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Stereoselective Synthesis and Antibacterial Evaluation of 4-Amido-isothiazolidinone Oxides

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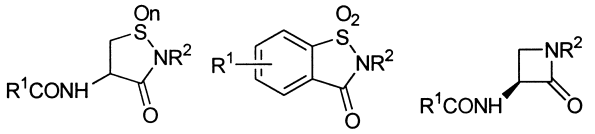
Abstract—Two well-defined oxidative chlorination–cyclization processes have been developed for the stereoselective synthesis of a variety of 4-amido-isothiazolidinone oxide derivatives. The stereochemistry of the cyclization products was confirmed by X-ray crystallography. These new compounds were designed as bacterial serine protease inhibitors. In tests, some of them showed weak antibacterial activity.

Several classes of serine proteases are covalently inhibited by β-lactam compounds.[1] Penicillin binding proteins (PBPs) are bacterial serine transpeptidases/carboxypeptidases involved in bacterial cell wall synthesis. Inhibition of PBPs by β-lactams produces the antibiotic action of these drugs. Bacterial β-lactamases hydrolyze a variety of β-lactam antibiotics, but can be inhibited with appropriately designed β-lactam derivatives. Human leukocyte elastase (HLE), on the other hand, is a mammalian serine protease known to be inhibited by various β-lactam compounds, such as cephalosporins.[2] The inhibition of these enzymes by β-lactams involves acylation of the active site serine residue of protein by the β-lactam amide.

This common mechanism of protease inhibition suggests a general strategy for drug design involving the selective delivery of a reactive warhead to react with the catalytic serine of the target enzyme by exploiting its unique structural, mechanistic, and substrate preferences. In an effort to develop a new class of antibacterial agent, we designed 4-amido-isothiazolidinone oxides **I** as potential PBP inhibitors. We based our design on known inhibitors of HLE, including various derivatives of saccharin **II** (benzisothiazolidinone).[3] Their inhibition mechanism involves covalent acylation of HLE by the activated amide carbonyl of the benzisothiazolidinone derivatives. The new isothiazolidinone targets **I** incorporate 4-amido and R^2 groups designed to resemble the corresponding substituents in known PBP inhibitors, such as monocyclic β-lactams **III**. These substituents should impart the structural specificity required by bacterial PBPs. In this communication, we report new stereoselective synthetic methods to compounds **I** and their biological testing results.



I **II** **III**

There are only few literature reports describing the synthesis of 4-amino-isothiazolidinone derivatives: a cycloaddition protocol[4] and a halogenation–cyclization strategy starting from cystine derivatives.[5] Although this latter strategy was ultimately useful for us, the reported halogenation–cyclization procedures were initially not reproducible in our hands. We speculated that our problems with these procedures stemmed from the absence of water when the halogenation reactions were run in halogenated solvents, or from insufficient solubility of the substrates when the reactions were performed in water or acetic acid. Based on the reaction's mechanism, we developed a well-defined halogenation–cyclization process. Thus, reaction of L-cystine ester **1** with 7–8 equiv Cl_2 and 4 equiv H_2O in ethyl acetate at −78 °C provided sulfonyl chloride **2**, which upon exposure to saturated ammonium hydroxide, formed sulfonamide **3** in 57% yield from **1**. The extra 2 equiv Cl_2 was consumed in the undesired, but inconsequential, chlorination of the activated phenoxy group. Efficient

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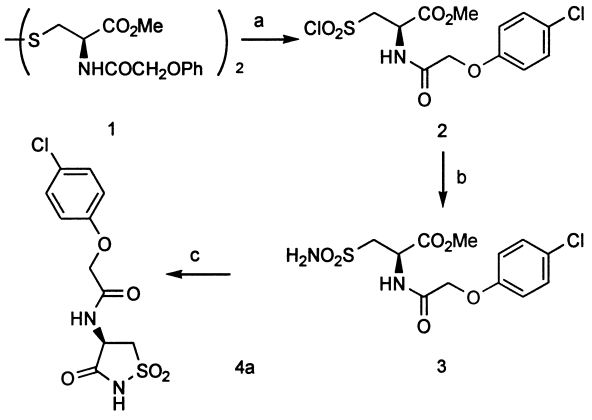
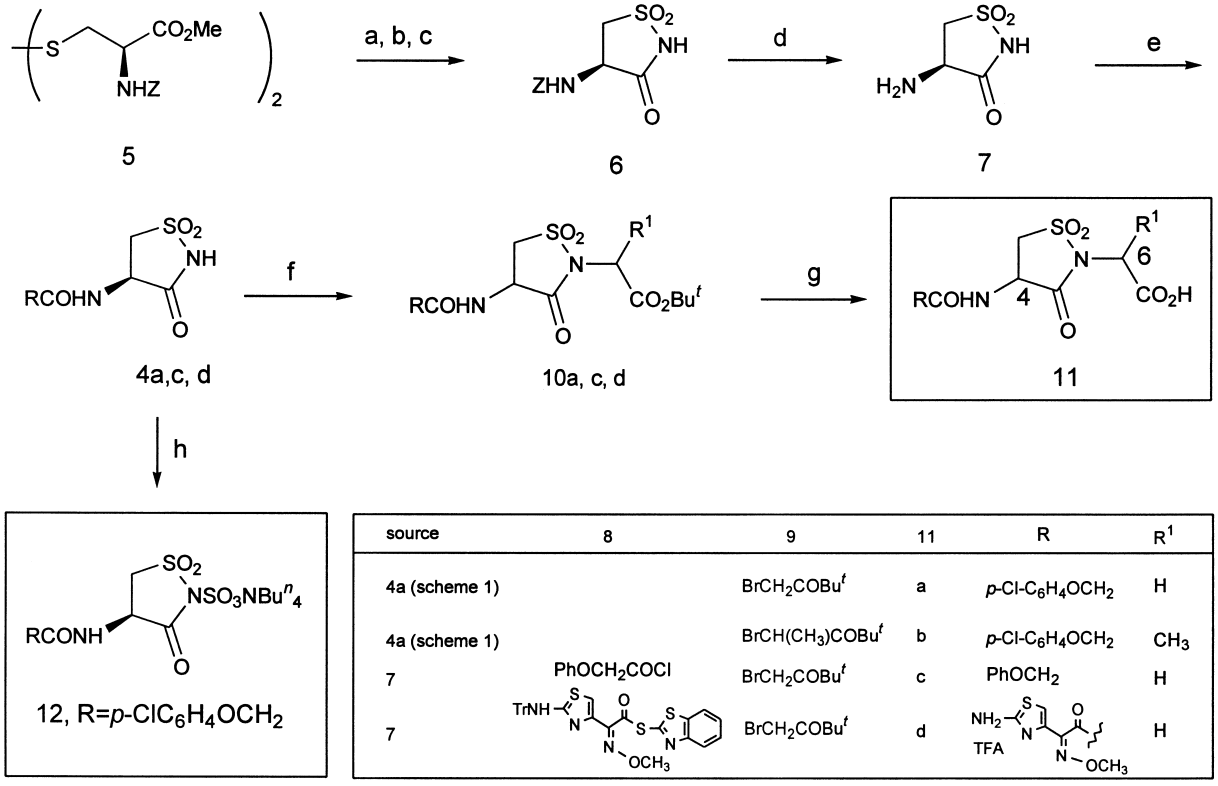
cyclization of sulfonamide **3** to isothiazolidinone **4a** was effected by treatment with sodium methoxide followed by acidification with Dowex 50WX2-100 resin (Scheme 1).

Following the same sequence, an *N*-benzyloxycarbonyl (Z) protected isothiazolidinone dioxide **6** was prepared from L-Z-cystine ester **5** (Scheme 2). Deprotection via hydrogenolysis provided the parent isothiazolidinone dioxide **7**.[6] Acylation of the 4-amino group, followed by alkylation at the sulfonamido position, and acidic deprotection, formed isothiazolidinone carboxylic acids **11**[7] as stereoisomeric mixtures (60/40 dr for **11b** and 3% ee for **11c**) at C-4 and at C-6 as determined by ^{1}H NMR and chiral HPLC. The stereoisomerization probably occurred during the alkylation step. In addition, dioxide **4a** was also converted to sulfamic acid salt **12** following a known method for monocyclic β-lactams **II**.[8]



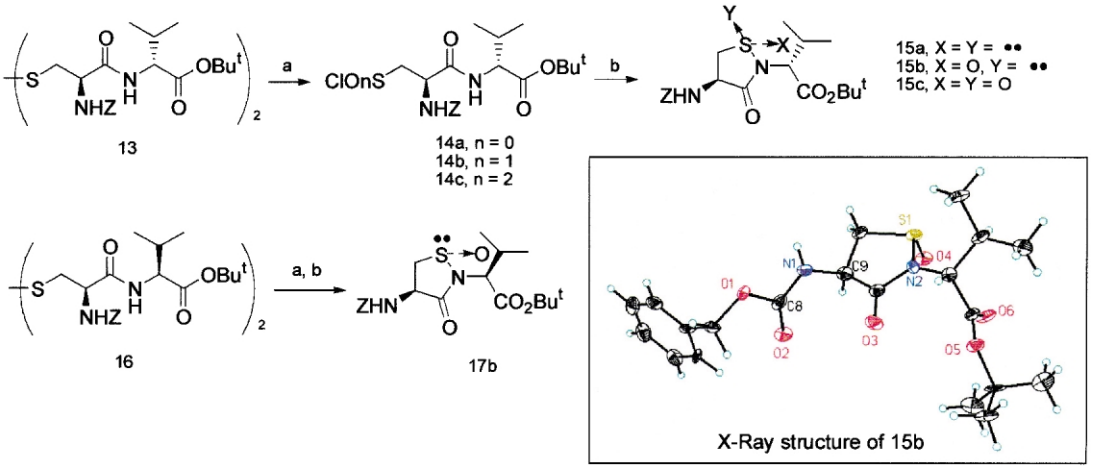
Scheme 1. (a) 7–8 equiv Cl_2, 4 equiv H_2O, EtOAc, −78 to 0 °C; (b) NH_4OH, 67% from **1**; (c) NaOMe, then Dowex resin, 100%.

The above oxidative chlorination–cyclization process involved amide formation in the cyclization step. A more general strategy for the chlorination–cyclization process involved in situ sulfonamide formation to effect the cyclization and provided *N*-alkylated isothiazolidinone oxides in one pot. When dipeptide **13**, prepared from L-Z-cystine and D-valine-OBu^t, was titrated with the respective amount of chlorine and water, as indicated in Scheme 3, sulfenyl chloride **14a**, sulfinyl chloride **14b**, or sulfonyl chloride **14c** was formed selectively and cleanly as observed by ^{1}H NMR. Upon addition of pyridine, isothiazolidinone **15a** was formed from **14a** in low yield; and the isothiazolidinone mono-oxide **15b**[9] was formed from **14b** in 56% yield with >95/5 dr. The formation of isothiazolidinone dioxide **15c** from sulfonyl chloride **14c** on treatment with pyridine was not observed, presumably due to steric hindrance. Following a similar process, diastereomeric isothiazolidinone mono-oxide **17b** was obtained in 62% yield as a single stereoisomer from dipeptide **16**, an epimer of dipeptide **13**. Both **15b** and **17b** had the *S*-configuration on sulfur as determined by X-ray crystallography analysis of **15b** (Scheme 3) and a derivative of **17b** (vide infra). The observed stereoselectivity seemed to be controlled primarily by the Z-protected 4-amino group, which may prefer to be oriented *trans* to the oxygen of the evolving sulfinamide group, thus avoiding a 1,3 interaction during the cyclization step.



source	8	9	11	R	R^1
4a (scheme 1)		$BrCH_2COBu^t$	a	*p*-Cl-$C_6H_4OCH_2$	H
4a (scheme 1)		$BrCH(CH_3)COBu^t$	b	*p*-Cl-$C_6H_4OCH_2$	CH_3
7	$PhOCH_2COCl$	$BrCH_2COBu^t$	c	$PhOCH_2$	H
7		$BrCH_2COBu^t$	d		H

Scheme 2. (a), (b), (c) as in Scheme 1 48–65% from **5**; (d) Pd–C, H_2, MeOH, 57–71%; (e) **8**, Py, DMF, 0 °C to rt, 43–60%; (f) **9**, *i*-Pr_2NEt, DMF, rt or 90 °C, 39–63%; (g) TFA, 48–83%; (h) (1) NaH; (2) SO_3Py, *n*-Bu_4NHSO_4, 45%.

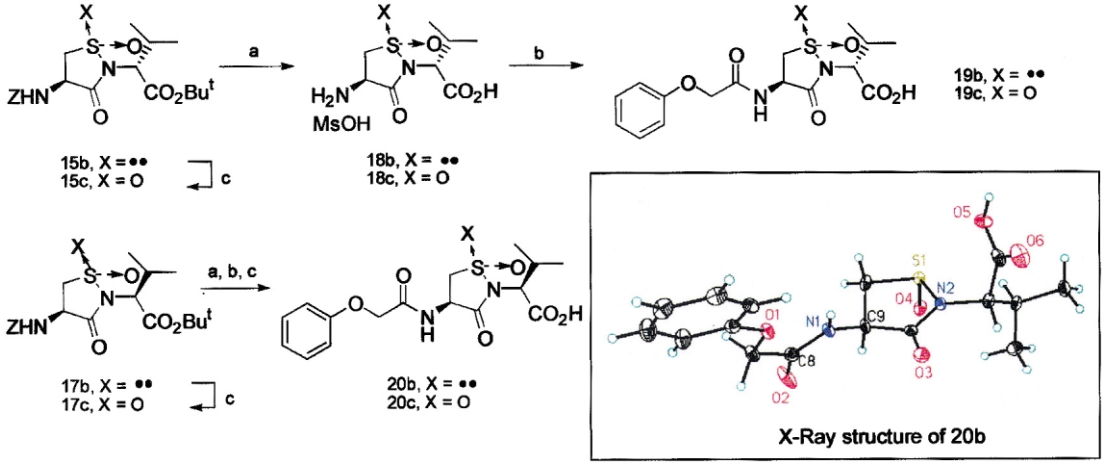


Scheme 3. (a) EtOAc, −78 to 0 °C, 1–3 equiv Cl_2 for **14a**; 3.5 equiv Cl_2, 2 equiv H_2O for **14b**; 7–8 equiv Cl_2, 4 equiv H_2O for **14c**; (b) Py, −78 °C to rt, 56%, dr >95/5 for **15b**, 62%, dr 100/0 for **17b**.

A mild, one-pot procedure was developed to transform intermediates **15b** or **17b** into isothiazolidinone acids **19b,c** or **20b,c** as shown in Scheme 4. Both the Z and the BOC groups of compound **15b** were removed successfully by treatment with a 1:5 mixture of methanesulfonic acid and CH_2Cl_2. This new method removed the protecting groups cleanly without ring-opening side reactions.[10] The amine, generated from amine salt **18b**, was acylated in the presence of 2-6-di-*tert*-butylpyridine to produce acid **19b** after aqueous workup. Partial epimerization at the carbon α to the carboxylic acid was observed when pyridine was used in the reaction. Oxidation of compound **15b** with MCPBA formed dioxide **15c**. Following the same reaction sequences, compounds **15c** and **17b** were converted to the corresponding acids **19c**[11] and **20b,c** in 61–92% overall yield. Also shown in Scheme 4 is the X-ray structure of mono-oxide **20b**.

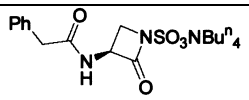
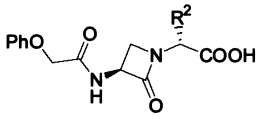
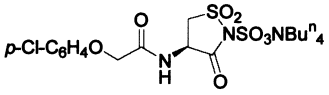
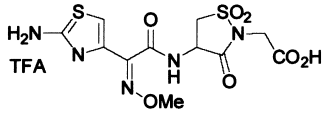
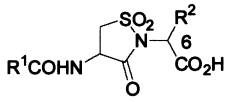
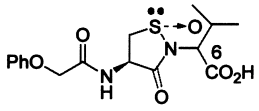
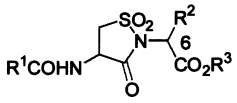
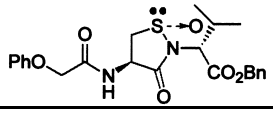
The prepared isothiazolidinone oxides were evaluated for in vitro antibacterial activity. Table 1 summarizes the range of minimum inhibition concentration (MIC) against multiple strains of bacteria[12] of these new compounds and three reference monocyclic β-lactams **21**–**23**.[8,13] With the exception of compound **19c**, no antibacterial activity was observed for the isothiazolidinone acids, whereas antibacterial activity was observed for the reference β-lactams. Weak antibacterial activity, however, was observed for acid **19c** and all isothiazolidinone esters (**24**–**26**[14] and **15b**), mostly against Gram-negative bacteria (*Enterobacter cloacae, Moraxella catarrhalis*, and/or *Klebsiella pneumoniae*). In a standard PBP binding assay,[15] all new compounds exibited IC_{50} values of >250 μg/mL for PBP1, 2 or 3 from *Staphylococcus aureus*. These compounds, especially the mono-oxides **19b** and **20b**, had much shorter half lives (see last column of Table 1) than their β-lactam counterparts in pH 7.4 phosphate buffer. The chemical instability could potentially contribute to the poor antibacterial activity observed.

In summary, we have developed stereoselective methods for the preparation of a class of 4-amido-isothiazolidinone oxides through two well defined oxidative chlorination–cyclization processes. Some of these



Scheme 4. (a) CH_3SO_3H/CH_2Cl_2 (1:5), anisole; (b) (1) $CF_3CON(CH_3)TMS$, CH_2Cl_2; (2) $PhOCH_2COCl$, 2,6-di-*tert*-Bu-Py, 0 °C, 61–92%; (c) MCPBA, $NaHCO_3$, CH_2Cl_2, 88%.

Table 1. Antibacterial activity and stability of isothiazolidinone oxides

Compd	Structure				MIC range (μg/mL)[a]	$t_{1/2}$ (h)[b]
	Core	R^1	R^2	R^3		
21[8]		—	—	—	1.6–>100	NT
22[13]		—	Pr^i	—	2–>32	>48
23[13]		—	H	—	62.5–>1000	>32
12		—	—	—	>64	<0.4
11d		—	—	—	>64	5.6
11c		$PhOCH_2$	H	—	>100	11.9
11b		*p*-Cl-C_6H_4O	CH_3	—	125–>1000	15.7
19c(6*R*)		$PhOCH_2$	Pr^i	—	16–>1000	22
19b(6*R*)		—	—	—	>1000	<1.4
20b(6*S*)		—	—	—	>1000	<3.3
24[14]		*p*-Cl-C_6H_4O	H	Bn	15.6–>1000	5.1
25		*p*-Cl-C_6H_4O	CH_3	Bu^t	31.3–>1000	NT
15b(6*R*)		$PhCH_2O$	Pr^i	Bu^t	31.3–>1000	15.8
26[14]		—	—	—	62.6–>1000	<0.5

[a]Determined for 24 strains of Gram+ and Gram− bacteria.[12] Low end MIC of β-lactams **21**–**23** is for *Staphylococcus aureus*, *Klebsiella pneumoniae* and/or *Streptococcus pneumoniae*, and of the active isothiazolidinone oxides is for *Enterobacter cloacae, Moraxella catarrhalis* and/or *Klebsiella pneumoniae*.
[b]Measured in pH 7.4 phosphate buffer; NT, not tested.

new compounds exhibited weak antibacterial activity, but are not PBP inhibitors.

Acknowledgements

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References and Notes

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6. Compound **7**: mp 228 °C (dec.); $[\alpha]_D^{20}$ +1° (*c* 0.65, CH_3OH/H_2O 1:1); ^1H NMR (CD_3OD, 300 MHz) δ 3.12 (dd, *J* = 13.0, 8.0 Hz, 1H), 3.66 (dd, *J* = 13.0, 8.0 Hz, 1H), 4.07 (dd, *J* = 8.0, 8.0 Hz, 1H); ^{13}C NMR (CD_3OD, 75 MHz) δ 57.27, 57.90,

182.19; MS *m/z* (ESI, negative): 149 ($M-H^+$); FTIR (cm^{-1}, KBr pellet): 3400 (br.), 1634 (br.), 972. When reacted with (*R*)-phenethylisocyanate, **7** formed only one urea diastereomer (by ^1H NMR and HPLC).

7. Alternatively, **11** was prepared from **6** via the following sequence: alkylation with bromide **9**, removal of the Z group using CH_3SO_3H/CH_2Cl_2 (1:5) as in Scheme 4, and acylation with compound **8** followed by acidic deprotection.

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9. Compound **15b**: to disulfide **13** (5.328 g, 6.505 mmol) in anhydrous ethyl acetate (65 mL) and water (0.234 mL, 13.0 mmol) at −78 °C was added chlorine in carbon tetrachloride (0.825 M in CCl_4, 27.6 mL, 22.8 mmol). The mixture was stirred at 0 °C for 30 min. At −78 °C pyridine (4.2 mL, 52.0 mmol) was added. The resulting white slurry was stirred at 0 °C for 15 min and at room temperature for 18 h, filtered, washed with ethyl acetate twice. The filtrate was concentrated, and the residue was purified via silica gel chromatography (30% and 40% EtOAc in hexane) to provide **15b** as a white solid (3.018 g, 54.7%): mp 115–116 °C; $[\alpha]_D^{20}$ +131° (*c* 0.49, CH_2Cl_2); ^1H NMR ($CDCl_3$, 300 MHz) δ 0.92 (d, *J* = 6.5 Hz, 3H), 1.06 (d, *J* = 6.5 Hz, 3H), 1.52 (s, 9H), 2.35 (m, 1H), 3.34 (m, 1H), 3.58 (dd, *J* = 12.5, 6.5 Hz, 1H), 4.51 (d, *J* = 9.0 Hz, 1H), 4.98 (m, 1H), 5.15 (s, 2H), 5.46 (m, 1H), 7.30–7.40 (m, 5H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 19.21, 19.29, 27.82, 30.50, 44.92, 49.82, 53.31, 57.31, 63.57, 67.44, 83.12, 128.19, 128.40, 128.60, 135.64, 155.52, 167.85, 173.35; MS *m/z* (ESI, positive) 447 ($M+Na^+$), 442 ($M+NH^+$), 425 ($M+H^+$), 369. Anal. calcd for $C_{20}H_{28}N_2O_6S$: C, 56.59; H, 6.65; N, 6.60. Found: C, 56.7; H, 6.63; N, 6.46; FTIR (cm^{-1}, KBr pellet): 3283, 1737, 1712, 1554, 1146, 1090. For a successful reaction, it is essential to control the amounts of water and chlorine and to convert the relatively less stable sulfonyl chloride **14b** directly, without isolation, to the mono-oxide **15b**.

10. The use of TfOH (Yajima, H.; Fuji, N.; Ogawa, H.; Kawatani, H. *J. Chem. Soc., Chem. Commun*. **1974**, 107) resulted in ring-opening products.

11. Compound **19c**: To dioxide **15c** (400 mg, 0.908 mmol) in anhydrous dichloromethane (6.0 mL) and anisole (0.197 mL, 1.82 mmol) was added methanesulfonic acid (1.18 mL, 18.2 mmol). The pink solution was stirred at room temperature for 18 h. Anhydrous diethyl ether (60 mL) was added and the mixture was stirred for 10 min. The resulting white slurry was filtered and the residue was washed with a 1/10 mixture of dichloromethane and ether (16.5 mL) twice and dried in vacuo. To the resulting white powder was added anhydrous dichloromethane (12 mL) and *N*-methyl-*N*-(trimethylsilyl)trifluoroacetamide (0.673 mL, 3.63 mmol). The mixture was stirred for 15 min and then cooled to 0 °C, treated with 2,6-di-*tert*-butylpyridine (0.254 mL, 1.09 mmol), and phenoxyacetyl chloride (0.125 mL, 0.908 mmol). The mixture was stirred at 0 °C for 2 h and concentrated. The resulting residue was treated at 0 °C with 0.1 N $NaHCO_3$ (100 mL) and brine (20 mL), and was extracted with ethyl acetate three times. The aqueous solution was further treated at 0 °C with 1 N HCl (ca. 10 mL until aqueous pH 2–3), and was extracted again with ethyl acetate three times. The combined organic extract from the second extraction was washed with brine two times and dried over anhydrous Na_2SO_4. Removal of the organic solvent via rotary evaporation provided compound **19c** as a white solid (321 mg, 92% yield). Mp 63–65 °C; $[\alpha]_D^{20}$ +71° (*c* 0.47, CH_2Cl_2); ^1H NMR ($CDCl_3$, 300 MHz) δ 0.97 (d, *J* = 7.0 Hz, 3H), 1.14 (d, *J* = 7.0 Hz, 3H), 2.74 (m, 1H), 3.86 (dd, *J* = 10.5, 12.5 Hz, 1H), 4.16 (d, *J* = 8.5 Hz, 1H), 4.23 (m, 1H), 4.59 (m, 2H), 5.24 (m, 1H), 6.94–7.09 (m, 3H), 7.30–7.37 (m, 2H), 7.84 (br. d, 1H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 19.91, 20.91, 27.69, 49.13, 51.91, 60.40, 67.12, 115.04, 122.58, 130.04, 157.14, 166.24, 170.61, 172.16; MS *m/z* (ESI, positive): 402 ($M+NH^+$), 4385 ($M+H^+$); HR-MS: Anal. calcd for $C_{16}H_{21}N_2O_7S$: 385.1069. Found: 385.1073; FTIR (cm^{-1}, KBr pellet): 3370, 1752, 1673, 1535, 1496, 1350, 1241, 1168.

12. National Committee for Clinical Laboratory Standards. *Method for Dilution Antimicrobial Susceptibility Test for Bacteria that Grow Aerobically*. DOC. M7-A4, 1997.

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14. Compound **24** prepared in 25% from **11a** with $BrCH_2CO_2Bn$/Hunig's base/DMF; compound **26** prepared in 29% from **19b** with BnOH/WSC/DMF.

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