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PROBING THE BINDING SITE OF THE PENICILLIN SIDE-CHAIN BASED ON THE TIPPER-STROMINGER HYPOTHESIS

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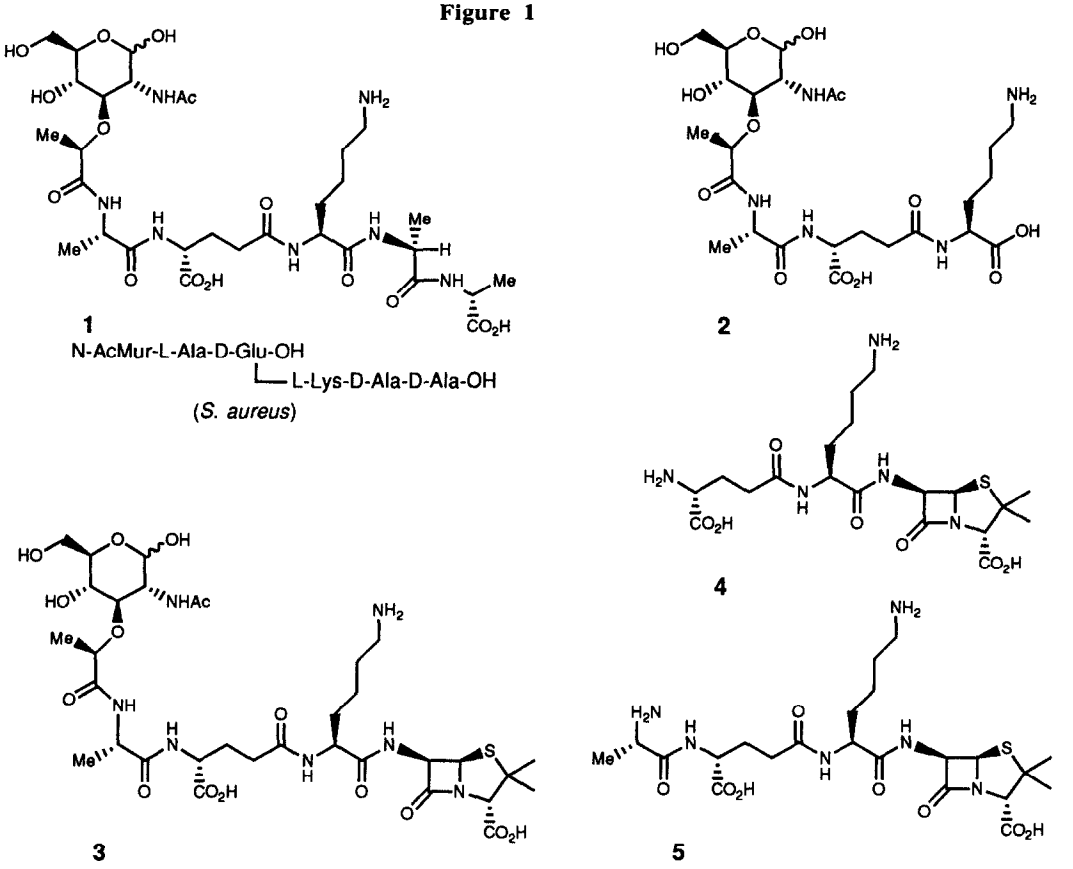
Abstract: A series of 6-aminoacyl penams were prepared in order to simulate the D-ala-D-ala-OH portion of the donor muramyl peptide involved in the transpeptidation reaction during the formation of the bacterial cell wall. (*R*)-, and (*S*)-4-Aminoacyl-α-butyl α-benzyl penicillins were prepared as models, and their binding to PBPs was studied.

In 1965, Tipper and Strominger[1] advanced the thought-provoking hypothesis that penicillin exerts its antibacterial action in part by mimicking the D-ala-D-ala moiety of a donor muramyl peptide **1** (Figure 1) in the transpeptidation reaction during the formation of the bacterial cell wall.[2] Extensive studies over the past 25 years have supported with the notion that inactivation of the transpeptidase involves a mechanism-based acylation of penicillin which acts like a suicide substrate.[3] X-ray crystallographic studies of an acyl-enzyme complex involving a cephalosporin and the R61 D,D-peptidase from Streptomyces,[4] and of Pen-G with the RTEM-1 β-lactamase,[5] show covalently bound Serine-62 and Serine-70, respectively, to the β-lactam carbonyl. These remarkable results have provided much insight into the possible mode of inactivation of other bacterial serine enzymes generally grouped as penicillin-binding proteins.[6] Over the years, extensive SAR studies involving literally hundreds of penicillins with modified 6-aminoacyl substituents have led to the conclusion that the target enzymes have definitive binding sites capable of recognizing conformational, functional, stereochemical and topological features of these substituents.[7] In particular a putative hydrophobic S_2 subsite on the enzymes is suggested to dock the aromatic ring of benzyl penicillin, while a closely related subsite binds lysine (or diaminopimelic acid), which represent near-terminal hydrophobic amino acid residues in the muramyl peptide.[8]

Intrigued by the original Tipper-Strominger hypothesis[1] we reasoned some time ago that the attachment of a 6-aminopenicillanic acid moiety as the terminal amide-linked subunit to a *N*-AcMur-L-ala-D-Glu-L-lys OH unit **2**, would lead to a novel derivative **3** in which the penam effectively replaces the original D-ala-D-ala portion of the natural substrate. While our work was in progress, Bentley and Stachulski,[9] reported the synthesis of a number of peptidyl-6-aminoacyl penicillanic acids, including the dipeptide **4**. Unfortunately, the *in vitro* antibacterial activity of the Beecham analogs[9] was disappointing, detracting from an otherwise creative opportunity to explore the Tipper-Strominger hypothesis by chemical means.

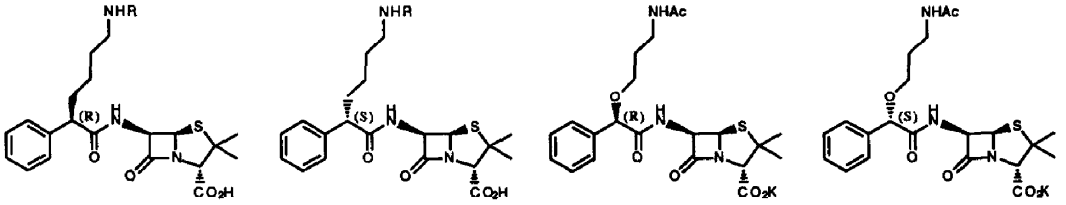
Figure 1



While pursuing our original goals toward the synthesis of the intended muramyl peptide analog **3**, we deemed it necessary to also bridge the peptide gap between **3** and the Beecham analog **4**, by synthesizing the des-*N*-AcMur-peptidyl-6-APA analog **5** as well as a number of lysyl 6-APA analogs. These compounds, in addition to **4**, represent a significant complement of natural muramyl peptide analogs containing a terminal 6-aminoacyl penicillanic acid moiety as a potential D-ala-D-ala mimic.

Preliminary results on the antibacterial activity of **2, 3** and **5** in *E.coli and S.aureus* were negative, and it became clear that a reassessment of the model was needed. It occurred to us that perhaps the aromatic moiety, which is an important functional and structural component of Pen G, was missing from our models. This prompted us to consider a hybrid structure in which a 4-aminobutyl chain (a lysine mimic) was appended to the α-benzylic carbon atom of Pen G as shown in **6** and **7** representing the (*R*)- and (*S*)-isomers respectively (Figure 2). Such molecules combine the requisite benzyl subunit in Pen G, as might be recognized by the hydrophobic binding site of target enzymes, with a lysine-like tether much the same as in the L-lys-D-ala-D-ala terminal tripeptide of the natural substrate **1**. The oxa-analogs **8** and **9**, which are readily available from D- and L-mandelic acids respectively, were also considered. Inasmuch as the analogs **6-9** are α-substituted benzyl penicillins, they also bear a structural relationship to ampicillin and carbenicillin which are potent antibacterial agents.

Figure 2



6a, R=H, mp 144-148°C
$[\alpha]_D$ +231.4° (c 1.03, MeOH)

6b, R=Ac, K salt, mp 140°C (dec.)
$[\alpha]_D$ +184.2° (c 1.2, MeOH)

7a, R=H, mp 178°C (dec.)
$[\alpha]_D$ +199.6° (c 1.05, MeOH)

7b, R=Ac, K salt; mp >160°C (dec.)
$[\alpha]_D$ +169.6° (c 1.71, MeOH)

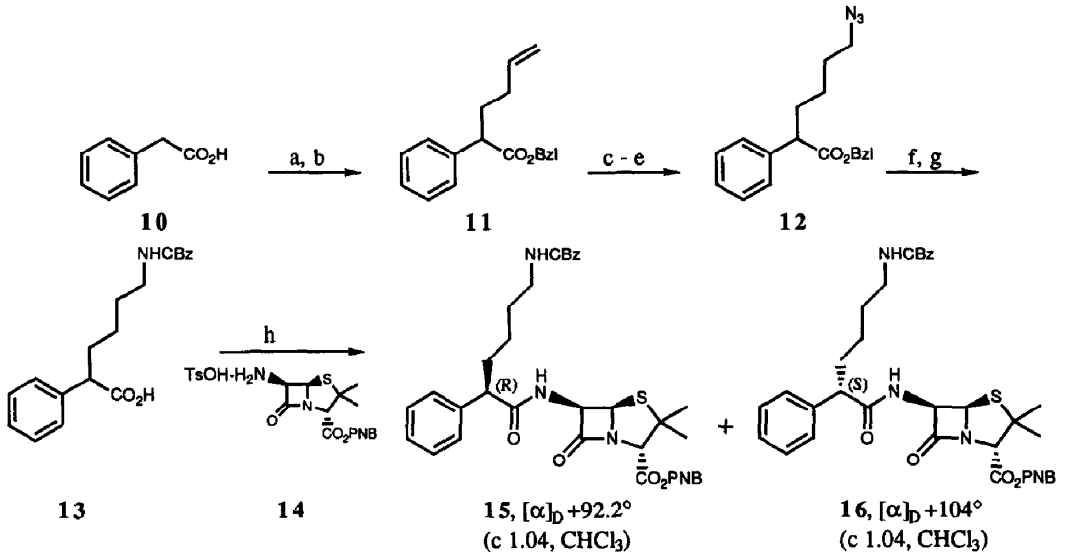
8, $[\alpha]_D$ +61.3° (c 1.09, MeOH)

9, $[\alpha]_D$ +150° (c 1.04, MeOH)

Chemistry

Racemic 2-(4-benzyloxycarbonylaminobutyl)-phenylacetic acid **13** was synthesized using standard methodology from the parent acid **10** as shown in Scheme 1. When the mixture was coupled to *p*-nitrobenzyl penicillin *p*-toluenesulfonate **14**, the two diastereomeric products **15** and **16** could be separated by column chromatography. Each product was sequentially deprotected and *N*-acetylated to ultimately give the intended hybrid structures **6a, 6b** and **7a,7b**. Since the stereochemical identity at the newly created stereogenic benzylic

Scheme 1



10 **11** **12**

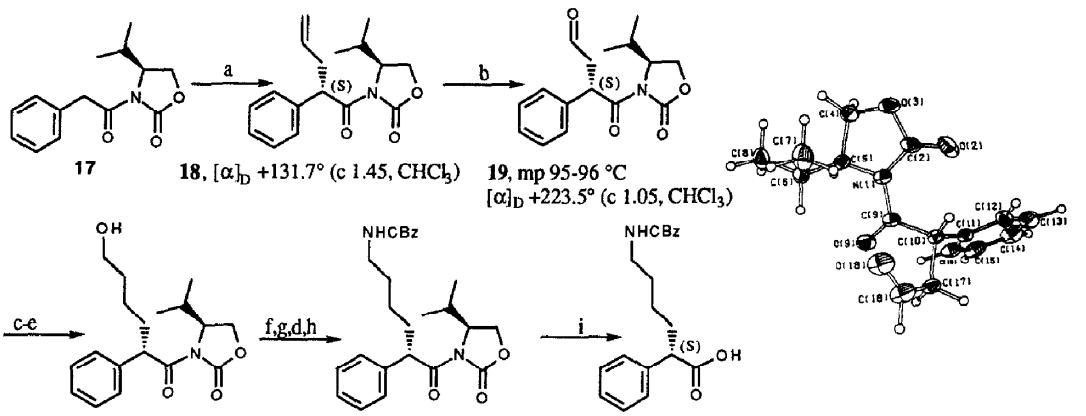
13 **14** **15**, $[\alpha]_D$ +92.2° (c 1.04, $CHCl_3$) **16**, $[\alpha]_D$ +104° (c 1.04, $CHCl_3$)

a. (i) LDA, THF, HMPA, 0°C, (ii) CH_2=$CHCH_2Br$, 91%; b. $PhCH_2OH$, DCC, DMAP, 86%; c. Disamylborane, THF, 0°C, 2h, then NaOH, H_2O_2, 96%; d. PPh_3, CBr_4, CH_2Cl_2 89%; e. NaN_3, DMSO, 90°C, quant.; f. 20% $Pd(OH)_2/C$, H_2, EtOAc, 96%; g. CbzCl, $NaHCO_3$, toluene, 86%; h. *i*-BuOCOCl, *N*-Me morpholine, THF, -15°C; add **14**, Et_3N, -15° to 25°C, 31%.

carbon in **15** and **16**, was not known, we chose to prepare one of the isomers by an unambiguous method adopting the Evans asymmetric alkylation method[10] as shown in Scheme 2. Thus, allylation of the oxazolidone **17** under standard conditions gave a mixture of **18** and its (*R*)-diastereomer in a ratio of 24:1 respectively (^1H

NMR). Oxidative cleavage of the terminal double bond in **18** gave a crystalline aldehyde **19** whose structure and absolute configuration was secured by single crystal X-ray analysis.[11] Having confirmed the sense of C-allylation we proceeded to extend the chain uneventfully via intermediates **20-22**. The acid **22** was coupled to **14** as before, and the product was found to be identical in all respects with the "*S*" isomer **16** prepared from the separation of diastereomers (Scheme 1). The N-Cbz derivatives were then transformed to the respective derivatives **6a, 6b** and **7a, 7b** using conventional methodology.

Scheme 2



17 **18**, $[\alpha]_D$ +131.7° (c 1.45, $CHCl_3$) **19**, mp 95-96 °C $[\alpha]_D$ +223.5° (c 1.05, $CHCl_3$)

20, mp 54°C $[\alpha]_D$ +116.7° (c 1.11, $CHCl_3$) **21**, $[\alpha]_D$ +67.5° (c 1.02, $CHCl_3$) **22**, $[\alpha]_D$ +38.7° (c 1.04, $CHCl_3$)

a. (i) LDA, THF, 0°C; (ii) allyl bromide, -78°C → -10°C, 66%; b. (i) O_3 pyr., EtOAc, (ii) Et_3N, 64%; c. Ph_3P = $CHCO_2Bn$, toluene, reflux, quant., d. 20% $Pd(OH)_2/C$, H_2, EtOAc, 96%; e. (i) EtOCOCl, *N*-Me morpholine, (ii). aq. $NaBH_4$, 0°C, 91%; f. PPh_3, CBr_4, CH_2Cl_2, 85%; g. NaN_3, DMSO, 90°C, quant.; h. CBzCl, aq. $NaHCO_3$, toluene, 85%; i. LiO_2H, THF, H_2O.

Since our intention was to study the effect of branching on the benzylic carbon in Pen G-type structures, we thought it would be of interest to consider the "oxa" analogs **8** and **9**. The (*R*)- and (*S*)-isomers were prepared in straightforward manner starting from the appropriate mandelic acid.

Results

Tables 1 and 2 list the results of binding of selected derivatives to penicillin-binding proteins from *E. coli* DCO and *S. aureus* ATCC25293.[12] The 4 aminobutyl analogs **6a** and **7a** showed equal binding activity; however, there appeared to be a distinct preference for the (*S*)-isomer in the 4-*N*-acetyl analog **7b** (Table 1). There was also a preference for the (*S*)-isomers **7a** and **7b** in the case of *S. aureus*, where no binding was observed with the (*R*)-isomers **6a** and **6b**. Interestingly, the oxa-analogs **8** and **9** did not exhibit any binding activity. Unfortunately, little if any antibacterial activity could be demonstrated with the hybrid analogs **6-9**, compared to Pen G.

We therefore conclude that while the Tipper-Strominger hypothesis remains a viable explanation for the mode of action of penicillins at the level of bacterial cell wall transpeptidases, our attempts to chemically simulate the structure of the reactive species have fallen short of our original expectations. Clearly, with the Pen G structure, an aromatic side chain is needed for *in vitro* and *in vivo* antibacterial activity.[13] An α-benzylic amino

Table 1: Binding to *E. coli* DCO PBPs

Analog	Concn. (μg/ml) Required for 90% Inhibitionof [^{14}C] Pen G Binding						MIC (μg/ml)[a]	
	PBP1a 90 kDa	PBP1b 90 kDa	PBP2 66 kDa	PBP3 60 kDa	PBP4 49 kDa	PBP5/6 40 kDa	DC0	DC2
6a	30	100	30	10	10	> 100	> 100[b]	> 100[b]
6b	>100	>100	>100	100	100	> 100	> 100[b]	> 20[b]
7a	**10**	**100**	**30**	**100**	**30**	**>100**	**100[b]**	**5[b]**
7b	30	100	30	30	30	> 100	> 100[b]	> 100[b]
Pen G	> 100	10	10	2	2	> 100	50	12
6-Epi-Pen G	>100	>100	>100	100	100	> 100	ND	ND
Ampicillin	2	10	0.5	2	0.1	100	2	0.4

ND, not determined. a. Inoculum 1%. b. Inoculum 0.01%.

and carboxyl group are tolerated if not optimal for activity (ampicillin, carbenicillin), and result in considerable improvement in the biological profile. Other changes as incorporated in the hybrid structures **6-9** that make the side-chain resemble part of the natural peptidoglycan are apparently not tolerated. It is not clear if this is due to the nature of the new side-chain itself, to the unfavorable or altered disposition of the aromatic ring vis-a-vis the penam ring system compared to Pen G, or to a combination of these and other more subtle effects.[14,15]

Table 2: Binding to *S. aureus ATCC 25293 PBPs*

Analog	Concn. (μg/ml) Required for 90% Inhibition of [^{14}C] Pen G Binding				MIC (μg/ml)[a]
	PBP1 87 kDa	PBP2 80 kDa	PBP3 75 kDa	PBP4 41 kDa	
6a	100	100	>100	ND	>100[b]
6b	100	>100	>100	ND	> 20[b]
7a	**30**	**30**	**>100**	**ND**	**5[b]**
7b	30	100	>100	ND	20[b]
Pen G	0.1	0.1	0.1	10	<0.05
6-Epi-Pen G	2	>100	ND	>100	> 20
Ampicillin	0.1	2	0.1	>100	1.6

ND, not determined. a. Inoculum 1%; b. Inoculum, 0.01%.

Acknowledgement

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