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Synthesis and biological evaluation of reversible inhibitors of IdeS, a bacterial cysteine protease and virulence determinant

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ABSTRACT

Analogues of the irreversible protease inhibitors TPCK and TLCK have been synthesized and tested as inhibitors of the bacterial cysteine protease IdeS excreted by *Streptococcus pyogenes*. Eight compounds were identified as inhibitors of IdeS in an in vitro assay. The most potent compounds contained an aldehyde function, thus acting as efficient reversible inhibitors, nitrile and azide derivatives showed moderate activity.

1. Introduction

Streptococcus pyogenes, a Gram-positive bacterium, is the causative agent for common and relatively mild human diseases such as strep throat (pharyngitis) and skin infections (impetigo, erysipelas), but it is also responsible for the life-threatening conditions streptococcal toxic shock syndrome and necrotizing fasciitis. In addition, *S. pyogenes* infections give rise to clinically important sequelae, and acute rheumatic fever following a throat or skin infection is the most common cause of heart disease in children world-wide. Several *S. pyogenes* virulence factors have been described and among these is a secreted cysteine protease called IdeS or Mac-1.[1–3] IdeS (*I*gG-*de*grading *e*nzyme of *S. pyogenes*) is a 35 kDa enzyme that cleaves IgG by hydrolyzing the peptide bond between two glycine residues in the hinge region of IgG, generating one $F(ab')_2$ fragment and two 1/2 Fc fragments. IgG antibodies represent the dominating class of antibodies in human blood, and they play an important role in immunity by recognizing invading microorganisms and promoting their phagocytosis and killing. The cleavage of IgG antibodies bound to the surface of *S. pyogenes* by IdeS, therefore represents a sophisticated mechanism by which the bacterium overcomes IgG attack.[4] Moreover, a recent study has demonstrated that the Fc fragments generated by IdeS cleavage of IgG also interfere with human defences.[5] IdeS has a unique degree of specificity and apart from IgG no other substrate has been found, which is explained by the requirement for a specific protein–protein interaction between IdeS and IgG before cleavage can occur.[2,6] In many autoimmune conditions (rheumatoid arthritis, systemic lupus, myasthenia gravis, immune thrombocytopenic purpura (ITP), etc.) IgG autoantibodies binding to human molecules contribute to the disease development, and they also cause acute transplant rejection. Given this background and the unique specificity of IdeS for IgG, the effect of IdeS in animal models of autoimmune diseases has been investigated, and the enzyme was found to prevent the development of rheumatoid arthritis in mice and to cure mice from lethal ITP.[7,8] So far IdeS has not been used to treat autoimmune disease in humans, but if and when this happens, there could be a need for compounds that block and control the activity of the enzyme. Moreover, the fact that IdeS protects *S. pyogenes* against IgG attack, suggests that blocking the activity of IdeS during *S. pyogenes* infection could be a new way of treating these sometimes hyper-acute and life-threatening infections. The present investigation was therefore undertaken to synthesize and identify inhibitors of IdeS.

As mentioned above, IdeS is a cysteine protease for which IgG is the only known substrate.[2] IdeS is inhibited by well known protease inhibitors such as iodoacetate, Z-LVG-CHN_2, *t*osyl-L-*p*henylalanine *c*hloromethyl *k*etone (TPCK) and *t*osyl-L-*l*ysine *c*hloromethyl *k*etone (TLCK), but not by E-64, another common cysteine protease inhibitor (Fig. 1).[2,9] These electrophilic inhibitors have been used to characterize IdeS and bind covalently via the catalytically active cysteine, acting as irreversible inhibitors.[10,11]

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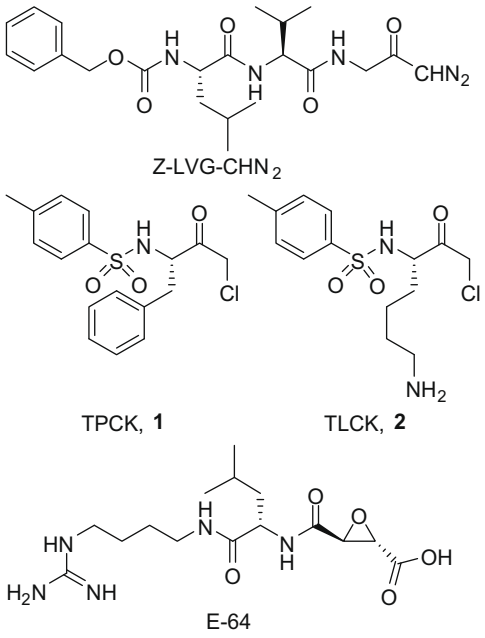


Figure 1. Irreversible inhibitors of cysteine proteases.

Inhibitors containing aldehyde or nitrile functionalities can react reversibly with the cysteine residue forming covalent complexes which differ in geometry (Fig. 2).[12]

Up to now there are only a few known non-covalently bound inhibitors for cysteine proteases. For example, development of inhibitors to cathepsin S and K have been reported in the literature.[13,14] These inhibitors are non-peptidic and are based on either a pyrimidine scaffold or a piperidine–pyrazole fused system. They are not chemically reactive and depend instead on other strong interactions with the active site. Such unreactive inhibitors are prone to be more selective, less toxic and therefore more useful as drugs.

TPCK and TLCK derived from the tosyl-substituted amino acids phenylalanine and lysine (Fig. 1), have shown inhibitory activity against IdeS (Mac-1).[9] They are also reported to have different potency and binding modes towards papain, another cysteine protease.[15] These differences imply that the side chain is of importance for the recognition and interaction between the inhibitor and the active site of the enzyme. Since the two side chains are different in structure and also have different physicochemical properties, further variation of the substitution pattern in this position could lead to new, more selective and potent inhibitors. Improved interaction via the side chain would make the reactive warhead less important and possibly new inhibitors binding reversibly or non-covalently could be developed.

TPCK and TLCK act via the highly reactive α-chloro ketone warhead. In the literature, the position where the α-chloro ketone is

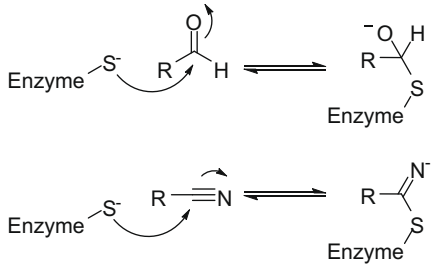


Figure 2. The aldehyde and nitrile functionalities are attacked by the cysteine residue to form covalent inhibitor–enzyme complexes.

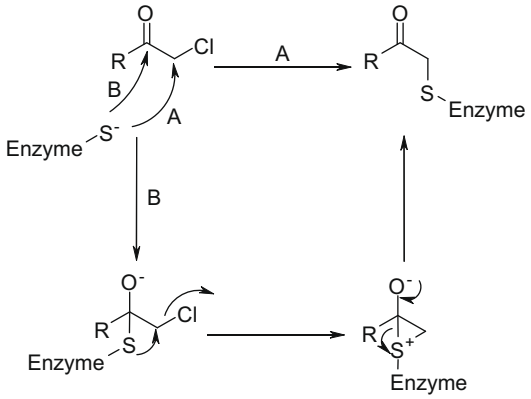


Figure 3. Nucleophilic attack by cycteine can occur at two positions in the irreversible α-chloro ketone inhibitor giving the covalent-enzyme-inhibitor complex via either route A or route B.

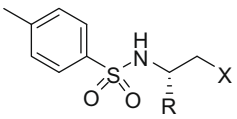
attacked by the cysteine is discussed (Fig. 3).[11] Attack can occur either at the α-position forming a complex through route A or at the carbonyl carbon forming the same complex via the intermediates in route B. The inhibitor–enzyme thioether complex has been crystallized, but the cyclic sulfonium intermediate has not been detected.[16]

We have synthesized analogues of TLCK and TPCK with variations in the side chain and in the warhead, and tested their ability to act as inhibitors of IdeS in an in vitro assay. The analogues were designed to provide indications whether reactivity of the warhead alone is responsible for the inhibitory activity, or if side chain interactions with the active site also contribute to the inhibition.

2. Results and discussion

2.1. Design

The design of novel potential IdeS inhibitors was based on the structures of TLCK and TPCK. The structures of the warhead X and the side chain R were varied (Fig. 4). If the warhead is less reactive, the impact of the side chain should increase. Instead of the α-chloro ketone moiety other electrophilic functional groups such as aldehyde and nitrile were introduced. In addition, a tosyloxy functionality was used as warhead to investigate if it could act as a leaving group in a reaction with the active site cysteine. The warhead was also exchanged for alcohol and azide functionalities. Furthermore, the side chain structures were varied. The benzyl and 4-aminobutyl groups corresponding to TPCK and TLCK, and also the less flexible and sterically demanding isopropyl group, were used. To analyse if the amino group in TLCK contributes to the interaction, the unfunctionalized *n*-butyl group was also used as side chain. We aimed for all possible combinations of warheads and side chains, and after synthesis 23 different test compounds were obtained.



X = OH, OTs, N_3, CN, CHO

R = $PhCH_2$-, $CH_3(CH_2)_3$-, $(CH_3)_2CH$-, $H_2N(CH_2)_4$-, $BocNH(CH_2)_4$-

Figure 4. Compounds synthesized as potential inhibitors of IdeS.

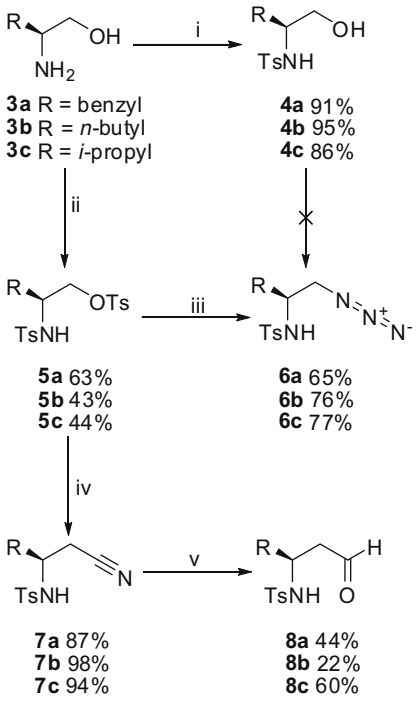
2.2. Synthesis

The synthetic route to compounds **4a**–**8c** in which the side chain R is either benzyl, *n*-butyl or *i*-propyl is shown in Scheme 1.

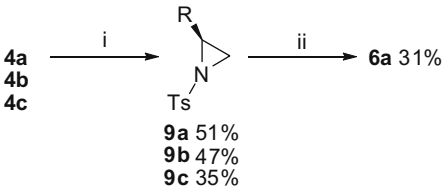
Initially, the amino alcohol derivatives of L-phenylalanine, L-norleucine and L-valine **3a**–**c** were reacted with tosylchloride and triethylamine in dichloromethane to form N-tosylated alcohols (**4a**–**c**) in good yields (86–95%) (Scheme 1). Attempts to convert the alcohols to the corresponding azides (**6a**–**c**) using Mitsunobu conditions resulted in aziridine formation (**9a**–**c**) (Scheme 2).

However, addition of water to the reaction mixture facilitated aziridine ring opening affording **6a** in low yield (31%) from **9a**. Trying to compete with the intramolecular ring formation by using an excess of NaN_3 (3 or 10 equiv), still gave the aziridine as the only product (35–47%). Aziridine formation has been reported earlier, either when treating amino alcohols under Mitsunobu conditions or the corresponding mesylate or tosylate using Et_3N, KOH or K_2CO_3 as the base.[17,18]

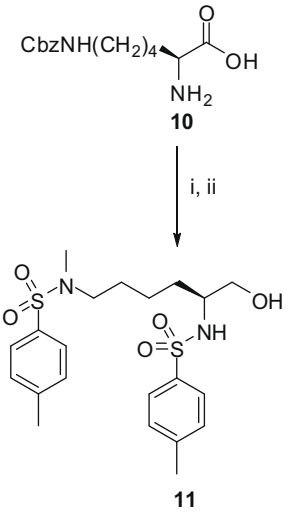
Instead, ditosylated intermediates (**5a**–**c**) were synthesized using two equivalents of tosylchloride in reactions with the amino alcohols (**3a**–**c**) to afford products in low to moderate yields (43–63%). Pyridine was used as base and no aziridine formation was detected. This is in agreement with the literature which states that aziridine formation had been favoured if triethylamine had been used.[19] Using azide and cyanide ion as nucleophiles to expel the O-tosyl group, gave the corresponding azides (**6a**–**c**) and nitriles (**7a**–**c**) in moderate or excellent yields, respectively, and with high purities when DMSO was used as the solvent. Using KCN in DMF did not result in any conversion of the starting material, due to the low solubility of KCN in DMF. The nitriles **7a**–**c** were reduced by DIBAL in toluene at −65 °C followed by hydrolysis of the formed imines to give the corresponding aldehydes **8a**–**c** in low to moderate yields (22–60%).[20] All 15 compounds (**4a**–**8c**) synthesized by this highly convergent route were tested for inhibitory activity against IdeS.



Scheme 1. Reagents and conditions: (i) TsCl, Et_3N, DMAP, DCM 0 °C→rt, o.n.; (ii) TsCl, pyridine, DCM 0 °C→rt, 6 h; (iii) NaN_3, DMSO, 35 °C, 6 h or o.n.; (iv) NaCN, DMSO, 35 °C, 6 h or o.n.; (v) DIBAL in toluene, −65 °C, 50 to 90 min.



Scheme 2. Reagents and conditions: (i) NaN_3, PPh_3, DIAD, THF, 0 °C→rt, o.n.; (ii) Water, rt, o.n.



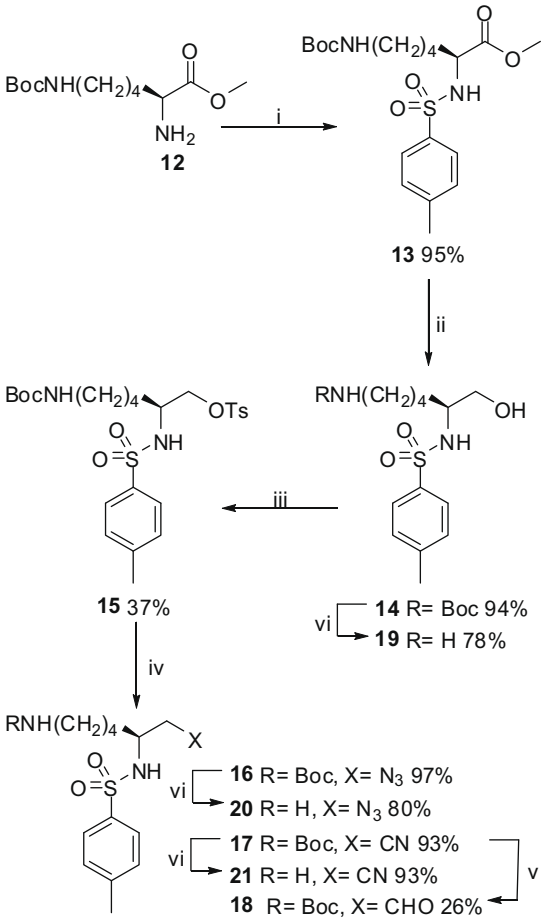
Scheme 3. Reagents and conditions: (i) $LiAlH_4$, THF; (ii) TsCl, Et_3N, DMAP, DCM. 0 °C→rt, o.n.

For the amino butyl derivatives **14–21** the synthetic route described above had to be modified. The amino alcohol derivative of ε-protected lysine is not commercially available. Using the experience from synthesis of compounds **4–8** we initially aimed for the ditosylated derivative **5** (R = CbzNH$(CH_2)_4$). However, reduction of the carboxylic acid group of ε-Cbz-protected lysine (**10**) using $LiAlH_4$ followed by tosylation gave instead the N-methylated derivative **11** as the sole product (Scheme 3).[21]

Therefore, the protecting groups were changed and reduction of ε-Boc-protected lysine methyl ester (**12**) using $NaBH_4$ was performed. This resulted in a low yield of the corresponding alcohol (**3,** R = BocNH$(CH_2)_4$) due to difficulties monitoring the reaction and the work-up of the polar product. As shown in Scheme 4, the route was instead started by N-tosylation of **12** to obtain **13** in excellent yield (95%). Now the reduction could be monitored by TLC and afforded a high yield (94%) of alcohol **14**. The alcohol was O-tosylated in a rather low yield (37%) as pyridine had to be used as base to avoid aziridine formation. The ditosylated product (**15**) was further reacted with either sodium azide or sodium cyanide in DMSO to give compounds **16** and **17**, respectively, in high yields (97% and 93%, respectively). The nitrile (**17**) was converted to the corresponding aldehyde (**18**) in the same manner as described above, again in a low yield (26%). Finally, the Boc-protected alcohol (**14**), azide (**16**) and nitrile (**17**) derivatives were deprotected by TFA in dichloromethane to give **19–21** in the yields 78%, 80% and 93%, respectively. Unfortunately, the unprotected aldehyde derivative could not be isolated with a satisfying purity.

2.3. Inhibitory activity of the synthesized compounds

IgG is composed of two heavy (56 kDa each) and two light chains (25 kDa each) that are held together by disulfide bonds.



Scheme 4. Reagents and conditions: (i) TsCl, Et_3N, DCM, 0 °C→rt, o.n.; (ii) $NaBH_4$, THF/ethanol, 0 °C→rt, o.n.; (iii) TsCl, pyridine, DMAP, DCM, 0 °C→rt, o.n.; (iv) NaN_3, DMSO, rt, o.n. or NaCN, DMSO, 35 °C, o.n.; (v) DIBAL in toluene, THF, −65 °C, 45 min; (vi) TFA in DCM, rt, 60–90 min.

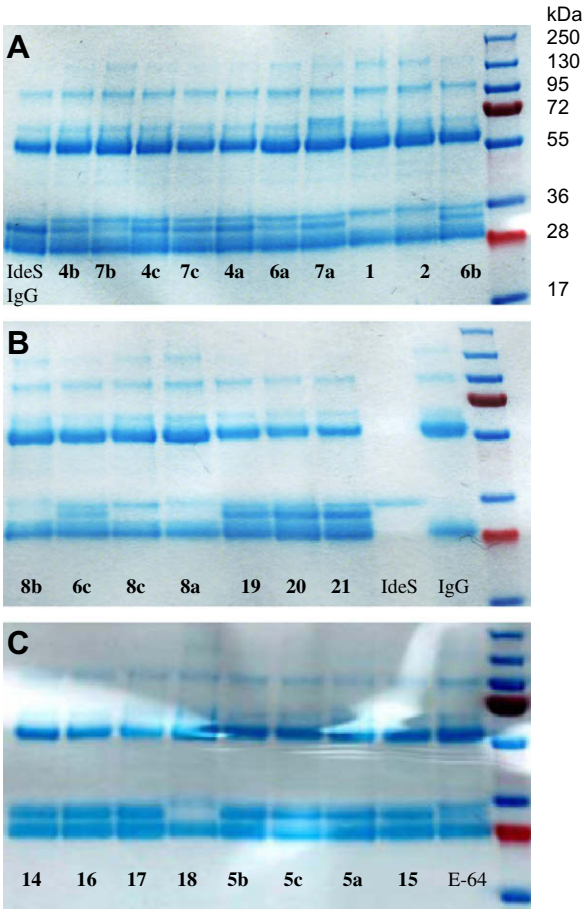


Figure 5. A–C Cleavage of IgG by IdeS in the presence of test compounds **4a**–**8c**, **14**–**21** and controls. The positive controls (**1** and **2**) are shown in 5A and the negative control (E-64) is shown in 5C.

Thus, when run on SDS–PAGE under reducing conditions, IgG gives rise to two bands of approximately 25 and 56 kDa (see Fig. 5B). IdeS with a molecular weight of 35 kDa (see Fig. 5B), cleaves IgG in the heavy chain generating two heavy chain fragments of 25 and 31 kDa, respectively. Therefore, incubation of IgG with IdeS followed by separation on SDS–PAGE under reducing conditions, will generate a new 31 kDa band from the heavy chain (the other 25 kDa peptide from the heavy chain is hidden in the light chain band), and the SDS–PAGE analysis is based on the appearance of this 31 kDa band. As mentioned above, IdeS is uniquely specific for IgG. This is explained by the fact that the enzyme has to bind to a site in Fc before cleavage can occur in the hinge region of the heavy chain. The requirement for this initial protein–protein interaction has made it impossible to develop a quantitative and practical screening assay for the proteolytic activity of IdeS, which is based on the hydrolysis of synthetic and natural peptides covering the cleavage site in the hinge.[5] However, to identify inhibitors of IdeS the qualitative SDS–PAGE analysis used in this work, was found to be sufficient.

Purified IdeS was preincubated with the test compounds **4a**–**8c**, **14–21,** or **1**, **2** and E-64 as positive and negative controls, respectively. Following the addition of polyclonal human IgG and incubation, the samples were separated by SDS–PAGE, and the results are shown in Figure 5A–C (for experimental details, see Experimental Section 4.10).

In the in vitro assay, eight out of the 23 compounds tested were able to inhibit IdeS. Compounds containing an aldehyde function as warhead were shown to efficiently inhibit the hydrolyzing activity of IdeS. In fact, all aldehydes tested (**8a**–**c**, **18**), substituted by benzyl, *n*-butyl, *i*-propyl and 4-*tert*-butyloxycarbonylaminobutyl showed as strong inhibitory activity as the positive controls TPCK (**1**) and TLCK (**2**). Apparently, the different side chains did not seem to contribute to the binding as the same degree of inhibition was observed for all derivatives.

Only one nitrile derivative (**7b**), substituted with an *n*-butyl group, showed some inhibition of IdeS (Fig. 5A). This implies that among these test compounds the *n*-butyl substituted derivative is interacting strongest with the active site of IdeS.

Three of the tested azide derivatives (**6a**–**c**) were shown to be moderate inhibitors of IdeS. Some of the first azides reported to be potent inhibitors of various cysteine proteases were recently shown to decompose into aldehydes when exposed to light.[22,23] These aldehydes were suggested to be the active species. Therefore the stability of our azide derivatives was tested and they were found to be stable in the solid state as no impurities of the corresponding aldehyde could be detected by ^1H NMR spectroscopy. The azides were also found to be stable in buffer solutions (pH 6.9 and 7.4) for 48 h, as determined by analytical HPLC (see the Experimental section 4.9.)

Both covalent and non-covalent interactions between azides and nucleophiles have been proposed but their mode of action as inhibitors has so far not been studied in detail. Nucleophilic attack of the cysteine residue as well as electrostatic interaction via the dipolar resonance structures of the azide functionality have been suggested.[24] Furthermore, thiols such as dithiothreitol, mercap-

toethanol and even cysteine, are reported to reduce azides to amines via a covalent intermediate, which could explain a reversible inhibition of IdeS.[25,26]

Compounds with alcohol (**4a**–**c**, **14**, **19**) and *O*-tosyl (**5a**–**c**, **15**) groups as warheads were not able to inhibit IdeS, irrespectively of the side chains used. Neither the lysine-derived azides (**16**, **20**) were active. The aziridine derivatives with *n*-butyl (**9b**) and *i*-propyl (**9c**) groups as side chains were also tested (results not shown), but did not result in any inhibition, even though aziridines have been reported to act as inhibitors for other cysteine proteases.[27] Reasons for the inactivity of *O*-tosyl and aziridine derivatives could either be low reactivity, steric hindrance or unfavourable spatial orientation of the ligand in the active site of IdeS.

3. Conclusion

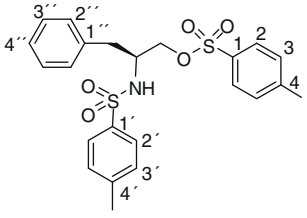
To the best of our knowledge, no compounds have been published with the specific aim to develop potential inhibitors of IdeS as previously reported inhibitors of IdeS have been used only for characterizing purposes.[2,6,9] In the present study we have used a highly convergent synthetic route to compounds which were tested for their ability to inhibit IdeS. The results showed that reversible binding of warheads, such as the aldehyde, can be sufficient to efficiently inhibit IdeS. In addition, nitrile and azide derivatives showed moderate inhibitory activity. For these inhibitors the binding of the warhead is still of major importance, but irreversible binding is not necessary. Finally, interactions between IdeS and other substituents than the warhead of the inhibitor were found to be of importance for the inhibition. The identification of reversible inhibitors of IdeS could be an important step towards a potential treatment of acute and severe *S. pyogenes* infections.

4. Experimental

4.1. General methods

Melting points were determined with a Büchi B-545 apparatus and are uncorrected**.** Optical rotations were measured with a Perkin Elmer 341 LC Polarimeter at 20 °C. ^{1}H and ^{13}C NMR spectra were recorded on a JEOL Eclipse 400 spectrometer at 400 MHz and 100 MHz, respectively, in $CDCl_3$ if nothing else is stated. Chemical shifts are reported in ppm with the solvent residual peak as internal standard ($CHCl_3$ δ^H 7.26, δ^C 77.00, CH_3OH δ^H 3.30, δ^C 49.00). Infrared spectra were recorded on a Perkin Elmer 16 PC FTIR spectrometer. Only the major peaks are listed. HRMS-analyses were run at BioAnSer, Gothenburg, Sweden. Analytical TLC was performed on Merck Silica Gel, grade 60 F_{254} and the spots were visualized by UV light (254 nm). Flash chromatography was performed on Merck Silica Gel 60. The hexane used was a mixture of isomers. Analytical HPLC was performed on a Waters 2690 system (Photodiode Array Detector at 254 nm and flow rate 1.5 mL/min) equipped with a Genesis Lightning C8 4 μm, 50 mm, ID 4.6 mm column. Starting materials, reagents, polyclonal IgG and molecular mass markers were purchased from Sigma–Aldrich and were used as such.

For the assignment of ^{1}H NMR signals, the numbering used is shown in the structure below.



4.2. General synthetic procedure for monotosylation

A solution of tosyl chloride (1.05 equiv) in dichloromethane (6 mL) was added dropwise over 90 min to a mixture of the amino alcohol (4 mmol), DMAP (0.1 equiv) and Et_3N (2 equiv) in dichloromethane (10 mL) at 0 °C. The reaction mixture was stirred over night at room temperature, and then diluted with dichloromethane. The solution was washed with water (3×) and brine (1×), dried ($MgSO_4$) and filtered. The solvent was removed in vacuo. Purification by flash chromatography (dichloromethane/methanol 95:5) gave the product.

4.2.1. (*S*)-3-Phenyl-2-tosylamino-1-propanol (4a)

White solid; Yield 91%; Mp 66–69 °C (lit.[28] 73–74 °C); $[\alpha]_D^{20}$ −47.5 (*c* 1.93, EtOH) (lit.[28] −38.8); ^{1}H NMR δ 7.57 (d, *J* = 8.1 Hz, 2H, *H2′*), 7.23–7.16 (m, 5H, *H3′*, *H2″*, *H4″*), 6.99–6.94 (m, 2H, *H3″*), 4.67 (d, *J* = 5.5 Hz, 1H, N*H*), 3.68–3.49 (m, 2H, CH_2OH), 3.49–3.39 (m, 1H, C*H*), 2.78 (dd, *J* = 7.0, 13.9 Hz, 1H, PhCH_A), 2.68 (dd, *J* = 7.3, 13.9 Hz, 1H, PhCH_B), 2.42 (s, 3H, CH_3), 2.04–1.96 (m, 1H, O*H*); ^{13}C NMR data were in agreement with those reported.[28] IR (KBr) *ν* 3445, 3159, 1316, 1157 cm^{-1}.

4.2.2. (*S*)-2-Tosylamino-1-hexanol (4b)

White solid, Yield 95%, Mp 86–88 °C (lit.[29] 81–83 °C); $[\alpha]_D^{20}$, ^{1}H and ^{13}C NMR data were in agreement of those reported.[29]

4.2.3. (*S*)-3-Methyl-2-tosylamino-1-butanol (4c)

White solid; Yield 86%; Data were in agreement of those reported.[30]

4.3. General synthetic procedure for ditosylation

A solution of tosyl chloride (2.1 equiv) in dichloromethane (5 mL) was added dropwise to a mixture of the amino alcohol (6 mmol) and pyridine (2.1 equiv) in dichloromethane (7 mL) at 0 °C. The reaction mixture was stirred for 6 h at room temperature, and then diluted with dichloromethane. The solution was washed with water (3×) and brine (1×), dried ($MgSO_4$) and filtered. The solvent was removed in vacuo. Purification by flash chromatography (hexane/ethyl acetate 3:1) gave the product.

4.3.1. (*S*)-3-Phenyl-2-tosylamino-propyl-1-toluenesulfonate (5a)

White solid; Yield 63%; Mp 100–102 °C (lit.[31] 97–98 °C); $[\alpha]_D^{20}$ −51.2 (*c* 1.0, MeOH) (lit.[31] −57.4); ^{1}H NMR δ 7.76 (d, *J* = 8.4 Hz, 2H, *H2′*), 7.52 (d, *J* = 8.4 Hz, 2H, *H2*), 7.36 (d, *J* = 8.4 Hz, 2H, *H3*), 7.22–7.09 (m, 5H, *H3′*, *H2″*, *H4″*), 6.92–6.83 (m, 2H, *H3″*), 4.61 (d, *J* = 8.1 Hz, 1H, N*H*), 4.16–3.81 (m, 2H, CH_2O), 3.61–3.52 (m, 1H, C*H*), 2.82 (dd, *J* = 7.3, 13.9 Hz, 1H, PhCH_A), 2.67 (dd, *J* = 7.0, 13.9 Hz, 1H, PhCH_B), 2.47 (s, 3H, PhCH_3), 2.41 (s, 3H, PhCH_3); ^{13}C NMR δ 145.2, 143.4, 136.7, 135.6, 132.2, 130.0, 129.7, 129.1, 128.7, 128.0, 126.9, 70.1, 53.5, 37.5, 21.7, 21.5; IR (KBr) *ν* 3447 (broad), 3291, 1364, 1174 cm^{-1}.

4.3.2. (*S*)-2-Tosylamino-hexyl-1-toluenesulfonate (5b)

White solid; Yield 43%; Mp 95–98 °C; $[\alpha]_D^{20}$ −44.4 (*c* 1.0, $CHCl_3$); ^{1}H NMR δ 7.73 (d, *J* = 8.4 Hz, 2H, *H2′*), 7.69 (d, *J* = 8.0 Hz, 2H, *H2*), 7.35 (d, *J* = 8.4 Hz, 2H, H3′), 7.30–7.23 (m, 2H, *H3*), 4.56 (d, *J* = 8.4 Hz, 1H, N*H*), 3.98–3.80 (m, 2H, CH_2O), 3.40–3.30 (m, 1H, C*H*), 2.46 (s, 3H, PhCH_3), 2.42 (s, 3H, PhCH_3), 1.53–1.30 (m, 2H, CH_2CH_2CH), 1.16–0.90 (m, 4H, $CH_3(CH_2)_2$), 0.74 (t, *J* = 7.0 Hz, 3H, CH_3CH_2); ^{13}C NMR δ 145.1, 143.6, 137.4, 132.2, 129.9, 129.7, 127.9, 127.0, 71.1, 52.3, 31.2, 27.2, 22.0, 21.6, 21.5, 13.7; IR (KBr) *ν* 3276, 2949, 1600, 1438, 1357, 1174 cm^{-1}; HRMS (FT-ICR-MS) Calcd for $C_{20}H_{27}NO_5S_2$ $[M+H]^+$ 426.1402, found 426.1390.

4.3.3. (*S*)-3-Methyl-2-tosylamino-butyl-1-toluenesulfonate (5c)

White solid; Yield 44%; Data were in agreement of those reported.[31,32]

4.4. General synthetic procedure for azide derivatives 6a–c

The ditosylated amino alcohol (0.3 mmol) was dissolved in DMSO (3 mL). Sodium azide (2 equiv) was added and the reaction mixture was heated to 35 °C over night. The mixture was diluted with water (5 mL) and extracted with ethyl acetate (3 × 5 mL). The combined organic phases were washed with brine, dried ($MgSO_4$) and filtered. The solvent was removed in vacuo to give the product.

4.4.1. (*S*)-1-Azido-3-phenyl-2-tosylamino-propane (6a)

Pale yellow oil; Yield 65%; $[\alpha]_D^{20}$ −29.4 (*c* 1.0, $CHCl_3$); IR, ^1H and ^{13}C NMR data were in agreement with those reported.[33]

4.4.2. (*S*)-1-Azido-2-tosylamino-hexane (6b)

Transparent oil; Yield 76%; $[\alpha]_D^{20}$ −38.8 (*c* 1.0, $CHCl_3$); ^1H NMR and IR data were in agreement with those reported.[34] ^{13}C NMR δ 143.6, 137.7, 129.7, 127.0, 54.8, 53.2, 32.2, 27.4, 22.1, 21.5, 13.7.

4.4.3. (*S*)-1-Azido-3-methyl-2-tosylamino-butane (6c)

White solid; Yield 77%; Mp 78–80 °C; $[\alpha]_D^{20}$ −57.1 (*c* 1.0, $CHCl_3$); ^1H NMR δ 7.76 (d, *J* = 8.1 Hz, 2H, H2′), 7.31 (d, *J* = 8.4 Hz, 2H, H3′), 4.59 (d, *J* = 7.7 Hz, 1H, N*H*), 3.37 (dd, *J* = 4.0, 12.4 Hz, 1H, CH_AN$_3$), 3.25 (dd, *J* = 5.1, 12.4 Hz, 1H, CH_BN$_3$), 3.13–3.05 (m, 1H, C*H*NH), 2.43 (s, 3H, PhCH_3), 1.86–1.73 (m, 1H, $(CH_3)_2$C*H*), 0.82 (d, *J* = 7.0 Hz, 6H, $(CH_3)_2$CH); ^{13}C NMR δ 143.6, 137.7, 129.7, 127.0, 58.4, 52.7, 29.7, 21.5, 19.0, 18.1; IR (KBr) *v* 3261, 2962, 2094, 1461, 1314, 1162 cm^{-1}; HRMS (FT-ICR-MS) Calcd for $C_{12}H_{18}N_4O_2S$ $[M+H]^+$ 283.1223, found 283.1223.

4.5. General synthetic procedure for nitrile derivatives 7a–c

The different nitrile derivatives were synthesized as described for the azides using sodium cyanide (2 equiv) instead of sodium azide.

4.5.1. (*S*)-4-Phenyl-3-tosylamino-butane-1-nitrile (7a)

White solid; Yield 87%; Mp 90–92 °C (lit.[33] 102–103 °C); $[\alpha]_D^{20}$ −43.0 (*c* 1.0, $CHCl_3$); IR, ^1H and ^{13}C NMR data were in agreement with those reported.[33]

4.5.2. (*S*)-3-Tosylamino-heptane-1-nitrile (7b)

White solid; Yield 98%; Mp 83–86 °C (lit.[34] 73–74 °C); $[\alpha]_D^{20}$ −57.9 (*c* 1.1, $CHCl_3$); IR, ^1H and ^{13}C NMR data were in agreement with those reported.[34]

4.5.3. (*S*)-4-Methyl-3-tosylamino-pentane-1-nitrile (7c)

White solid; Yield 94%; Mp 91–95 °C; $[\alpha]_D^{20}$ −77.6 (*c* 1.0, $CHCl_3$); ^1H NMR δ 7.77 (d, *J* = 8.4 Hz, 2H, *H2*′), 7.33 (d, *J* = 8.4 Hz, 2H, *H3*′), 4.95 (d, *J* = 8.4 Hz, 1H, N*H*), 3.33–3.17 (m, 1H, C*H*NH), 2.59 (d, *J* = 5.1 Hz, 2H, CH_2CN), 2.44 (s, 3H, PhCH_3), 1.92 (oct, 1H, *J* = 6.6, Hz, $(CH_3)_2$C*H*), 0.83 (d, *J* = 7.0 Hz, 3H, CH_{3A}CH), 0.81 (d, *J* = 7.3 Hz, 3H, CH_{3B}CH); ^{13}C NMR δ 143.9, 137.0, 129.8, 127.1, 116.9, 55.5, 30.9, 22.6, 21.5, 19.1, 17.5; IR (KBr) *v* 3247, 2961, 2251 w, 1451, 1328, 1154 cm^{-1}; HRMS (FT-ICR-MS) Calcd for $C_{13}H_{18}N_2O_2S$ $[M+H]^+$ 267.1162, found 267.1164.

4.6. General synthetic procedure for aldehyde derivatives 8a–c[35]

The nitrile (0.64 mmol) was dissolved in dry THF (3 mL) under nitrogen atmosphere at −65 °C. A solution of DIBAL in toluene (1 M, 5 equiv) was added in portions over 15 min and the reaction mixture was stirred at −65 °C for 50–90 min. The excess of reagent was quenched by acetone (2 mL) at −65 °C. The mixture was syringed into an aqueous HCl solution (5%) (3 mL) at 0 °C. The phases were separated and the organic phase was washed twice with brine, dried ($MgSO_4$) and filtered. The solvents were removed in vacuo at room temperature. Purification by flash chromatography (hexane/ethyl acetate 1:1) gave the product.

4.6.1. (*S*)-4-Phenyl-3-tosylamino-1-butanal (8a)

Pale yellow oil; Yield 44%; $[\alpha]_D^{20}$ −31.8 (*c* 0.6, CH_2Cl_2) (lit.[36] $[\alpha]_D^{23}$ −18); IR, ^1H and ^{13}C NMR data were in agreement with those reported.[36]

4.6.2. (*S*)-3-Tosylamino-1-heptanal (8b)

Transparent oil; Yield 22%; $[\alpha]_D^{20}$ +26.7 (*c* 1.0 , $CHCl_3$) (lit.[37] +24.8); IR, ^1H and ^{13}C NMR data were in agreement with those reported.[37]

4.6.3. (*S*)-4-Methyl-3-tosylamino-1-pentanal (8c)

Pale yellow oil; Yield 60%; $[\alpha]_D^{20}$ +34.6 (*c* 1.0, $CHCl_3$); ^1H NMR δ 9.58 (s, 1H, C*H*O), 7.73 (d, *J* = 8.4 Hz, 2H, *H2*′), 7.29 (d, *J* = 8.4 Hz, 2H, *H3*′), 4.76 (d, *J* = 8.8 Hz, 1H, N*H*), 3.51–3.39 (m, 1H, C*H*NH), 2.64–2.48 (m, 2H, CH_2CHO), 2.42 (s, 3H, PhCH_3), 1.86–1.76 (m, 1H, $(CH_3)_2$C*H*), 0.80 (d, *J* = 7.0 Hz, 3H, CH_{3A}CH), 0.78 (d, *J* = 7.0 Hz, 3H, CH_{3B}CH); ^{13}C NMR δ 200.6, 143.5, 137.6, 129.7, 127.1, 54.6, 45.9, 31.7, 21.5, 18.7, 18.2; IR (KBr) *v* 3284, 2964, 1721, 1325, 1157 cm^{-1}; HRMS (FT-ICR-MS) Calcd for $C_{13}H_{19}NO_3S$ $[M+H]^+$ 270.1158, found 270.1159.

4.7. Synthetic procedures to lysine derivatives

4.7.1. (*S*)-Methyl 6-*tert*-butoxycarbonylamino-2-tosylamino hexanoate (13)

A solution of tosyl chloride (2.12 g, 11.1 mmol) in dichloromethane (30 mL) was added dropwise over 40 min to a mixture of H-Lys(Boc)-OMe * HCl (3.0 g; 10.1 mmol) and Et_3N (3.08 g, 30.4 mmol) in dichloromethane (45 mL) at 0 °C. The reaction mixture was stirred over night at room temperature, and then diluted with dichloromethane. The solution was washed with water (2 × 25 mL) and brine (2 × 25 mL), dried ($MgSO_4$) and filtered. The solvent was removed in vacuo. Purification by flash chromatography (hexane/ethyl acetate 5:1) gave **13** as a white solid (3.98 g, 95%). Mp 85–86 °C; $[\alpha]_D^{20}$ +21.9 (*c* 0.6, $CHCl_3$); ^1H NMR δ 7.70 (d, *J* = 8.1 Hz, 2H, *H2*′), 7.28 (d, *J* = 8.1 Hz, 2H, *H3*′), 5.21 (d, *J* = 9.2 Hz, 1H, N*H*), 4.54 (br s, 1H, CON*H*), 3.92–3.83 (m, 1H, C*H*), 3.47 (s, 3H, OCH_3), 3.13–2.97 (m, 2H, NHCH_2), 2.41 (s, 3H, PhCH_3), 1.78–1.56 (m, 2H, CH_2CH), 1.49–1.22 (m, 13H, $(CH_3)_3$; CH_2CH_2); ^{13}C NMR δ 172.1, 155.9, 143.6, 136.6, 129.6, 127.2, 79.1, 55.4, 52.4, 40.0, 32.7, 29.2, 28.3, 22.0, 21.5; IR (KBr) *v* 3383, 2930, 1737, 1692, 1531, 1343, 1167 cm^{-1}.

4.7.2. (*S*)-6-*tert*-Butoxycarbonylamino-2-tosylamino-1-hexanol (14)

A solution of **13** (1.8 g, 4.3 mmol) in THF/ethanol (2:3) (30 mL) was added to a slurry of $NaBH_4$ (0.49 g, 13.0 mmol) in THF (4 mL) at 0 °C. More $NaBH_4$ (0.1 g, 2.6 mmol) was added after 6 h and the reaction mixture was stirred at room temperature over night. The reaction was quenched by careful addition of water (3 mL) while stirring. A white precipitate was removed by filtration and the filtrate was concentrated. The residue was redissolved in dichloromethane (100 mL). Aqueous NaOH (25%) (1 mL) and water (25 mL) were added and the water phase was extracted twice with dichloromethane (20 mL). The combined organic phases were washed twice with brine, dried ($MgSO_4$) and filtered. The solvent was removed in vacuo to yield **14** as a white solid (1.58 g, 94%). Mp 159–161 °C; $[\alpha]_D^{20}$ −5.0 (*c* 1.0, MeOH); ^1H NMR δ 7.76 (d,

J = 8.1 Hz, 2H, $H2'$), 7.29 (d, J = 8.1 Hz, 2H, $H3'$), 4.90 (d, J = 7.3 Hz, 1H, SO_2NH), 4.50 (br s, 1H, CONH), 3.52–3.38 (m, 2H, NHCH_2), 3.26–3.15 (m, 1H, CH), 3.15–3.02 (m, 1H, CH_AOH), 3.02–2.91 (m, 1H, CH_BOH), 2.42 (s, 3H, PhCH_3), 1.47–1.04 (m, 6H, (CH_2)$_3$CH), 1.43 (s, 9H, (CH_3)$_3$CO); ^{13}C NMR δ 156.4, 143.5, 137.8, 129.7, 127.1, 79.4, 64.0, 55.3, 39.3, 30.8, 29.8, 28.4, 22.0, 21.5; IR (KBr) ν 3530, 3283, 2933, 1681, 1530, 1162 cm^{-1}; HRMS (FT-ICR-MS) Calcd for $C_{18}H_{30}N_2O_5S$ [M+H]$^+$ 387.1947, found 387.1944.

4.7.3. (*S*)-6-*tert*-Butoxycarbonylamino-2-tosylamino-hexyl-1-toluenesulfonate (15)

A solution of tosyl chloride (0.81 g, 7.12 mmol) in dichloromethane (10 mL) was added dropwise to a mixture of **14** (1.5 g, 3.8 mmol), pyridine (0.92 g, 19.4 mmol) and DMAP (47 mg, 0.65 mmol) in dichloromethane (30 mL) at 0 °C. The reaction mixture was stirred over night at room temperature, and then diluted with dichloromethane. The solution was washed with water and brine, dried ($MgSO_4$) and filtered. The solvent was removed in vacuo. Purification by flash chromatography (hexane/ethyl acetate 2:1) gave **15** as a transparent oil (0.78 g, 37%). $[\alpha]_D^{20}$ −31.4 (c 1.0, $CHCl_3$); ^{1}H NMR δ 7.72 (d, J = 8.4 Hz, 2H, $H2'$), 7.69 (d, J = 8.1 Hz, 2H, $H2$), 7.34 (d, J = 8.4 Hz, 2H, $H3'$), 7.27–7.24 (m, 2H, $H3$), 5.08 (d, J = 7.7 Hz, 1H, SO_2NH), 4.50 (br s, 1H, CONH), 3.96 (dd, J = 3.3, 9.9 Hz, 1H, CH_AO), 3.80 (dd, J = 5.1, 9.9 Hz, 1H, CH_BO), 3.39–3.27 (m, 1H, CH), 2.99–2.88 (m, 2H, NHCH_2), 2.45 (s, 3H, PhCH_3), 2.42 (s, 3H, PhCH_3), 1.43 (s, 9H, (CH_3)$_3$CO), 1.34–0.94 (m, 6H, (CH_2)$_3$CH); ^{13}C NMR δ 156.2, 145.2, 143.6, 137.3, 132.2, 130.0, 129.7, 128.0, 127.1, 79.4, 71.1, 52.2, 30.8, 29.5, 28.4, 21.9, 21.7, 21.5; IR (KBr) ν 3290, 2870, 1692, 1359, 1167 cm^{-1}; HRMS (FT-ICR-MS) Calcd for $C_{25}H_{36}N_2O_7S_2$ [M+H]$^+$ 541.2035, found 541.2025.

4.7.4. (*S*)-1-Azido-6-*tert*-butoxycarbonylamino-2-tosylamino-hexane (16)

Sodium azide (55 mg, 0.85 mmol) was added to a solution of **15** (230 mg, 0.42 mmol) in DMSO (5 mL) and the reaction mixture was stirred at room temperature over night. The mixture was diluted with water (12 mL) and extracted with ethyl acetate (3 × 7 mL). The combined organic phases were washed with brine (4 × 7 mL), dried ($MgSO_4$) and filtered. The solvent was removed in vacuo to give **16** as a white solid (0.17 g, 97%). Mp 74–77 °C; $[\alpha]_D^{20}$ −28.8 (c 0.22, $CHCl_3$); ^{1}H NMR δ 7.76 (d, J = 8.1 Hz, 2H, $H2'$), 7.31 (d, J = 7.7 Hz, 2H, $H3'$), 4.83 (br s, 1H, SO_2NH), 4.49 (br s, 1H, CONH), 3.36–3.22 (m, 3H, CH, CH_2N$_3$), 3.05–2.95 (m, 2H, NHCH_2), 2.44 (s, 3H, PhCH_3), 1.44 (s, 9H, (CH_3)$_3$CO), 1.40–1.06 (m, 6H, (CH_2)$_3$CH); ^{13}C NMR δ 156.1, 143.6, 137.7, 129.7, 127.0, 79.3, 54.8, 53.0, 39.8, 31.9, 29.6, 28.4, 22.3, 21.5; IR (KBr) ν 3368, 3286, 2938, 2094, 1681, 1527, 1316, 1159 cm^{-1}; HRMS (FT-ICR-MS) Calcd for $C_{18}H_{29}N_5O_4S$ [M+H]$^+$ 412.2011, found 412.2010.

4.7.5. (*S*)-7-*tert*-Butoxycarbonylamino-3-tosylamino-heptane-1-nitrile (17)

Sodium cyanide (91 mg, 1.85 mmol) was added to a solution of **15** (0.5 g, 0.93 mmol) in DMSO (13 mL) and the reaction mixture was heated to 35 °C over night. The mixture was diluted with water (20 mL) and extracted with ethyl acetate (3 × 15 mL). The combined organic phases were washed with brine (3 × 10 mL), dried ($MgSO_4$) and filtered. The solvent was removed in vacuo to give **17** as a transparent oil (0.34 g, 93%). No further purification was needed. $[\alpha]_D^{20}$ −39.0 (c 0.5, $CHCl_3$); ^{1}H NMR δ 7.76 (d, J = 8.4 Hz, 2H, $H2'$), 7.33 (d, J = 8.4 Hz, 2H, $H3'$), 5.22 (d, J = 7.0 Hz, 1H, SO_2NH), 4.51 (br s, 1H, CONH), 3.53–3.33 (m, 1H, CH), 3.10–2.93 (m, 2H, NHCH_2), 2.69–2.53 (m, 2H, CH_2CN), 2.44 (s, 3H, PhCH_3), 1.44 (s, 9H, (CH_3)$_3$CO), 1.71–1.02 (m, 6H, (CH_2)$_3$CH); ^{13}C NMR δ 156.4, 143.9, 137.0, 129.8, 127.0, 116.9, 79.5, 49.9, 39.4, 32.8, 29.4, 28.4, 24.8, 21.9, 21.5; IR (neat) ν 3280, 2924, 2251, 1696, 1517, 1452, 1329, 1159 cm^{-1}; HRMS (FT-ICR-MS) Calcd for $C_{19}H_{29}N_3O_4S$ [M+H]$^+$ 396.1951, found 396.1954.

4.7.6. (*S*)-7-*tert*-Butoxycarbonylamino-3-tosylamino-1-heptanal (18)

A solution of **17** (0.3 g, 0.79 mmol) in dry THF (4 mL) under nitrogen atmosphere was cooled to −65 °C. A solution of DIBAL in toluene (1 M) (4 mL, 3.95 mmol) was added in portions over 15 min and the reaction mixture was stirred at −65 °C for an additional 30 min. Excess of reagent was quenched by addition of acetone (1.5 mL) and the mixture was syringed into an aqueous HCl solution (5%) (4 mL) at 0 °C. The phases were separated and the organic phase was washed twice with brine, dried ($MgSO_4$) and filtered. The solvent was removed in vacuo at room temperature. Purification by flash chromatography (hexane/ethyl acetate 1:1) gave **18** as a transparent oil (80 mg, 26%). $[\alpha]_D^{20}$ −10.6 (c 1.0, $CHCl_3$); ^{1}H NMR δ 9.62 (s, 1H, CHO), 7.74 (d, J = 8.1 Hz, 2H, $H2'$), 7.30 (d, J = 8.1 Hz, 2H, $H3'$), 5.08 (br s, 1H, SO_2NH), 4.47 (br s, 1H, CONH), 3.63–3.51 (m, 1H, CH), 3.05–2.90 (m, 2H, NHCH_2), 2.70–2.52 (m, 2H, CH_2CHO), 2.43 (s, 3H, PhCH_3), 1.44 (s, 9H, (CH_3)$_3$CO), 1.38–1.02 (m, 6H, (CH_2)$_3$CH); ^{13}C NMR δ 200.7, 156.2, 143.7, 137.8, 129.8, 127.2, 49.4, 48.7, 39.9, 34.3, 29.5, 28.5, 22.7, 21.6; IR (neat) ν 3279, 2931, 1691, 1519, 1327, 1161 cm^{-1}; HRMS (FT-ICR-MS) Calcd for $C_{19}H_{30}N_2O_5S$ [M+H]$^+$ 399.1947, found 399.1947.

4.8. Synthetic procedures for Boc-deprotection

4.8.1. (*S*)-6-Amino-2-tosylamino-1-hexanol*TFA (19)

TFA (0.2 mL, 2.7 mmol) was added to a solution of **14** (80 mg, 0.19 mmol) in dichloromethane (3 mL). The mixture was stirred at room temperature for 1 h. Excess of reagent and solvent were removed in vacuo to give **19** as a transparent oil (60 mg, 78%). $[\alpha]_D^{20}$ +9.0 (c 1.1, MeOH); ^{1}H NMR (CD_3OD) δ 7.74 (dd, J = 8.1, 8.4 Hz, 2H, $H2'$), 7.36 (d, J = 8.1 Hz, 2H, $H3'$), 4.23–4.10 (m, 1H, CH), 3.35–3.14 (m, 2H, NH_3CH_2), 2.86–2.75 (m, 2H, CH_2OH), 2.42 (s, 3H, CH_3), 1.66–1.20 (m, 6H, (CH_2)$_3$CH); ^{13}C NMR (CD_3OD) δ 144.6, 140.2, 130.68, 128.0, 65.0, 56.2, 40.5, 32.0, 28.2, 23.5, 21.4; IR (neat) ν 3258 (broad), 2921, 1683, 1430, 1319, 1156 cm^{-1}; HRMS (FT-ICR-MS) Calcd for $C_{13}H_{22}N_2O_3S$ [M+H]$^+$ 287.1423, found 287.1411.

4.8.2. (*S*)-6-Azido-5-tosylamino-hexylamine*TFA (20)

TFA (0.2 mL, 2.7 mmol) was added to a solution of **16** (150 mg, 0.36 mmol) in dichloromethane (3 mL). The mixture was stirred at room temperature for 90 min. Excess of reagent and solvent were removed in vacuo to give **20** as a pale yellow oil (124 mg, 80%). $[\alpha]_D^{20}$ −5.0 (c 1.0, MeOH); ^{1}H NMR (CD_3OD) δ 7.76 (d, J = 8.4 Hz, 2H, $H2'$), 7.38 (d, J = 8.8 Hz, 2H, $H3'$), 3.36–3-27 (m, 1H, CH), 3.19 (dd, J = 5.1, 12.4 Hz, 1H, CH_AN$_3$), 3.14 (dd, J = 5.1, 12.4 Hz, 1H, CH_BN$_3$), 2.82 (t, J = 7.7 Hz, 2H, NH_3CH_2), 2.43 (s, 3H, CH_3), 1.62–1.22 (m, 6H, (CH_2)$_3$CH); ^{13}C NMR (CD_3OD) δ 144.7, 140.1, 130.8, 128.0, 56.0, 54.3, 40.6, 33.2, 28.2, 23.5, 21.4; IR (neat) ν 3169 (broad), 2930, 2105, 1679, 1526, 1433, 1319, 1152 cm^{-1}; HRMS (FT-ICR-MS) Calcd for $C_{13}H_{21}N_5O_2S$ [M+H]$^+$ 312.1488, found 312.1480.

4.8.3. (*S*)-7-Amino-3-tosylamino-heptane-1-nitrile*TFA (21)

TFA (0.2 mL, 2.7 mmol) was added to a solution of **17** (130 mg, 0.33 mmol) in dichloromethane (3 mL). The mixture was stirred at room temperature for 1 h. Excess of reagent and solvent were removed in vacuo to give **21** as a pale yellow oil (130 mg, 93%). $[\alpha]_D^{20}$ −14.0 (c 1.0, MeOH); ^{1}H NMR (CD_3OD) δ 7.76 (d, J = 8.1 Hz, 2H, $H2'$), 7.38 (d, J = 8.4 Hz, 2H, $H3'$), 3.50–3.40 (m, 1H, CH), 2.78 (t, J = 7.3 Hz, 2H, NH_3CH_2), 2.40–2.61 (m, 2H, CH_2CN), 2.42 (s, 3H, CH_3), 1.64–1.45 (m, 4H, CH_2CH_2), 1.39–1.14 (m, 2H, CH_2CH); ^{13}C NMR (CD_3OD) δ 145.0, 139.8, 130.9, 128.0, 118.3, 51.1, 40.4, 34.4,

27.9, 25.2, 23.4, 21.4; IR (neat) ν 3173 (broad), 2920, 2254, 1674, 1531, 1428, 1326, 1154 cm^{-1}; HRMS (FT-ICR-MS) Calcd for $C_{14}H_{21}N_3O_2S$ $[M+H]^+$ 296.1426, found 296.1414.

4.9. Azide stability test

Azide **6c** (10 mg) was dissolved in acetonitrile (0.5 mL) and either phosphate buffer (0.5 mL) (pH 7.4) or ammonium acetate buffer (pH 6.9) and samples were exposed to ambient laboratory light. Aliquots (0.1 mL) were taken out at $t = 0$, 2, 4, 6, 8, 24, 48 h, analyzed twice directly or diluted with ammonium acetate buffer/acetonitrile (1:1) (0.9 mL) and analyzed in duplicate on analytical HPLC.

4.10. IdeS inhibition assay

IdeS was produced in *E. coli* and purified as previously described.[2] IdeS (2 μL; 1 mg/mL) diluted in phosphate buffered saline (PBS) (pH 7.4) was incubated with the test compounds dissolved in DMSO (10 mg/mL; 100× excess) for 30 min at room temperature in the dark. Human polyclonal IgG (5 μL, 1 mg/mL) in PBS was added, followed by incubation at 37 °C for 1 h. The samples were boiled with an equal volume of SDS–PAGE sample buffer containing 4% SDS and 5% 2-mercaptoethanol, and subjected to SDS–PAGE (4–20% Precise Gels from Pierce). Gels were stained with Coomassie Blue.

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