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Design and synthesis of bridged γ-lactams as analogues of β-lactam antibiotics

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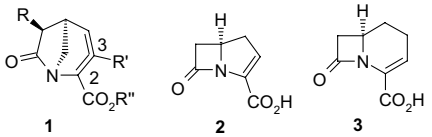
Abstract—Anti-Bredt bridged bicyclo[3.2.1] γ-lactams were designed as inhibitors of penicillin binding proteins (PBPs). The compounds were prepared by a carbenoid insertion into a lactam N–H bond. Their weak antibacterial activity could either be explained by a poor chemical stability or by unfavorable steric interactions of the methylene bridge of the γ-lactam with the targeted enzymes.

β-Lactam antibiotics are the most important class of antibacterial agents. They irreversibly inhibit the last step of the bacterial cell wall biosynthesis mediated by the serine transpeptidase activity of the penicillin binding proteins (PBPs).[1] However, the extensive use of β-lactams has given rise to an increasing development of the resistance mainly by modification of the target PBPs or by the production of β-lactamases. Despite the development of resistance, PBPs still remain an attractive target as they are both essential and specific to bacteria. Furthermore, they are extracellular and their inhibition has a bactericidal effect. We believe that the design of a novel class of nonβ-lactam PBP inhibitors could overcome these resistance mechanisms.

β-Lactams are supposed to be reactive mimics of the D-alanyl-D-alanine dipeptide substrate of PBPs.[1] Both a molecular shape mimicking the dipeptide (i.e., a carboxylic acid located at a given distance of the lactam C=O)[2] and an acylating ability due to an enhanced reactivity compared to a normal lactam are the basic requirements for biological activity of inhibitors.[3] In many attempts to move away from the β-lactam scaffold, several research groups were interested in γ-lactams.[4] However, only pyrazolidinone[5] and lactivicin[6] scaffolds led to a significant antibacterial activity through the inhibition of the PBPs.

As part of our program to discover a novel class of PBP inhibitors, we were interested in the synthesis of bridged anti-Bredt lactams. The first anti-Bredt lactam designed to target the PBPs was a 1,3-bridged β-lactam described by Williams and co-workers in 1986.[7] However, the high strain of the bicyclo[4.1.1] β-lactam led to unstable compounds with poor antibacterial activity. From the work of Greenberg,[8] and Hall and El-Shekeil[9] it was anticipated that bridged anti-Bredt γ-lactams could have a reasonable chemical stability. The bridged bicyclo[3.2.1] γ-lactam scaffold of type **1** was thus designed to mimic carbapenem **2** or carbacephem **3** scaffolds.



1 **2** **3**

	1	1a	1b	1c	1d	1e	1f	1g	1h
R	H	H	H	H	H	Et	Et	Et	Et
R'	H	OH	OCH_3	OCH_3	OCH_3	OH	OCH_3	OCH_3	OCH_3
R''	H	PNB	PNB	PNB	H	PNB	PNB	H	PNB
Δ	2	2	2	3	3	2	2	2	3

All type **1** compounds prepared are racemic.

To our knowledge there is only one example of bicyclo[3.2.1] γ-lactam described.[10] In order to synthesize such strained bicyclo[3.2.1] system we decided to use

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carbenoid insertion reaction into an N–H lactam bond. This methodology already proved to be very fruitful in the synthesis of strained molecules.[7,11]

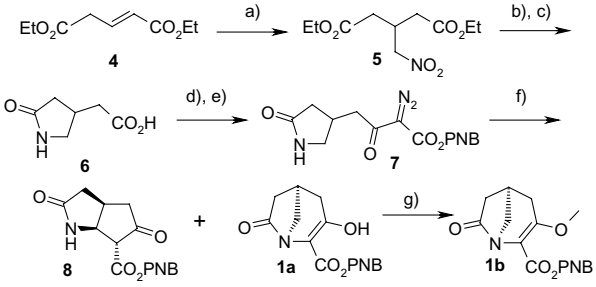
Compound **1b** was prepared according to Scheme 1. In the key rhodium catalyzed carbenoid ring closure, the desired NH insertion product **1a** was obtained in 40% yield together with 42% of the CH insertion product **8**. Enol **1a** proved to be rather unstable during purification on silica gel and was treated with an excess of diazomethane to yield 88% of **1b**.[12] The methylenolether **1b** showed a high IR absorption at 1765 cm^{-1} characteristic of the carbonyl of the bridged lactam. The cleavage of the *p*-nitrobenzyl (PNB) ester **1b** could not be achieved due to the rapid degradation of the product during hydrogenolysis.

As the double bond in **1b** could either contribute to the ring strain and to some extent to the delocalization of the nitrogen lone pair, it was anticipated that deconjugation of the double bond could improve the chemical stability as it was observed for carbapenems.[13]

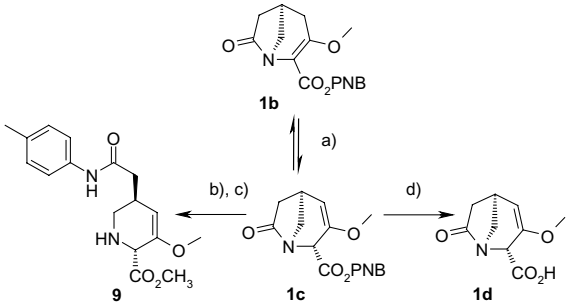
When **1b** was exposed to DBU in dichloromethane a 68/32 mixture of **1b**/**1c** was obtained in solution (Scheme 2). Compound **1c** was then isolated in 15% yield. The stereochemistry of the ester group of **1c** on the less hindered α face of the molecule was inferred from carbapenem chemistry.[13] As expected, **1c** displayed a reduced IR absorption for the carbonyl lactam 22 cm^{-1} lower than **1b** probably indicating a reduction of the ring strain. A first attempt of debenzylation of **1c** in AcOEt followed by methylation with diazomethane afforded 16% yield of **9**. Surprisingly this compound resulted from the ring opening of the lactam by the poorly nucleophilic *p*-toluidine formed by hydrogenolysis of the PNB ester. When the reaction was carried out in d_6-acetone, *p*-toluidine was converted in isopropyl amine, and the desired acid **1d** was obtained and characterized by ^1H NMR.[14] However, it was not possible to isolate this compound as a solid, either as a free acid, or as its sodium salt.

In order to further improve the chemical stability, compounds **1e**–**h** bearing a *trans* ethyl substituent were prepared (Schemes 3 and 4). The *trans* ethyl substituent is found in carbapenem PS5[15] and should not affect the biological activity. However due the increased steric hindrance of the carbonyl and the positive inductive effect of the ethyl substituent, chemical stability of **1e**–**h** should be improved compared to **1a**–**d**.

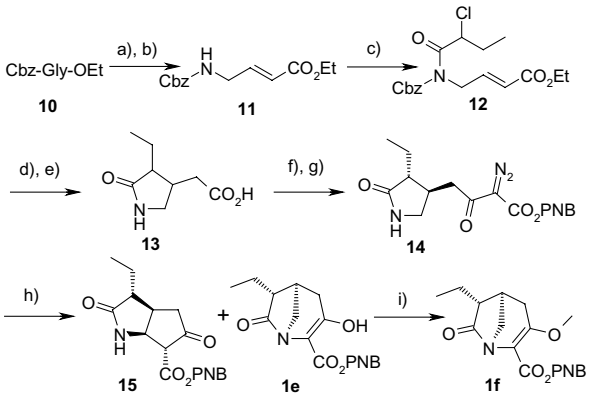
The key lactam intermediate **13** (*trans*/*cis* = 85/15) was obtained by a free radical cyclization of imide **12**



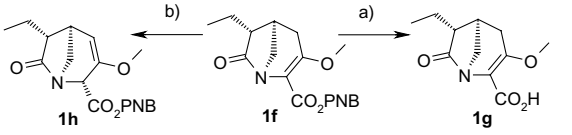
Scheme 1. Synthesis of bridged bicyclo[3,2,1] γ-lactam. Reagents and conditions: (a) CH_3NO_2, cat. DBU, 80 °C, 84%; (b) Pd/C, H_2, MeOH, then toluene reflux, 70%; (c) KOH, MeOH, rt then IR 120 H^+ form, 95%; (d) CDI, THF, rt then Mg ($PNBO_2CCHCO_2$), $2H_2O$, THF, rt, 89%; (e) *p*-dodecylsulfonylazide, TEA, CH_3CN, rt, 89%; (f) cat. $Rh_2(OAc)_4$, toluene reflux, **8**: 42%, **1a**: 40%; (g) CH_2N_2, CH_2Cl_2, 88%.



Scheme 2. Reagents and conditions: (a) 1.1 equiv DBU, CH_2Cl_2, rt, 15%; (b) Pd/C, H_2, AcOEt; (c) CH_2N_2, CH_2Cl_2; (d) Pd/C, H_2, acetone, 66% by NMR with Cl_2CH–$CHCl_2$ as internal standard.



Scheme 3. Reagents and conditions: (a) DIBAH toluene, −50 °C; (b) $(EtO)_2POCH_2CO_2Et$, NaH, THF, −30 °C, 75% overall; (c) 5 equiv 2-chloro-butyryl chloride, 4 Å M.S., 120 °C, 62%; (d) Bu_3SnH, AIBN, toluene 80 °C, 79%; (e) 3.5 equiv KOH, MeOH, rt; (f) CDI, THF, rt then Mg ($PNBO_2CCHCO_2$), $2H_2O$, THF, rt, 66% overall; (g) *p*-dodecylsulfonylazide, TEA, CH_3CN, rt 78%; (h) $Cu(acac)_2$, toluene reflux, 30%; (i) CH_2N_2, CH_2Cl_2, 60%.



Scheme 4. Reagents and conditions: (a) Pd/C, H_2, acetone, 100% by NMR with Cl_2CH–$CHCl_2$ as internal standard; (b) 1.1 equiv DBU, CH_2Cl_2, rt, 28% + 32% of **1f**.

followed by simultaneous saponification of the Cbz and ethyl ester protecting groups. Separation of *cis* and *trans* isomers could be easily achieved by chromatography of the β-ketoester intermediate. Surprisingly, rhodium-catalyzed cyclization of diazo-β-ketoester **14** afforded **1e** in only 5% yield together with 80% of CH insertion product **15**. The yield of the desired NH insertion product **1e** was improved up to 30% by using 1 equiv of $Cu(acac)_2$ in refluxing toluene.

The PNB ester of **1f** could be cleaved under the same conditions used for the preparation of **1d**. Acid **1g** was stable enough to be characterized in solution in d_6-acetone.[16] As previously described for **1c**, the double bond was deconjugated away from the nitrogen to afford **1h** in 32% yield.

The antibacterial activity of the two acid derivatives **1d** and **1g** was first evaluated using broth dilution methodology on a panel of Gram-positive and Gram-negative bacteria. Both compounds displayed weak antibacterial activity against Gram-negative bacteria *P. aeruginosa*, *S. marcescens*, *E. cloacae* with MICs ranging from 40 to 80 μg/mL. MICs for compound **1d** against *S. aureus* was 80 μg/mL. Furthermore, the esters **1b**,**c**,**f** and **1h** did not show any antibacterial activity.

In order to ascertain whether the antibacterial activity was due to inhibition of PBPs, binding of **1g** to PBPs of *P. aeruginosa*[17] was determined (Table 1). Compound **1g** displayed affinity for essential PBP 2 and PBP 3 with IC_{50} of 50 and 5 μM, respectively, which could account for the observed antibacterial activity.

As β-lactams may either be substrates or inhibitors of β-lactamases, we have determined the interaction of **1b–d** and **1f–h** with TEM-1 and P99 β-lactamases. Depending on the compound and the enzyme, a time-independent or time-dependent inhibition IC_{50} from 25 to 700 μM was observed. These results indicate that the compounds

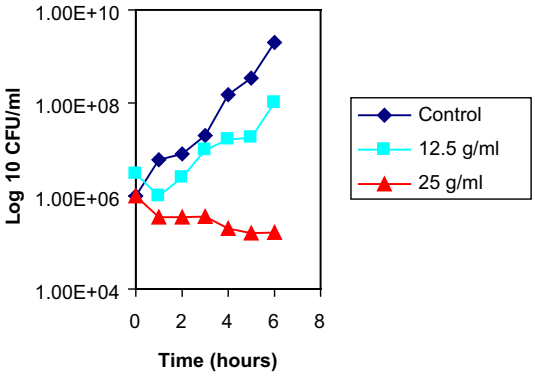
Table 1. Binding[17] to the PBPs of *P. aeruginosa* IC_{50} μM

Compds	PBP 1a	PBP 1b	PBP 2	PBP 3	PBP 4	PBP 5
1g	>50	>50	50	5	5	50
Imipenem	0.3	0.6	<0.6	Nd	<0.1	2.3

Table 2. Chemical hydrolysis and inhibition of β-lactamases

Compds	Half life (min) at pH = 9, 37 °C NaCl 0.08 M	TEM-1 (class A) inhibition IC_{50}, μM[a]	P99 (class C) inhibition IC_{50}, μM[a]
1b	6	54	160[b]
1c	Nd	45	25[b]
1d	14	280	140
1f	42	>1000	300
1g	25	700[b]	100
1h	55	420[b]	210[b]
Clavulanic acid	120	0.065[b]	>1000

[a] Inhibition of hydrolysis of nitrocephin after 5 min pre-incubation with the enzyme.[18]
[b] Time dependent inhibition.



Scheme 5. Time kill curve *S. aureus* 011HT1.

are either poor substrates or poor inhibitors of β-lactamases (Table 2).

As chemical reactivity was shown to be important for biological activity of inhibitors,[3] the rate of base catalyzed hydrolysis of **1b–d** and **1f–h** was determined (Table 2).[19] As expected, the ethyl substituted series **1f–h** proved to be more stable than the unsubstituted one **1b–d**. All the compounds were more reactive than clavulanic acid, which showed that their chemical reactivity was sufficient for interaction with the target enzymes. Acid **1d** and **1g** displayed short half-lives of 14 and 25 min that could explain the observed weak antibacterial activity. Determination of time kill curves could better estimate the antibacterial activity of unstable compounds. The time kill curve of **1d** against *S. aureus* was measured (Scheme 5). A stable one-log decrease of the bacterial cell concentration was maintained over a 6 h period at the highest concentration whereas growth resumed after 1 h at 12.5 μg/mL concentration.

Besides reactivity, the molecular shape of the inhibitor is another important requirement for activity. Compound **1** was designed to fulfill Cohen's distance criteria (3.0–3.9 Å): distance between the oxygen atom of the lactam and the carbon of the carboxyl group was indeed 3.30 Å. In order to determine whether there was a difference with the modeled structure **1**, the 3D structure of **1h** was determined by single crystal X-ray analysis (Fig. 1).[20] The α-stereochemistry of ethyl and ester substituents was unambiguously confirmed. As expected, the nitrogen atom proved to be highly pyramidal with a sum of the angles around the nitrogen of 322.2°. The N–C and C=O bond length were 1.44 and 1.22 Å, respectively. The distance between oxygen of the lactam and the carbon of the ester group of **1h** was 4.26 Å in good agreement with the distance 4.17 Å measured on the modeled compound **1d** and outside the range defined by

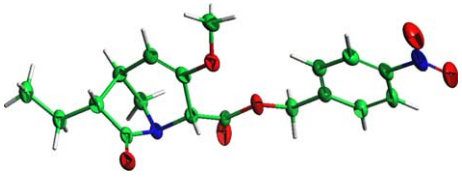


Figure 1.

Cohen. This could explain the poor activity of **1d**. On the other hand, acid **1g** with a distance of 3.22 Å should have been more active.

As there is almost no difference of activity between **1d** and **1g** and that both compounds displayed comparable short half-lives, we think that the chemical stability is probably an important limiting factor for the activity. Furthermore, the data of inhibition of the PBPs and the β-lactamases recorded after a short pre-incubation period with the enzymes indicated that the compounds were poorly recognized by these enzymes. Therefore, we assumed that chemical stability is not the only factor that could account for the weak activity of the compounds. We think that the presence of a methylene bridge of the γ-lactam could create unfavorable steric interaction with the enzyme at the Michaelis complex stage.

In summary we have designed a novel family of anti-Bredt bicyclo[3.2.1] γ-lactams as PBP and β-lactamase inhibitors. Work is in progress to clarify and solve the above-mentioned issues namely: chemical stability and unfavorable steric interaction with the targeted enzymes. Two solutions are currently being explored by substituting a carbon atom for a heteroatom, oxygen, or nitrogen. On one hand, the introduction of a heteroatom α to the carbonyl of the lactam should lead to a more stable carbamoyl or ureido linkage. On the other hand, replacement of the methylene bridge by a heteroatom should reduce steric hindrance.

Acknowledgements

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12. IR data ($CHCl_3$) **1b**: 1498, 1524, 1589, 1608, 1709, 1765 cm^{-1}. **1c**: 1495, 1525, 1709, 1743 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ (ppm) **1b**: 2.35 (2H, m), 2.74 (1H, ddd, $J = 2.0$, 5.0, 18.0 Hz), 2.85–2.95 (3H, m), 3.28 (1H, m), 3.82 (3H, s), 5.26 (1H, d, $J = 14.0$ Hz), 5.48 (1H, d, $J = 14.0$ Hz), 7.63 (2H, d, $J = 8.5$ Hz), 8.20 (2H, d, $J = 8.5$ Hz). **1c**: 2.48 (1H, dd, $J = 4.0$, 16.0 Hz), 2.70 (1H, dd, $J = 4.0$, 16.0 Hz), 2.96 (1H, m), 3.11 (1H, ddd, $J = 1.0$, 2.0, 11.5 Hz), 3.51 (3H, s), 3.58 (1H, ddd, $J = 1.0$, 4.0, 11.5 Hz), 4.71 (1H, d, $J = 1.0$ Hz), 5.11 (1H, dt, $J = 5.0$, 1.0 Hz), 5.32 (2H, AB, $J = 13.5$ Hz), 7.53 (2H, d, $J = 8.5$ Hz), 8.22 (2H, d, $J = 8.5$ Hz).
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14. ^1H NMR (200 MHz, d_6-acetone) δ (ppm) **1d**: 2.05 (1H, dd, $J = 4.0$, 16.0 Hz), 2.60 (1H, dd $J = 4.0$, 16.0 Hz), 2.80 (1H, m), 2.95 (1H, dd, $J = 2.0$, 11.0 Hz), 3.35 (3H, s), 3.45 (1H, dd, $J = 4.0$, 11.0 Hz), 4.30 (1H, s), 5.10 (d, $J = 6.0$ Hz, 1H).
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16. IR data ($CHCl_3$) **1f**: 1496, 1524, 1588, 1608, 1707, 1761 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$) δ (ppm) **1f**: 1.10 (3H, t, $J = 7.5$ Hz), 1.58 (1H, m), 1.80 (1H, m), 2.25 (1H, ddd, $J = 2.5$, 6.0, 9.0 Hz), 2.36 (1H, d, $J = 18.0$ Hz), 2.58 (1H, m), 2.81 (1H, dd, $J = 3.0$, 12.0 Hz), 2.96 (1H, dd, $J = 5.5$, 18.0 Hz), 3.39 (1H, dd, $J = 2.5$, 12.0 Hz), 3.82 (3H, s), 5.25 (1H, d, $J = 14.0$ Hz), 5.51 (1H, d, $J = 14.0$ Hz), 7.64 (2H, d, $J = 8.5$ Hz), 8.21 (2H, d, $J = 8.5$ Hz). ^1H NMR (300 MHz, d_6-acetone) δ (ppm) **1g**: 1.07 (3H, t, $J = 7.5$ Hz), 1.66 (2H, m), 2.32 (1H, m), 2.56 (1H, dt, $J = 18.5$, 1.0 Hz), 2.65 (1H, m), 2.80 (1H, dd, $J = 2.5$, 11.5 Hz), 3.07 (1H, dd, $J = 5.5$, 18.5 Hz), 3.31 (1H, dd, $J = 2.5$, 11.5 Hz), 3.83 (3H, s).
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