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Sulfation made simple: a strategy for synthesising sulfated molecules†

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The study of organosulfates is a burgeoning area in biology, yet there are significant challenges with their synthesis. We report the development of a tributylsulfoammonium betaine as a high yielding route to organosulfates. The optimised reaction conditions were interrogated with a diverse range of alcohols, including natural products and amino acids.

Organosulfates play a variety of important roles in biology, from xenobiotic metabolism to the downstream signalling of steroidal sulfates in disease states.[1] Sulfate groups on glycosaminoglycans (GAGs) such as heparin, heparan sulfate and chondroitin sulfate, facilitate molecular interactions and protein ligand binding at the cellular surface,[2] an area of interest in drug discovery.[3]

Heparin (an anticoagulant),[4] avibactam® (a β-lactamase inhibitor),[5] sotradecol (a treatment for varicose veins),[6] the sulfate metabolite of paracetamol (an analgesic),[7] and the glycomimetic **C3** (used to study atherosclerosis),[8] contain organosulfate motifs (Fig. 1). There are many other natural sources of bioactive sulfated compounds.[9]

The incorporation of polar hydrophilic organosulfate groups onto drug-like molecules is timely to facilitate research investigating sulfated GAGs as potential new therapies.[10] However, the insertion and isolation of sulfate groups into target molecules remains a challenging aspect of their synthesis,[11] prompting recent advances into sulfate revealing pro-drugs.[12]

The presence of one or more sulfate group makes chemical synthesis and purification of (per)sulfated compounds challenging, primarily due to their poor solubility in organic solvents.[13] Therefore the insertion of organosulfate groups is typically the final step in a synthetic method, limiting further chemical modifications.[14]

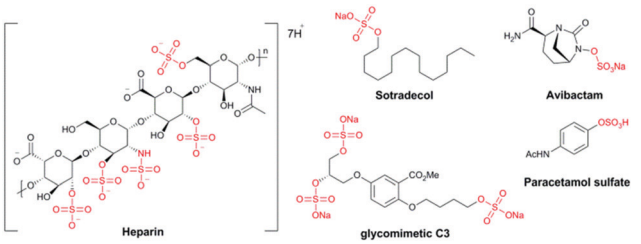

Fig. 1 Examples of drug molecules containing organosulfates: *heparin*, *sotradecol*® and *avibactam*®; a chemical tool compound (**C3**) and a metabolite of paracetamol.

A variety of methods to prepare organosulfates are shown in Chart 1.

The preparation of organosulfate esters include a microwave assisted approach to the sulfation of alcohols, using Me_3NSO_3 and $PySO_3$, Chart 1(i)).[15,16] The addition of catalytic diaryl borinic acid can be used with Me_3NSO_3 to sulfate carbohydrates.[17] The limitations with these routes include the need for a stoichiometric excess of the reagent per alcohol group (up to 10 eq. per hydroxyl group) and difficulties with purification. Poor solubility in organic solvents makes aqueous purification protocols and ion-exchange chromatography a standard procedure, limiting the practicality. An alkyl chlorosulfate ester with subsequent deprotection has proven a reliable method, but limitations include the need to use a strong base and a deprotection step leads to side products (Chart 1(ii)).[18] A DCC/H_2SO_4-sulfate coupling has been demonstrated but is not amenable to acid sensitive substrates (Chart 1(iii)).[19] A sulfitylation–oxidation protocol (Chart 1(iv)) involves the synthesis of a protected sulfite ester, oxidation to the protected sulfate ester and cleavage to the sodium sulfate salt.[20] However, the use of multiple steps and purification sequences is limiting. A process route to Avibactam[21] (Fig. 1 and Chart 1(v) involved sulfation of the hydroxylamine intermediate using Me_3NSO_3, followed by cation exchange with tetrabutylammonium acetate, gave the organosulfate as its tetrabutylammonium salt. The sodium salt was obtained by precipitation in 77% yield over 2 steps on a multi-kg scale. Similarly, the use of a sulfate bis(tributylammonium) salt for the preparation for

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† Electronic supplementary information (ESI) available: Preparative routes, compound characterisation, copies of ^1H and ^{13}C spectra and the cif file for the X-ray crystal structure of **1**. CCDC 1894165 (**1**). For ESI and crystallographic data in CIF or other electronic format see DOI: 10.1039/c9cc01057b

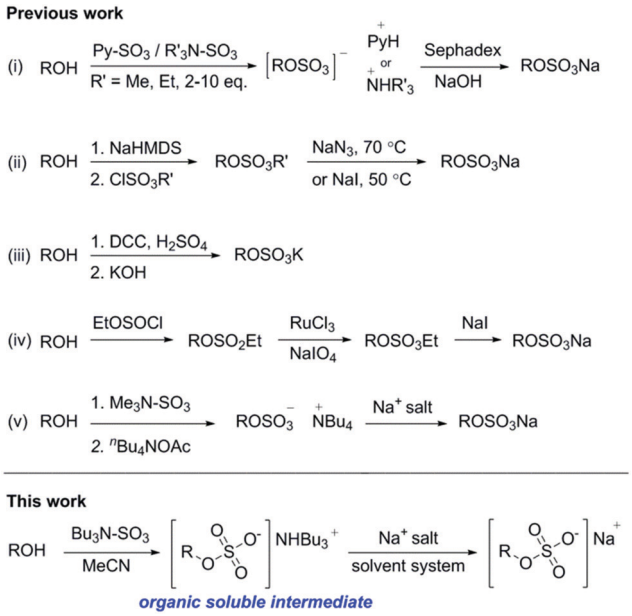
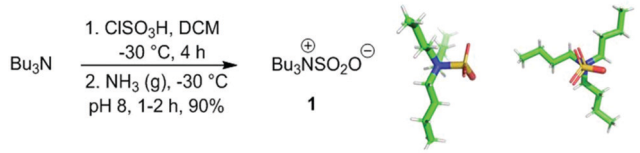


Chart 1 Previous strategies to the synthesis of sulfate esters: this work: sulfate ester formation using tributylsulfoammonium betaine gives direct access to an organic soluble organosulfate intermediate that can be readily ion exchanged.

nucleoside phosphosulfates[22] highlighted that the solubility of the organosulfate ester can be modulated by increasing the lipophilicity of the corresponding cation.

During a medicinal chemistry programme we encountered difficulties using the established sulfation methods to persulfate compounds, due to the poor solubility of the organosulfate intermediates and their resulting purification. We sought to develop an all in one reagent, to improve the solubility of the intermediate organosulfate ester. Combining tributylamine (pK_a, 10.89) with SO_3, we envisioned this would create a complex (Bu_3NSO_3, **1**) for the persulfation of alcohols, with increased lipophilicity of the intermediate sulfate ester, improving the overall solubility of the organosulfates in organic solvents. We rationalised that **1** would retain similar activity to SO_3 complexes with Et_3N and Me_3N, due to their similar Lewis base strengths, pK_a = 11.01 and 10.63, respectively. Overall, **1** may permit sequential chemical steps in organic solvents, with a simple purification method to the corresponding sodium salt, streamlining the synthesis of organosulfates.

The complexation of sulfur trioxide to nitrogen or oxygen containing molecules (such as pyridine, NMe_3, NEt_3, DMF, THF and dioxane) is well known,[11,15] the use of an organic solubilising partner, tributylamine, is however not. To the best of our knowledge, the only literature report of the synthesis and physical study of **1** was by Moede in 1949.[23] It was not until 1976 that Parshikov and co-workers[24] studied **1** as a sulfating agent on simple aliphatic alcohols (without spectroscopic characterisation) and found that **1** reacts *via* an S_N2 mechanism driven by the hydrogen-bonding propensity of the alcohol under study. However, to the best of our knowledge no further use or development of this reagent has been reported.

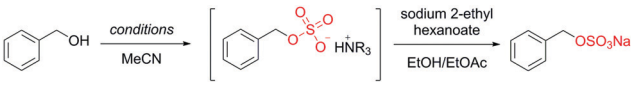


Scheme 1 (a) Synthesis of Bu_3NSO_3 (**1**); (b) alternative views of the crystal structure of **1** obtained from small molecule single crystal X-ray diffraction.

We synthesised **1** by reaction of tributylamine with chlorosulfonic acid, affording a 90% yield on a 60 g scale (Scheme 1(a)).[25] For the first time both NMR spectral data and the crystal structure of **1**, obtained from small molecule single crystal X-ray diffraction, was determined (Scheme 1(b)).[26] Bu_3NSO_3 (**1**) adopts a *Gauche* conformation within an asymmetric unit cell caused by hydrogen bonding between the methylene hydrogen atoms α to the nitrogen and the oxygens of SO_3. The measured N–S bond length in **1** is 1.886(3) Å, a comparable bond length to a single N–S bond (typically: 1.73–1.83 Å *versus* 2.06 Å for a donor–acceptor system),[27] suggesting that **1** exists as a betaine in the solid state which may have implications for the other unsolved amine–SO_3 complexes and their associated mechanisms.

Benzyl alcohol (**2a**) was selected for the optimisation study (Chart 2) due to a distinct down-field shift (+0.35 ppm) of the benzylic signal after sulfation (by ^{1}H-NMR spectroscopy).

We examined the sulfation of **2a** with varying equivalents of **1** (entries 1 to 4). It was found that 2.0 equivalents of **1** was optimal for high conversions (>99%) and isolated yields (95%, entry 3).



Entry	amine-SO_3	Eq.	T °C	t (h)	Additive	Conversion at 0.5 h (%)	Conversion at end (%)	Isolated yield (%)
1	NBu_3	1.1	90	8.0	-	48	83	41
2	NBu_3	1.5	90	4.0	-	26	69	62
3	NBu_3	2.0	90	1.0	-	90	>99	95
4	NBu_3	4.0	90	0.5	-	>99	>99	95
5	NBu_3	2.0	70	6.0	-	22	>99	96
6	NBu_3	2.0	50	8.0	-	9	60	58
7	NBu_3	2.0	30	120	-	2	>99	96
8	NBu_3	2.0	20	18	-	1	9	[a]
9	Py	2.0	70	0.5	-	>99	>99	17[b]
10	Py	2.0	70	0.5	Bu_4NI[c]	>99	>99	47
11	Py	2.0	70	0.5	Bu_4NOAc[c]	>99	>99	76
12	NMe_3	2.0	70	6.0	-	3	29	[a]
13	NMe_3	2.0	70	6.0	Bu_4NI	-	88	[a]
14	NBu_3	2.0	105[d]	0.5	-	68	68	[a]
15	NBu_3	2.0	120[d]	1.0	-	68	68	[a]
16	NBu_3	2.0	70	2.0	Na_2CO_3	16	57	[a]
17	NBu_3	2.0	70	2.0	K_2CO_3	8	8	[a]
18	NBu_3	2.0	70	2.0	Et_3N	15	34	[a]
19	NBu_3	2.0	70	6.0	Bu_3N	48	60	[a]

Chart 2 Optimisation of a model system. [a] Not isolated; [b] isolated as the pyridinium salt; [c] additive used during work-up; [d] microwave irradiation.

Less than 2.0 equivalents of **1** (entries 1 and 2) gave incomplete conversion to **3a**. We next surveyed the effect of temperature (entries 5–8 *vs.* entry 3). 90 °C and 2.0 equivalents of **1** gave complete conversion to **3a** within 2 h. Notably, reaction completion was achieved, even at 30 °C (with increased reaction time) demonstrating that **1** could be a suitable reagent for temperature sensitive substrates such as proteins.[13]

We compared the use of two commercially available amine–SO_3 complexes, namely pyridine and trimethylamine (entries 9 and 12, respectively). Py–SO_3 was more reactive than **1**, giving complete conversion to **3a** in 0.5 h but only a 17% isolated yield of the pyridinium species. As a control experiment, the sequential exchange of the pyridinium salt with different Bu_4N cations afforded the isolation of **4a** in 47% (Bu_4NI) and 76% (Bu_4NOAc) yield, respectively (entries 10 and 11). The use of Me_3N–SO_3 gave poor results (0% isolated yield of **4a** from a 29% conversion to **3a**). The addition of Bu_4NI to the reaction mixture significantly improved the reaction, affording an 88% conversion to **3a** (entry 13). Unfortunately, a more complex reaction mixture was detrimental to the isolation of **4a**. These results demonstrate the isolation benefits associated with the protocol developed with **1**.

We observed that **1** can achieve high conversions of **2a** to **3a**/**4a** without microwave irradiation (entries 1–7 *vs.* 14 and 15) unlike other reported sulfating agents. The addition of a hetero or homogenous base (entries 16–19) was investigated and in all cases this led to a decrease in conversion to **3a**. Most notably, the addition of tributylamine (entry 19) initially increased the rate conversion to **3a**, but reduced the overall conversion contradicting the original report.[24] We rationalised that the addition of Et_3N (with a higher pK_a value than Bu_3N) competes in the reaction, forming Et_3NSO_3 *in situ*. This amine exchange has also been observed in the reaction of Et_3N with $PySO_3$.[21]

We applied the optimal conditions to the synthesis of a range of sulfate esters (Chart 3). In all examples (Chart 3) we observed a near-quantitative conversion to the corresponding sulfate ester as the tributylammonium salt, independent of

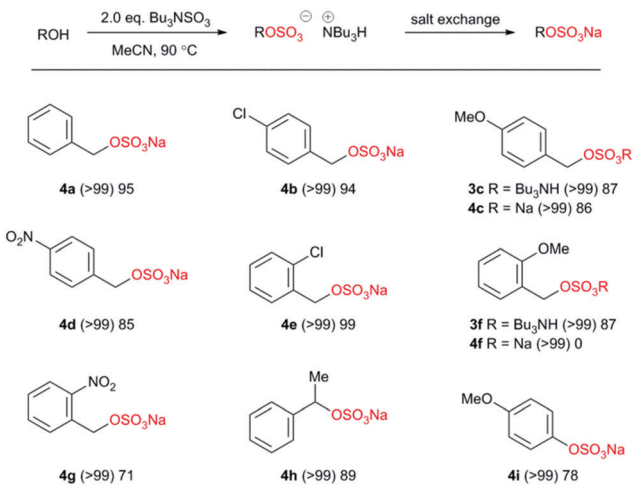


Chart 3 Application of methodology to benzylic and phenolic alcohols. (Parentheses indicates reaction conversion as measured by ^{1}H NMR spectroscopy.)

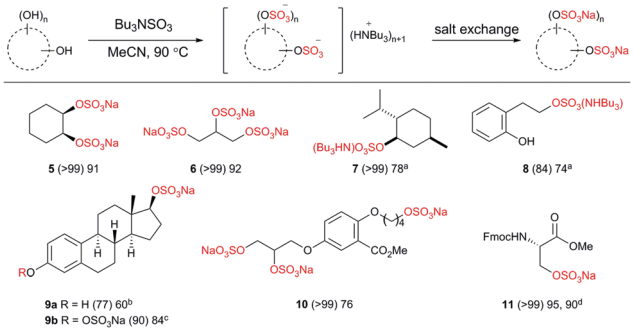


Chart 4 Reaction scope on diverse alcohols (2.0 eq. of **1** per OH group except where indicated). [a] Isolated as NBu_3H salt; [b] 1.5 eq. of **1**; [c] 5.0 eq. of **1**; [d] reaction performed at 38 °C using 4.0 eq. of **1** in DMF. (Parentheses indicate reaction conversion as measured by ^{1}H NMR spectroscopy.)

both electronic and steric factors. Differences occurred when converting the intermediate into a crystalline sodium salt; most likely due to the variability in precipitation of the sodium salt. Therefore differences of electron-withdrawing and electron-donating factors are difficult to draw with certainty. Steric bulk adjacent to the reacting centre was accommodated with ease (**4h**) and the reaction could also be applied to phenolic alcohols (**4i**).

Driving an organosulfate ester reaction to completion, by sulfating all possible hydroxyl sites in a structure remains a synthetic challenge. This is influenced by anionic crowding, making per-sulfation progressively more difficult.[16] Therefore we applied the conditions to examples of compounds requiring two or more sulfation events, including natural products, and compounds containing different hydroxyl moieties within the same structure to probe the selectivity profile (Chart 4).

Cyclohexane-1,2-diol afforded the disulfate in high conversion and isolated yield, likewise with glycerol, the tri-sulfated analogue was prepared with ease (**5** and **6**, respectively). The reaction of **1** with menthol (**7**) delivered the $NHBu_3$ salt with ease.

To probe the alcohol selectivity profile, a primary alcohol was functionalised in preference to a phenol in **8**. Therefore, β-estradiol afforded 17-β-estradiol sulfate[28] over the more common metabolite 3-β-esteradiol in 60% isolated yield (**9a**) using 1.5 eq. **1**. Using 5.0 eq. of **1** both the 17- and 3-positions were sulfated in 84% isolated yield (**9b**).

We then applied the methodology to the original medicinal chemistry challenge, the sodium salt of glycomimetic **C3** (**10**).[8] High conversion and an isolated yield of 76% on a 500 mg scale was achieved. The methodology was applied to the Fmoc-protected amino acid, serine, resulting in excellent yields (95% under normal conditions) and 90% at a non-denaturing temperature (**11**) both using 4.0 eq. of **1**. Importantly, no loss in enantiomeric ratio was observed upon sulfation (>99 : 1).[29]

In summary, we have reported the first scalable preparation and reaction scoping study of **1** as a mild, bench-stable, and chromatography-free method to access organic sulfate esters as their ammonium or sodium salts. The reaction holds promise with the ability to install up to three sulfate groups on complex scaffolds including examples where sterics would limit other

methods. Further work to elucidate the scope of the reaction on human sulfate metabolites is ongoing.

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Conflicts of interest

There are no conflicts to declare.

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26 CCDC 1894165 (**1**) contains the supplementary crystallographic data for this paper. Crystal data for **1**: $C_{12}H_{27}NO_3S$ (M = 265.40 g mol^{-1}): trigonal, space group *R3c* (no. 161), a = 14.3352(2) Å, c = 12.2455(2) Å, V = 2179.28(8) Å^3, Z = 6, T = 100.01(10) K, μ(CuKα) = 1.969 mm^{-1}, D_{calc} = 1.213 g cm^{-3}, 8776 reflections measured (16.14° ≤ 2Θ ≤ 147.692°), 977 unique (R_{int} = 0.0273, R_{sigma} = 0.0115) which were used in all calculations. The final R_1 was 0.0209 ($I > 2\sigma(I)$) and wR_2 was 0.0570 (all data).
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29 We thank reviewer 2 for this suggestion and the insight it provided. Chiral HPLC traces are provided in the ESI†.