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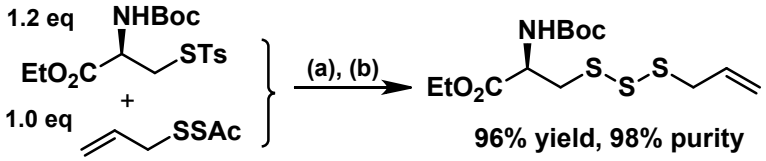
Unsymmetrical Organotrisulfide Formation via Low Temperature Disulfanyl Anion Transfer to an Organothiosulfonate

Doaa Ali,[a] Roger Hunter,*[,a] Catherine H. Kaschula,[b] Stephen De Doncker,[a] and Sophie C. M. Rees-Jones[a]

[a]Department of Chemistry, University of Cape Town, Rondebosch 7701, South Africa

[b]Department of Chemistry and Polymer Science, Stellenbosch University, Stellenbosch, 7600 South Africa

TOC



(a) THF, -78 °C; (b) NaOMe (1M in MeOH), 1 eq, 30 secs

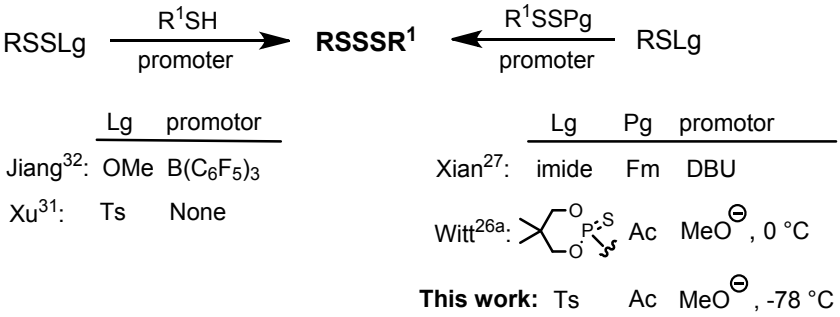
ABSTRACT: New methodology is presented for formation of unsymmetrical organotrisulfides in high yield and purity, relatively free of polysulfide by-products. The highlight of the method is the low temperature (-78 °C) deprotection of a disulfanyl acetate with sodium methoxide in THF to form a disulfanyl anion, which reacts rapidly *in situ* with an organothiosulfonate (*S*-aryl or *S*-alkyl) within thirty seconds followed by quenching. The discovery of these new reaction conditions, together with the relative greenness of the chemistry overall makes for an efficient protocol, from which a range of organotrisulfides covering aliphatic, aromatic as well as cysteine and sugar groups can be accessed in high yield and purity.

INTRODUCTION

As with their disulfide[1] counterparts, organotrisulfides are well represented in Nature, appearing as protective agents in a range of natural products[2] such as the varacins,[3] the epipolythiodiketopiperazine alkaloids[4] and the ene-diyne antibiotics[5] such as the shishijimicins,[6] esperamicin[7] and calicheamicin,[8] as well as protein trisulfides.[9] Undoubtedly the most prevalent natural source of organotrisulfides is to be found in the *Alliaceae* family[10] of which garlic is a key member.[11] A much more recent discovery is their application as high-energy catholytes for rechargeable lithium ion batteries.[12] Aliphatic organotrisulfides are generally quite stable in both aqueous and biological systems, and diallyl trisulfide (DATS) from garlic has been shown to be involved with glutathione redox-exchange reactions[13] that generate the relatively recently discovered gasotransmitter hydrogen sulfide,[14] which itself is involved in signal transduction *in vivo*. Importantly, it has been found[15] that while endogenous hydrogen sulfide is cancer-cell protective, exogenously generated hydrogen sulfide from an external organotrisulfide such as diallyl trisulfide (DATS) exerts the opposite effect as cytotoxic, and this discovery has created a lot of interest in developing synthetic organotrisulfides as potential anti-cancer agents (DATS),[16] as well as antibacterial agents.[17]

Regarding synthesis methodology for producing symmetrical organotrisulfides, most[18] of the methods have focused on carrying out a double substitution on the central organotrisulfide sulfur, with the latter represented as either a nucleophilic metal sulfide reacting with a suitable electrophilic partner, eg Bunte salts[19] or thiosulfonates,[20] or as an electrophilic one of the type $(Lg)_2S$, where Lg is Cl,[21] imidazole[22] or phthalimido[23] reacting with a suitable nucleophilic partner as either the thiol or an appropriately activated surrogate such as $(TMS)_2S$ (with a thiosulfinate).[24] Base-mediated treatment of a sulfenylthiocarbonate producing a disulfanyl anion has also been reported for symmetrical organotrisulfide production.[25] However, none of these approaches are suitable for producing unsymmetrical

organotrisulfides efficiently, since either they produce a fair degree of symmetrical trisulfide as a by-product that is difficult to remove chromatographically, or they are geared towards formation of the symmetrical organotrisulfide exclusively. By comparison, effective methods reported for producing unsymmetrical trisulfides rely on prepreparing a disulfanyl derivative and reacting accordingly, Scheme 1.



Scheme 1. A Summary of Recent Methods for Unsymmetrical Trisulfide Synthesis

Hence, for the nucleophilic variants of this approach, while Witt and coworkers have demonstrated[26a] that a hydrodisulfide RSSH[26] can be generated from the corresponding disulfanyl acetate using methoxide ion and reacted *in situ* cleanly with a sulfenyl derivative bearing a phosphorodithioate leaving group, Xian has shown[27] that 9-fluorenylmethyl disulfides under base-mediated elimination conditions (DBU) at room temperature can also act as suitable surrogates for hydrodisulfide production for reaction with sulfenylimides to afford organotrisulfides. Working things the other way round via a RSSLg species enjoys the advantage of being able to use a thiol as the nucleophile, in which several leaving groups have been explored for the electrophilic disulfanyl partner including from the older literature thiocarbonate,[28] phthalimido,[29] and chloride.[30] In much more recent research though, both tosylate[31] (only *t*-butyl was used as the R group of the RSSTs though) and methoxy[32] as leaving groups have been reported, the latter using tris(perfluorophenylborane) as a catalytic activator in which significant scope in the product range was demonstrated. However,

sulfenylthiotosylates (RSSTs) have been shown generally to be unstable and extrude S in polar solvents.[33]

Although the synthesis options for producing unsymmetrical organotrisulfides have significantly improved in recent times, a number of challenges still exist regarding target scope, product purity and the relative greenness of the method. Notably, the more recent methods illustrated in Scheme 1[26a,27,31,32] still either use harsh reagents (Br_2, $SOCl_2$)[26a,31] to prepare sulfenyl halide intermediates, are limited in scope,[31] involve long exchange sequences to arrive at the precursor for coupling,[27] or involve additional catalysts to promote coupling.[32] Furthermore, none of those methodologies just mentioned have satisfactorily demonstrated an efficiency for producing aromatic-aromatic trisulfides as the most difficult class of the group. In this paper we demonstrate a novel, extremely straightforward-to-use and relatively green-efficient methodology that allows access to all three general classes of aliphatic-aliphatic, aliphatic-aromatic and aromatic-aromatic products including some cysteine and sugar trisulfides.

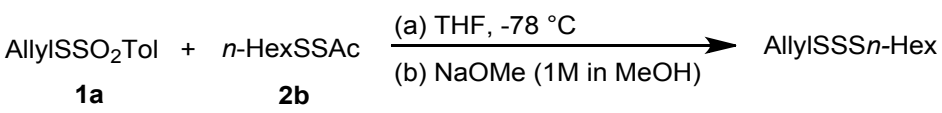
RESULTS AND DISCUSSION

From the outset, we were intent on developing a disulfanyl transfer agent that met the above reaction efficiency criteria, in which we eventually opted for a nucleophilic disulfanyl anion with a thiosulfonate as the electrophilic source, for which, somewhat surprisingly, there were no literature examples. As an S1 electrophilic source, thiosulfonates have excellent handling characteristics as well as a facile green access via alkyl halide substitution with commercially available sodium thiotosylate[34] or from a disulfide with iodine and sodium benzenesulfinate,[35] both methods avoiding the use of a toxic halogenating agent. The nucleophilic S2 partner was chosen as an organohydrodisulfide (RSSH), which we appreciated posed a more difficult challenge regarding its generation.[26b] Current literature suggests that the role of this

interesting species is still not fully understood in biological systems, although the general consensus is that in the form of Cys-SSH it likely provides a storage species or surrogate for H_2S as a gasobiotransmitter.[36] For generation of RSSH as a synthesis reagent in solution, a proven and convenient precursor is undoubtedly the corresponding disulfanyl acetate RSSAc, which can be deprotected in either acid or base media. However, according to current literature[36] neither medium is ideal; RSSH is relatively stable in acid,[36a,37] deprotection is slow,[37] while in basic medium the opposite is true in which S_0 extrusion to form the thiolate occurs,[36a,] which in the present context would lead to disulfide products. However, the fact that disulfanyl acetates can be easily accessed from thiosulfonates via reaction with potassium thioacetate (KSAc) in DCM,[38] ultimately persuaded us that investing time and effort into improving on the troublesome base-promoted deprotection conditions was warranted. Our quest to achieve this was given a boost by taking note of the somewhat intriguing findings reported by Witt[26a] in his sulfenylphosphorodithioate methodology (Scheme 1), in which he showed good aliphatic-aliphatic unsymmetrical trisulfide outcomes using 2 equivalents of sodium methoxide in methanol to deprotect his RSSAc in situ at 0 °C degrees in MeOH as solvent. This set us thinking about fine tuning these conditions for carrying out a one-pot reaction for our case by also adding the methoxide last, in the anticipation that the hardness of the methoxide as well as the greater stability of the disulfanyl anion (as leaving group) over that of the thiolate (from the thiosulfonate) would result in chemoselective substitution (S_NAc) and deprotection of the acetate over that of the thiosulfonate. A model reaction was carried out between AllylSTs **1a** and *n*-HexSSAc **2b**, the latter chosen in view of its easy access via substitution of the corresponding thiosulfonate (1 equiv) with potassium thioacetate (1.3 equiv) in DCM.[38] As representative of an aliphatic disulfanyl acetate, **2b** was perfectly stable towards silica gel chromatography and subsequent handling. In the event (see Table 2 for a complete numbering set), we were overjoyed to find

that adding the methoxide as a 1M solution in methanol to a mixture of the two reactants at -78 °C in THF gave the desired result. Furthermore, interestingly, experiments carried out to establish both the optimal stoichiometry of reactants as well as the minimum reaction time for minimising potential exchange reactions leading to polysulfides revealed that a ratio of thiosulfonate to disulfanyl acetate of 1.2:1 together with a time of only thirty seconds after methoxide addition (with moderate stirring) with 1 eq. of methoxide achieved full conversion (TLC; entry 3 in Table 1) to afford the organotrisulfide cleanly, after which the reaction was quenched with aqueous ammonium chloride. Following chromatography the desired trisulfide product was obtained in excellent yield and purity (HPLC) in each run. The product (allylSSS*n*-hex), as a typical representative of this class of organotrisulfide, was completely stable towards chromatography and leaving out on the bench for a few days, as well as the recording of NMR spectra in $CDCl_3$. Each set of conditions gave a single clean and less polar spot on the TLC plate, with no indication of a by-product, even leaving for a relatively longer reaction time (entries 2 and 4), as reflected in the yields (Table 1). By comparison, using the thiosulfonate as limiting reagent with a slight excess of the disulfanyl acetate gave more by-products, presumably due to subsequent exchange reactions involving the excess nucleophilic disulfanyl anion. Interestingly, full conversion could be achieved with less than a stoichiometric amount of methoxide promotor (entries 2 and 4 in Table 1), suggesting that the sulfinate leaving group from the S-S coupling can also exert a level of acetyl deprotection, Table 1.

Table 1: Optimised Conditions for Trisulfide Model Reaction

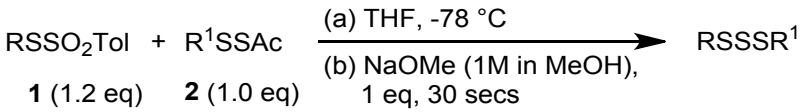


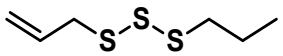
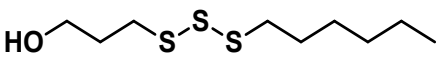
Entry	Eq. 1a	Eq. 2b	Eq. NaOMe	Time	Yield %[a]	Purity %[b]
1	1.5	1	1	30 sec	80	93
2	1.5	1	0.7	5 min	90	93
3	**1.2**	**1**	**1**	**30 sec**	**88**	**96**
4	1.2	1	0.7	5 min	92	95

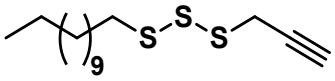
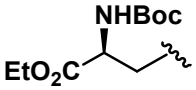
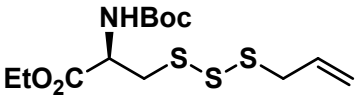
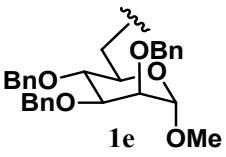
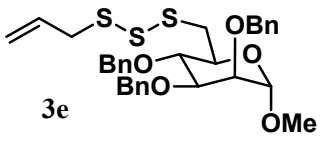
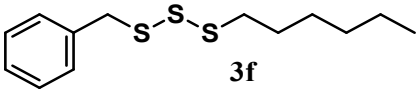
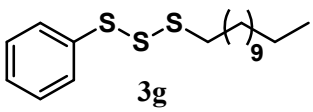
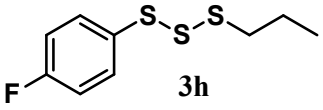
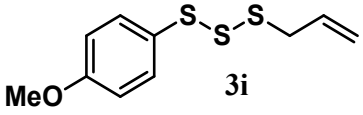
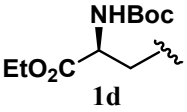
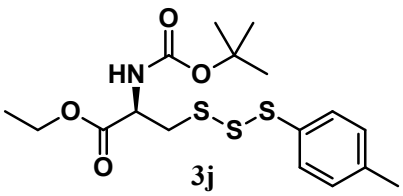
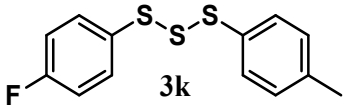
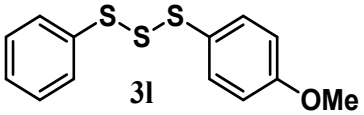
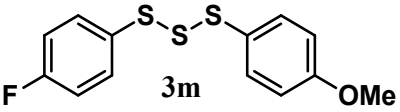
[a] After column chromatography. [b] Measured by HPLC on a C18 column.

With a set of model conditions in hand we next set about applying them to synthesizing the three different classes of trisulfide mentioned previously as aliphatic-aliphatic, aliphatic-aromatic and aromatic-aromatic. The results are shown in Table 2 for organotrisulfide products **3a-m**.

Table 2. Synthesis of Unsymmetrical Trisulfides 3a-3m



Entry	R	R^1	$RSSSR^1$	Yield (%)[a]	Purity (%)[b]
1	**Allyl, 1a**	***n*-Pr, 2a**	 **3a**	**95**	**96**
2	**-$(CH_2)_3OH$, 1b**	***n*-Hex, 2b**	 **3b**	**90**	**98**

3	*n*-Dodec, 1c	Propargyl, 2c	 3c	52	96
4	 1d	Allyl, 2d	 3d	96	98
5	 1e	Allyl, 2d	 3e	66	89
6	Bn, 1f	*n*-Hex, 2b	 3f	85	99
7	Ph, 1g	*n*-Dodec, 2e	 3g	59	94
8	*p*-FC_6H_4, 1h	*n*-Pr, 2a	 3h	61	96
9	*p*-$MeOC_6H_4$, 1i	Allyl, 2d	 3i	64	97
10	 1d	*p*-Tolyl, 2f	 3j	70	87
11	*p*-FC_6H_4, 1h	*p*-Tolyl, 2f	 3k	89	60[c]
12	Ph, 1g	*p*-$MeOC_6H_4$, 2g	 3l	64	78
13	*p*-FC_6H_4, 1h	*p*-$MeOC_6H_4$, 2g	 3m	81	91

[a]After column chromatography. [b]Measured by HPLC on a C18 column. [c]92% after preparative chromatography.

The organotrisulfide R groups were varied in terms of lipophilicity, functionality and substitution on the aromatic ring. The thiosulfonate reactants $RSSO_2Tol$ **1** with R as an aliphatic group could be accessed in a routine fashion using nucleophilic substitution of RBr with commercially available sodium *p*-tolylthiosulfonate;[34] for aromatic counterparts, reaction of the corresponding homodisulfide with iodine and sodium *p*-tolylsulfinate was used.[35] A bonus of the methodology was that the disulfanyl acetates **2** could be accessed from their corresponding thiosulfonates **1** via reaction with potassium thioacetate in DCM.[38] Gratifyingly, the model reaction time of 30 secs held throughout in each case, and products were stable following column chromatography. All of the trisulfides except **3g** (1-dodecyl-3-phenyltrisulfane) were new compounds and were fully characterised by NMR and IR spectroscopy, HPLC (C18 column) and HRMS. ^{1}H NMR spectroscopy for products containing an alkyl R group could be used to corroborate trisulfide formation (from disulfide) by virtue of the downfield shift of the methylene adjacent to S compared to that of the disulfide (~0.2 ppm to around 2.8 ppm).[26a] For the aliphatic-aliphatic group (entries 1-6), the choice of which R group presented as thiotosylate and which as disulfanyl acetate was arbitrary, and the trisulfide yields were generally excellent and above 85%, except for the propargylic trisulfide **3c** (52%) and the protected mannose trisulfide **3e** (66%). Similarly, their purities, as ascertained by HPLC, were also excellent (above 96%) except for the sugar case **3e** (89%). For the cysteine case (**3d**), coupling only worked having the amino acid as its thiotosylate, while the mannose case **3e** also had the sugar moiety as a thiotosylate but only because this was the easier way round to achieve synthetically. This class of organotrisulfide proved to be very stable even towards leaving on the bench for a few days. Moving to the aliphatic-aromatic group (entries 7-10), the aromatic partner was always presented as a

thiotosylate in view of the propensity of aromatic disulfanyl anions to extrude sulfur to form the corresponding thiolate ion,[36a] as well as the superior stability (for isolation) of the thiotosylate over the corresponding aromatic disulfanyl acetate. Yields in this series dropped down a little to around 60-70%, while purities remained in the 90s except for the cysteine derivative **3j** (87%). Synthesis of 2-(*tert*-butyltrisulfanyl)pyridine (with 2-pyrido and *t*-butyl as the two R groups) was successful and the compound could be chromatographed, but it proved to be unstable in solution when recording the NMR spectra, presumably due to S-S exchange reactions promoted through the pyridine lone pair. Generally, though, this class of organotrisulfide, though less stable than aliphatic-aliphatic variants, proved also to be quite stable for a day or so on the bench after chromatography. Finally, for the aromatic-aromatic trisulfide group (entries 11-13), firstly it should be mentioned that these are hardly ever mentioned in the literature, presumably in view of their propensity to undergo S-S exchange reactions; testimony to this was that the three cases **3k-3m** are all new compounds, in spite of their relatively simple substitution patterns. Importantly, one of the reactants now had to be presented as an aromatic disulfanyl acetate, in which once again *S*-substitution of the corresponding thiotosylate with potassium thiotosylate in DCM was used, rather than the older and less green method involving reaction of an aromatic thiol with acetyl sulfenyl chloride.[37] In this case, the appropriate aromatic thiotosylate was accessed using reaction of the corresponding homodisulfide with iodine and *p*-tolylsulfinate.[35] Importantly, though, compared to the aliphatic cases (rt required), reactions between the thiotosylate and KSAc were much quicker in the aromatic series but needed to be carried out at -40 °C in order to achieve a clean conversion (by TLC). However, unlike the aliphatic cases, the aromatic disulfanyl acetates (**2f** and **2g**) were unstable on silica gel, and instead were used crude following the filtration of residual acetate and thiotosylate salts followed by solvent evaporation. Importantly, it was pleasing to note that ^{1}H NMR spectroscopic analysis of the

crude aromatic disulfanyl acetate products showed a very high product purity, with no residual acetate or thiotosylate resonances. Once synthesized, these products were used as soon as possible. Looking at the reaction results from Table 2 (entries 11-13), while yields of organotrisulfides **3k-3m** were on a par with the aliphatic-aromatic group, purities were generally lower (60-91%), which was attributed to their higher reactivity towards S-S exchange reactions. These aromatic organotrisulfides (**3k-3m**) were stable towards work-up and chromatography. Aromatic rings bearing an electron-withdrawing group (eg entry 13 with *p*-fluorophenyl to give **3m**) needed to be reacted as their thiotosylates and not as disulfanyl acetates, in which expulsion of sulfur to afford a stabilised aromatic thiolate anion emerged as a problem. In this regard, all attempts to prepare pyridine-containing heteroaromatic-aromatic organotrisulfides failed, even using the pyridine moiety as its thiotosylate, again presumably due to the extremely high reactivity towards S-S exchange reactions in the reaction medium Aromatic-aromatic organotrisulfides **3k-3m** were relatively labile and needed to be kept in the refrigerator at -20 °C following chromatographic purification.

CONCLUSION

In summary, we have developed a new method for producing various classes of organotrisulfides in high yield and purity involving low temperature sulfenylation of a disulfanyl anion by an organothiotosylate. Particularly pleasing is that the method holds up to a fair degree of yield and purity for aromatic-aromatic variants, which have been under-represented in other studies. The method is extremely straightforward to carry out and uses inexpensive chemicals. Furthermore, the discovery of low temperature generation and usage of the disulfanyl anion RSS^-, in which R can be varied broadly, opens up the way for application of this important biosynthon synthetically. Future Chemical Biology studies

towards studying the mode of action of trisulfides in cancer cells will no doubt benefit from the results reported herein from the aliphatic-aliphatic and aromatic-aliphatic classes.

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EXPERIMENTAL SECTION

Unless otherwise specified, all reagents were purchased from commercial sources and used without further purification. THF was freshly distilled over sodium wire and benzophenone. Reactions were carried out in oven-dried glassware with a magnetic stirrer, and performed under a nitrogen atmosphere unless otherwise stated. The low temperature (-78 °C) of the trisulfide reactions was achieved with acetone/liquid nitrogen mixtures in a low temperature bath. Aqueous solutions were prepared using deionized water. ^{1}H NMR and ^{13}C{H} NMR spectra were recorded on a Bruker 300 MHz (^{1}H, 300 MHz; ^{13}C, 75.5 MHz) or a Bruker 400 MHz (^{1}H, 400 MHz; ^{13}C, 101 MHz) instrument. All spectral data were acquired at 295 K. Chemical shifts are reported in parts per million (ppm, δ), downfield from tetramethylsilane (TMS, δ = 0.00 ppm) and are referenced to residual solvent ($CDCl_3$, δ = 7.26 ppm (^{1}H) and 77.16 ppm (^{13}C)). Coupling constants (*J*) are reported in Hertz (Hz). The multiplicity abbreviations used are: br broad, s singlet, d doublet, t triplet, q quartet, m multiplet. Infrared (IR) spectra were recorded on a Perkin Elmer Spectrum 100 FT-IR Spectrometer. High-resolution mass-spectra were obtained from the University of Stellenbosch Mass Spectrometry Service and recorded in electrospray positive mode (ES^+) or atmospheric solids analysis probe ($ASAP^+$) with a time-of-flight analyser system on a Waters Synapt G2 machine. Thin layer chromatography was carried out on Merck silica gel $60F_{254}$ pre-coated aluminium foil sheets and were visualised using UV light (254 nm), while non-UV active compounds were sprayed with a 2.5% solution of *p*-anisaldehyde in a mixture of sulfuric acid and ethanol (1:10 v/v), iodine vapour, or ceric ammonium sulfate solution and then heated. Column chromatography was carried out using silica gel 60 (Merck 7734), eluting with the

specified solvent system. Optical rotations were measured at 20 °C and are reported as $[\alpha]_D^{20}$ (*c* g/ml, solvent). The determination of melting points was carried out using a hot-stage microscope (HSM) and are uncorrected. HPLC analyses were carried out with a C18 reverse-phase column.

General Procedure for Synthesis of Thiotosylates 1a-i.

Aliphatic thiosulfonates **1a,**[39] **1b,**[40] **1c,**[41] **1e and 1f**[42] were all prepared from the corresponding alkyl bromide or iodide by substitution with sodium 4-methylbenzenesulfonothioate in acetonitrile at room temperature according to literature procedures.[34] Aromatic thiosulfonates **1g**,[43] **1h and 1i**,[44] as well as **1d** were all prepared from the corresponding disulfide and sodium *p*-tolylsulfinate and iodine, also according to a literature procedure.[35]

*(R)-Ethyl 2-((tert-butoxycarbonyl)amino)-3-(tosylthio)propanoate, (**1d**),* (86.0 mg, 88%): mp 52–54 °C; $[\alpha]_D^{20}$ = -2.5 (*c* = 1.0, $CHCl_3$); IR (ν_{max}/cm^{-1}) 3363, 1735, 1695; ^1H NMR (300 MHz, $CDCl_3$) δ 7.80 (d, *J* = 8.3 Hz, 2H), 7.34 (d, *J* = 8.3 Hz, 2H), 5.30 (br s, 1H), 4.51 (m, 1H), 4.19 (q, *J* = 7.2 Hz, 2H), 3.57–3.32 (m, 2H), 2.44 (s, 3H), 1.43 (s, 9H), 1.26 (t, *J* = 7.2 Hz, 3H); $^{13}C\{H\}$ NMR (101 MHz, $CDCl_3$) δ 169.8, 155.1, 145.2, 141.9, 130.1, 127.3, 80.6, 62.3, 53.1, 37.9, 28.4, 21.8, 14.2; HRMS (ESI^+) *m/z* $[M + H]^+$ calcd for $C_{17}H_{26}NO_6S_2$ 404.1202, found 404.1207.

Prepared from the corresponding tribenzyl-protected α-methylmannopyranoside,[45] *S-(((2S,3S,4S,5S,6S)-3,4,5-tris(benzyloxy)-6-methoxytetrahydro-2H-pyran-2-yl) methyl) 4-methylbenzenesulfonothioate (**1e**),* (30.0 mg, 73%): $[\alpha]_D^{20}$ = +66.3 (*c* = 1.0, $CHCl_3$); IR (ν_{max}/cm^{-1}) 1138, 698; ^1H NMR (300 MHz, $CDCl_3$) δ 7.73 (d, *J* = 8.3 Hz, 2H), 7.41–7.27 (m, 15H), 7.18 (d, *J* = 8.3 Hz, 2H), 4.94 (d, *J* = 11.1 Hz, 1H), 4.75–4.50 (m, 6H), 3.85–3.64 (m, 4H), 3.49–3.40 (m, 1H), 3.21 (s, 3H), 3.15–3.05 (m, 1H), 2.39 (s, 3H); $^{13}C\{H\}$ NMR (101 MHz, $CDCl_3$) δ 144.6, 142.1, 138.4, 138.4, 138.3, 129.8, 128.5, 128.5, 128.5, 128.5,

128.2, 128.0, 127.9, 127.8, 127.8, 127.4, 99.1, 80.2, 77.6, 75.2, 74.8, 73.1, 72.3, 70.5, 55.0, 38.3, 21.7; HRMS (ESI$^+$) *m/z* [M + H]$^+$ calcd for $C_{35}H_{39}O_7S_2$ 635.2137, found 635.2130.

General Procedure for Synthesis of Disulfanyl Acetates 2a-g.

Disulfanyl acetates **2a**,[46] **2b**[38] **2c**, **2d**,[46] **2e**,[26a] **2f**,[37] **2g**,[37] were all synthesized by reaction of the corresponding thiosulfonate (1 equiv) with potassium thioacetate (1.3 equiv) in DCM at rt for a few hours for aliphatic cases, and -40 °C for aromatic variants, the method modelled on a recent literature procedure.[38] Here, the thiotosylate was always used as the limiting reagent and checked for total consumption (by TLC). While the aliphatic variants could be column chromatographed, the aromatic ones needed to be used as crude, in both cases following the filtration of salts and the evaporation of solvent.

*1-(prop-2-yn-1-ylsulfinothioyl)ethanone (**2c**),* (131.0 mg, 81%): IR (ν_{max}/cm^{-1}) 3287, 1727; ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.48 (d, *J* = 2.7 Hz, 2H), 2.49 (s, 3H), 2.30 (t, *J* = 2.7 Hz, 1H); ^{13}C{H} NMR (101 MHz, $CDCl_3$) δ 194.2, 78.4, 73.1, 29.0, 26.8; HRMS (ASAP$^+$) *m/z* [M + H]$^+$ calcd for $C_5H_7OS_2$ 146.9938, found 146.9943.

General Procedure for Synthesis of Unsymmetrical Trisulfides 3a-3m.

The thiosulfonate **1** (1.2 eq) and disulfanyl acetate (1.0 eq) were taken up in THF (0.1-0.2 M) under N_2 and cooled to -78 °C for 10 minutes. Sodium methoxide (1 M, 1.0 eq) was added slowly dropwise via syringe (10-20 sec) and the resultant solution stirred rapidly for 30 sec following the methoxide addition. The reaction was then quenched with saturated aqueous ammonium chloride (5 mL), and the product extracted into EtOAc (3 x 10 mL). Following drying ($MgSO_4$) and evaporation of solvent, the residue was purified via silica column chromatography using Hexane as eluent for all organotrisulfides (except **3b**, **3d**, **3e** and **3j**, in which case up to 10% EtOAc in Hexane was used), to afford organotrisulfides **3a-3m**.

*1-Allyl-3-propyltrisulfane, (**3a**).* From **1a** (127.8 mg, 0.560 mmol) and **2a** (70.0 mg, 0.467 mmol) in THF (3 mL) and sodium methoxide (0.47 mL, 1M, 0.47 mmol) to afford trisulfide

3a (80.0 mg, 95 %) as an oil. IR (ν_{max}/cm^{-1}) 722, 476; ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.93–5.83 (m, 1H), 5.26–5.18 (m, 2H), 3.51 (d, *J* = 7.2 Hz, 2H), 2.86 (t, *J* = 7.2 Hz, 2H), 1.85–1.72 (m, 2H), 1.02 (t, *J* = 7.2 Hz, 3H); ^{13}C{H} NMR (101 MHz, $CDCl_3$) δ 133.0, 119.1, 41.7, 41.2, 22.3, 13.3; HRMS (ASAP$^+$) *m/z* $[M + H]^+$ calcd for $C_6H_{13}S_3$ 181.0179, found 181.0186.

*3-(Hexyltrisulfanyl)propan-1-ol, (**3b**).* From **1b** (119.0 mg, 0.484 mmol and disulfanyl acetate **2b** (77.0 mg, 0.400 mmol) in THF (3 mL) and sodium methoxide (0.4 mL, 1M, 0.40 mmol) to afford trisulfide **3b** (86.0 mg, 90%) as a colourless oil. IR (ν_{max}/cm^{-1}) 3325, 788, 475; ^{1}H NMR (300 MHz, $CDCl_3$ δ 3.78 (t, *J* = 6.2 Hz, 2H), 3.00 (t, *J* = 7.1 Hz, 2H), 2.88 (t, *J* = 7.4 Hz, 2H), 2.07–1.96 (m, 2H), 1.78–1.68 (m, 2H), 1.54 (s, OH, 1H), 1.47–1.28 (m, 6H), 0.89 (t, *J* = 6.9 Hz, 3H); ^{13}C{H} NMR (101 MHz, $CDCl_3$) δ 61.1, 39.1, 35.4, 31.6, 31.5, 28.9, 28.3, 22.6, 14.1; HRMS (ASAP$^+$) *m/z* $[M]^+$ calcd for $C_9H_{20}OS_3$ 240.0676, found 240.0677.

*1-Dodecyl-3-(prop-2-yn-1-yl)trisulfane (**3c**).* From thiotosylate **1c** (107.0 mg, 0.300 mmol) and disulfanyl acetate **2c** (37.0 mg, 0.253 mmol) in THF (2 mL) and sodium methoxide (0.25 mL, 1M, 0.25 mmol) to afford trisulfide **3c** (40.0 mg, 52%) as a colourless oil. IR (ν_{max}/cm^{-1}) 635, 477; ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.62 (d, *J* = 2.7 Hz, 2H), 2.91 (t, *J* = 7.4 Hz, 2H), 2.33 (d, *J* = 2.7 Hz, 1H), 1.80–1.68 (m, 2H), 1.45–1.23 (m, 18H), 0.88 (t, *J* = 6.8 Hz, 3H); ^{13}C{H} NMR (101 MHz, $CDCl_3$) δ 79.1, 72.8, 39.5, 32.1, 29.8, 29.8, 29.7, 29.6, 29.5, 29.3, 29.0, 28.7, 26.8, 22.8, 14.2; HRMS (ASAP$^+$) *m/z* $[M + H]^+$ calcd for $C_{15}H_{29}S_3$ 305.1431, found 305.1429.

*(R)-Ethyl 3-(allyltrisulfanyl)-2-((tert-butoxycarbonyl)amino)propanoate (**3d**).* From thiosulfonate **1d** (98.0 mg, 0.243 mmol) and disulfanyl acetate **2d** (30.0 mg, 0.203 mmol) in THF (2 mL) and sodium methoxide (0.20 mL, 1M, 0.20 mmol, 1 eq) to afford trisulfide **3d** (69.0 mg, 96%) as a colourless oil. $[\alpha]_D^{20}$ = +98.3 (*c* = 1.0, $CHCl_3$); IR (ν_{max}/cm^{-1}) 635, 477; ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.96–5.80 (m, 1H), 5.38 (br s, 1H), 5.29–5.18 (m, 2H), 4.64 (m, 1H), 4.22 (q, *J* = 7.1 Hz, 2H), 3.50 (d, *J* = 7.2 Hz, 2H), 3.42–3.25 (m, 2H), 1.45 (s, 9H),

1.30 (t, J = 7.1 Hz, 3H); $^{13}C\{H\}$ NMR (101 MHz, $CDCl_3$) δ 170.6, 155.2, 132.7, 119.5, 80.4, 62.0, 53.2, 41.7, 41.5, 28.5, 14.3; HRMS (ESI^+) *m/z* $[M + H]^+$ calcd for $C_{13}H_{24}NO_4S_3$ 354.0867, found 354.0869.

*(2S, 3S, 4S, 5S, 6S)-2-((allyltrisulfanyl)methyl)-3,4,5-tris(benzyloxy)-6-methoxytetrahydro-2H-pyran (**3e**).* From thiosulfonate **1e** (130.0 mg, 0.205 mmol) and disulfanyl acetate **2d** (25 mg, 0.169 mmol) in THF (4 mL) and sodium methoxide (0.17 mL, 1M, 0.17 mmol) to afford trisulfide **3e** (65.0 mg, 66%) as a colourless thick oily liquid. $[\alpha]_D^{20}$ = +138.9 (c = 1.0, $CHCl_3$); IR (ν_{max}/cm^{-1}) 477; ^1H NMR (300 MHz, $CDCl_3$) δ 7.40–7.27 (m, 15H), 5.94–5.78 (m, 1H), 5.25–5.14 (m, 2H), 4.97 (d, J = 10.8 Hz, 1H), 4.75–4.60 (m, 6H), 3.94–3.75 (m, 4H), 3.49 (d, J = 7.2 Hz, 2H), 3.42 (dd, J =13.4 , 2.1 Hz, 1H), 3.34 (s, 3H), 3.07 (dd, J = 13.4, 9.0 Hz, 1H); $^{13}C\{H\}$ NMR (101 MHz, $CDCl_3$) δ 138.6, 138.5, 138.4, 132.8, 128.6, 128.6, 128.5, 128.5, 128.1, 128.0, 127.9, 127.8, 127.8, 119.2, 99.2, 80.4, 77.9, 75.3, 75.0, 73.0, 72.4, 70.7, 55.1, 42.0, 41.7; HRMS (ESI^+) *m/z* $[M + H]^+$ calcd for $C_{31}H_{37}O_5S_3$ 585.1803, found 585.1813.

*1-Benzyl-3-hexyltrisulfane (**3f**).* From thiosulfonate **1f** (86.8 mg, 0.312 mmol) and disulfanyl acetate **2b** (50.0 mg, 0.260 mmol) in THF (2 mL) and sodium methoxide (0.26 mL, 1M, 0.26 mmol) to afford trisulfide **3f** (60.0 mg, 85%) as a colourless oil. IR (ν_{max}/cm^{-1}) 696, 468; ^1H NMR (300 MHz, $CDCl_3$) δ 7.38–7.25 (m, 5H), 4.11 (s, 2H), 2.83 (t, J = 7.2 Hz, 2H), 1.80–1.65 (m, 2H), 1.47–1.25 (m, 6H), 0.91 (t, J = 6.8 Hz, 3H); $^{13}C\{H\}$ NMR (101 MHz, $CDCl_3$) δ 136.8, 129.6, 128.8, 127.7, 43.3, 39.2, 31.5, 29.0, 28.3, 22.7, 14.1; HRMS ($ASAP^+$) *m/z* $[M + H]^+$ calcd for $C_{13}H_{21}S_3$ 273.0805, found 273.0793.

*1-Dodecyl-3-phenyltrisulfane (**3g**).* From thiosulfonate **1g** (184.0 mg, 0.697 mmol) and disulfanyl acetate **2e** (160.0 mg, 0.579 mmol) in THF (4 mL) and sodium methoxide (0.57 mL, 1M, 0.57 mmol) to afford trisulfide **3g**[26a] (117.0 mg, 59%) as a yellow oil. IR (ν_{max}/cm^{-1}) 470; ^1H NMR (300 MHz, $CDCl_3$) δ 7.65–7.58 (m, 2H), 7.39–7.28 (m, 3H), 2.82 (t, J = 7.4

Hz, 2H), 1.73–1.61 (m, 2H), 1.39–1.22 (s, 18H), 0.89 (t, J = 6.8 Hz, 3H); $^{13}C\{H\}$ NMR (101 MHz, $CDCl_3$) δ 137.4, 130.4, 129.2, 128.3, 39.2, 32.1, 29.8, 29.8, 29.7, 29.6, 29.5, 29.3, 29.1, 28.7, 22.8, 14.2.

*1-(4-Fluorophenyl)-3-propyltrisulfane (**3h**).* From thiosulfonate **1h** (135.4 mg, 0.480 mmol) and disulfanyl acetate **2a** (60.0 mg, 0.400 mmol) in THF (3 mL) and sodium methoxide (0.40 mL, 1M, 0.40 mmol) to afford trisulfide **3h** (57.0 mg, 61%) as a yellow oil. IR (ν_{max}/cm^{-1}) 473; ^1H NMR (300 MHz, $CDCl_3$) δ 7.64–7.57 (m, 2H), 7.10–7.02 (m, 2H), 2.81 (t, J = 7.4 Hz, 2H), 1.80–1.65 (m, 2H), 0.98 (t, J = 7.4 Hz, 3H); $^{13}C\{H\}$ NMR (101 MHz, $CDCl_3$) δ 163.3 (d, J_{CF} = 248.5 Hz), 133.3 (d, J_{CF} = 8.0 Hz), 132.7, 116.4, (d, J_{CF} = 22.1 Hz), 41.4, 22.4, 13.2; HRMS (ASAP$^+$) *m/z* $[M]^+$ calcd for $C_9H_{11}FS_3$ 234.0007, found 234.0010.

*1-Allyl-3-(4-methoxyphenyl) trisulfane (**3i**).* From thiosulfonate **1i** (262.0 mg, 0.891 mmol) and disulfanyl acetate **2d** (110.0 mg, 0.743 mmol) in THF (5 mL) and sodium methoxide (0.74 mL, 1M, 0.74 mmol) to afford trisulfide **3i** (116.0 mg, 64%) as a yellow oil. IR (ν_{max}/cm^{-1}) 467; ^1H NMR (300 MHz, $CDCl_3$) δ 7.57 (d, J = 8.9 Hz, 2H), 6.89 (d, J = 8.9 Hz, 2H), 5.91–5.72 (m, 1H), 5.19–5.02 (m, 2H), 3.82 (s, 3H), 3.42 (d, J = 7.2 Hz, 2H); $^{13}C\{H\}$ NMR (101 MHz, $CDCl_3$) δ 160.8, 134.2, 132.7, 128.0, 119.2, 114.9, 55.5, 41.8; HRMS (ASAP$^+$) *m/z* $[M + H]^+$ calcd for $C_{10}H_{13}OS_3$ 245.0129, found 245.0129.

*Ethyl N-(tert-butoxycarbonyl)-S-(p-tolyldisulfaneyl)-L-cysteinate (**3j**).* From thiosulfonate **1d** (146.0 mg, 0.362 mmol) and disulfanyl acetate **2f** (60.0 mg, 0.303 mmol) in THF (2 mL) and sodium methoxide (0.30 mL, 1M, 0.30 mmol) to afford trisulfide **3j** (86.0 mg, 70%) as a colourless solid. mp 80-82 °C; $[\alpha]_D^{20}$ = +115.0 (c = 1.0, $CHCl_3$); IR (ν_{max}/cm^{-1}) 3369, 1735, 481; ^1H NMR (300 MHz, $CDCl_3$) δ 7.50 (d, J = 8.1 Hz, 2H), 7.17 (d, J = 8.1 Hz, 2H), 5.36 (br s, 1H), 4.59 (m, 1H), 4.22 (q, J = 7.2 Hz, 2H), 3.42–3.21 (m, 2H), 2.36 (s, 3H), 1.46 (s, 9H), 1.29 (t, J = 7.2 Hz, 3H); $^{13}C\{H\}$ NMR (101 MHz, $CDCl_3$) δ 170.6, 155.1, 139.2, 133.0,

131.5, 130.1, 80.3, 62.0, 53.1, 41.4, 28.5, 21.3, 14.3; HRMS (ESI^+) *m/z* $[M + H]^+$ calcd for $C_{17}H_{26}NO_4S_3$ 404.1024, found 404.1018.

*1-(4-Fluorophenyl)-3-(p-tolyl)trisulfane (**3k**).* From thiosulfonate **1h** (51.0 mg, 0.181 mmol) and disulfanyl acetate **2f** (30.0 mg, 0.151 mmol) in THF (1 mL) and sodium methoxide (0.15 mL, 1M, 0.15 mmol) to afford trisulfide **3k** (38.0 mg, 89%) as a yellow to green oil. IR (ν_{max}/cm^{-1}) 502; ^1H NMR (300 MHz, $CDCl_3$) δ 7.53–7.46 (m, 2H), 7.43–7.39 (m, 2H), 7.12–7.07 (m, 2H), 7.00–6.93 (m, 2H), 2.35 (s, 3H); $^{13}C\{H\}$ NMR (101 MHz, $CDCl_3$) δ 163.3 (d, *J* = 249.5 Hz), 139.1, 133.4, (d, *J* = 9.1 Hz), 133.0, 132.0, 131.3, 130.0, 116.3 (d, *J* = 22.1 Hz), 21.3; HRMS ($ASAP^+$) *m/z* $[M]^+$ calcd for $C_{13}H_{11}FS_3$ 282.0007, found 282.0011.

*1-(4-Methoxyphenyl)-3-phenyltrisulfane (**3l**).* From thiosulfonate **1g** (148.0 mg, 0.561 mmol) and disulfanyl acetate **2g** (100.0 mg, 0.467 mmol) in THF (3 mL) and sodium methoxide (0.47 mL, 1M, 0.47 mmol) to afford trisulfide **3l** (84.0 mg, 64%) as a green oil. IR (ν_{max}/cm^{-1}) 469; ^1H NMR (300 MHz, $CDCl_3$) δ 7.53–7.45 (m, 4H), 7.32–7.23 (m, 3H), 6.85–6.78 (m, 2H), 3.81 (s, 3H); $^{13}C\{H\}$ NMR (101 MHz, $CDCl_3$) δ 160.8, 136.8, 134.4, 130.0, 129.2, 128.1, 127.4, 114.9, 55.6; HRMS ($ASAP^+$) *m/z* $[M]^+$ calcd for $C_{13}H_{12}OS_3$ 280.0050, found 280.0053.

*1-(4-Fluorophenyl)-3-(4-methoxyphenyl) trisulfane (**3m**).* From thiosulfonate **1h** (63.0 mg, 0.223 mmol) and disulfanyl acetate **2g** (40.0 mg, 0.187 mmol) in THF (2 mL) and sodium methoxide (0.18 mL, 1M, 0.18 mmol) to afford trisulfide **3m** (45.0 mg, 81%) as a green to yellow oil. IR (ν_{max}/cm^{-1}) 463; ^1H NMR (300 MHz, $CDCl_3$) δ 7.52–7.44 (m, 4H), 7.00–6.93 (m, 2H), 6.83–6.78 (m, 2H), 3.81 (s, 3H); $^{13}C\{H\}$ NMR (101 MHz, $CDCl_3$) δ 163.0 (d, *J* = 165.0 Hz), 160.8, 134.3, 132.9 (d, *J* = 5.0 Hz), 132.0, 127.1, 116.3 (d, *J* = 15.1 Hz), 114.9, 55.6; HRMS ($ASAP^+$) *m/z* $[M]^+$ calcd for $C_{13}H_{11}FOS_3$ 297.9956, found 297.9957.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publication website at DOI:

^{1}H and ^{13}NMR spectra and HPLC traces of all compounds (PDF).

AUTHOR INFORMATION

Corresponding Author

*Fax: +27 21 650 5195. E-mail: Roger.Hunter@uct.ac.za

ORCID

Roger Hunter: 0000-0001-8775-083X

Notes

The authors declare no competing financial interest.

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REFERENCES

(1) (a) Liu, H.; Jiang, X. Transfer of Sulfur: From Simple to Diverse. *Chem. Asian J*. **2013**, *8*, 2546-2563. (b) Wang, M.; Jiang, X. Sulfur-Sulfur Bond Construction. *Top. Curr. Chem*. **2018**, *376:14*, 1-40.

(2) Pluth, M. D.; Bailey, T. S.; Hammers, M. D.; Hartle, M. D.; Henthorn, H. A.; Steiger, A. K. Natural Products Containing Hydrogen Sulfide Releasing Moieties. *Synlett* **2015**, *26*, 2633-2643.

(3) Makarieva, T. N.; Stonik, V. A.; Dmitrenok, A. S.; Grebnev, B. B.; Isakov, V. V.; Rebachyk, N. M. Varacin and Three New Marine Antimicrobial Polysulfides from the Far-Eastern Ascidian *Polycitor sp*. *J. Nat. Prod*. **1995**, 58, 254-58.

(4) (a) Adams, T. C.; Payette, J. N.; Cheah, J. H.; Movassaghi, M. Concise Total Synthesis of (+)-Luteoalbusins A and B. *Org. Lett*. **2015**, *17*, 4268-4271. (b) Kajula, M.; Ward, J. M.; Turpeinen, A.; Tejesvi, M. V.; Hokkanen, J.; Tolonen, A.; Häkkänen, H.; Picart, P.; Ihalainen, J.; Sahl, H.-G.; Pirttilä, A. M.; Mattila, S. Bridged Epipolythiodiketopiperazines from Penicillium raciborskii, an Endophytic Fungus of *Rhododendron tomentosum Harmaja*. *J. Nat. Prod*. **2016**, *79*, 685-690.

(5) Boryczka, S.; Mol, W.; Jowasa, A. Enediyne Anticancer Antibiotics. *Wiadomosci Chemiczne* **2007**, *61*, 445-471.

(6) (a) Oku, N.; Matsunaga, S.; Fusetani, N. Shishijimicins A-C, Novel Enediyne Antitumor Antibiotics from the Ascidian *Didemnum proliferum*. *J. Am. Chem. Soc*. **2003**, *125*, 2044-2045. (b) Nicolaou, K. C.; Li, R.; Lu, Z.; Pitsinos, E. N.; Alemany, L. B.; Aujay, M.; Lee, C.; Sandoval, J.; Gavrilyuk, J. Streamlined Total Synthesis of Shishijimicin A and its Application to the Design, Synthesis, and Biological Evaluation of Analogues thereof and Practical Syntheses of PhthNSSMe and Related Sulfenylating Reagents. *J. Am. Chem. Soc.* **2018**, *140*, 12120-12136.

(7) Lam, K. S.; Veitch, J. A.; Golik, J.; Krishnan, B.; Klohr, S. E.; Volk, K. J.; Forenza, S.; Doyle, T. W. Biosynthesis of Esperamicin A1, an Enediyne Antitumor Antibiotic. *J. Am. Chem. Soc*. **1993**, *115*, 12340-12345.

(8) Nicolaou, K. C. Organic Synthesis: the Art and Science of Replicating the Molecules of Living Nature and Creating Others like them in the Laboratory. *Proc. Math. Phys. Eng. Sci*. **2014**, *470*, 20130690/1-20130690/17.

(9) Nielsen, R. W.; Tachibana, C.; Hansen, N. E.; Winther, J. R. Trisulfides in Proteins. *Antioxid. Redox Sign.* **2011**, *15*, 67-75.

(10) Kubec, R.; Krejčová, P.; Mansur, L.; García, N. Flavor Precursors and Sensory-Active Sulfur Compounds in *Alliaceae* Species Native to South Africa and South America. *J. Agr. Food Chem*. **2013**, *61*, 1335-1342.

(11) (a) Baltes, A-K. Organic Polysulfides. *Dtsch. Apoth. Ztg.* **2013**, *153*, 56-61. (b) Ikram, R.; Low, K. H.; Hashim, N. B.; Ahmed, W.; Nasharuddin. M. N. A. Characterization of Sulfur-Compounds as Chemotaxonomic Markers in the Essential Oils of *Allium* Species by Solvent-Free Microwave Extraction and Gas Chromatography-Mass Spectrometry *Anal. Lett.* **2018**, *ahead of print*.

(12) Wu, M.; Cui, Y.; Bhargav, A.; Losovyj, Y.; Siegel, A.; Agarwal, M.; Ma, Y.; Fu, Y. Organotrisulfide: A High Capacity Cathode Material for Rechargeable Lithium Batteries. *Angew. Chem. Int. Ed*. **2016**, *55*, 10027-10031.

(13) Benavides, G. A.; Squadrito, G. L.; Mills, R. W.; Patel, H. D.; Isbell, T. S.; Patel, R. P.; Darley-Usmar, V. M.; Doeller, J. E.; Kraus, D. W. Hydrogen Sulfide Mediates the Vasoactivity of Garlic. *PNAS* **2007**, *104*, 17977-17982.

(14) (a) Pluth, M. D.; Bailey, T. S.; Hammers, M. D.; Hartle, M. D.; Henthorn, H. A.; Steiger, A. K. Natural Products Containing Hydrogen Sulfide Releasing Moieties. *Synlett* **2015**, *26*, 2633-2643. (b) Zhao, Y.; Henthorn, H. A.; Pluth, M. D. Kinetic Insights into

Hydrogen Sulfide Delivery from Caged-Carbonyl Sulfide Isomeric Donor Platforms. *J. Am. Chem. Soc*. **2017**, *139*, 16365-16376.

(15) (a) Kashfi, K. The Dichotomous Role of H_2S in Cancer Cell Biology. *Biochem. Pharmacol*. **2018**, *149*, 205-223. (b) Oláh, G.; Módis, K.; Töro, G.; Hellmich. M. R.; Szczesny, B.; Szabo, C. Role of Endogenous and Exogenous Nitric Oxide, Carbon Monoxide and Hydrogen Sulfide in HCT116 Colon Cancer Cell Proliferation. *Biochem. Pharmacol*. **2018**, *149*, 186-204.

(16) (a) Zhu, S.-J.; Ying, H.-Z.; Wu, Y.; Qiu, N.; Liu, T.; Yang, B.; Dong, X.-W.; Hu, Y.-Z. Design, Synthesis and Biological Evaluation of Novel Podophyllotoxin Derivatives Bearing 4β-Disulfide/Trisulfide Bond as Cytotoxic Agents. *RSC Adv*. **2015**, *5*, 103172-103183. (b) Kiesel, V. A.; Stan, S. D. Diallyl Trisulfide, a Chemopreventive Agent from Allium Vegetables, Inhibits Alpha-Secretases in Breast Cancer Cells. *Biochem. Biophys. Res. Commun*. **2017**, *484*, 833-838. (c) Wei, Z.; Shan, Y.; Tao, L.; Liu, Y.; Zhu, Z.; Liu, Z.; Wu, Y.; Chen, W.; Wang, A.; Lu, Y. Diallyl Trisulfides, a Natural Histone Deacetylase Inhibitor, Attenuate HIF-1α Synthesis, and Decreases Breast Cancer Metastasis. *Mol. Carcinogenesis*, **2017**, *56*, 2317-2331.

(17) Wu, T.; Huang, Y.; Chen, Y.; Zhang, M. Antibacterial Effect of (2*E*,2*E*)-4,4-Trisulfanediylbis(but-2-enoic acid) against *Staphylococcus aureus*. *PLoS One*, **2018**, 13, e0197348/1-e0197348/9.

(18) For miscellaneous methods, see: (a) Kresze, G.; Patzschke, H. P. The Structure of Organosulfur Compounds. III. Addition of Thiophenols to Thionylanilines; a Route to Alkyl Aryl Trisulfides. *Chem. Ber.* **1960**, *93*, 380-386. (b) Hou, Y.; Abu-Yousef, I. A.; Doung, Y.; Harpp, D. N. Sulfur-Atom Insertion into the S-S Bond - Formation of Symmetric Trisulfides. *Tetrahedron Lett*. **2001**, *42*, 8607-8610. (c) Arisawa, M.; Tanaka, K.; Yamaguchi, M. Rhodium-Catalyzed Sulfur Atom Exchange Reaction between Organic Polysulfides and

Sulfur. *Tetrahedron Lett*. **2005**, *46*, 4797-4800. (d) Lach, S.; Witt, D. TBAF-Promoted Formation of Symmetrical Trisulfides. *Heteroatom Chem.* **2014**, *25*, 10-14.

(19) (a) Milligan, B.; Saville, B.; Swan, J. M. Trisulfides and Tetrasulfides from Bunte Salts. *J. Chem. Soc*. **1963**, 3608-3614. (b) For an interesting case of a Pd-catalysed C-S bond formation involving a Bunte salt to afford a cyclic sulfide, see: Qiao, Z.; Liu, H.; Xiao, X.; Fu, Y.; Wei, J.; Li, Y.; Jiang, X. Efficient Access to 1,4-Benzothiazine: Palladium-Catalysed Double C-S Bond Formation Using $Na_2S_2O_3$ as Sulfurating Reagent. *Org. Lett*. **2013**, *15*, 2594-2597.

(20) Bowman, G. T.; Field, L. Biologically Oriented Organic Sulfur Chemistry. 22. 2-(3-Chloropropyl)-5-chloropentanol as a Prototype for Synthesis of Functionalized Polysulfides and Prodrugs. *J. Org. Chem*. **1982**, *47*, 222-226.

(21) (a) Derbesy, G.; Harpp, D. N. A Simple Method to Prepare Unsymmetrical Di-, Tri-, and Tetrasulfides. *Tetrahedron Lett*. **1994**, *35*, 5381-5384. (b) Zysman-Colman, E.; Harpp, D. N. Optimization of the Synthesis of Symmetric Aromatic Tri- and Tetrasulfides. *J. Org. Chem*. **2003**, *68*, 2487-2489.

(22) (a) Feher, F.; Degen, B. Preparation of Diimidazolylsulfanes and Di(aziridinyl)-sulfanes. *Angew. Chem. Int. Ed. Engl.* **1967**, *6*, 703-704. (b) Banerji, A.; Kalena, G. P. A New Synthesis of Organic Trisulfides. *Tetrahedron Lett*. **1980**, *21*, 3003-3004.

(23) Harpp, D. N.; Steliou, K.; Chan, T. H. Organic sulfur chemistry. 26. Synthesis and Reactions of Some New Sulfur Transfer Reagents. *J. Am. Chem. Soc*. **1978**, *100*, 1222-1228.

(24) Capozzi, G.; Capperucci, A.; Degl'Innocenti, A.; Del Duce, R.; Menichetti, S. Silicon in Organosulfur Chemistry. Part 1. Synthesis of Trisulfides. *Tetrahedron Lett*. **1989**, *30*, 2991-2994.

(25) (a) Harpp, D. N.; Granata, A. Organic Sulfur Chemistry, XXI. Trisulfide Formation by Alkoxide Decomposition of Sulfenylthiocarbonates. *Tetrahedron Lett*. **1976**, *17*, 3001-3004.

(b) Bolton, S. G.; Cerda, M. M.; Gilbert, A. K.; Pluth, M. D. Effects of Sulfane Sulfur Content in Benzyl Polysulfides on Thiol-Triggered H_2S Release and Cell Proliferation. *Free Rad. Biol. Med*. **2019**, *131*, 393-398.

(26) (a) Lach, S.; Witt, D. Efficient Synthesis of Functionalized Unsymmetrical Dialkyl Trisulfanes. *Synlett* **2013**, 24, 1927-1930. (b) See also a recent acid-catalysed deprotection of a disulfanyl acetate for unsymmetrical disulfide formation: Dai, Z.; Xiao, X.; Jiang, X. Nucleophilic Disulfurating Reagents for Unsymmetrical Disulfides Construction via Copper-Catalyzed Oxidative Cross Coupling. *Tetrahedron* **2017**, *73*, 3702-3706.

(27) (a) Park, C. M.; Johnson, B. A.; Duan, J. C.; Park, J. J.; Day, J. J.; Gang, D.; Qian, W. J.; Xian, M. 9-Fluorenylmethyl (Fm) Disulfides: Biomimetic Precursors for Persulfides. *Org. Lett*. **2016**, *18*, 904-907. (b) Xu, S.; Wang, Y.; Radford, M. N.; Ferrell, A. J.; Xian, M. Synthesis of Unsymmetric Trisulfides from 9-Fluorenylmethyl Disulfides. *Org. Lett*. **2018**, *20*, 465-468.

(28) Mott, A.W.; Barany, G. A New Method for the Synthesis of Unsymmetrical Trisulfanes. *Synthesis*, **1984**, 657-660.

(29) Harpp, D. N.; Ash, D. K. Organic sulfur chemistry. XIII. Synthesis of Unsymmetric Trisulfides. *Int. J. Sulfur Chem*. Part A, **1971**, *1*, 211-214.

(30) (a) Feher, F.; Kruse, W. The Chemistry of Sulfur. LIII. Unsymmetrical Sulfane Derivatives of the Types RS_nCl and RS_nH *Chem. Ber*. **1958**, *91*, 2528-2531. (b) Bohme, H.; van Ham, G. Synthesis and Reactions of Alkyldisulfuryl Chlorides. *Lieigs Ann. Chem*. **1958**, *617*, 62-70.

(31) Wang, W.; Lin, Y.; Ma, Y.; Tung, C.-H.; Xu, Z. Cu-Catalyzed Electrophilic Disulfur Transfer: Synthesis of Unsymmetrical Disulfides. *Org. Lett*. **2018**, *20*, 3829-3832.

(32) By Xiao, X.; Xue, J.; Jiang, X. Polysulfurating Reagent Design for Unsymmetrical Polysulfide Construction *Nat. Commun*. **2018**, 9, 1-9.

(33) (a) Harpp, D. N.; Ash, D. K.; Smith, R. A. Organic Sulfur chemistry. 33. Chemistry of Sulfenic Sulfonic Thioanhydrides. Solvent-dependent Sulfur Extrusion. *J. Org. Chem*. **1979**, *44*, 4135-4140. (b) Williams, C. R.; Macdonald, J. G.; Harpp, D. N.; Steudel, R.; Foerster, S. A Likely Mechanism of Solvent-induced Sulfur Loss from Sulfenylthiosulfonates. *Sulf. Lett.* 1**992**, *13*, 247-55.

(34) (a) Takano, S.; Hiroya, K.; Ogasawara, K. Facile and Efficient Synthesis of Alkyl Thiotosylates using Anion Exchange Resin. *Chem. Lett*. **1983**, 255-6. (b) Scholz, D. New Synthetic Methods. 9. S-Alkyl 4-methylbenzenethiosulfonates, Excellent Reagents for α-Thiolation of Cyclic Ketones. *Liebigs Ann. Chem.* **1984**, 259-63.

(35) (a) Fujiki, K; Tanifuji, N.; Sasaki, Y.; Yokoyama, T. New and Facile Synthesis of Thiosulfonates from Sulfinate/Disulfide/I_2 System. *Synthesis* **2002**, 343-348. (b) Taniguchi, N. Oxidative Coupling of Dichalcogenides with Sodium Sulfinates via Copper-Catalyzed Cleavage of S-S and Se-Se Bonds. *J. Org. Chem*. **2015**, *80*, 1764-1770.

(36) (a) Bailey, T. S.; Zakharov, L. N.; Pluth, M. D. Understanding Hydrogen Sulfide Storage: Probing Conditions for Sulfide Release from Hydrodisulfides. *J. Am. Chem. Soc*. **2014**, *136*, 10573-10576. (b) Iciek, M.; Kowalczyk-Pachel, D.; Bilska-Wilkosz, A.; Kwiecień, I.; Górny, M.; Wlodek, L. *S*-Sulfhydration as a Cellular Redox Regulation. *Biosci. Rep*. **2016**, *36*, e00304. 1-17. (c) Filipovic, M. R.; Zivanovic, J.; Alvarez, B.; Banerjee, R. Chemical Biology of H_2S Signaling through Persulfidation. *Chem. Rev*. **2018**, 118, 1253-1337.

(37) For original work on aromatic hydrodisulfides from acid hydrolysis of ArSSAc, see: Kawamura, S.; Horii, T.; Tsurugi, J. Aryl Hydrodisulfides. *J Org. Chem*. **1971**, *36*, 3677-3680.

(38) Xiao, X.; Feng, M.; Jiang, X. New Design of a Disulfurating Reagent: Facile and Straightforward Pathway to Unsymmetrical Disulfanes by Copper-Catalyzed Oxidative Cross-Coupling. *Angew. Chem. Int. Ed*. **2016**, *55*, 14121-14125.

(39) Silva, F.; Khokhar, S. S.; Williams, D. M.; Saunders, R.; Evans, G. J. S.; Graz, M.; Wirth. T. Short Total Synthesis of Ajoene. *Angew. Chemie. Int. Ed*. **2018**, *57*, 12290-12293.

(40) Su, Z.; Yang, S.; Long, Z. Preparation of Novel Cyclosporin Derivatives for the Treatment and Prevention of especially a Viral Infection. PCT Int. Appl. **2017**, WO 2017200984 A1 Nov 23, 2017.

(41) Carrigan, C. N.; Imperiali, B. The Engineering of Membrane-Permeable Peptides. *Anal. Biochem*. **2005**, *341*, 290-298.

(42) Kaschula, C. H.; Hunter, R.; Stellenboom, N.; Caira, M. C.; Winks, S.; Ogunleye, T.; Richards, P.; Cotton, J.; Zilbeyaz, K.; Wang, Y.; Siyo, V.; Ngarande, E.; Parker, M. I. Structure-Aactivity Studies on the Anti-Proliferation Activity of Ajoene Analogues in WHCO1 Oesophageal Cancer Cells. *Eur. J. Med. Chem*. **2012**, *50*, 236-254.

(43) Bulman Page, P. C.; Goodyear, R. L.; Horton, A. E.; Chan, Y.; Karim, R.; O'Connell, M. A.; Hamilton, C.; Slawin, A. M. Z.; Buckley, B. R.; Allin, S. M. Formal Total Synthesis of (+)-C9-Deoxyomuralide from L-Leucine using a Double Sacrificial Chirality Transfer Approach. *J. Org. Chem*. **2017**, *82*, 12209-12223.

(44) Zhang, G.-Y.; Lv, S.-S.; Shoberu, A.; Zou, J.-P. Copper-Catalyzed TBHP-Mediated Radical Cross-Coupling Reaction of Sulfonylhydrazides with Thiols Leading to Thiosulfonates. *J. Org. Chem*. **2017**, *82*, 9801-9807.

(45) Liang, L.; Vincent, S. P. Synthesis of D-Glycero-D-manno-heptose 1,7-bisphosphate (HBP) Featuring a β-Sstereoselective bis-Phosphorylation. *Tetrahedron Lett.* **2017**, *58*, 3631-3633.

(46) Field, L.; Hanley, W. S.; McVeigh, I. Organic Disulfides and Related Substances. 32. Preparation and Decomposition of β-Ssubstituted Ethyl Acetyl Disulfides. *J. Org. Chem*. **1971**, *36*, 2735-2739.