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Synthesis and antibacterial activity of 6″-decanesulfonylacetamide-functionalised amphiphilic derivatives of amikacin and kanamycin

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ABSTRACT

Aminoglycoside antibiotics represent the first class of successful drugs in the treatment of tuberculosis; however, mycobacteria and other bacterial species possess several drug resistance mechanisms to inactivate these natural products. In the past 15 years, a variety of amphiphilic aminoglycosides have been shown to have improved activity against infectious microorganisms and to subvert resistance mechanisms. Here, we report on four novel synthetic compounds derived from two existing potent antitubercular compounds and describe their activity against both *Mycobacterium tuberculosis* and *Staphylococcus aureus*. It was found that a decanesulfonylacetamide-based conjugate of amikacin displayed promising preliminary antitubercular activities, warranting further investigation to assess the therapeutic potential of these unique antimicrobials.

Keywords: amide–triazole conjugates, amikacin, amphiphilic aminoglycosides, antibiotics, antimicrobial agents, kanamycin, *Mycobacterium tuberculosis*, *n*-decanesulfonylacetamide.

Introduction

Structural modification of the aminoglycoside (AG) scaffold has historically been a successful approach towards creating novel antibiotics. This approach led to the discovery of amikacin (AMI), a potent AG that is used for the treatment of tuberculosis (TB) today.[1] Recently, a new class of antibiotics derived from the polycationic AG scaffold has produced several promising lead compounds that have revived activities against drug-resistant bacterial species of clinical importance. This new class of antimicrobial agent is derived from conjugation of hydrophobic groups such as amphiphilic alkyl chains, amino acids, peptides and aromatics to AG scaffolds, creating semi-synthetic amphiphilic AGs (AAGs). AAGs have shown promising preliminary results against several drug-resistant bacterial strains by evading common mechanisms of resistance and exerting novel mechanisms of action.[2–5]

Polycationic anti-bacterials (PAs) are a structurally diverse class of antibacterial agents having broad-spectrum activities and multiple modes of action. Amphiphilic PAs such as cationic antimicrobial peptides act on bacterial membranes, whereas non-amphiphilic PAs such as AGs act by binding to *16S* rRNA (ribosomal RNA).[6] However, mode of action studies reveal AAGs predominately target bacterial membranes, thereby leading to depolarisation and increased permeability.[7–10] Amphiphilic cationic bacterial membrane disrupters interact with negatively charged components of the bacterial cell membrane such as phosphatidylglycerol, lipopolysaccharides and teichoic acids orders of magnitude more tightly than the bivalent cations that hold these negatively charged components together.[11,12] Through displacement of bivalent cations, disruption of the outer membrane occurs, leading to uncontrolled cell permeability and cell death. Bacterial anionic lipids are attractive targets in the design of antibacterial agents as they can exert broad-spectrum effects while displaying potent activities towards resistant bacterial strains. Furthermore, owing to their mode of action, which results in the

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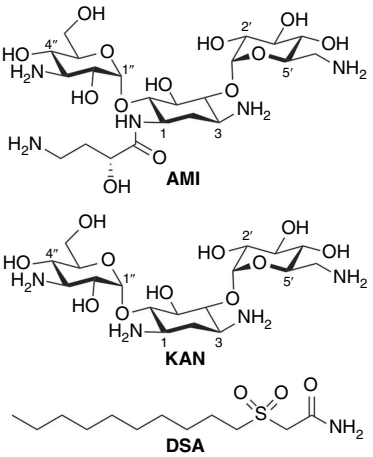


Fig. 1. Chemical structure of amikacin (AMI), kanamycin (KAN) and *n*-decanesulfonylacetamide (DSA).

depolarisation of bacterial membranes through the formation of pores, *in vitro* resistance to cationic amphiphiles is rarely observed.[13] AAGs have also demonstrated the potential to boost innate immune responses, inducing immunomodulatory responses, offering new perspectives for the treatment of persistent and dormant infections.[14]

In this work, we have chemically conjugated an amphiphilic sulfonylacetamide-based moiety to the scaffolds of AMI and kanamycin (KAN) (Fig. 1). Long alkyl chained sulfonylacetamides have been previously reported to display potent antitubercular activities.[15,16] The most active of these, *n*-decanesulfonylacetamide (DSA), has a reported minimum inhibitory concentration (MIC) of 0.75–1.5 μg mL^{-1} towards *Mycobacterium tuberculosis*, which is comparable with first-line agents used to treat TB.[15] These simple alkyl sulfonylacetamides have been shown to significantly decrease mycolic acid levels in mycobacteria, providing promising drug leads due to the importance of lipid biosynthesis to the virulence and intracellular survival of *M. tuberculosis*.[16,17] However, simple alkyl sulfonylacetamides displayed poor pharmacokinetics within an *in vivo* model, resulting in suboptimal bioavailability. In addition, we have previously shown conversion of the amide in DSA into a thioamide afforded reduced antitubercular activity and improved activity against *Staphylococcus aureus*.[18]

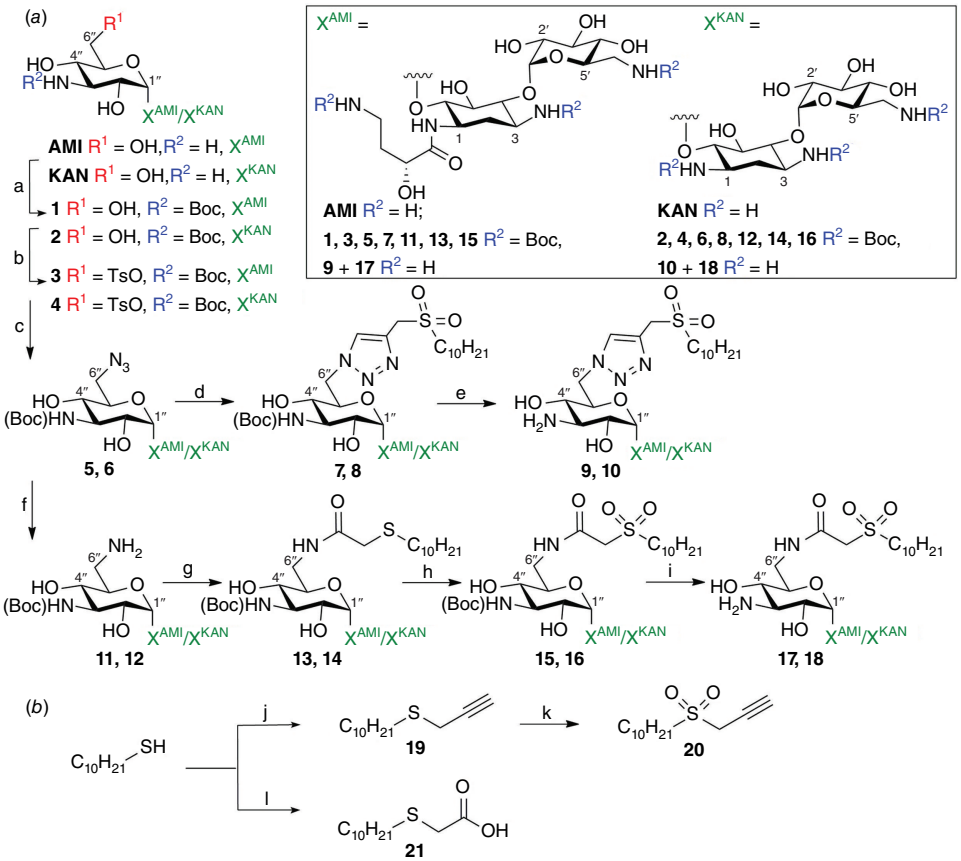
Within this new work, a series of AAGs were synthesised from the scaffolds of AMI and KAN utilising the active functionality of the sulfonylacetamide DSA. We were interested in investigating the conversion of AGs into antibiotic hybrids possessing sulfonylacetamide functionality to then explore potential structural candidates for further development of antimicrobial agents. Amphiphilic derivatisation of the AMI and KAN scaffold at 6″ was achieved through straightforward single-site modifications as outlined in Scheme 1. Triazole AAG conjugates were prepared by copper-catalysed click chemistry, while amide linkers were synthesised utilising carbodiimide coupling protocols. The resulting conjugates were evaluated for their antimicrobial activities towards *M. tuberculosis* and *S. aureus*.

Results and discussion

The synthesis of amphiphilic AMI and KAN-based sulfonylacetamide conjugates is described in Scheme 1. To determine the importance and durability of the amide linkage, the decanesulfonyl group was also added by a triazole linker. Derivatisation of the 6″ position of the aminoglycoside scaffolds was achieved following a straightforward synthetic approach involving subsequent functionalisation of the 6″ position, as outlined in Scheme 1. The 6″-azido-functionalised AG derivatives were prepared following synthetic strategies previously described for neomycin.[19] Briefly, the amines of the AG scaffolds had Boc protecting groups installed to allow selective tripsylation of the 6″ primary alcohol. Subsequent displacement of the tripsyl leaving group allowed installation of an azide at the 6″ position, affording azide–alkyne cycloaddition with a previously prepared lipid alkyne **20**. Finally, Boc functionality was removed to afford the final targets **9** and **10** as TFA salts. Synthesis of the amide conjugates was achieved by reduction of the 6″ azide to an amine by hydrogenation, followed by 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC) coupling with a previously prepared carboxylic acid derivative of DSA (**21**). Following conjugation, the sulfide was oxidised to a sulfone and Boc functionality was removed to afford **17** and **18** as TFA salts.

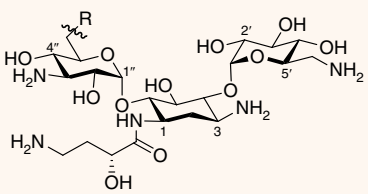
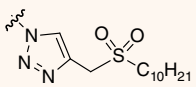
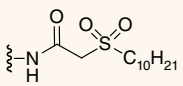
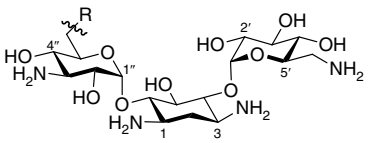
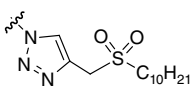
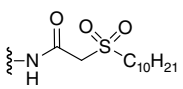
The synthesised AAGs were evaluated for their antitubercular and antistaphylococcal activities (Table 1). In general, conjugation of DSA lipid moieties to the AG scaffold reduced antibacterial activity when compared with the parent scaffold. The most active compound was the amide-conjugated AMI derivative **17**, which displayed an MIC_{90} of 22 μg mL^{-1} towards *M. tuberculosis*. Although less efficient when compared with the parent scaffold, **17** displayed a modest level of inhibition, suggesting DSA derivatives of AGs with increased lipophilicity can attain antitubercular activities (Fig. 2).

Although the amide derivative **17** demonstrated antitubercular activities, the corresponding triazole conjugate **9** displayed low inhibitory efficiency, suggesting the amide linker utilised in **17** influences antitubercular activity. Interestingly, membrane-targeting AAG derivatives reported by Herzog *et al.*[20] demonstrated lipid conjugates utilising both amide and triazole linkers exhibited similar antimicrobial activities across several bacterial species. Although it has been reported that the length and nature of the amphiphilic moiety can greatly influence antibacterial inhibition,[21] the polycationic scaffold utilised can also affect antimicrobial activities.[2] The latter falls in line with data presented here: the AMI conjugate **17** displays antitubercular activities whereas the corresponding KAN derivative **18** has an abrupt loss of killing efficiency while maintaining the



Scheme 1. (*a*) Synthesis of 6″ AMI and KAN sulfonylacetamide-based amide and triazole conjugates **9**, **10**, **17** and **18**; (*b*) synthesis of structural building blocks **20** and **21**. Reagents and conditions: (a) Boc_2O, triethylamine (TEA), H_2O, MeOH, 50°C, 24 h; (b) 2,4,6-TPSCl, pyridine, room temperature (rt), 18 h; (c) NaN_3, DMF, 70°C, 18 h; (d) **20**, sodium ascorbate, $CuSO_4$, H_2O, *i*-PrOH, 40°C, 2–4 h; (e) TFA, DCM, rt, 1–2 h; (f) $H_{2(g)}$, $Pd(OH)_2/C$, MeOH, rt, 18 h; (g) **21**, 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC), *N*-hydroxysuccinimide (NHS), THF, H_2O, rt to 50°C, 15–24 h; (h) $KHSO_5$, MeOH, H_2O, 18 h; (i) TFA, DCM, rt, 1–2 h; (j) propargyl bromide, K_2CO_3, acetone, rt, 24 h; (k) $KHSO_5$, MeOH, H_2O, rt, 18 h; (l) bromoacetic acid, K_2CO_3, MeCN, 50°C, 24 h. (Boc, *t*-butyloxycarbonyl)

Table 1. Inhibitory effects of the synthesised library of AAGs towards *M. tuberculosis* (H37Rv) reported as an MIC_{90} ($n = 2$) and *S. aureus* (American Type Culture Collection ATCC 25923) reported as MIC ($n = 3$).

Compound	R	H37Rv MIC_{90} (μg mL^{-1})	ATCC25923 MIC (μg mL^{-1})
AMI	–	0.6	–
KAN	–	1.3	–
	**9** 	>50	100–200
	17 	22	200
	**10** 	>50	100–200
	18 	>50	>200

same amphiphilic functionality. This could imply that **17** is exerting an inhibitory effect by hitting a specific molecular target(s), rather than a broad interaction with the bacterial membrane, particularly considering the importance of lipid metabolism in *M. tuberculosis*.

As AAGs can act through binding of bacterial membranes as opposed to targeting rRNA, lipophilicity has a significant influence on the membrane permeabilisation and killing efficiency of the therapeutic.[10,21–24] Lipophilicity may be an influential determinant towards the activity of the AMI

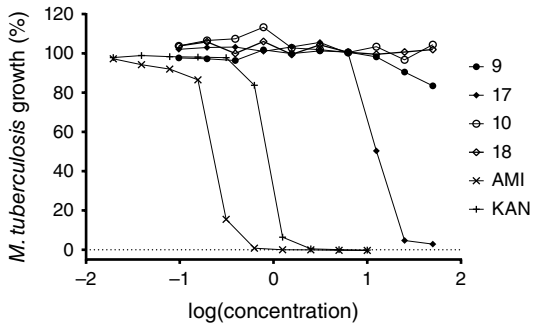


Fig. 2. Percentage growth of *M. tuberculosis* following exposure to AMI alkylsulfonyl conjugates **9** and **17**, and KAN alkylsulfonyl conjugates **10** and **18**, tested at a serial dilution of 50–0.098 μg mL^{-1} (log of concentration in micrograms per millilitre). AMI and KAN controls serial diluted at a concentration of 10–0.020 μg mL^{-1}.

amide conjugate **17** compared with KAN corresponding derivative **18**. The AMI derivative **17** is the most active within this study, whereas the corresponding KAN derivative **18** displayed no antitubercular activity (MIC_{90} > 50 μg mL^{-1}). AMI is a semi-synthetic derivative of KAN, structurally differing by an (*S*)-4-amino-2-hydroxybutanoyl (AHB) substituent at C1. Although AMI is more active towards *M. tuberculosis* compared with KAN, the difference in activities between **17** and **18** is unexpected given their structural similarities in terms of their lipophilic moieties, AG scaffold and net positive charge.

Although amphiphilic AMI and KAN-based lipid conjugates have reported antistaphylococcal activities, the small series reported here displays low inhibitory efficiency. AMI and KAN-derived polycarbamates and polyethers reported by Bera *et al.*[2] were assessed against a spectrum of bacterial species, including for antistaphylococcal activities and were found to have a broad spectrum effect. When compared with the antistaphylococcal activities of **9**, **10**, **17** and **18** reported here, the influence of amphiphilicity is apparent, as most poly-modified conjugates provided enhanced antibacterial activities towards *S. aureus*. Results reported by Zhang *et al.*[25] also found amphiphilicity to be influential as di-conjugates of KAN were demonstrated to be more active compared with mono-conjugates, which often displayed no activity towards *S. aureus*. A KAN 4″,6″-di-*O*-nonyl derivative was the most active towards *S. aureus* (MIC 8 μg mL^{-1}) whereas single-site modification of *O*-nonyl at either 4″ or 6″ resulted in inactive compounds (≥ 250 μg mL^{-1}). Polyconjugates have also demonstrated inhibitory efficiency towards *S. aureus* and other bacterial pathogens when constructed from the scaffolds of other AGs, such as tobramycin and neomycin.[2,26] Mechanistic studies suggest the improved activities of di-conjugates in comparison with mono-conjugates is due to the complementary molecular shape allowing insertion within bacterial membranes.[8]

Few AMI-based AAGs have been reported and to our knowledge, none have been assessed for their inhibitory effect towards *M. tuberculosis*. Most of the functionalised AGs assessed and reported for antimycobacterial efficacy are conjugates of the tobramycin scaffold, along with KAN, netilmicin and sisomicin.[27–29] The activities of a series of 6″-thioether and sulfone-linked alkyl chained conjugates of KAN towards *M. smegmatis* has been previously described.[27] The optimal chain length found for mycobacterial inhibition was C_{11}–C_{13}, in line with the lipid length of conjugates reported here. Interestingly, oxidation of the thioether linkage to a sulfone decreased inhibitory effect towards *S. aureus*, while improving mycobacterial activity.[27] This suggests that sulfonylacetamide-based conjugates may provide superior Gram-negative activities with unoxidised lipid moieties as opposed to the sulfone utilised in the inhibitor's design. Additionally, the structural position of AHB appendages on the scaffolds of AMI and KAN have been previously shown to influence mycobacterial activities against *M. smegmatis*.[29] In line with results presented here, the most active AMI derivative reported in the study towards mycobacterial species displayed no effectiveness towards Gram-positive species, including *S. aureus*.[29]

It has been found that amphiphilic derivatisation is capable of reducing the effect of AG modifying enzymes (AMEs).[29] Enzymatic modification of AGs is the most prevalent mechanism of bacterial resistance. Owing to structural changes resulting from amphiphilic conjugation of the AG scaffold, AAGs have the ability to reduce affinity and evade the action of AMEs.[27,29,30] A putative benefit of the DSA-conjugated AGs may be a means to circumvent structural changes from AMEs resulting in restored activity towards resistant bacterial strains. Alkyl chain 6″ conjugates of tobramycin have demonstrated antibacterial activities across an array of organisms including resistant strains through membrane interruption.[30] Most notable were activities against bacterial strains expressing Eis, an acetyltransferase that causes high levels of resistance to AGs in extensively drug-resistant TB.[31–33] Within the reported library, sulfur-linked alkyl conjugates were found to be poor substrates for several AMEs, including Eis, compared with tobramycin. It has been established that AAGs circumvent resistant mechanisms and exert antimicrobial inhibition through cell wall disruption; therefore, exploring activities towards resistant strains in addition to mechanistic studies would provide further insight into how valuable these derivatives are as lead compounds for further drug development.

Conclusion

In this work, a series of AAGs were synthesised utilising various synthetic strategies to chemically conjugate lipophilic moieties with previously reported antitubercular activities to the 6″ position of AMI and KAN by amide or triazole functionality. The antibacterial activities of the small series of amphiphilic AMI and KAN-based conjugates

is described. Notably, the amide-linked sulfonylacetamide conjugate of AMI (**17**) was identified to have promising preliminary activity against *M. tuberculosis* (MIC_{90} 22 μg mL^{-1}). The activities of the AAGs synthesised here was influenced by the AG scaffold, lipid moiety and structural composition of the linker utilised. Although **17** displayed antitubercular efficiency, the target of action remains unknown and it could be exerting its effect by a membranolytic interaction or exhibiting a prodrug-dependent mode of action following the release of free AMI and a lipid moiety post-cleavage of the amide linker. Mechanistic studies would assess the therapeutic potential of these unique antimicrobials while providing further insight into how valuable these derivatives are as lead compounds for further, more refined, drug design and development.

Experimental procedures

Determination of minimum inhibitory concentration against *Mycobacterium tuberculosis* and *Staphylococcus aureus*

M. tuberculosis (H37Rv) was grown in Middlebrook 7H9S media, with 10% albumin dextrose catalase (ADC) supplementation, 1% tryptone, 0.05% glycerol and 0.05% Tween 80. Freshly seeded cultures were grown at 37°C for ~14 days to mid-exponential phase (optical density measured at a wavelength of 600 nm, OD_{600} = 0.4–0.8) for the inhibitory assay. The microbial culture was diluted to OD_{600} = 0.001 in 7H9S media and 100 μL (2×10^4 colony forming units mL^{-1}) was added to each well, as well as the serial-diluted compound. Each 96-well plate prepared for the assay included rifampicin at a range of 0.4–200 nM as a positive control. The plates were incubated for 5 days at 37°C in a humidified incubator prior to the addition of resazurin (30 μL of a 0.02% solution) and 12.5 μL of Tween-80 to each well. Following 24-h incubation, the fluorescence was measured on a Fluorostar Omega fluorescent plate reader with an excitation wavelength of 530 nm and emission read at 590 nm.

S. aureus (ATCC 25923) was grown in lysogeny broth (LB) at 37°C with 160 rpm shaking. The MIC was determined using *S. aureus* cultures grown overnight in LB media and subcultured 1:100 the following morning. When cells were in mid-log phase, 10^5 cells were added to a microtitre plate for MIC determination containing the appropriate therapeutic that was serial-diluted. Bacterial cells were grown overnight for 18 h at 37°C in aerobic conditions and cell viability was determined by measuring absorbance at 600 nm on a Tecan Infinite 200 Pro plate reader.

General synthetic procedures

Chemicals for which synthesis is not reported were obtained from commercial suppliers. All solvents used were either reagent grade, distilled or dried and distilled prior to use. ^1H and distortionless enhancement by polarisation transfer ^{13}C NMR spectra were obtained using a Bruker Avance III HD Ascend 400 MHz spectrometer operating at 400 and 100 MHz respectively, or a Bruker Avance III HD 600 MHz operating at 600and 150 MHz. Signals are recorded to two decimal places in terms of chemical shifts (δ, ppm) relative to MeOD and D_2O. The abbreviations for multiplicities used are: s, singlet; d, doublet; t, triplet and m, multiplet. Low-resolution mass spectrometry (LRMS) spectra were recorded with a Bruker Esquire 3000, and high-resolution (HR) MS with a Bruker maXis II ETD. Flash chromatography was conducted using flash chromatography grade silica, 200–400 mesh, purchased from Sigma–Aldrich with distilled solvents. Thin layer chromatography (TLC) was used to monitor reactions using Merck TLC plates. TLC plates were visualised under UV light and developed with appropriate TLC stains such as H_2SO_4 and charring with heat.

Synthesis of 1-(4-*N*-*tert*-butoxycarbonyl-2-hydroxybutyryl)-3,6′,3″ tri-*N*-(*tert*-butoxycarbonyl)-kanamycin [amikacin] (1)

To a stirred solution of amikacin sulfate (1.50 g, 2.60 mmol) dissolved in 1:1 H_2O:MeOH (50 mL) was added TEA (15 equiv.) followed by di-*tert*-butyldicarbonate (10 equiv.). The resulting reaction mixture was stirred at 50°C for 24 h. On completion, the MeOH was evaporated, and the residue was partitioned between EtOAc (100 mL) and H_2O (50 mL), extracted with EtOAc (3 × 50 mL), dried over Na_2SO_4 and concentrated under vacuum. Purification by flash chromatography with 9:1 DCM:MeOH afforded **1** as white solid in a yield of 63% (1.62 g, 1.60 mmol). LRMS (electrospray ionisation, ESI): *m/z* calculated for $[M + Na]^+$ $C_{42}H_{75}N_5NaO_{21}$, 1009.1; found 1009.1.

Synthesis of 1,3,6′,3″-tetra-*N*-(*tert*-butoxycarbonyl)-kanamycin (2)

A solution of kanamycin sulfate (2.00 g, 3.43 mmol) was reacted following the synthetic conditions outlined for the synthesis of compound **1**. Compound **2** was afforded as a white solid in a yield of 72% (2.13 g, 2.40 mmol). LRMS (ESI): *m/z* calculated for $[M + Na]^+$ $C_{38}H_{68}N_4NaO_{19}$, 907.4; found 907.8.

Synthesis of 6″-(4-methylbenzene-1-sulfonyl)-tetra-*N*-(*tert*-butoxycarbonyl)-amikacin (3)

Compound **1** (650 mg, 0.66 mmol) was dissolved in anhydrous pyridine (30 mL) and reacted with 2,4,6-TPSCl (28 equiv.) under an atmosphere of argon for 18 h. Following completion, pyridine was removed under vacuum by co-evaporation with toluene and the remaining residue was partitioned between H_2O (100 mL) and EtOAc (100 mL), then extracted with EtOAc (3 × 50 mL). The organic layers were combined, dried over Na_2SO_4 and concentrated under reduced pressure. Product **3** was purified on

flash silica in a gradient of isocratic DCM → 98:2 DCM:MeOH affording **3** as a white solid in a yield of 78% (640 mg, 0.51 mmol). LRMS (ESI): *m/z* calculated for $[M + Na]^+$ $C_{57}H_{97}N_5NaO_{23}$, 1274.6; found 1275.2.

Synthesis of 6″-(4-methylbenzene-1-sulfonyl)-1,3,6′,3″-tetra-*N*-(*tert*-butoxycarbonyl)-kanamycin (4)

A solution of **2** (2.13 g, 2.40 mmol) was reacted following the same methodology outlined for the synthesis of **3**. Purification of **4** was achieved by a gradient elution with isocratic DCM → 98:2 → 95:5 DCM:MeOH on flash silica, affording **4** as a white solid with a yield of 48% (1.31 g, 1.14 mmol). LRMS (ESI): *m/z* calculated for $[M + Na]^+$ $C_{53}H_{90}N_4NaO_{21}S$, 1173.6; found 1174.2.

Synthesis of 6″-azido-tetra-*N*-(*tert*-butoxycarbonyl)-amikacin (5)

A solution of **3** (640 mg, 0.51 mmol) was dissolved in anhydrous DMF (10 mL), to which NaN_3 (4 equiv.) was added; the resulting mixture was heated to 70°C and reacted for 18 h under an atmosphere of argon. On completion, the sample was partitioned between EtOAc (100 mL) and $NaHCO_3$ (50 mL) and extracted into EtOAc (3 × 50 mL). The organic fractions were combined, dried over Na_2SO_4 and concentrated under vacuum. The resulting residue was further purified by flash chromatography; purification of **5** in 12:1 DCM:MeOH afforded the target as an amorphous white solid with a yield of 29% (148 mg, 0.15 mmol). LRMS (ESI): *m/z* calculated for $[M + Na]^+$ $C_{42}H_{74}N_8NaO_{20}$, 1033.5; found 1033.5.

Synthesis of 6″-azido-1,3,6′,3″-tetra-*N*-(*tert*-butoxycarbonyl)-kanamycin (6)

Compound **4** (1.31 g, 1.14 mmol) was reacted following the synthetic conditions reported for the synthesis of **5**. Compound **6** was purified by flash chromatography with a gradient solvent system of DCM:MeOH 95:5 → 12:1, affording a white solid with a yield of 81% (840 mg, 0.92 mmol). LRMS (ESI): *m/z* calculated for $[M + Na]^+$ $C_{38}H_{67}N_7NaO_{18}$, 932.4; found 932.9.

Synthesis of 6″-(4-((decylsulfonyl)methyl)-1,2,3-triazole)-tetra-*N*-(*tert*-butoxycarbonyl)-amikacin (7)

A solution of **5** (100 mg, 0.10 mmol) was stirred with the lipid acetylene **20** (1.2 equiv.) dissolved in a 1:1 solution of H_2O and *i*-PrOH (5 mL). To the resulting reaction mixture were added sodium ascorbate (0.2 equiv.) and $CuSO_4$ (0.1 equiv.). The resulting solution was heated to 40°C and stirred for 2–4 h. Following completion, as indicated by TLC, the crude reaction mixture was concentrated under vacuum and the crude residue was eluted with 10:1 DCM:MeOH, affording **7** as a white solid at a yield of 40% (50 mg, 0.04 mmol). LRMS (ESI): *m/z* calculated for $[M + Na]^+$ $C_{55}H_{98}N_8NaO_{22}S$, 1277.6; found 1277.9.

6″-(4-((Decylsulfonyl)methyl)-1,2,3-triazole)-1,3,6′,3″-tetra-*N*-(*tert*-butoxycarbonyl)-kanamycin (8)

Synthesis of **8** was achieved by reacting **6** (100 mg, 0.11 mmol) with the reaction conditions reported for the synthesis of **7**. Compound **8** was obtained as a white solid with a yield of 60% (76 mg, 0.07 mmol). LRMS (ESI): *m/z* calculated for $[M + Na]^+$ $C_{51}H_{91}N_7NaO_{20}S$, 1176.6; found 1176.9.

Synthesis of 6″-(4-((decylsulfonyl)methyl)-1,2,3-triazole)-amikacin (9)

The conjugated intermediate **7** (50 mg, 0.04 mmol) was dissolved in DCM (1 mL) and stirred at 0°C, to which TFA (0.5 mL per 50 mg (w/v%)) was added dropwise. The reaction was carried out for 1–2 h at ambient temperature, concentrated under reduced pressure and azeotroped with toluene to afford the final deprotected conjugate **9** as a white solid at a yield of 97% (45 mg, 0.04 mmol). ^{1}H NMR (400 MHz, D_2O) δ 8.24 (s, 1H), 5.46 (s, 1H), 5.14 (s, 1H), 4.64–4.54 (m, 2H), 4.33–4.22 (m, 2H), 4.18 (dd, J = 10.4, 2.6 Hz, 1H), 4.14–3.98 (m, 2H), 3.90–3.57 (m, 6H), 3.56–3.37 (m, 3H), 3.37–3.21 (m, 2H), 3.23–3.09 (m, 2H), 2.32–2.06 (m, 3H), 2.07–1.66 (m, 2H), 1.58–1.38 (m, 2H), 1.42–1.07 (m, 12H), 0.86 (t, J = 6.5 Hz, 3H). ^{13}C NMR (100 MHz, D_2O) δ 175.4, 163.1, 162.8, 97.3, 96.8, 86.9, 79.8, 78.5, 72.6, 72.2, 70.8, 70.7, 69.6, 69.5, 68.7, 67.8, 66.9, 55.1, 52.1, 50.6, 48.9, 48.8, 48.0, 40.3, 36.9, 31.2, 30.8, 30.2, 28.6, 28.4, 28.4, 28.1, 27.4, 22.0, 21.2, 13.4. HRMS **9** (ESI): *m/z* calculated for $[M + 2H]^{2+}$ $C_{35}H_{68}N_8O_{14}S$, 428.2288; found 428.2284.

Synthesis of 6″-(4-((decylsulfonyl)methyl)-1,2,3-triazole)-kanamycin (10)

Synthesis of **10** was achieved by reacting **8** (76 mg, 0.07 mmol) with the synthetic methodology reported for compound **9**. Compound **10** was isolated as a white solid with a yield of 99% (73 mg, 0.07 mmol). ^{1}H NMR (600 MHz, MeOD) δ 8.13 (s, 1H), 5.38 (d, J = 3.9 Hz, 1H), 5.09 (d, 1H), 4.62–4.55 (m, 3H), 4.30–4.25 (m, 1H), 4.10–4.04 (m, 1H), 3.85–3.74 (m, 2H), 3.73–3.67 (m, 2H), 3.65–3.54 (m, 3H), 3.49–3.39 (m, 4H), 3.23 (t, J = 9.4 Hz, 1H), 3.18 (t, 2H), 3.06 (dd, J = 13.0, 8.9 Hz, 1H), 2.48 (s, 1H), 1.89–1.82 (m, 2H), 1.51–1.44 (m, 2H), 1.40–1.25 (m, 12H), 0.90 (t, J = 6.9 Hz, 3H). ^{13}C NMR (150 MHz, MeOD) δ 129.9, 129.2, 102.1, 96.9, 85.5, 81.1, 74.4, 73.2, 73.1, 73.0, 72.8, 70.4, 69.9, 68.7, 56.5, 53.3, 52.1, 51.7, 50.4, 48.7, 42.3, 33.0, 30.6, 30.5, 30.4, 30.2, 29.5, 28.9, 23.7, 22.9, 14.4. HRMS (ESI): *m/z* calculated for $[M + 2H]^{2+}$ $C_{31}H_{61}N_7O_{12}S$, 377.7050; found 377.7027.

Synthesis of 6″-amino-tetra-*N*-(*tert*-butoxycarbonyl)-amikacin (11)

To a solution of **5** (148 mg, 0.15 mmol) stirring in MeOH (3–10 mL) was added $Pd(OH)_2/C$ 20 wt-% (1.2 equiv.). The reaction system was degassed and flushed with $H_{2(g)}$ several times. Hydrogenation was then carried out under 1 atm (101.3 kPa) $H_{2(g)}$ overnight for 18 h. The reaction was filtered over Celite 545 and concentrated under vacuum, affording **11** as a white solid with a mass yield of 78% (113 mg, 0.11 mmol). LRMS (ESI): *m/z* calculated for $[M + Na]^+$ $C_{42}H_{76}N_6NaO_{20}$, 1007.5; found 1007.7.

Synthesis of 6″-amino-1,3,6′,3″-tetra-*N*-(*tert*-butoxycarbonyl)-kanamycin (12)

Synthesis of **12** was achieved by reacting **6** (639 mg, 0.70 mmol) with the synthetic methodology reported for **11**. Compound **12** was isolated as a white solid with a yield of 71% (438 mg, 0.50 mmol). LRMS (ESI): *m/z* calculated for $[M + Na]^+$ $C_{38}H_{69}N_5NaO_{18}$, 906.5; found 905.9.

Synthesis of 6″-(2-(decylthio)acetamide)-tetra-*N*-(*tert*-butoxycarbonyl)-amikacin (13)

To a solution of **11** (113 mg, 0.11 mmol) dissolved in 9:1 THF:H_2O (5–10 mL) were added the decyl acid **21** (1.5 equiv.), EDC (1.5 equiv.) and *N*-hydroxysuccinimide (NHS) (1.5 equiv.). The reaction was carried out at 50°C for 24 h. Once it was complete, the solvent was removed under reduced pressure and the resulting residue was purified by flash chromatography. Elution of the crude material in DCM:MeOH 15:1 → 9:1 afforded **13** in a yield of 27% (37 mg, 0.03 mmol) as a white solid. LRMS (ESI): *m/z* calculated for $[M + Na]^+$ $C_{54}H_{98}N_6NaO_{21}S$, 1221.6; found 1222.4.

Synthesis of 6″-(2-(decylthio)acetamide)-1,3,6′,3″-tetra-*N*-(*tert*-butoxycarbonyl)-kanamycin (14)

Synthesis of **14** was achieved by reacting **12** (438 mg, 0.50 mmol) with the synthetic conditions reported for the synthesis of **13**. Following a reaction time of 15 h at ambient temperature, the crude reaction mixture was eluted in DCM:MeOH 95:5 → 12:1, isolating **14** as a white solid in a yield of 43% (233 mg, 0.21 mmol). LRMS (ESI): *m/z* calculated for $[M + Na]^+$ $C_{50}H_{91}N_5NaO_{19}S$, 1120.6; found 1120.9.

Synthesis of 6″-(2-(decylsulfonyl)acetamide)-tetra-*N*-(*tert*-butoxycarbonyl)-amikacin (15)

Compound **13** (37 mg, 0.03 mmol) was dissolved in MeOH:H_2O (8:3) and reacted with $KHSO_5$ (3 equiv.). The reaction was carried out for 18 h, and on completion, the reaction mixture was partitioned in EtOAc (50 mL) and H_2O (50 mL) and extracted in EtOAc (5 × 30 mL). The organic layers were combined, dried over Na_2SO_4 and concentrated. Purification of **15** in a gradient elution of 95:5 → 9:1 DCM:MeOH yielded the target as a white solid at 42% (16 mg, 0.01 mmol). LRMS (ESI): *m/z* calculated for $[M + Na]^+$ $C_{54}H_{98}N_6NaO_{23}S$, 1253.6; found 1254.8.

Synthesis of 6″-(2-(decylsulfonyl)acetamide)-1,3,6′,3″-tetra-*N*-(*tert*-butoxycarbonyl)-kanamycin (16)

Synthesis of **16** was achieved by reacting **14** (233 mg, 0.21 mmol) with the synthetic conditions reported for the synthesis of **15**. Elution of **16** in 12:1 DCM:MeOH afforded the target as a white solid at 53% (127 mg, 0.11 mmol). LRMS (ESI): *m/z* calculated for [M] $C_{50}H_{91}N_5O_{20}S$, 1129.6; found 1128.7.

Synthesis of 6″-(2-(decylsulfonyl)acetamide)-amikacin (17)

Compound **15** (16 mg, 0.01 mmol) was reacted following the TFA deprotection conditions reported for the synthesis of compound **9**. Compound **17** was isolated as a white solid at a yield of 95% (14 mg, 0.01 mmol). ^{1}H NMR (400 MHz, D_2O) δ 5.64 (d, J = 3.8 Hz, 1H), 5.23 (d, J = 3.9 Hz, 1H), 4.39–4.27 (m, 4H), 4.25–4.08 (m, 1H), 3.99–3.94 (m, 3H), 3.92–3.81 (m, 2H), 3.77 (dd, J = 9.9, 3.7 Hz, 1H), 3.73–3.59 (m, 4H), 3.58–3.42 (m, 5H), 3.33–3.21 (m, 3H), 2.36–2.21 (m, 3H), 2.11–1.97 (m, 1H), 1.97–1.83 (m, 3H), 1.63–1.49 (m, 2H), 1.48–1.25 (m, 12H), 0.94 (t, J = 6.7 Hz, 3H). ^{13}C NMR (150 MHz, MeOD) δ 177.4, 164.8, 99.6, 96.4, 81.9, 81.4, 74.6, 73.7, 73.0, 73.0, 71.9, 71.1, 70.4, 70.1, 69.2, 56.9, 54.8, 50.4, 42.2, 38.3, 33.1, 30.7, 30.7, 30.6, 30.5, 30.4, 30.3, 30.2, 29.8, 29.4, 23.7, 22.9, 14.4. HRMS (ESI): *m/z* calculated for $[M + 2H]^{2+}$ $C_{34}H_{68}N_6O_{15}S$, 416.2232; found 416.2209.

Synthesis of 6″-(2-(decylsulfonyl)acetamide)-kanamycin (18)

Compound **16** (127 mg, 0.11 mmol) was reacted following the TFA deprotection conditions reported for the synthesis of compound **9**. Compound **18** was isolated as a white solid in a yield of 98% (111 mg, 0.11 mmol). ^{1}H NMR (600 MHz, MeOD) δ 5.46 (d, J = 3.9 Hz, 1H), 5.07 (d, J = 3.6 Hz, 1H), 4.06 (td, J = 9.5, 3.0 Hz, 1H), 4.03–3.98 (m, 1H), 3.88–3.79 (m, 3H), 3.77–3.72 (m, 1H), 3.70 (t, J = 9.2 Hz, 1H), 3.64–3.55 (m, 3H), 3.49–3.43 (m, 2H), 3.44—3.39 (m, 2H), 3.37 (t, J = 10.3 Hz, 1H), 3.21 (dd, J = 9.4, 0.8 Hz, 1H), 3.02 (dd, J = 13.0, 9.2 Hz, 1H), 2.50 (dt, J = 12.3, 4.2 Hz, 1H), 1.99–1.91 (m, 1H), 1.87–1.79 (m, 2H), 1.51–1.43 (m, 2H), 1.41–1.23 (m, 12H), 0.90 (t, J = 7.0 Hz, 3H). ^{13}C NMR (150 MHz, MeOD) δ 165.0, 102.2, 97.0, 85.4, 81.2, 74.4, 73.5, 73.0, 73.0, 72.6, 70.5, 70.1, 68.8, 56.4, 54.7, 51.9, 48.9, 42.2, 41.5, 33.0, 30.6, 30.5, 30.4, 30.2, 29.4, 29.2, 23.7, 22.8, 14.4. HRMS (ESI): *m/z* calculated for $[M + 2H]^{2+}$ $C_{30}H_{61}N_5O_{13}S$, 365.6994; found 365.7003.

Synthesis of decyl-(prop-2-yn-1-yl)-sulfane (19)

To a solution of 1-decanethiol (1.03 mL, 5.00 mmol) and K_2CO_3 (2.07 g, 15.0 mmol) dissolved in anhydrous acetone (10 mL) was added 80% propargyl bromide in toluene (0.67 mL, 7.50 mmol). The reaction proceeded under an atmosphere of argon at ambient temperature for 24 h. Following completion, the reaction was quenched with H_2O and the mixture partitioned between 1 M HCl (100 mL) and EtOAc (100 mL), then extracted with EtOAc (2 × 50 mL). The organic layers were combined and washed with 1 M HCl (3 × 30 mL), H_2O (30 mL) and brine (30 mL), dried over Na_2SO_4 and concentrated under vacuum to afford **19** as an amber oil in a yield of 70% (1.09 g, 5.13 mmol). ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.23 (d, J = 2.6 Hz, 2H), 2.67 (t, J = 7.4 Hz, 2H), 2.21 (t, J = 2.6 Hz, 1H), 1.61 (p, J = 7.4 Hz, 2H), 1.47–1.34 (m, 2H), 1.34–1.18 (m, 12H), 0.87 (t, J = 6.8 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 80.3, 70.9, 32.0, 31.8, 29.7, 29.6, 29.4, 29.3, 29.1, 29.0, 22.8, 19.3, 14.2. LRMS (ESI): m/z calculated for $[M + H]^+$ $C_{13}H_{25}S$, 213.2; found 213.2.

Synthesis of 1-(prop-2-yn-1-ylsulfonyl)-decane (20)

Compound **19** (1.09 g, 5.13 mmol) was dissolved in MeOH (30 mL), the solution cooled to 0°C, and $KHSO_5$ (4.75 g, 15.4 mmol) dissolved in H_2O (15 mL) was added dropwise. The reaction was carried out at ambient temperature for 18 h. On completion, the MeOH solution was partially concentrated under vacuum and the residue was partitioned between H_2O (100 mL) and DCM (100 mL) and extracted into DCM (3 × 30 mL). The combined organic layers were dried over Na_2SO_4 and concentrated under reduced pressure to afford **20** as a white solid in a yield of 98% (1.23 g, 5.03 mmol). ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.83 (d, J = 2.7 Hz, 2H), 3.19 (t, J = 7.9 Hz, 2H), 2.50 (t, J = 2.7 Hz, 1H), 1.93–1.80 (m, 2H), 1.50–1.40 (m, 2H), 1.39–1.20 (m, 12H), 0.88 (t, J = 6.6 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 76.5, 71.8, 51.5, 45.0, 32.7, 29.6, 29.4, 29.4, 29.1, 28.5, 22.8, 22.1, 14.2. LRMS (ESI): m/z calculated for $[M + Na]^+$ $C_{13}H_{24}NaO_2S$, 267.1; found 267.2.

Synthesis of 2-(decylthio)-acetic acid (21)

To a solution of 1-decanethiol (5 mL, 24.3 mmol) and K_2CO_3 (10.0 g, 72.4 mmol) dissolved in MeCN (40 mL) was added bromoacetic acid (4.17 g, 30.0 mmol). The resulting solution was stirred at 50°C for 24 h under an atmosphere of argon. Following reaction completion, the sample was dried under reduced pressure and partitioned between 1 M HCl (100 mL) and EtOAc (100 mL), extracted into EtOAc (3 × 50 mL), and washed with 1 M HCl (1 × 30 mL) and brine (1 × 30 mL). The combined organic layers were dried over Na_2SO_4 and concentrated under vacuum. Compound **21** was isolated as a white solid in a yield of 94% (5.31 g, 22.9 mmol). ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.25 (s, 2H), 2.65 (t, J = 7.42 Hz, 2H), 1.59 (p, J = 7.7 Hz, 2H), 1.42–1.27 (m, 2H), 1.27 (s, 12H), 0.87 (t, J = 6.81 Hz 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 174.9, 33.6, 33.0, 32.0, 29.7, 29.6, 29.4, 29.3, 29.1, 28.9, 22.4, 13.8. LRMS **21** (ESI): m/z calculated for $[M–H]^-$ $C_{12}H_{23}O_2S$, 231.1; found 231.1.

Supplementary material

Supplementary material is available online.

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Data availability. The data that support this study will be shared upon reasonable request to the corresponding author.

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