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Preparation and separation of antimicrobial agents derived from capreomycin

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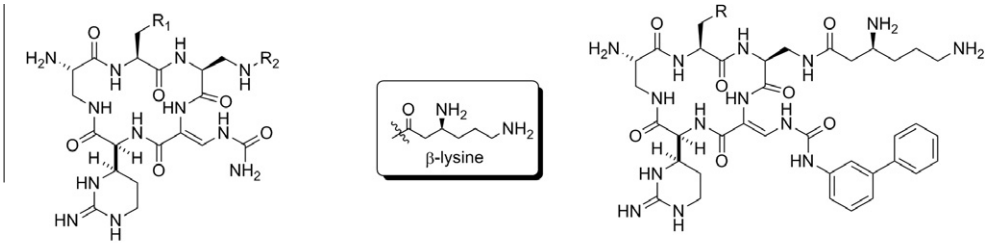
ABSTRACT

A practical method for the preparation of single component biaryl-urea analogs of capreomycin is described. The protocol involves derivatization of capreomycin followed by global protection of the capreomycin analog mixture. The individual components were separated using simple flash column chromatography followed by deprotection to give the desired analogs with high purity.

Various analogs of the capreomycins have been prepared semi-synthetically and have shown unique antimicrobial activity.[1] Capreomycin, isolated by Herr and co-workers at Lilly[2] from fermentations of *Streptomyces capreolus*, is a mixture of four components; capreomycins IA (**1a**) and IB (**1b**) comprise ~90% of the mixture and capreomycins IIA (**1c**) and IIB (**1d**) account for ~10% of the mixture (Fig. 1). We were interested in preparing biaryl-urea analogs of capreomycin which exhibited potent *in vitro* activity against MRSA (Methicillin-Resistant *Staphylococcus aureus*) and other Gram positive bacteria. Utilizing the previously reported ureido exchange reaction,[3] our colleagues had determined that a mixture comprised primarily of the two analogs shown in Figure 1, AR380559 (**2a**) and AR380547 (**2b**), displayed potent in vitro activity against MRSA as well as other Gram positive bacteria.[4]

Although the ureido exchange reaction proceeds quite cleanly giving a similar ratio of capreomycin analogs relative to the ratio of constituents present in the starting capreomycin sulfate (**1a–d**), we required a scalable semi-synthetic process that would provide the single components in high purity. To achieve this goal we either needed to purify the initial capreomycin sulfate mixture to give one individual starting material or develop a method to purify the product mixture. The isolated product mixture from the ureido exchange reaction (primarily comprised of **2a** and **2b**) exhibits similar physical properties to capreomycin sulfate, namely, it is an amorphous powder with low organic solubility. After encountering significant issues developing an analytical HPLC method to separate **2a** and **2b** it was determined that preparative HPLC was not viable. Furthermore, simple flash chromatography of either the initial capreomycin sulfate mixture (**1a–d**) or the biaryl-urea mixture was not pragmatic due to the similar retention times observed for the individual components. In turn, we looked to modify the product mixture using protecting groups to give favorable



Components of capreomycin sulfate
1a: capreomycin IA; R_1 = OH, R_2 = β-lysine
1b: capreomycin IB; R_1 = H, R_2 = β-lysine
1c: capreomycin IIA; R_1 = OH, R_2 = H
1d: capreomycin IIB; R_1 = H, R_2 = H

Major (>90%) components of analog mixture
2a: AR380559; R = OH
2b: AR380547; R = H

Figure 1. Capreomycin constituents and related analogs.

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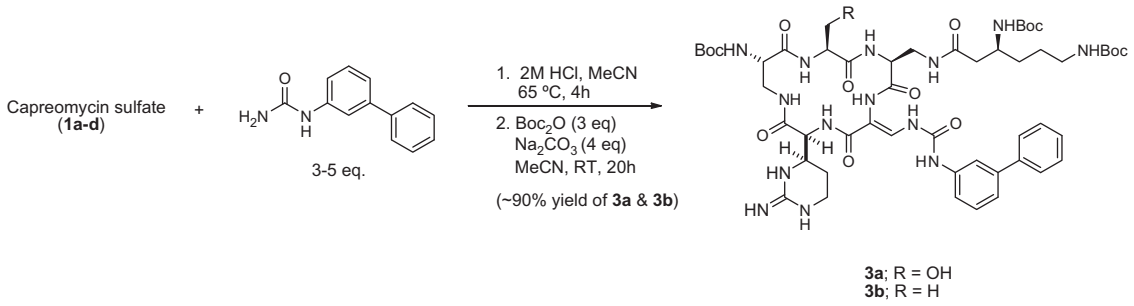


Scheme 1. Ureido exchange reaction and Boc protection.

physical properties that would allow us to separate the mixture of **2a** and **2b**. The most obvious solution was to exploit the serine handle of **2a** as a means of separation, via either crystallization, solid-phase capture of the hydroxyl moiety or chromatography.

Conversion of capreomycin to the biaryl-urea analogs was performed employing the ureido exchange reaction using similar conditions to that previously reported[1a] for the preparation of phenyl urea analogs (Scheme 1). After screening various co-solvents (dioxane, MeOH, EtOH, IPA, MeCN, and THF) and acids (HCl, H_2SO_4, H_3PO_4, and HOAc) we determined that an equal mixture of 2 N HCl and MeCN was the optimal solvent system for conversion of capreomycin to the desired biaryl ureas. As this is a reversible process, it was observed that using an excess of 1-(biphenyl-3-yl)urea favored formation of the desired products. Under optimal reaction conditions we determined that it was necessary to employ 3–5 equiv of the urea to obtain >90% conversion to the ureido exchange products. The products could be isolated using the resin capture strategy previously reported,[1a] however, we were able to employ an extractive protocol for isolation of the product mixture.[5] Upon reaction completion, the unreacted biaryl urea was removed (and recycled) by extraction using EtOAc and the product-rich aqueous layer was lyophilized to give the product mixture as a white powder. Treatment of the crude mixture with di-*tert*-butyl-dicarbonate (Boc_2O) gave quantitative conversion of all capreomycin-related components to the globally protected analogs which could be extracted into EtOAc and concentrated to give a mixture composed primarily of compounds **3a** and **3b**.

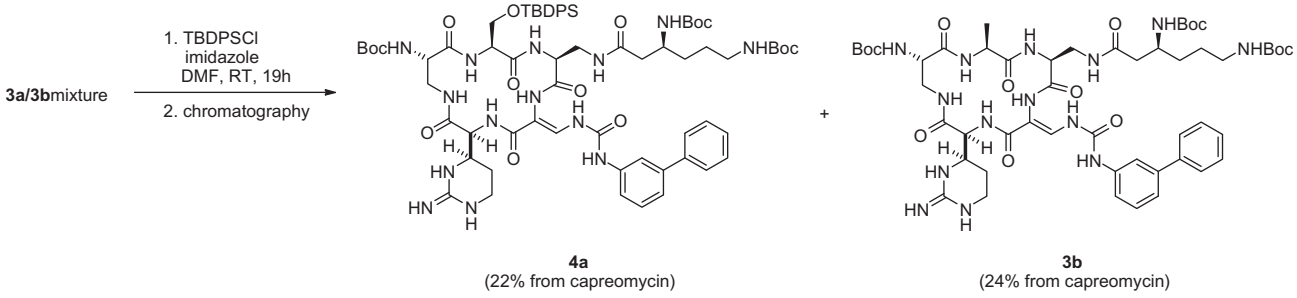
Table 1
Screen of silyl-protecting groups

Entry	HPLC retention times for various analogs (min)			
	Silyl chloride	IA silyl ether	IIB analog (3b)	Difference
4a	*t*BuPh$_2$SiCl	3.46	2.57	0.89
4b	Ph$_2$MeSiCl	3.24	2.63	0.61
4c	Ph$_3$SiCl	3.44	2.63	0.81
4d	*t*BuMe$_2$SiCl	3.16	2.65	0.51
4e	Et$_3$SiCl	3.15	2.62	0.53

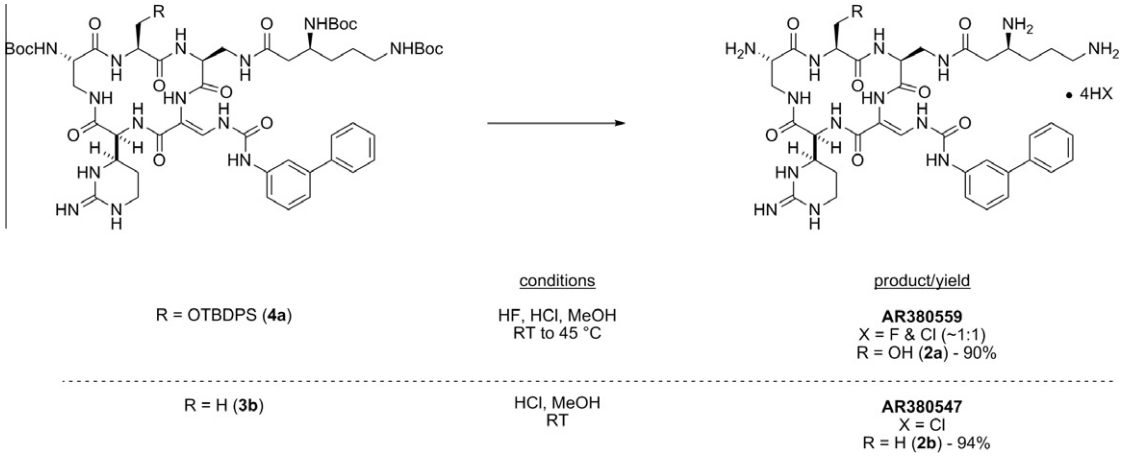
Attempts to separate **3a** from **3b** by simple crystallization or solid-phase capture using a resin technology proved fruitless. We discovered, after evaluating hydroxyl-protecting groups including acylation with acid chlorides and formation of silyl ethers, that protection of the serine hydroxyl moiety with a silyl group sufficiently altered the polarity of the IA and IB components so that they could be separated using simple flash column chromatography. An additional benefit of a silyl ether-protecting group was that complete deprotection of the IA component could potentially be accomplished in the same step as deprotection of the Boc-protected amines. In order to determine the most practical silyl ether for this purpose, a screen was performed utilizing several commercially available silyl chlorides (Table 1). To evaluate the polarity difference between various silyl ethers the **3a/b** mixture was reacted with the silyl chloride identified in Table 1 and the crude reaction mixtures were analyzed using a trivial HPLC method.[6] The difference in retention times between **4a–e** and **3b** was then used as an indication of the polarity difference between the silyl ether analog and **3b**. Based on the results summarized in Table 1, the TBDPS group (entry **4a**) gave the greatest difference in retention time indicating that it would be most easily separated from **3b**. Additionally, the TBDPS ether was sufficiently stable to enable a chromatographic separation to be executed and therefore it was chosen for preparative use (Scheme 2). The overall yield (of both congeners) for the analog formation, protection, and chromatographic separation of the two main components (**4a** and **3b**) was 46%.[5]

The desired single component products were easily isolated after removal of the protecting groups (Scheme 3). Deprotection of **3b** was accomplished using anhydrous HCl in MeOH to cleanly give the tetra-HCl salt of AR380547 (**2b**) in 94% yield.[7] Similarly, treatment of **4a** with HF in methanol followed by anhydrous HCl in methanol cleanly liberated a mixed HF/HCl salt of AR380559 (**2a**) in 90% yield.[8]

In conclusion, this protocol provides a synthetically useful and potentially scalable method for the preparation of single component biaryl-urea capreomycin analogs such as AR380559 (**2a**) and



Scheme 2. Protection with TBDPS and chromatographic separation.



Scheme 3. Deprotection of biaryl analogs.

AR380547 (**2b**) in good yields and high purities using standard laboratory techniques.

Acknowledgments

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2010.04.136.

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5. *Procedure for preparation of* **3b** *and* **4a**: To a mixture of capreomycin sulfate (10.0 g, 13.5 mmol) and 3-biphenylurea (11.2 g, 52.7 mmol) was added 100 mL of acetonitrile followed by 100 mL of freshly prepared 2 N HCl. The mixture was then heated at 65 °C with stirring for 24 h. The reaction mixture was cooled to room temperature and neutralized (pH 8) using 2.5 N NaOH solution. The mixture was filtered to recover unreacted 3-biphenylurea and the filtrate was washed with EtOAc (2 × 100 mL). The product-rich aqueous layer was lyophilized to give 22.2 g of an off-white powder. The solid was suspended in 200 mL of MeCN and treated with Boc_2O (8.9 g, 41 mmol) and Na_2CO_3 (6.0 g, 56 mmol) and stirred at ambient temperature for 20 h. The reaction mixture was diluted with 200 mL of water and extracted with EtOAc (2 × 100 mL). The combined organic phases were dried over $MgSO_4$ and concentrated under reduced pressure to give 11.5 g of a light yellow oil which was used directly in the next step without purification. The oil was dissolved in 60 mL of DMF and treated with imidazole (2.0 g, 29 mmol) and TBDPSCl (5.6 g, 20 mmol). The mixture was stirred under N_2 at ambient temperature for 19 h. The reaction mixture was partitioned between 5% NaCl solution and EtOAc (400 mL each) and the aqueous layer was extracted with EtOAc (200 mL). The combined organic phases were washed with 0.1 N HCl, 10% $NaHCO_3$, brine, and saturated NaCl solution (150 mL each), dried over $MgSO_4$, and concentrated under reduced pressure to give a foam. The foam was purified by flash chromatography on silica gel (elution with 0–15% MeOH in dichloromethane) to give 3.7 g (24%) of **3b** (R_f = 0.23 in 10% MeOH in DCM) and 4.1 g (22%) of **4a** (R_f = 0.44 in 10% MeOH in DCM), both as white powders.
6. *HPLC conditions*: YMC ODS-AQ 4.6 × 50 mm S-5μ 120 Å column; eluent A = water with 1% IPA and 0.01% HFBA (heptafluorobutyric acid); eluent B = MeCN with 1% IPA and 0.01% HFBA; 4 min gradient with 5% to 95% B; flow rate = 2 mL/min.; UV detection at 254 nm.
7. *Procedure for preparation of* **2a**: To a solution of **4a** (0.53 g, 0.39 mmol) in MeOH (5 mL) was added concentrated aqueous HF (0.57 mL, 15.6 mmol) and the mixture was stirred at 60 °C for 30 min and then cooled to ambient temperature. A solution of 3.0 M anhydrous HCl in MeOH (5.2 mL, 15.6 mmol) was added to the reaction mixture and the resulting mixture was stirred at ambient temperature overnight. The vessel was opened to the atmosphere and heated until most of the MeOH had been removed. The resulting solid was triturated with MTBE (20 mL) and the product was isolated by vacuum filtration and dried to give 0.34 g (90%) of **2a** as a white powder. ^{1}H NMR (500 MHz, D_2O) δ 1.59 (dt, J = 14.6, 14.3, 8.6, 6.8 Hz, 1H) 1.67 (br m, 4H), 1.97 (dt, J = 14.6, 5.5, 5.1 Hz, 1H), 2.55 (dd, J = 16.4, 8.3 Hz, 1H) 2.67 (dd, J = 16.4, 4.6 Hz, 1H), 2.90 (br m, 2H), 3.13 (dd, J = 13.7, 9.6 Hz, 1 H), 3.22 (br t, J = 6.8, 5.5 Hz, 2H), 3.56 (br dt, J = 13, 4.7 Hz, 1H), 3.66 (d, J = 7.2 Hz, 2H,), 3.74 (d, J = 5.2 Hz, 1H), 3.98 (dd, J = 13.7, 5.2 Hz, 1H), 4.21 (dd, J = 9.6, 5.2 Hz, 1H), 4.30 (t, J = 7.2 Hz, 1H), 4.34 (ddd, J = 8.6, 5.5, 2.4 Hz, 1H), 4.69 (t, J = 5.1 Hz, 1H), 4.91 (d, J = 2.4 Hz, 1H), 7.28 (br m, 1H), 7.34 (t, J = 7.8 Hz, 1H), 7.39–7.37 (m, 2H), 7.42 (t, J = 7.8 Hz, 2H), 7.56 (m, 1H), 7.58 (d, J = 8.0 Hz, 2H), 8.03 (br s, 1H); ^{13}C NMR (125 MHz, D_2O) δ 22.8, 22.8, 28.9 36.1, 36.5, 38.0, 38.8, 39.4, 48.3, 53.4, 49.0, 50.9, 54.5, 54.9, 62.0, 105.1, 119.2, 119.9, 123.2, 126.9, 127.9, 129.1, 129.8, 134.7, 137.4, 139.9, 141.5, 153.0, 154.3, 167.0, 167.2, 171.0, 171.6, 172.0, 172.3; HRMS calcd m/z for $C_{37}H_{53}N_{14}O_8$ 821.4165 $(M+H)^+$, found 821.4185.
8. *Procedure for preparation of* **2b**: To a solution of **3b** (1.01 g, 0.91 mmol) in MeOH (10 mL) was added a solution of 3.4 M HCl in MeOH (10 mL, 34 mmol) and the mixture was stirred at ambient temperature for 3 h. The reaction mixture was concentrated to give 0.82 g (94%) of **2b** as a white powder. ^{1}H NMR (500 MHz, D_2O) δ 1.33 (d, J = 7.3 Hz, 3H), 1.62 (m, 1H), 1.70 (m, 2H), 1.70 (m, 2H), 1.97 (m, 1H), 2.56 (dd, J = 16.2, 8.7 Hz, 1H), 2.67 (dd, J = 16.8, 4.7 Hz, 1H), 2.94 (m, 2H), 3.24 (m, 2H), 3.25 (m, 1H), 3.55 (penta, J = 7.4 Hz, 1H), 3.61 (dd, J = 14.2, 10.5 Hz, 1H), 3.76 (dd, J = 14.7, 10.5 Hz, 1H), 4.04 (dd, J = 14.0, 5.1 Hz, 1H), 4.22 (dd, J = 7.3, 4.6 Hz, 1H), 4.26 (m, 1H), 4.32 (q, J = 7.3 Hz, 1H), 4.32 (dd, J = 10.0, 5.6 Hz, 1H), 4.84 (dd, J = 2.4 Hz, 1H), 7.30 (m, 1H), 7.32 (m, 1H), 7.35 (m, 1H), 7.35 (m, 1H), 7.41 (m, 1H), 7.42 (m, 1H), 7.49 (d, J = 7.3 Hz, 1H), 8.01 (s, 1H); ^{13}C NMR (125 MHz, D_2O) δ 18.1, 22.9, 23.1, 29.3, 36.2, 36.6, 38, 39, 39.6, 48.3, 49, 49.2, 51.4, 53.4, 55.1, 105.4, 118.6, 119.5, 123.3, 127.0, 128.1, 129.2, 130, 134.8, 137.6, 139.9, 141.6, 152.1, 154.3, 166.8, 167.1, 171, 172, 175.8. HRMS calcd m/z for $C_{37}H_{53}N_{14}O_7$ 805.4216 $(M+H)^+$, found 805.4233.