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ORIGINAL ARTICLE

Novel semisynthetic antibiotics from caprazamycins A–G: caprazene derivatives and their antibacterial activity

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Acidic treatment of a mixture of caprazamycins (CPZs) A–G isolated from a screen of novel antimycobacterial agents gave caprazene, a core structure of CPZs, in high yield. Chemical modification of the resulting caprazene was performed to give its various derivatives. The structure–activity relationships of the caprazene derivatives against several mycobacterial species and pathogenic Gram-positive and Gram-negative bacteria were studied. Although caprazene showed no antibacterial activity, the antibacterial activity was restored for its 1‴-alkylamide, 1‴-anilide and 1‴-ester derivatives. Compounds 4b (CPZEN-45), 4d (CPZEN-48), 4f and 4g (CPZEN-51) exhibited more potent activities against *Mycobacterium tuberculosis* and *M. avium* complex strains than CPZ-B. These results suggest that caprazene would be a good precursor from which novel semisynthetic antibacterial antibiotics can be designed for the treatment of mycobacterial diseases such as tuberculosis and *M. avium* complex infection.
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Keywords: acid-fast bacteria; antibacterial activity; caprazamycin; caprazene; CPZEN-45; mycobacteria; tuberculosis

INTRODUCTION

The excessive and inappropriate use of antibacterial drugs has brought about the emergence of resistant bacteria, and as a result, the therapeutic effects of many existing antibiotics have decreased. In the fight against an increasing number of drug-resistant pathogens, renewed interest in the isolation of novel antibiotics containing a new structure derived from natural products has increased. Moreover, the development of potent analogs and/or semisynthetic derivatives of the newly discovered antibiotics would have the potential to be superior to the original materials.

We have screened for a novel antimycobacterial substance having a specific and effective spectrum of activity with a new mode of action from microbial products. As a part of this program, we discovered a structurally analogous mixture of caprazamycins (CPZs), a group of novel liponucleoside antibiotics containing many different alkyl side-chains, in a culture broth of Actinomycete strain *Streptomyces* sp. MK 730-62F2.[1] CPZs are composed of an uridine moiety, a 5-amino-D-ribose moiety, a diazepinone ring system, a 3-methylglutaric acid moiety, an *O*-methylated-L-rhamnose moiety and a fatty acid moiety. Based on the difference in the contained fatty acid, CPZs are classified into seven components (A–G).

Its major component, caprazamycin B (CPZ-B), showed excellent antimycobacterial activity *in vitro* against drug-susceptible and multi-drug-resistant *Mycobacterium tuberculosis* strains.[2,3] Furthermore, it showed superior therapeutic efficacy in a pulmonary tuberculosis (TB) model induced in mice with no significant toxicity.[4]

CPZs inhibit the bacterial translocase I, which is one of the key enzymes for peptidoglycan biosynthesis. The inhibition of the translocase I is not recognized as being the mode of action of current anti-TB drugs. As translocase I is an essential enzyme among bacteria, it has been intensely investigated as a prime target for new antibacterial agents. A number of translocase I inhibitors[5] such as liposidomycins[6,7] that are structurally related to CPZs, mureidomycins,[8] pacidamycins,[9] napsamycins,[10] tunicamycin,[11] capuramycins,[12,13] muraymycins,[14] muraminomicins,[15] A-102395,[16] A-94964,[17,18] A-97065 complex[19] and A-90289 complex[20] have been reported.

Based on its excellent biological properties, CPZ-B was considered to be a promising anti-TB drug candidate. However, in spite of its potential, certain obstacles complicate its development as a new anti-TB drug, including its onerous separation from a complex mixture by using HPLC and its extremely poor water-solubility profile.

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During a structural elucidation study, we recently discovered that caprazene (CPZEN, **1**)[21] can be obtained quantitatively from CPZ-B. CPZEN is composed of a uridine moiety, a 5-amino-D-ribose moiety and a diazepinone ring system, and it benefits from not having a fatty acid moiety that contributes to the generation of a multicomponent mixture. We hypothesized that we could use CPZEN as a basis to create new derivatives with better antibacterial activity than CPZ-B and to overcome the limitations of isolating CPZ-B from a mixture of CPZs. Herein, we describe the efficient acquisition of CPZEN from a mixture of CPZs and the synthesis and structure–activity relationships of CPZEN derivatives, which might have utility in the design of new antimycobacterial agent.

RESULTS AND DISCUSSION

As shown in Scheme 1, we successfully produced CPZEN from a mixture of CPZs by replicating the reaction conditions used for the acidic hydrolysis of CPZ-B. Briefly, after the mixture of CPZs was obtained from a fermentation broth, it was treated with aqueous acetic acid, which cleaved the ester portion containing different alkyl side-chains, simultaneously producing a carbon–carbon double bond to afford CPZEN containing the CPZ core structure. Varying the ratios of the components had no influence on the results. CPZEN was obtained in crystal form at ~64% yield. Unfortunately, CPZEN exhibited no antibacterial activity. However, as the molecule is structurally similar to the CPZs, we anticipated that introducing a lipophilic side-chain into a suitable position of the molecule would effectively restore the antibacterial activity.

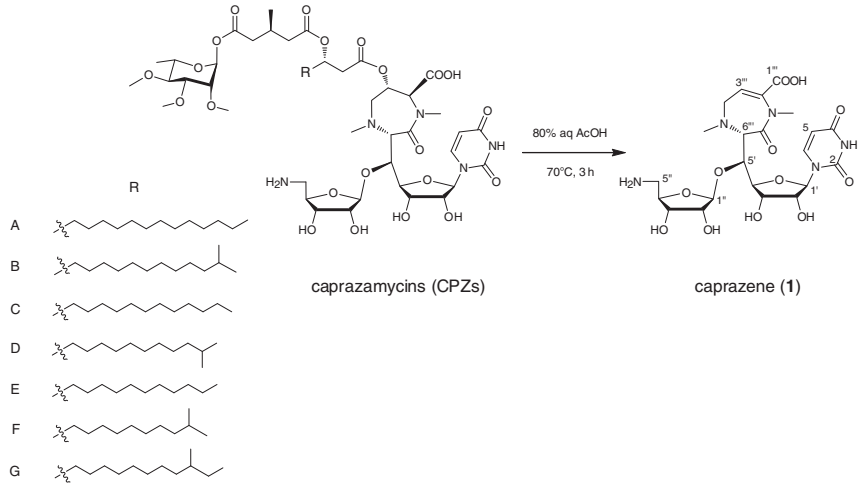
In CPZEN, there are two functional groups that differ in terms of the strength of their basicity (or acidity), that is, the primary amino group of D-ribose located in the southwest region and the carboxyl group on the diazepinone ring. As the carboxyl group is located at the adjacent position of the fatty acid-binding site, we considered that it is a suitable point for the introduction of the side-chain. In addition, we planned to improve water solubility by forming the acid salts of the basic molecules, thereby maintaining a free amino group.

Treating CPZEN with Boc_2O in the presence of triethylamine gave the triethylamine salt of the *N*-Boc derivative **2**. We examined the effect of introducing a side-chain into the carboxyl group using this compound. As outlined in Scheme 2, the derivatives of the alkylamide-type compounds (**3a–s**) and the anilide-type compounds (**4a–h**) were prepared by condensing the carboxyl group of **2** with various amines or anilines, followed by removing the protecting group (Boc) from the primary amino group. Furthermore, the ester derivatives **5a–e** were obtained by condensing **2** with various alcohols followed by deprotection of the *N*-Boc group. The condensation reactions were effectively attained by using bis(2-oxo-3-oxazolidinyl)-phosphinic chloride (BOP-Cl)[22] or 4-(4, 6-dimethoxy-1, 3, 5-triazin-2-yl)-4-methylmorpholinium chloride (DMT-MM).[23,24]

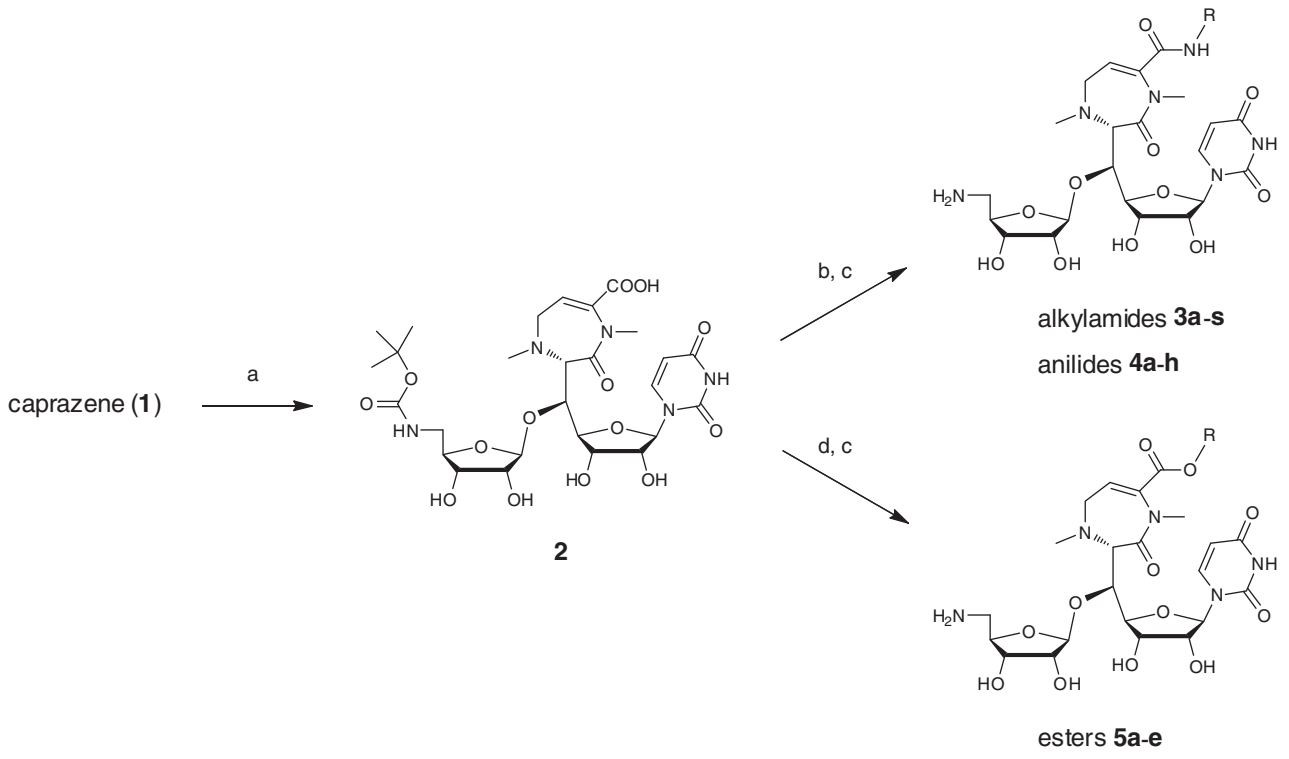
Table 1 presents a summary of the antibacterial activities against various mycobacteria and hemolytic effects of alkylamides **3a–s**, anilides **4a–h**, esters **5a–e** and CPZ-B. As expected, these results showed that introducing a lipophilic side-chain at the carboxyl group of CPZEN leads to the recovery of its antibacterial activity. In addition, the trifluoroacetic acid salts of the alkylamide, anilide and ester derivatives had good water solubility. The strains in Table 1, *M. tuberculosis*, *M. bovis* and *M. avium* complex (MAC; consists of two species: *M. avium* and *M. intracellulare*) are important pathogens that cause serious infectious diseases in human. The recent increase in multidrug-resistant TB, extensively drug-resistant TB and refractory TB/HIV co-infection cases are crucial public health problems.[25,26] In addition, MAC is a common opportunistic human pathogen that is known to possess natural resistance against existing anti-TB drugs, and for which there is a general lack of treatment options.[27–29]

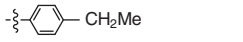
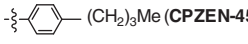
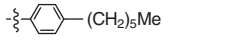
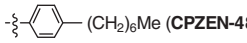
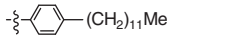
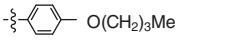
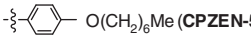
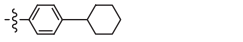
Examination of the relationship between the inhibition of mycobacteria by simple alkylamides **3a–q** suggested that the antibacterial activities of the derivatives are influenced by the length of their carbon chain. Interestingly, *in vitro* activity against *M. tuberculosis* H37Rv (international standard strain) was enhanced rapidly when the carbon atoms of the side-chain reached six. The antibacterial activity was further increased to levels more than that of CPZ-B when the side-chain was from 9 to 21 carbons. A similar trend was observed for *M. tuberculosis* Kurono, *M. bovis* Ravenel and *M. avium* complex. On the other hand, the optimal carbon-chain lengths to achieve excellent antibacterial activity against *M. smegmatis*, *M. vaccae* and *M. phlei* were somewhat narrow. Based on the results from all the bacteria, the optimal length of the carbon-chain was from 11 to 15 (Table 1).

Strong hemolytic effects were also observed when the carbon-chain was elongated in these compounds, whereas the direct correlation of carbon-chain length with the antibacterial activities was not clear. Upon comparing the derivatives with a side-chain consisting of the same number of carbons, an interesting phenomenon was observed about the hemolytic action, which is an indicator of a side effect. Derivative **3h** with a linear side-chain had a stronger hemolytic effect, whereas **3s** containing a cyclic side-chain had a lower effect.



Scheme 1 Preparation of caprazene (CPZEN, **1**) from a mixture of CPZs A–G.



3a R = $(CH_2)_4Me$
3b R = $(CH_2)_5Me$
3c R = $(CH_2)_6Me$
3d R = $(CH_2)_7Me$
3e R = $(CH_2)_8Me$
3f R = $(CH_2)_9Me$
3g R = $(CH_2)_{10}Me$
3h R = $(CH_2)_{11}Me$
3i R = $(CH_2)_{12}Me$ (**CPZEN-6**)
3j R = $(CH_2)_{13}Me$
3k R = $(CH_2)_{14}Me$
3l R = $(CH_2)_{15}Me$
3m R = $(CH_2)_{16}Me$
3n R = $(CH_2)_{17}Me$
3o R = $(CH_2)_{18}Me$
3p R = $(CH_2)_{19}Me$
3q R = $(CH_2)_{20}Me$
3r R = $(CH_2)_8CH{=}CH(CH_2)_7Me$ (*Z*-isomer)
3s R = cyclododecyl
4a R =  CH_2Me
4b R =  $(CH_2)_3Me$ (**CPZEN-45**)
4c R =  $(CH_2)_5Me$
4d R =  $(CH_2)_6Me$ (**CPZEN-48**)
4e R =  $(CH_2)_{11}Me$
4f R =  $O(CH_2)_3Me$
4g R =  $O(CH_2)_6Me$ (**CPZEN-51**)
4h R = 
5a R = $(CH_2)_9Me$
5b R = $(CH_2)_9CH{=}CH_2$
5c R = $(CH_2)_{12}Me$
5d R = $(CH_2)_{10}CH{=}CHCH_2Me$ (*Z*-isomer)
5e R = $(CH_2)_{17}Me$

Scheme 2 Synthesis of 1‴-alkylamide (**3a–s**), anilide (**4a–h**) and ester (**5a–e**) derivatives of caprazene. Reagents and conditions: (a) Boc_2O, Et_3N, aqueous 1,4-dioxane, room temperature; (b) RNH_2, bis(2-oxo-3-oxazolidinyl)phosphinic chloride (BOP-Cl), Et_3N, THF, room temperature or RNH_2, 4-(4,6-dimethoxy-1,3,5-triazin-2-yl)-4-methylmorpholinium chloride (DMT-MM), aqueous 2-propanol, room temperature; (c) TFA, MeOH, room temperature; (d) ROH, BOP-Cl, pyridine, room temperature.

Interestingly, **3s** demonstrated potent antibacterial activity against *M. intracellulare*. As shown by a comparison of **3n** and **3r**, the presence or absence of an unsaturated bond in the carbon-chain did not significantly affect the antimicrobial activity and the hemolytic effects.

The anilide derivatives, which had a side-chain containing a benzene ring, also exhibited excellent antibacterial activities. Derivatives **4c**, **4d** (CPZEN-48) and **4g** (CPZEN-51) showed excellent activity against both strains of *M. tuberculosis* H37Rv and MAC, but they also possessed strong hemolytic effects. However, 4-butylanilide **4b** (CPZEN-45) as well as 4-butoxyanilide **4f** and 4-cyclohexylanilide **4h** were more potent than CPZ-B *in vitro* against *M. tuberculosis* H37Rv, while maintaining minimal hemolytic effects.

From a comparison between **4b** and **4f** and between **4d** and **4g**, it was shown that the existence of an oxygen atom in the terminal alkyl side-chain slightly reduced the antibacterial activity toward all strains.

Table 1 Antimycobacterial activities and hemolytic effects of CPZEN derivatives

	MIC ($\mu g\,ml^{-1}$)									
Compound	*M. tuberculosis* H37Rv	*M. tuberculosis* Kurono	*M. bovis* Ravenel	*M. avium subsp.avium* ATCC 25291	*M. avium subsp. paratuberculosis* ATCC 43015	*M. intracellulare* JCM 6384	*M. smegmatis* ATCC 607	*M. vaccae* ATCC 15483	*M. phlei*	Hemolytic activity ($\mu g\,ml^{-1}$)
3a	12.5	6.25	6.25	—	—	—	100	100	100	1000
3b	3.13	1.56	3.13	12.5	0.78	1.56	50	100	100	1000
3c	3.13	1.56	1.56	—	—	—	25	25	50	1000
3d	3.13	0.78	1.56	—	—	—	3.13	25	12.5	1000
3e	1.56	0.78	1.56	1.56	0.20	0.20	0.78	12.5	3.13	500
3f	1.56	1.56	1.56	—	—	—	0.39	6.25	0.78	250
3g	1.56	1.56	1.56	3.13	0.20	0.20	<0.20	1.56	0.39	125
3h	1.56	1.56	1.56	3.13	—	0.20	0.39	1.56	0.39	62.5
3i (CPZEN-6)	1.56	1.56	1.56	3.13	0.10	0.20	0.39	0.78	0.78	31.3
3j	1.56	1.56	1.56	3.13	0.39	0.39	1.56	1.56	0.78	31.3
3k	1.56	1.56	1.56	—	—	—	3.13	0.78	1.56	62.5
3l	1.56	1.56	3.13	—	—	—	3.13	3.13	3.13	31.3
3m	0.78	1.56	1.56	—	—	—	3.13	6.25	1.56	31.3
3n	1.56	1.56	1.56	0.78	0.39	0.78	6.25	3.13	3.13	62.5
3o	1.56	1.56	1.56	1.56	0.78	0.78	12.5	3.13	25	31.3
3p	1.56	1.56	0.78	—	—	—	25	50	>100	62.5
3q	1.56	0.78	1.56	—	—	—	50	>100	>100	>250
3r	3.13	3.13	3.13	6.25	—	0.78	1.56	1.56	1.56	62.5
3s	1.56	1.56	1.56	3.13	0.05	<0.05	3.13	25	6.25	500
4a	—	—	—	6.25	3.13	1.56	100	>100	—	>1000
4b (CPZEN-45)	1.56	1.56	3.13	1.56	0.39	0.20	6.25	50	12.5	500
4c	0.78	—	—	0.78	0.20	0.20	0.78	6.25	—	250
4d (CPZEN-48)	0.78	0.78	0.78	1.56	0.05	0.10	<0.20	—	—	62.5
4e	3.13	3.13	3.13	6.25	0.39	0.78	6.25	6.25	—	31.3
4f	1.56	3.13	3.13	3.13	0.78	0.78	25	100	—	1000
4g (CPZEN-51)	0.78	0.78	0.78	0.78	0.20	0.10	0.78	6.25	—	125
4h	1.56	1.56	1.56	1.56	0.78	0.39	1.56	25	—	1000
5a	12.5	—	—	—	—	—	12.5	12.5	12.5	125
5b	12.5	12.5	12.5	—	—	—	12.5	6.25	12.5	125
5c	6.25	—	—	50	3.13	3.13	12.5	12.5	6.25	62.5
5d	12.5	6.25	6.25	—	—	—	25	6.25	12.5	62.5
5e	6.25	3.13	3.13	12.5	3.13	6.25	>100	>100	>100	62.5
CPZ-B	3.13	3.13	3.13	6.25	1.56	3.13	1.56	1.56	1.56	>1000
AMPH	—	—	—	—	—	—	—	—	—	31.3

Abbreviations: AMPH, amphotericin B; CPZ-B, caprazamycin B.

On the other hand, the presence of an oxygen atom proved to be effective in moderating the hemolytic action. Further, it was observed that the activity of **4c,** containing a linear chain, is better than that of **4h** having a cyclic side-chain, though **4c** shows a stronger hemolytic effect than **4h**.

On the whole, the levels of antibacterial activity of the ester derivatives **5a–e** were inferior to those of the alkylamide or anilide derivatives. The existence of an NH proton adjacent to the carbonyl group at the 1‴-position might be important for the strength of the antibacterial activity.

M. avium subsp. *paratuberculosis* is a pathogen that causes the contagious Johne's disease in cattle, sheep and other ruminants. However, effective drugs against Johne's disease have not yet been reported. As many CPZEN derivatives demonstrated strong activity against *M. avium* subsp. *paratuberculosis*, the expectation is that future drug development of these derivatives will lead to an active agent against this class of organisms.

Table 2 indicates the antibacterial activities of CPZEN derivatives against some pathogenic Gram-positive bacteria such as *Staphylococcus aureu*s and methicillin-resistant *S. aureus* and Gram-negative bacteria such as *Escherichia coli* and *Klebsiella pneumoniae*. The antibacterial activity toward Gram-positive bacteria was more potent when the length of the side-chain reached a certain specific range similar to what was observed for the acid-fast bacteria. However, against *S. aureus*, including methicillin-resistant *S. aureus*, that are particularly clinically important bacteria, the activities of all derivatives were weaker than that of CPZ-B. Against Gram-negative bacteria, the CPZEN derivatives did not have favorable activities. Interestingly, only derivatives with a side-chain of a particular length (from 12 to 14) showed intermediate activity against *K. pneumoniae*, whereas CPZ-B is not active against it.

The antibacterial activities of CPZ-B and **3i** (CPZEN-6) against various kinds of bacteria are shown in Figure 1. CPZ-B exhibits antimicrobial activities against the most important Gram-positive

Table 2 Antibacterial activities of CPZEN derivatives

Compound	MIC (μg ml^{-1})					
	S. aureus FDA 209P	*S. aureus* MRSA No.5	*S. aureus* MS16526 (MRSA)	*B. subtilis* PCI 219	*E. coli* K-12	*K. pneumoniae* PCI 602
3a	100	>100	>100	25	>100	>100
3b	>100	>100	>100	25	>100	>100
3c	25	>100	>100	6.25	>100	>100
3d	6.25	100	100	3.13	>100	>100
3e	6.25	50	50	1.56	>100	>100
3f	6.25	50	25	0.78	>100	>100
3g	6.25	25	12.5	0.39	>100	50
3h	3.13	12.5	6.25	0.78	100	6.25
3i (CPZEN-6)	3.13	6.25	3.13	0.39	100	6.25
3j	3.13	6.25	3.13	0.20	>100	12.5
3k	1.56	6.25	6.25	<0.20	>100	50
3l	3.13	6.25	3.13	0.78	>100	>100
3m	1.56	6.25	6.25	1.56	>100	>100
3n	6.25	6.25	6.25	<0.20	>100	>100
3o	6.25	6.25	6.25	0.78	>100	>100
3p	6.25	12.5	12.5	1.56	>100	>100
3q	6.25	12.5	12.5	1.56	>100	>100
3r	1.56	6.25	6.25	0.78	>100	>100
3s	100	>100	>100	1.56	>100	>100
4a	>100	>100	>100	12.5	>100	>100
4b (CPZEN-45)	25	100	100	3.13	>100	50
4c	1.56	12.5	12.5	0.39	>100	12.5
4d (CPZEN-48)	1.56	25	12.5	<0.20	50	6.25
4e	3.13	12.5	12.5	0.39	>100	>100
4f	>100	>100	>100	3.13	>100	>100
4g (CPZEN-51)	3.13	25	25	<0.20	50	3.13
4h	12.5	100	100	0.39	100	6.25
5a	12.5	50	50	0.78	>100	>100
5b	25	50	50	1.56	>100	>100
5c	3.13	25	12.5	0.78	>100	25
5d	6.25	25	12.5	0.78	>100	25
5e	25	50	50	3.13	>100	>100
CPZ-B	0.78	1.56	1.56	1.56	>100	>100

Abbreviations: CPZ-B, caprazamycin B; MRSA, methicillin-resistant *S. aureus*.

bacteria, that is, *S. aureus*, *Streptococcus pneumoniae* and *S. pyogenes* and some Gram-negative pathogens like *Haemophilus influenzae*. It is surprising that CPZ-B is effective against *H. influenzae*, whereas CPZ-B is virtually inactive against most Gram-negative pathogens.

In contrast, **3i**, a representative CPZEN derivative, showed reduced antibacterial activity against *S. aureus*, *S. pneumoniae* and *H. influenzae* compared with CPZ-B whereas it had improved antibacterial activity against *M. tuberculosis* H37Rv and *Enterococcus faecalis/faecium*. Thus, obvious differences existed in susceptibilities to pathogens between the CPZ-B and CPZEN derivatives. Consequently, it was possible that a new mode of action existed for CPZEN derivatives compared with CPZ-B.

TB and/or non-TB mycobacteriosis (NTM) generally requires at least $\geqslant$6 months of chemotherapeutic treatment period. Therefore, selectivity, a narrow-range antimicrobial spectrum and specificity toward mycobacterial species are the most preferable qualities for a novel anti-TB candidate. From this perspective, some of the CPZEN derivatives are considered to be promising for the treatment of TB and NTM infections. Moreover, their hemolytic effects are low enough, suggesting that they will have good safety profiles. However, further studies are necessary to confirm this point, especially in situations where long-term repeated doses are tested.

Table 3 includes the MIC range, MIC_{50} and MIC_{90} of some CPZEN derivatives whose hemolytic activities are sufficiently low. Although **3e**, **3s**, **4b** (CPZEN-45), **4f** and **4h** showed the same *in vitro* activity against *M. tuberculosis* H37Rv (MIC 1.56 μg ml^{-1}), they exhibited differences in their potencies toward drug-sensitive *M. tuberculosis* clinical isolates ($n=21$). CPZEN-45 was identified to be the most active CPZEN derivative, with a MIC range, MIC_{50} and MIC_{90} for drug-sensitive *M. tuberculosis* clinical isolates of 0.78–12.5, 1.56 and 3.13 μg ml^{-1}, respectively. CPZEN-45 exhibited excellent selectivity and antibacterial activity against *M. tuberculosis* H37Rv and drug-sensitive *M. tuberculosis* clinical isolates ($n=21$), and MAC. Moreover, CPZEN-45 showed negligible hemolysis and excellent water solubility (>200 mg ml^{-1}). Consequently, CPZEN-45 is promising as both a new anti-TB drug and a novel anti-MAC agent.

We studied the derivatization of CPZEN, a core structure of CPZs, for the purpose of overcoming the drawbacks of CPZ-B. We synthesized a number of derivatives by introducing various amines, anilines and alcohols at the carboxyl group of the diazepinone ring. Many obtained CPZEN derivatives had suitable water solubility and exhibited excellent antibacterial activity against mycobacteria compared with that of CPZ-B. It is noteworthy that we succeeded in obtaining derivatives with superior activity to CPZEN, which had no antibacterial activity. From the above-mentioned results, it was shown that CPZEN would be a good precursor for the development of novel semisynthetic antibacterial antibiotics. Recently, CPZEN-45 was found to have excellent *in vitro* antibacterial activity against several multidrug-resistant *M. tuberculosis* (multidrug-resistant-TB) strains. CPZEN-45 also showed excellent therapeutic efficacy in a murine TB model infected with an extensively drug-resistant *M. tuberculosis* (extensively drug-resistant-TB) strain. Our results strongly support the development of CPZEN-45 as a new anti-TB drug. The details of the *in vivo* antimycobacterial activity and a mode of action of CPZEN-45 will be reported in due course.

EXPERIMENTAL PROCEDURE

General methods

Melting points were determined on a Kofler block and are uncorrected. Optical rotations were determined with a 241 polarimeter (PerkinElmer Inc., Waltham, MA, USA). NMR spectra (^{1}H at 500, ^{13}C at 125.8 and ^{19}F at 376.5 MHz) were recorded with an AVANCE 500 and/or AVANCE 400 spectrometer (Bruker BioSpin, Rheinstetten, Germany) at 300 K, unless stated otherwise. Chemical shifts (δ) of ^{1}H, ^{13}C and ^{19}F spectra were measured downfield from internal Me_4Si (for ^{1}H and ^{13}C) or internal Freon 11 (for ^{19}F), and were confirmed, when necessary, by shift-correlated two-dimensional spectra. Mass spectra were recorded using a LTQ Orbitrap mass spectrometer (HR-MS) or a LTQ XL (ESI) spectrometer (Thermo Fisher Scientific, San Jose, CA, USA). TLC was performed on a Kieselgel 60 F_{254} (Merck, Darmstadt, Germany), and column chromatography was carried out on Wakogel C-200 (Wako Pure Chemical Industries, Ltd., Osaka, Japan). Elemental analysis was performed using a PE 2400 II analyzer (PerkinElmer Inc.).

(A) Preparation of caprazene (1) from a mixture of caprazamycins

A mixture (50.0 g) of CPZ-A, B, C, D, E, F and G was dissolved in 80% aqueous AcOH (500 ml) and the solution was heated for 3 h at 70 °C. TLC (4:7:2:7 n-PrOH–EtOH–$CHCl_3$–17% aqueous NH_3) of the solution showed spots at $R_F=0.75$ (degradation products), 0.35 (trace), 0.25 (caprazene, major) and 0.05 (trace) (cf. CPZs: $R_F=0.55$). The reaction solution was concentrated, and acetone was added to the resulting syrup. The precipitate obtained was thoroughly washed with acetone to give a brown solid. A solution of the solid in water–methanol (1:1, 1000 ml) was treated with active carbon (10 g), filtered and concentrated to afford caprazene (24.3 g) as a crude pale-yellow solid.

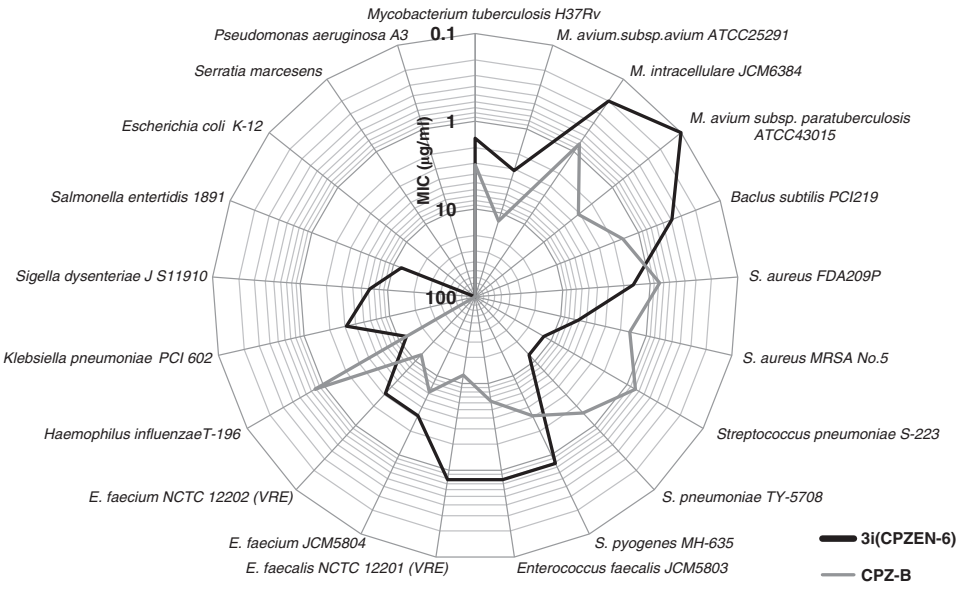


Figure 1 A radar chart of the antimicrobial spectrum of caprazamycin B and **3i** (CPZEN-6).

Table 3 MIC distribution of CPZEN derivatives against drug-sensitive *M. tuberculosis* clinical isolates (21 strains)

	M. tuberculosis (n = 21)		
Compound	Range (μg ml^{-1})	MIC_{50}	MIC_{90}
3e	1.56–12.5	6.25	12.5
3s	1.56–12.5	3.13	12.5
4a	3.13–25	12.5	25
4b (CPZEN-45)	0.78–12.5	1.56	3.13
4d (CPZEN-48)	0.78–25	3.13	6.25
4f	1.56–12.5	6.25	6.25
4h	0.78–6.25	3.13	6.25
CPZ-B	3.13–12.5	6.25	6.25
EMB	3.13–6.25	3.13	6.25
RFP	0.08–0.32	0.16	0.32

Abbreviations: CPZ-B, caprazamycin B; EMB, ethambutol; RFP, rifampicin.

Crystallization from water–acetone (1:1, 750 ml) gave caprazene (17.2 g) as colorless crystals. Yield 64% (when only CPZ-B was calculated as a starting material).

^{1}H NMR (500 MHz, DMSO-d_6) δ 2.31 (3H, s, 5‴-NCH_3), 2.71 (1H, br, 4‴a-H), ~2.96 (1H, 5″a-H), 2.97 (3H, s, 8‴-NCH_3), 3.14 (1H, br d, J = ~13 Hz, 5″b-H), ~3.33 (1H, 4‴b-H), 3.75–3.87 (3H, 3′, 2″ and 6‴-H), 3.79 (1H, slightly br d, J = ~3 Hz, 2″-H), 3.83 (1H, br t, J = ~6 Hz, 3′-H), 3.94 (1H, slightly br s, 2′-H), ~3.97 (1H, br, 4″-H), 4.10–4.23 (3H, 4′, 5′ and 3″-H), 4.13 (1H, d, J = ~10 Hz, 5′-H), 5.02 (1H, s, 1″-H), 5.51 (1H, d, J = ~1.5 Hz, 1′-H), 5.63 (1H, d, J = 8 Hz, 5-H), 6.41 (1H, t, J = 7 Hz, 3‴-H), 7.78 (1H, d, J = 8 Hz, 6-H), ~8.80 (2H, br, NH_2); ^{13}C NMR (125.8 MHz, DMSO-d_6) δ 32.1 (CH_3N-8‴), ~40 (C-5″), ~41 (CH_3N-5‴), 51.5 (C-4‴), 61.7 (broad, C-6‴), 68.9 (C-3′), 69.4 (C-3″), 74.0 (C-2′), 74.4 (C-2″), 76.4 (C-5′), 78.3 (C-4″), 82.2 (C-4′), 89.5 (C-1′), 100.9 (C-5), 109.4 (C-1″), 119.5 (C-3‴), 140.1 (C-6), 146.2 (C-2‴), 150.2 (C-2), 163.3 (C-4), 165.2 (C-1‴) and 168.4 (C-7‴). Anal. calc. for $C_{22}H_{31}N_5O_{12}$ $3H_2O$: C 43.21, H 6.10, N 11.45. The results were found to be:C 43.14, H 6.25, N 11.68.

The physicochemical data were identical to that of the authentic sample in Igarashi *et al.*[21]

(B) Preparation of 5″-*N*-*t*-butoxycarbonylcaprazene (2)

To a solution of caprazene (50.0 g, 81.8 mmol) in water–1, 4-dioxane (2:1, 600 ml), triethylamine (13.6 g, 134 mmol) and di-*t*-butyl dicarbonate (24.7 g, 113 mmol) were added and the mixture was stirred for 5 h at room temperature. After addition of 28% aqueous ammonia (5 ml), the resulting solution was concentrated to give a colorless solid of **2** (67.2 g) as an addition salt of triethylamine. This solid was used in the next reaction without further purification.

2 as an addition salt of triethylamine; ESI-MS *m/z* 759 $(M + Et_3N + H)^+$; ^{1}H NMR (500 MHz, DMSO-d_6) δ 1.07 [9H, t, $(CH_3CH_2)_3N$], 1.36 [9H, s, $(CH_3)_3$C-O], 2.32 (3H, s, 5‴-NCH_3), 2.94 (3H, s, 8‴-NCH_3), 5.01 (1H, s, 1″-H), 5.57 (1H, d, J = ~2 Hz, 1′-H), 5.59 (1H, d, J = 8 Hz, 5-H), 6.39 (1H, br t, 3‴-H), 7.26 (1H, br s, 5″-NH), 7.80 (1H, d, J = 8 Hz, 6-H), 11.30 (1H, br s, 3-NH).

Purification by silica-gel column chromatography (3:1 $CHCl_3$ −MeOH) and subsequent crystallization (1:2 water–acetone) gave **2** as colorless crystals; m.p. 238–240 °C (dec.); $[\alpha]_D^{23} + 29^\circ$ (*c* 1, 1:1 H_2O–pyridine); ESI-MS: *m/z* 680.3 $(M + Na)^+$; ^{1}H NMR (500 MHz, D_2O) δ 1.29 [9H, s, $(CH_3)_3$C-O], 3.08 (3H, s, 8‴-NCH_3), 3.12 (3H, slightly br s, 5‴-NCH_3), 3.14 (1H, br d, J = ~15 Hz, 5″a-H), 3.50 (1H, br d, J = 15 Hz, 5″b-H), 3.76 (1H, br, 4‴a-H), 4.02–4.05 (2H, m, 2″ and 3″-H), 4.14 (1H, br s, 4″-H), 4.17 (1H, d, J = 8 Hz, 4′-H), 4.30 (1H, dd, J = 2 and 5 Hz, 2′-H), ~4.31 (1H, br, 4‴b-H), 4.36 (1H, dd, J = 5 and 8 Hz, 3′-H), 4.44 (1H, d, J = 10 Hz, 6‴-H), 4.63 (1H, d, J = 10 Hz, 5′-H), 5.23 (1H, d, J = 4 Hz, 1″-H), 5.74 (1H, d, J = ~2 Hz, 1′-H), 5.84 (1H, d, J = 8 Hz, 5-H), 6.56 (1H, t, J = 7 Hz, 3‴-H), 7.77 (1H, d, J = 8 Hz, 6-H); ^{13}C NMR (125.8 MHz, D_2O) δ 28.1 [$(CH_3)_3$C-O], 33.8 (CH_3N-8‴), 40.6 (CH_3N-5‴), 42.6 (C-5″), 53.3 (C-4‴), 61.9 (C-6‴), 68.7 (C-3′), 72.4 (C-3″), 73.9 (C-2′), 74.7 (C-5′), 76.4 (C-2″), 81.5 [$(CH_3)_3$C-O], 81.6 (C-4′), 86.0 (C-4″), 91.4 (C-1′), 102.3 (C-5), 110.1 (C-1″), 119.5 (C-3‴), 142.5 (C-6), 147.5 (C-2‴), 151.6 (C-2), 159.6 [$(CH_3)_3$CO-*C*(O)-], 164.7 (C-7‴), 166.6 (C-4), 167.3 (C-1‴). Anal. calc. for $C_{27}H_{39}N_5O_{14}$ H_2O: C 48.00, H 6.12, N 10.37. Found: C 48.00, H 6.18, N 10.34.

(C) Preparation of caprazene-1‴-alkylamides

Caprazene tetradecylamide (3j). To a solution of **2** (a salt of triethylamine, 250 mg, 0.33 mmol) in THF (10 ml), triethylamine (100 mg, 1 mmol), bis(2-oxo-3-oxazolidinyl)phosphinic chloride (200 mg, 0.79 mmol) and tetradecylamine (117 mg, 0.55 mmol) were added and the resulting mixture was stirred for 2 h at room temperature. The resulting solution was concentrated to give a residue, which was extracted with $CHCl_3$. The organic solution was

washed with water and then concentrated. The residue obtained was purified by column chromatography (10:1 $CHCl_3$–MeOH) to give the 5″-*N*-Boc-protected intermediate of **3j** (158 mg, 61% from caprazene) as a colorless solid. Then, the solid was dissolved in a methanol solution of 80% trifluoroacetic acid (2.5 ml) and the solution was kept for 1 h at room temperature. The reaction solution was concentrated and diethyl ether was added to the resulting syrup. The precipitate obtained was thoroughly washed with diethyl ether and dried to give the colorless solid **3j** (178 mg, 59% from caprazene as an addition salt of bis-trifluoroacetic acid). ESI-MS: *m/z* 753.4 $(M+H)^+$; $[\alpha]_D^{20}+68^\circ$ (*c* 0.5, H_2O); ^1H NMR (500 MHz, DMSO-d_6) δ 0.86 (3H, t, $J=7$ Hz, $CH_3(CH_2)_{13}$–), 1.18–1.30 (22H, m, $CH_3(CH_2)_{11}CH_2CH_2-$), 2.35 (3H, br s, 5‴-NCH_3), 2.90 (3H, s, 8‴-NCH_3), 5.09 (1H, br s, 1″-H), 5.55 (1H, s, 1′-H), 5.63 (1H, d, $J=8$ Hz, 5-H), 6.29 (1H, br t, $J=\sim6$ Hz, 3‴-H), 7.68 (1H, br d, $J=\sim8$ Hz, 6-H), 11.32 (1H, s, 3-NH). Anal. calc. for $C_{36}H_{60}N_6O_{11}$ $2CF_3COOH$ H_2O: C 48.09, H 6.46, N 8.41. Found: C 47.75, H 6.82, N 8.34.

(D) Preparation of caprazene-1‴-anilides

Caprazene 4-butylanilide (4b, CPZEN-45). To a solution of **2** (a salt of triethylamine, 34.8 g, 45.9 mmol) in 2-propanol−water (19:1, 700 ml), 4-butylaniline (7.03 g, 47.1 mmol) and 4-(4, 6-dimethoxy-1,3,5-triazin-2-yl)-4-methylmorpholinium chloride n-hydrate (DMT-MM) (20.0 g, approximately 70 mmol) were added and the mixture was stirred for 6 h at room temperature. Concentration gave a residue, which was dissolved in 5% aqueous potassium hydrogen sulfate (800 ml). The aqueous solution was washed with EtOAc and neutralized with 10% aqueous sodium carbonate (300 ml). The precipitate obtained was extracted with EtOAc. The organic solution was washed with saturated aqueous sodium chloride, dried (Na_2SO_4) and concentrated to afford 5″-*N*-Boc protected intermediate of **4b** (32.2 g, 92% from caprazene) as a pale-yellow solid. This solid was used in the next reaction without further purification.

An analytical sample of the 5″-*N*-Boc-protected intermediate of **4b** was prepared by silica gel column chromatography (5:1 $CHCl_3$−MeOH), $[\alpha]_D^{21}+54^\circ$ (*c* 1, MeOH); ESI-MS: *m/z* 811.4 $(M+Na)^+$; ^1H NMR (500 MHz, DMSO-d_6) δ 0.89 [3H, t, $J=7$ Hz, $CH_3(CH_2)_3C_6H_4NH$], 1.31 [9H, s, $(CH_3)_3C$-O], 1.52 (2H, m, $CH_3CH_2CH_2CH_2-$), 2.35 (3H, s, 5‴-NCH_3), 2.93 (3H, s, 8‴-NCH_3), 5.59 (1H, d, $J=8$ Hz, 5-H), 5.63 (1H, slightly br d, $J=2$ Hz, 1′-H), 6.39 (1H, t, $J=6$ Hz, 3‴-H), 7.13 and 7.51 [each 2H, d, $J=8.5$ Hz, $CH_3(CH_2)_3C_6H_4NH$], 7.79 (1H, d, $J=8$ Hz, 6-H), 10.12 [1H, s, $CH_3(CH_2)_3C_6H_4NH$], 11.30 (1H, s, 3-NH); ^{13}C NMR (125.8 MHz, DMSO-d_6) δ 13.7 ($CH_3CH_2CH_2CH_2-$), 21.6 ($CH_3CH_2CH_2CH_2-$), 28.2 [$(CH_3)_3C$-O], 32.1 (CH_3N-8‴), 33.1 ($CH_3CH_2CH_2CH_2-$), 34.2 ($CH_3CH_2CH_2CH_2-$), ∼40 (CH_3N-5‴), ∼42 (C-5″), 52.6 (C-4‴), 61.0 (C-6‴), 69.1 (C-3′), 70.4 (C-3″), 74.1 (C-2′), 74.9 (C-2″), 75.3 (C-5′), 77.5 [$(CH_3)_3C$-O], 81.7 (C-4″), 82.7 (C-4′), 88.9 (C-1′), 101.1 (C-5), 109.5 (C-1″), 120.2, 128.2, 136.0 and 137.9 (aromatic), 122.8 (C-3‴), 139.6 (C-6), 140.4 (C-2‴), 150.2 (C-2), 155.9 [$(CH_3)_3CO$-*C*(O)-], 161.4 (C-1‴), 163.0 (C-4), 169.6 (C-7‴). Anal. calc. for $C_{37}H_{52}N_6O_{13}$ $2H_2O$: C 53.87, H 6.84, N 10.19. Found: C 54.10, H 6.98, N 10.29.

A solution of the 5″-*N*-Boc protected intermediate of **4b** (23.8 g, 28.9 mmol) in trifluoroacetic acid−methanol (4:1, 240 ml) was kept for 2 h at room temperature. The reaction solution was concentrated and diethyl ether was added to the resulting syrup. The precipitate obtained was thoroughly washed with diethyl ether to give **4b** bis-trifluoroacetate (27.3 g) as a pale yellow solid. The solid was dissolved in n-butanol (1200 ml) and the solution was washed successively with 5% aqueous sodium hydrogen carbonate and water. The resulting organic solution was decolorized with active carbon (4 g) and concentrated to afford a colorless solid (21.1 g). Crystallization from methanol (130 ml) −hexane (60 ml) gave **4b** trifluoroacetate (17.6 g, 66% from caprazene) as colorless crystals.

4b (**CPZEN-45**) trifluoroacetate; solubility in water (>200 mg/ml); m.p. 175∼177 °C (dec.); $[\alpha]_D^{22}+79^\circ$ (*c* 1, MeOH); HR-MS: *m/z* calc for $C_{32}H_{45}N_6O_{11}$ $(M+H)^+$ 689.3141, found 689.3132; ESI-MS: *m/z* 801 $(M+CF_3COOH-H)^-$; ^{19}F NMR (376.5 MHz, DMSO-d_6) δ −73.86 (s, CF_3); ^1H NMR (500 MHz, 10 mg/ml in D_2O) δ 0.85 (3H, t, $J=7$ Hz, $CH_3CH_2CH_2CH_2-$), 1.26 (2H, m, $CH_3CH_2CH_2CH_2-$), 1.53 (2H, m, $CH_3CH_2CH_2CH_2-$), 2.47 (3H, s, 5‴-NCH_3), 2.56 (2H, t, $J=7.5$ Hz, $CH_3CH_2CH_2CH_2-$), 2.98 (1H, dd, $J=7$ and 12 Hz, 4‴a-H), 3.05 (3H, s, 8‴-NCH_3), 3.18 (1H, dd, $J=\sim4$ and ∼14 Hz, 5″a-H), 3.35 (1H, dd, $J=\sim3$ and ∼14 Hz, 5″b-H), 3.48 (1H, dd, $J=7$ and 12 Hz, 4‴b-H), 4.07 (1H, d, $J=9.5$ Hz, 6‴-H), 4.15 (1H, slightly br s, 2″-H), 4.17−4.25 (4H, m, 2′, 3′, 3″ and 4″-H), 4.32 (1H, dd, $J=\sim2.5$ and ∼4 Hz, 4′-H), 4.44 (1H, dd, $J=2.5$ and 9.5 Hz, 5′-H), 5.27 (1H, s, 1″-H), 5.70 (1H, d, $J=\sim8$ Hz, 5-H), 5.70 (1H, d, $J=\sim5$ Hz, 1′-H), 6.67 (1H, t, $J=7$ Hz, 3‴-H), 7.18 and 7.26 (each 2H, d, $J=8$ Hz, aromatic), 7.48 (1H, d, $J=8$ Hz, 6-H); ^{13}C NMR (125.8 MHz, 10 mg/ml in D_2O) δ 13.6 ($CH_3CH_2CH_2CH_2-$), 22.0 ($CH_3CH_2CH_2CH_2-$), 32.6 (CH_3N-8‴), 33.4 ($CH_3CH_2CH_2CH_2-$), 34.7 ($CH_3CH_2CH_2CH_2-$), 39.8 (CH_3N-5‴), 40.4 (C-5″), 51.3 (C-4‴), 62.3 (broad, C-6‴), 70.0 (C-3′), 70.7 (C-3″), 73.8 (C-2′), 75.5 (C-2″), 77.0 (C-5′), 79.3 (C-4″), 84.3 (C-4′), 89.7 (C-1′), 102.6 (C-5), 110.1 (C-1″), 116.8 (CF_3, q, $J_{C,F}=292$ Hz), 122.0, 129.5, 133.9 and 142.0 (aromatic), 125.6 (C-3‴), 141.6 (C-6), 142.8 (C-2‴), 151.6 (C-2), 162.7 (C-1‴), 166.3 (C-4), 171.6 (C-7‴). ^1H NMR (500 MHz, 15 mg/ml in DMSO-d_6) δ 0.89 (3H, t, $J=7$ Hz, $CH_3CH_2CH_2CH_2-$), 1.29 (2H, m, $CH_3CH_2CH_2CH_2-$), 1.52 (2H, m, $CH_3CH_2CH_2CH_2-$), 2.37 (3H, s, 5‴-NCH_3), 2.53 (2H, t, $J=7.5$ Hz, $CH_3CH_2CH_2CH_2-$), 2.95 (3H, s, 8‴-NCH_3), ∼2.96 (1H, 4‴a-H), 3.06 (1H, dd, $J=\sim4$ and 13.5 Hz, 5″a-H), 3.19 (1H, dd, $J=\sim4$ and 13.5 Hz, 5″b-H), 3.52 (1H, slightly br dd, $J=\sim7$ and ∼12 Hz, 4‴b-H), 3.83−3.89 (3H, 3′, 2″ and 6‴-H), 3.96 (1H, dt, $J=2.5$, 5 and 5 Hz, 2′-H), 4.02 (1H, dt, $J=\sim4$, ∼4 and ∼8 Hz, 4″-H), 4.09 (1H, br s, 3″-H), 4.13 (1H, d, $J=7.5$ Hz, 4′-H), 4.21 (1H, d, $J=10$ Hz, 5′-H), 5.10 (1H, s, 1″-H), 5.52−5.28 (2H, 3′ and 3″-OH), 5.34 (1H, d, $J=4$ Hz, 2″-OH), 5.60 (1H, d, $J=2.5$ Hz, 1′-H), 5.62 (1H, d, $J=8$ Hz, 5-H), 5.63 (1H, d, $J=\sim5$ Hz, 2′-OH), 6.39 (1H, t, $J=6.5$ Hz, 3‴-H), 7.13 and 7.52 (each 2H, d, $J=8$ Hz, aromatic), 7.69 (1H, d, $J=8$ Hz, 6-H), 8.17 (3H, br s, NH_3^+), 10.17 (1H, s, 1‴-CONH), 11.30 (1H, br s, 3-NH); ^{13}C NMR (125.8 MHz, 15 mg ml^{-1} in DMSO-d_6) δ 13.7 ($CH_3CH_2CH_2CH_2-$), 21.6 ($CH_3CH_2CH_2CH_2-$), 31.8 (CH_3N-8‴), 33.1 ($CH_3CH_2CH_2CH_2-$), 34.2 ($CH_3CH_2CH_2CH_2-$), ∼39 (CH_3N-5‴), ∼40 (C-5″), 51.7 (C-4‴), 61.7 (broad, C-6‴), 68.8 (C-3′), 69.7 (C-3″), 73.9 (C-2′), 74.3 (C-2″), 75.5 (C-5′), 78.1 (C-4″), 82.3 (C-4′), 89.2 (C-1′), 101.1 (C-5), 109.3 (C-1″), 117.2 (CF_3, q, $J_{C,F}=301$ Hz), 120.2, 128.3, 135.9 and 138.1 (aromatic), 121.8 (C-3‴), 139.8 (C-6), 141.8 (C-2‴), 150.2 (C-2), 158.0 (CF_3COOH, q, $J_{C,F}=31$ Hz), 161.0 (C-1‴), 163.1 (C-4) and 168.9 (C-7‴). Anal. calc. for $C_{32}H_{44}N_6O_{11}\cdot CF_3COOH$ $3H_2O$: C 47.66, H 6.00, N 9.81. Found: C 47.72, H 6.04, N 9.77.

(E) Preparation of caprazene-1‴-esters

Caprazene tridecyl ester (5c). To a solution of **2** (a salt of triethylamine, 150 mg) in pyridine (5 ml), bis(2-oxo-3-oxazolidinyl)phosphinic chloride (120 mg, 0.55 mmol) and tridecan-1-ol (100 mg, 0.5 mmol) were added and the resulting mixture was stirred overnight at room temperature. The resulting solution was concentrated to give a residue, which was extracted with $CHCl_3$. The organic solution was washed with water and then concentrated. The residue obtained was purified by column chromatography (10:1 $CHCl_3$–MeOH) to give the 5″-*N*-Boc-protected intermediate of **5c** (95 mg, 62% from caprazene) as a colorless solid. Then, the solid was dissolved in a methanol solution of 80% trifluoroacetic acid (2.7 ml) and the solution was kept at room temperature for 1 h. The reaction solution was concentrated and diethyl ether was added to the resulting syrup. The precipitate obtained was thoroughly washed with diethyl ether and dried to give the colorless solid **5c** (110 mg, 59% as an addition salt of bis-trifluoroacetic acid from caprazene). ESI-MS: *m/z* 740.4 $(M+H)^+$; $[\alpha]_D^{19}+50^\circ$ (*c* 0.5, H_2O); ^1H NMR (500 MHz, DMSO-d_6) δ 0.86 (3H, t, $J=7$ Hz, $CH_3(CH_2)_{12}$–), 1.20–1.30 (20H, m, $CH_3(CH_2)_{10}CH_2CH_2$–), 2.36 (3H, br s, 5‴-NCH_3), 2.96 (3H, s, 8‴-NCH_3), 5.09 (1H, s, 1″-H), 5.53 (1H, d, $J=2$ Hz, 1′-H), 5.64 (1H, d, $J=8$ Hz, 5-H), 6.73 (1H, t, $J=7$ Hz, 3‴-H), 7.63 (1H, d, $J=8$ Hz, 6-H), 11.33 (1H, s, 3-NH). Anal. calc. for $C_{35}H_{57}N_5O_{12}$ $2CF_3COOH$ $3H_2O$: C 45.84, H 6.41, N 6.85. Found: C 45.68, H 6.04, N 6.56.

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