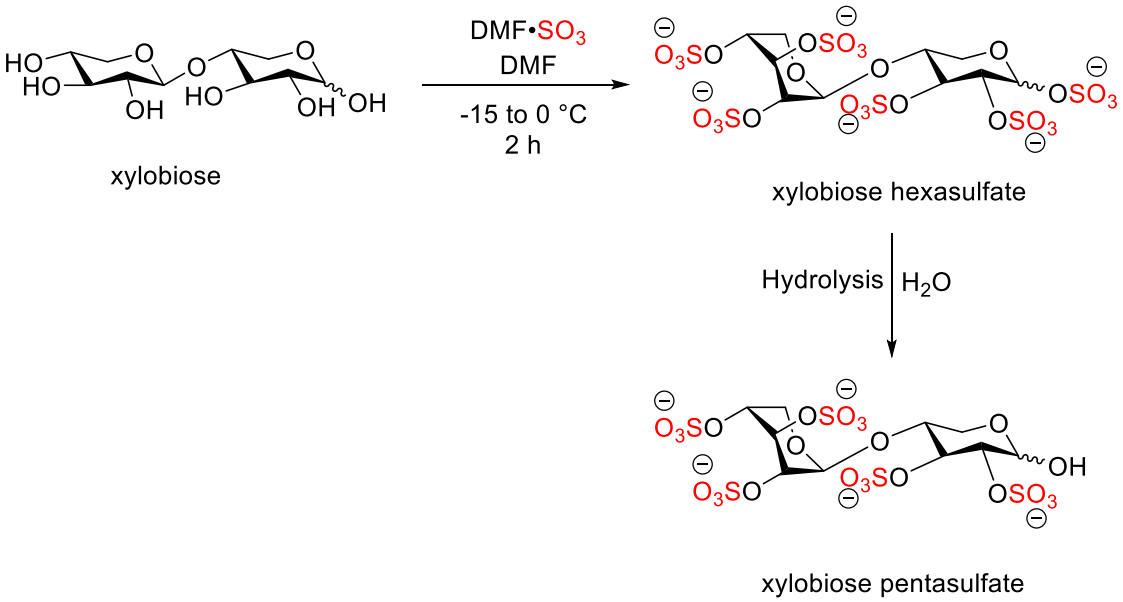
Figure 3. The structure of pentosan polysulfate sodium (Elmiron®) which was approved for the treatment of interstitial cystitis.[1]

An initial attempt for the sulfation of xylooligosaccharides was examined on different substrates such as xylose, methyl-β-xyloside, and xylobiose using Py·SO_3 complex at high temperature (60 °C) and 18 h reaction duration.[49] Unfortunately, the β-1→4 linkage of xylan derivatives was cleaved by the addition of pyridine as pyridinium contamination (**Scheme 13**).[51]



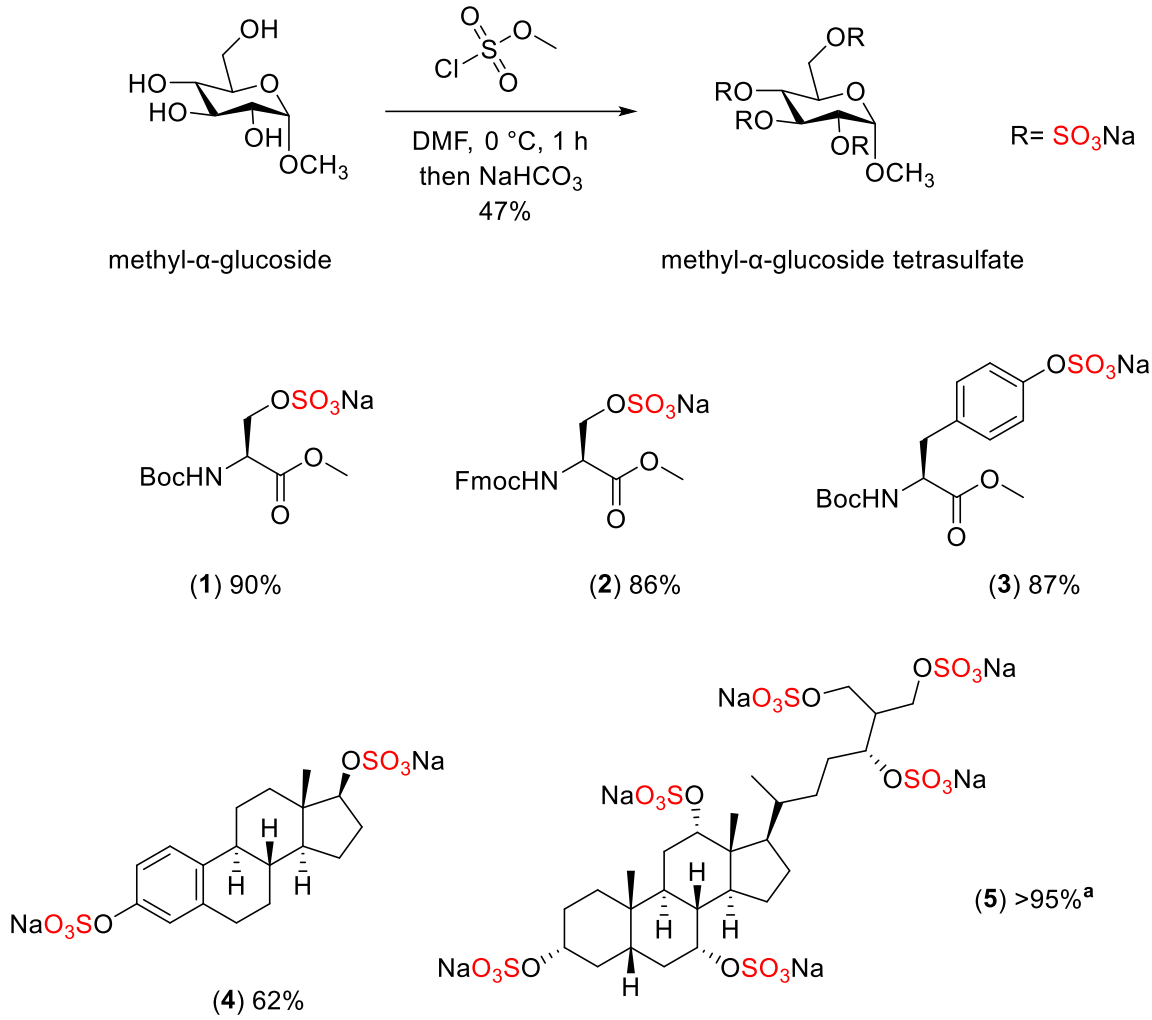
Scheme 13. The sulfation reaction of xylan derivatives using Py·SO_3 complex.

Due to the previous unsuccessful attempt with Py·SO_3, DMF·SO_3 complex was used for the sulfation of xylobios.[49] The sulfation of xylobios with DMF·SO_3 complex was carried out between −15 to 0 °C for two hours affording xylobiose hexasulfate and xylobiose pentasulfate, a reduced sugar, which was formed via hydrolysis of hexasulfate substrate (**Scheme 14**).



Scheme 14. The sulfation reaction of xylobios using DMF·SO_3 complex affording xylobiose hexasulfate and pentasulfate.

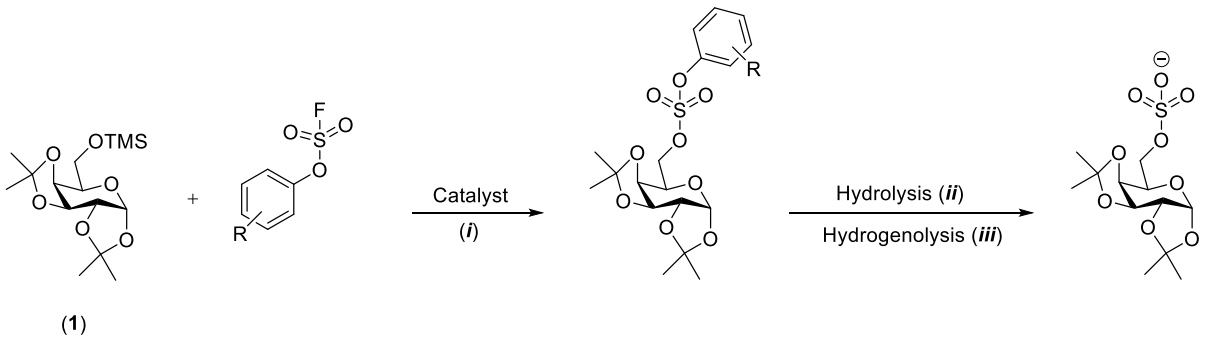
Malins. has also reported an *in situ* synthesis of DMF·SO_3 complex using the strategy of the addition of methyl chlorosulfate to DMF at 0 °C. This reaction is exothermic leading to the formation of chloromethane as a side product reaction.[49] This protocol was initially investigated on the methyl-α-glucoside following the optimised conditions; methyl chlorosulfate (1.1 eq./OH) at 0 °C for 1 h affording the methyl-α-glucoside tetrasulfate in 47% isolated yield. This protocol was further explored on a wide range of small molecules including amino acids, disaccharide, steroids, and acid-sensitive substrates (**Scheme 15**).



Scheme 15. The sulfation reaction of methyl-α-glucoside using methyl chlorosulfate and DMF protocol. Scope of sulfated small molecules with methyl chlorosulfate, sodium Boc-Serine methyl ester sulfate (**1**), sodium Fmoc-*L*-Serine methyl ester sulfate (**2**), sodium Boc-tyrosine methyl ester sulfate (**3**), sodium β-estradiol disulfate (**4**), and sodium scymnol persulfate (**5**). [a] conversion percentage calculated by ^{1}H NMR spectroscopy.

Sulfation Using Sulfur (VI) Fluoride Exchange (SuFEx) Reaction

Recently, early stage *O*-sulfation reaction between aryl fluorosulfates and silyl ethers was reported leading to the formation of sulfuric acid diesters, which are subsequently reduced to the target sulfates by hydrogenolysis step.[52] This strategy was applied to a wide range of small molecules such as monosaccharides, disaccharides, an amino acid, and a steroid.[52] Liu and co-workers have explored the SuFEx reaction between silyl ethers and aryl fluorosulfates to introduce sulfate diesters on carbohydrate and non-carbohydrate scaffolds.[52] This was followed by a deprotection step of the aryl sulfate monoester resulting in the formation of *O*-sulfate molecules (**Scheme 16**).



Scheme 16. General *O*-sulfation of protected galactopyranose (**1**) using SuFEx strategy. Conditions: (*i*) DBU, MeCN, 2 h, r.t.; (*ii*) 5 M sodium methoxide, 1 h, r.t.; (*iii*) $Pd(OH)_2/C$ and H_2, buffer solution (MeCN/MeOH/PBS) 2:2:1, 2 h, r.t.

This optimised method was applied to different small biomolecules such as monosaccharides, disaccharides, an amino acid, and a steroid (**Figure 4**).[52]

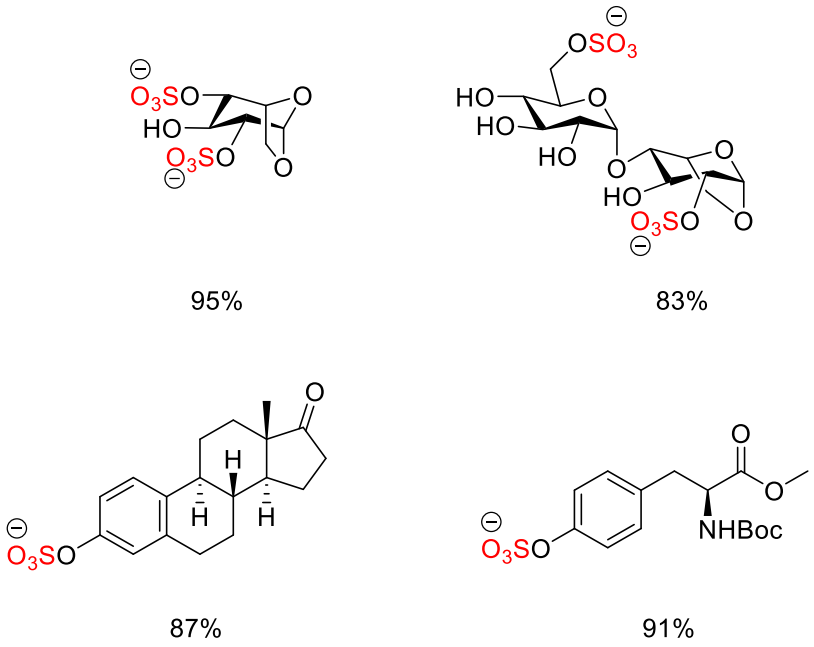
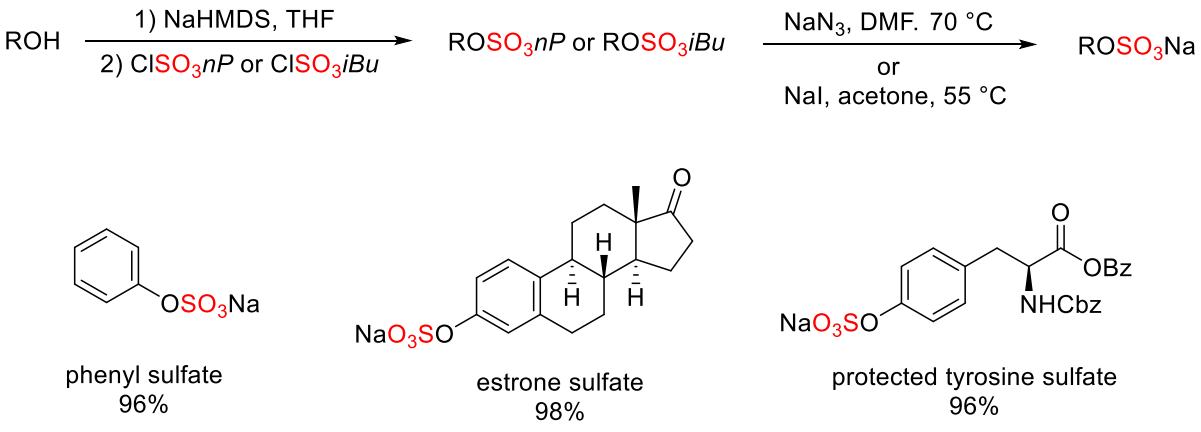


Figure 4. Scope of some sulfated biomolecules using SuFEx approach.

Protection/Deprotection Approaches

Despite the effectiveness of direct sulfation methods, the formation of complex sulfated molecules may be hampered by the poor solubility of sulfated molecules in organic solvents, stability issues, and purification challenges of sulfated molecules.[23] As a result, there was a growing interest in developing protection/deprotection strategy which involves the incorporation of sulfate group(s) in a masked form into the target scaffold followed by a deprotection step affording final sulfate molecules.[23][53] Simpson and co-workers have used alkyl protecting groups such as isobutyl and neopentyl groups for the preparation of several sulfate monoesters.[53] In this method, phenolic or alcoholic substrates were initially treated with a strong base such as sodium hydride (NaH) or sodium hexamethyldisilazide (NaHMDS) in THF solvent at -75 °C followed by the addition of isobutyl or neopentyl chlorosulfate affording the desired protected sulfate monoesters. Subsequent deprotection step using sodium azide or sodium iodide affording the desired sulfates (**Scheme 17**).[53]



Scheme 17: Protection/deprotection method using the protected sulfate neopentyl or isobutyl esters followed by the deprotection reaction with NaN_3 for the removal of neopentyl (*nP*) protecting group and sodium iodide (NaI) for the removal of isobutyl (*iBu*) protecting group affording the final desired sulfates. Scope of some sulfate monoester using protection/deprotection method.

Conclusions and outlook

Sulfation is one of the most important modifications that occur to a wide range of small biomolecules including polysaccharides, proteins, flavonoids, and steroids. Historically, the incorporation of sulfate moiety to an appropriate substrate, results in an increase of the substrate's hydrophilicity and therefore facilitate its elimination from the body. However, sulfated scaffolds such as polysaccharides, proteins, flavonoids, and steroids have significant biological and pharmacological roles such as cell signalling, modulation of immune and inflammation response, anticoagulation, antiatherosclerosis, and antiadhesive. It is imperative that sulfated molecules can be easily prepared for furthering the biological understanding of the role of sulfate in the body.

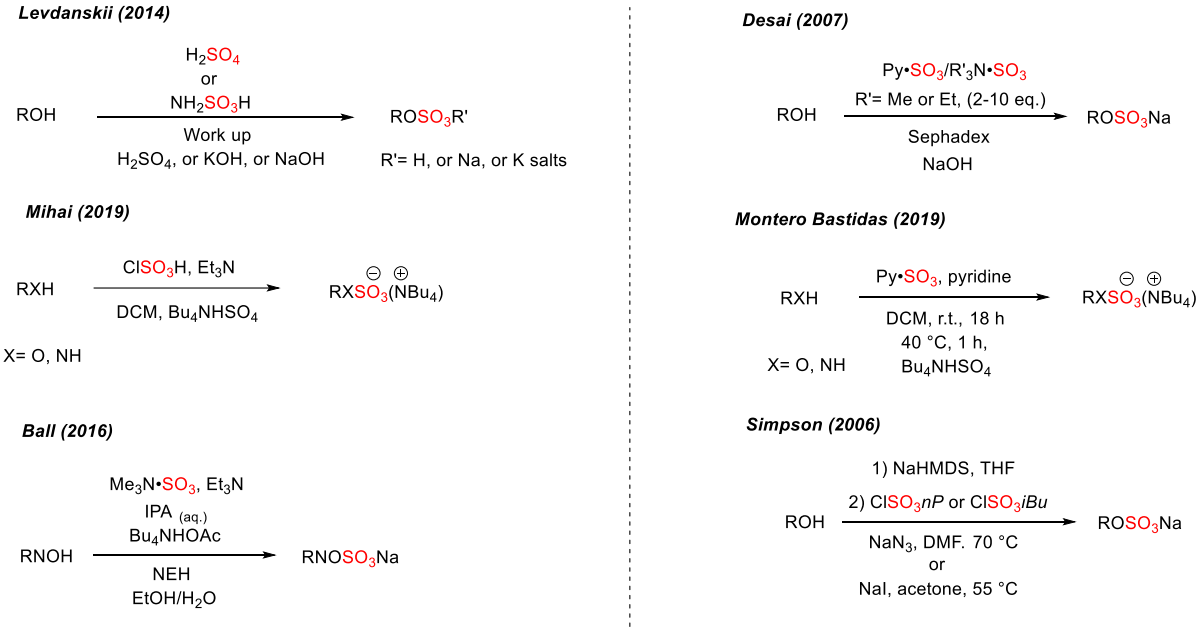


Figure 5: Previous conventional sulfation methods including sulfuric acid/sulfamic acid, chlorosulfonic acid, sulfur trioxide amine complexes, and the protection/deprotection methods which suffer from some issues such as multiple-purification steps reactions, toxicity issues (eg pyridine contamination), purification challenges, stoichiometric excess of reagents which leads to increase of a reaction cost, and intrinsic stability issues of both the reagent and product.

This *essay* summarised the most encountered chemical sulfation methods of small molecules (**Figure 5**). Traditional sulfation reactions have relied on sulfuric acid derivatives. Sulfation reaction using sulfur trioxide amine/amide complexes was the most used method for alcoholic or phenolic groups in carbohydrates, steroids, proteins, and aliphatic or alicyclic scaffolds. Despite the effectiveness of these methods, they suffer from some issues such as multiple-purification steps reactions, toxicity issues (eg pyridine contamination), purification challenges, stoichiometric excess of reagents which leads to increase of a reaction cost, and intrinsic stability issues of both the reagent and product.

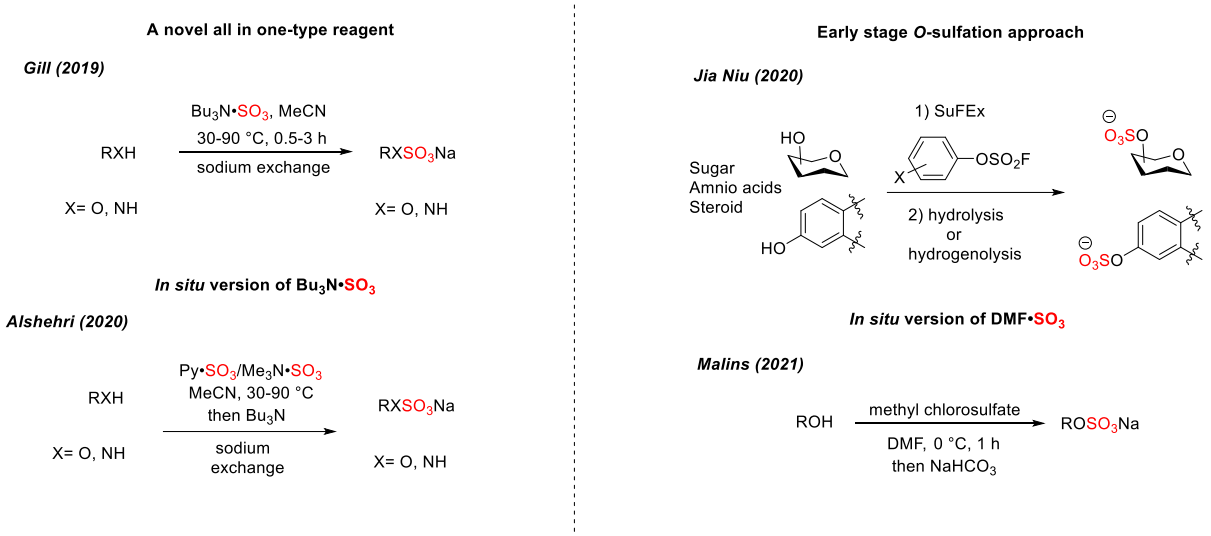


Figure 6: Recent chemical sulfation methods including the novel sulfating reagent, TBSAB-$Bu_3N{\cdot}SO_3$, *in situ* version of $Bu_3N{\cdot}SO_3$, an early *O*-sulfation stage using SuFEx, and the Malins *in situ* version $DMF{\cdot}SO_3$.

Recent advances (**Figure 6**) including SuFEx, the Malins *in situ* reagent approach and TBSAB show the widespread appeal of novel sulfating approaches that will enable a larger exploration of the field in the years to come by simplifying the purification and isolation to access bespoke sulfated small molecules.

Conflicts of Interest

None to declare.

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Author contribution

JAA and AMJ drafted and revised the manuscript.

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