SYNTHESIS AND BIOLOGICAL EVALUATION OF FUNCTIONALIZED EPOXIDES STRUCTURALLY RELATED TO THE CARBAPENEM FAMILY

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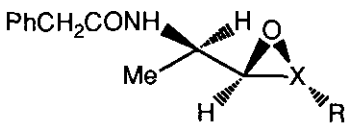
Abstract - Epoxides (**6**) and (**7**), topologically related to the carbapenem antibiotics, were designed as potential alkylating inhibitors of the bacterial D,D-peptidases. The olefinic precursors (**8**-**9**) were readily prepared, in three steps, by coupling the Wittig reagent (**13**) with the aldehyde synthons (**10**) or (**11**) resulting from diastereoselective aldol condensations. Epoxide (**7**) showed a weak anti-β–lactamase activity.

INTRODUCTION

The increasing bacterial resistance to penicillin and cephalosporin antibiotics stimulated intensive research aiming at the discovery of non β-lactam analogs[1] which would bypass the microorganisms' defenses. Some years ago, we became interested in the synthesis of alkylating agents capable of interacting irreversibly with penicillin binding proteins.[2] We first prepared oxaziridines (**1**-**2**)[3,4] and epoxides (**3**-**4**)[5,6] equipped with penicillin acylamino side-chains (Scheme 1). Unfortunately, the compounds were chemically too unstable to be used in any biological application. The easy cleavage of the strained heterocyclic ring probably resulted from an anchimeric assistance of the neighbouring amide group.



Oxaziridines : X = N

1 R = C $(Me)_2$ - CO_2R'

2 R = $(CH_2)_2$ - CO_2R'

Epoxides : X = CH

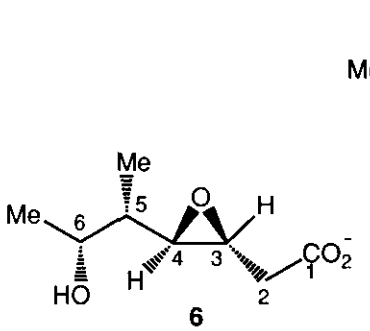
3 R = $(CH_2)_n$ - CO_2R' (n = 1, 2)

4 R = SO_3^- Na^+

Scheme 1

We therefore turned our attention to the synthesis of epoxides (**5-7**) bearing an hydroxyalkyl side-chain typical of the carbapenem family [7,8] (Scheme 2). The α, β–epoxysulphonic acid (**5**)[6] was prepared and found to be stable in aqueous solution. However it did not show any antibacterial or anti-β–lactamase activity.

The present paper describes the synthesis of the corresponding 3,4-*trans*-epoxides (**6**) (5,6-*syn*) and (**7**) (5,6-*anti*) (Scheme 2). The structural design of these potential inhibitors of bacterial serine D,D-peptidases has been discussed in previous papers.[3,6]



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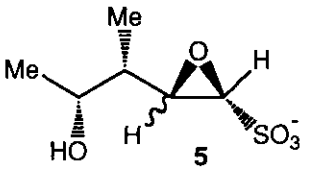

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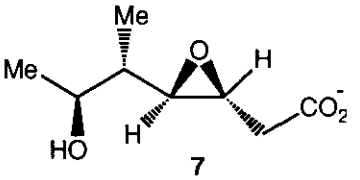

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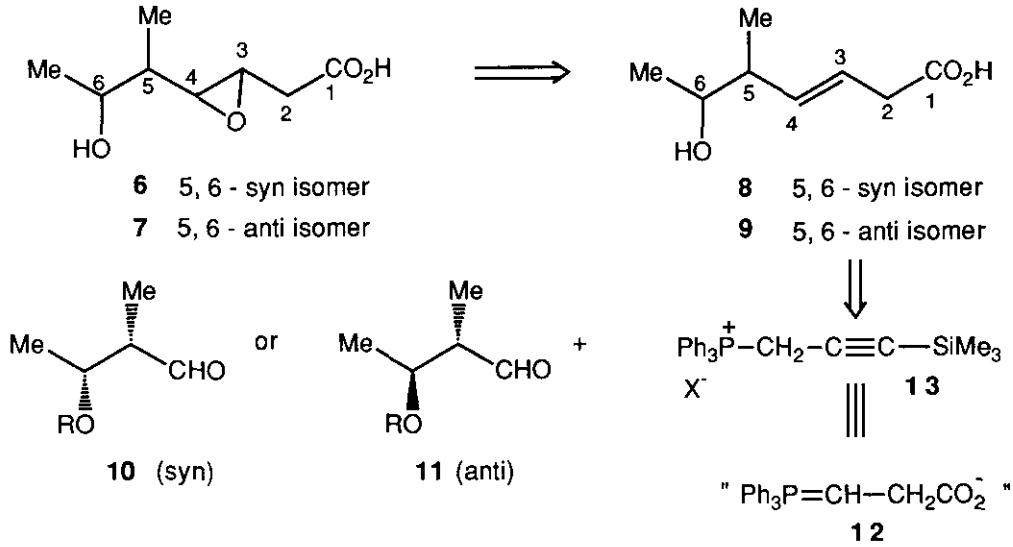
Stereochemistry is relative.

Scheme 2

It is worth mentioning that a reactive epoxide function is present in the structure of some naturally occurring antibiotics, like phosphonomycin,[9] methylenomycin A[10] and the pseudomonic acids.[11] Moreover, several synthetic substrate-like epoxides have been found to act as specific and irreversible inactivators of pepsin,[12] renin,[13] papain,[14] cathepsins,[15,16] subtilisin,[17] *chymotrypsin*[17] *and carboxypeptidase A.*[18]

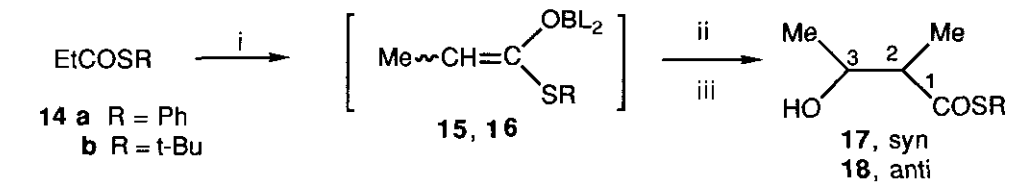
RESULTS

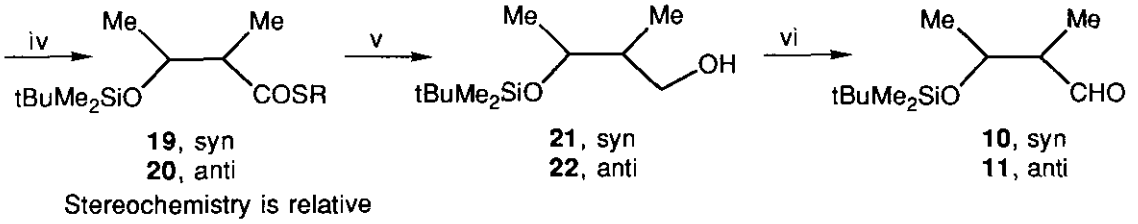
Our synthetic plan (Scheme 3) for the preparation of the target epoxides (**6-7**) was based on the oxidation of trans olefinic precursors (**8-9**) obtained by coupling aldehydes (**10-11**) with the Wittig reagent (**13**), used as synthetic equivalent of **12**.[5]



Scheme 3

Both *syn* and *anti* aldehydes (**10**) and (**11**) were readily obtained from a sequence of reactions involving a diastereoselective aldol condensation[19,20] of a boron enolate with acetaldehyde as a key step (Scheme 4 - Table I). S-Phenyl propanethiolate (**14a**) was converted into E-propenyloxyborane (**15a**) (Entry 1) by reaction with bicyclo[3.3.1]boron-9-nonane triflate according to Masamune's procedure.[21] The condensation of **15a** with acetaldehyde took place with high diastereoselectivity, as observed with other aldehydes.[19] The *syn* configuration of **17a** (Entry 1) was assigned on the basis of the value of the coupling constant for protons H-2 and H-3 (4.1 Hz).[19] The Z-propenyloxyborane (**16b**) (Entry 4) was generated from the reaction of S-t-butyl propanethiolate (**14b**) with the bulkier dicyclopentylboron triflate, again following Masamune's procedure.[22] The condensation of **16b** with acetaldehyde was also highly stereoselective in favour of the *anti* isomer (**18b**) (Entry 4); the J value for protons H-2 and H-3 was 6.5 Hz.[19] We also observed that the stereoselectivity of the aldol-type condensation significantly decreases





Stereochemistry is relative

Reagents and conditions: (i) L_2BOTf, $EtN(iPr)_2$, ether, 0°C or -78°C; (ii) MeCHO (excess), 20°C; (iii) H_2O_2, H_2O - MeOH, pH = 7; (iv) $tBuMe_2SiX$, Et_3N, ether, 20°C; (v) $NaBH_4$, EtOH, reflux or $LiBH_4$ THF,reflux; (vi) PCC, CH_2Cl_2, 20°C.

Scheme 4

when the boron enolate (**16a**) was generated from S-phenyl propanethiolate (**14a**) and dicyclopentylboron triflate (Entries 2 and 3). It can be assumed that this combination of reagents generated a mixture of Z and E enolates (**16a**) which led to the observed *syn* and *anti* aldol products (**17a**) and (**18a**). This observation further confirmed that S-t-butyl propanethiolate-dicyclopentylboron triflate constituted a unique combination for the production of Z enolate.

Table I : Diastereoselective aldol condensation

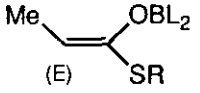
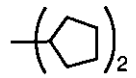
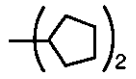
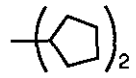
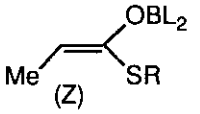
Entry	R	L_2	t°C	Enolates[21, 22]		Products Yield	Products syn : anti
1	Ph		0°C		**15a**	79%, **17a**	≥ 96 : 4
2	Ph		0°C	(E) + (Z)	**16a**	52%, **17a** : **18a**	50 : 50
3	Ph		-78°C	(E) + (Z)	**16a**	60%, **17a** : **18a**	37 : 63
4	tBu		-78°C		**16b**	89%, **18b**	≤ 4 : 96

Table II : Preparation of aldehydes (10 and 11)

Yields of *syn* isomers [a],[b]				Yields of *anti* isomers [a],[b]			
17a	**19a**	**21**	**10**	**18b**	**20b**	**22**	**11**
79%	92%	82%	79%	89%	98%	81%	82%
overall: 47% from **14a**				overall: 58% from **14b**			

(a) All products were purified by chromatography. (b) All compounds are racemic.

The transformation of thiol esters (**17a**) and (**18b**) into aldehydes (**10**) and (**11**) was accomplished in three steps with good overall yields (Scheme 4, Table II). After silylation of the β–hydroxyl functions, the thiol esters were reduced into primary alcohols by treatment with lithium borohydride in refluxing THF. This reagent gave better yields than sodium borohydride in ethanol or DIBAL-H in ether. Oxidation of **21-22** with pyridinium chlorochromate yielded aldehydes (**10-11**), The vicinal coupling constants in ^{1}H-nmr between H-2 and H-3 were respectively 4.3 and 6.0 Hz for the *syn* and *anti* isomers. In both cases, the formyl proton gave rise to a doublet near δ 9.7. The carbonyl function gave a typical line at 205 ppm in the ^{13}C-nmr spectra.

For the three-carbon homologation, we applied a Wittig strategy that we had already used in a previous synthesis.[5] Treatment of γ-trimethylsilylpropargyl(triphenyl)phosphonium bromide (**13**)[23,24] with butyllithium gave an ylide which was quenched with aldehydes (**10-11**) to yield the enynes (**23-24**) as mixtures of geometrical isomers (Scheme 5, Table III). The *trans* stereochemistry was assigned to the major isomers (J_{AB} for olefinic protons = 16.1 Hz). A high E selectivity (> 85%) was normally expected for such a stabilized ylide.[25] Further transformation of

Table III : Preparation of epoxides (6 and 7)[a]

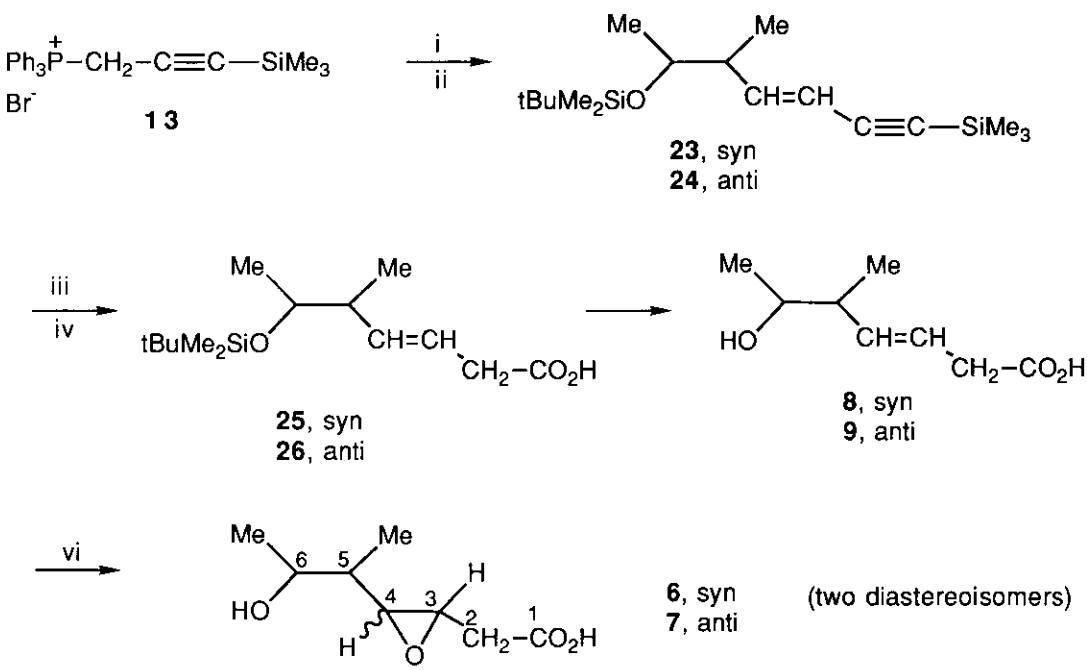
Yield of *syn* isomers					Yields of *anti* isomers				
23	(E : Z)[b]	**25**	**8**	**6**	**24**	(E : Z)[b]	**26**	**9**	**7**
93%	(85 : 15)	79%	84%	72%	75%	(87 : 13)	82%	96%	85%
Overall : 44% from **10**					Overall : 50% from **11**				

(a) All compounds are racemic. (b) The product was purified by chromatography.

the trimethylsilyl acetylenes (**23-24**) into the acid derivatives (**25-26**) was accomplished[26] in two steps by hydroboration followed by controlled oxidation. Removal of the *O*-t-butyldimethylsilyl protecting group[27] with aqueous HF in acetonitrile yielded the alcohols (**8-9**). These were easily oxidized[28] with *m*-chloroperbenzoic acid to give epoxides (**6-7**) (Table III) which were unstable under usual chromatographic conditions. However, they could be readily purified by extraction with cold water.

Epoxides (**6**) and (**7**) were obtained as mixtures of diastereoisomers (1.5 : 1 and 1 : 1 ratios) resulting from unselective formation of the new asymmetric centers C-3 and C-4 in the oxidation process. The ^{1}H-nmr spectra showed two ddd multiplets and two dd multiplets near δ 3.0-3.2 and δ 2.7-2.8, assigned to the H-3 and H-4 protons respectively on the small rings. The low value of the vicinal coupling constant is characteristic of a *trans* relationship (J = 2.5 Hz).[29] The H-2 protons of the methylene groups gave a typical ABX pattern near δ 2.3-2.6 (J_{AB} = 16.8 Hz).

Aqueous solutions of epoxides (**6**) and (**7**) were stable for several days when stored at 4° C in the dark. But they rapidly decomposed when neat, even at low temperature (- 60° C).



Reagents and conditions: (i) nBuLi, THF, -78 to 0°C; (ii) **10** or **11**, THF, -78 to 0°C; (iii) $HB(cC_6H_{11})_2$ THF, 0°C; (iv) H_2O_2, aq.NaOH , MeOH, 0 to 20°C; (v) aq.HF, MeCN, 20°C; (vi) MCPBA, CH_2Cl_2, 20°C.

Scheme 5

BIOLOGICAL EVALUATION

The olefins (**8**-**9**) and epoxides (**6**-**7**) were tested *in vitro* against representative gram-positive and gram-negative bacterial strains (*E. coli, Kl. pneumoniae, Ps. aeruginosa, Pr. mirabilis, S. marcescens, S. typhimurium, Sta. aureus, Str. pyogenes, Pr. vulgaris*). They were all devoid of antibiotic activity at concentrations up to 400 μM.[30] They were also inactive as inhibitors of isolated soluble D,D-peptidases.[31]

Compounds (**6**-**9**) were further tested against representative β-lactamases[32] from classes A, B and C. Interestingly, the epoxide (**7**) induced 40 % inhibition of *Bacillus cereus* β-lactamase at 1mM concentration. Thus, a weak biological activity was observed for an epoxide designed as a topological analog of carbapenem antibiotics.

EXPERIMENTAL

Ir spectra were taken with a Perkin-Elmer 297 instrument and calibrated with polystyrene. ^{1}H-nmr spectra were recorded on Varian T60 or Varian XL-200 spectrometers in $CDCl_3$ (unless otherwise mentioned) with TMS as internal standard. ^{13}C-nmr spectra were recorded on Varian XL-200 instrument in $CDCl_3$. Column chromatographies were performed with Merck silica gel K-60 (230-400 mesh) using distilled solvents. CH_2Cl_2 was dried over P_2O_5 at reflux. Ether and THF were dried over $LiAlH_4$ under argon atmosphere.

(*syn*)-Thiophenyl 3-hydroxy-2-methylbutanoate (**17a**) :**17a** was prepared according to reference 6; R_F = 0.25 (EtOAc-CH_2Cl_2 7 : 93); ir (film) 3420 (br), 1695, 1478, 1440, 1148, 942, 745 *cm*$^{-1}$; ^{1}H-nmr (200 MHz) δ 1.18 (3H, d, J = 6.4 Hz), 1.31 (3H, d, J = 7.0 Hz), 2.44 (1H, br s), 2.77 (1H, qd, J = 4.1 and 7.0 Hz), 4.14 (1H, qd, J = 4.1 and 6.4 Hz), 7.41 (5H, s); ^{13}C-nmr ppm 12.4, 20.5, 54.6, 68.4, 127.4, 129.2, 129.5, 134.4, 201.5.

(*anti*)-Thio-t-butyl 3-hydroxy-2-methylbutanoate (**18b**) : The solvent was removed under vacuum from 27 ml (13.5 mmol) of a solution of dicyclopentylboron triflate (0.5 M in CH_2Cl_2) and replaced with dry ether (21 ml) under argon atmosphere. At -78° C, under stirring, a solution of thiol ester **14b** (1.971 g, 13.5 mmol) and diisopropylethylamine (2.4 ml, 13.5 mmol) in dry ether (21 ml) was

added dropwise during 10 min. After 1 h stirring at 20° C, an excess of acetaldehyde was added (4.5 ml in 21 ml of ether) and the mixture was further stirred for 3 h. A solution of phosphate buffer (pH7, 100 ml) and hydrogen peroxide (30 % in water, 16 ml) in methanol (110 ml) was added, and the resultant mixture was stirred overnight. Concentration under vacuum, extraction with CH_2Cl_2 (3 x 75 ml), washing with aqueous $Na_2S_2O_3$, drying over $MgSO_4$ and evaporation gave crude **18b** which was purified by flash-chromatography on silica gel (EtOAc - CH_2Cl_2 5 : 95; R_F = 0.40) : yield 1.519 g (89 %, yellow oil); ir (film) 3420 (br), 1675, 1455, 1364, 1157, 1110, 955 cm^{-1}; ^{1}H-nmr (200 MHz) δ 1.18 (3H, d, J = 7.0 Hz), 1.21 (3H, d, J = 6.4 Hz), 1.47 (9H, s), 2.58 (1H, qd, J = 6.5 and 7.0 Hz), 2.61 (1H, qd, J = 6.5 and 6.4 Hz), 3.90 (1H, br s); ^{13}C-nmr ppm 14.5, 20.7, 29.6, 48.0, 55.3, 69.6, 204.6.

(*syn*)-Thiophenyl 3-(t-butyldimethyl)silyloxy-2-methylbutanoate (**19a**) : **19a** was prepared according to reference 6; ^{1}H-nmr (60 MHz) δ 0.03 (6 H, s), 0.85 (9H, s), 1.13 (3H, d, J = 6.0 Hz), 1.17 (3H, d, J = 6.8 Hz), 2.68 (1H, qd, J = 6.0 and 6.8 Hz), 4.05 (1H, qd, J = 6.0 and 6.0 Hz), 7.30 (5H, s).

(*anti*)-Thio-t-butyl 3-(t-butyldimethyl)silyloxy-2-methylbutanoate (**20b**) : The alcohol (**18b**) (1.519 g, 7.99 mmol) dissolved in dry ether (80 ml) was treated under argon atmosphere at 20° C with triethylamine (1.11 ml, 7.99 mmol) and t-butyldimethylsilyl triflate (1.83 ml, 7.99 mmol). After 30 min stirring, the mixture was allowed to stand for 15 min and the upper ether layer was decanted. The residue was further washed with ether (2 x 30 ml). The combined ether solutions were evaporated and run through a short column of silica gel (hexane - ether 9 : 1) to furnish **20b** as a colourless oil : yield 2.390 g (98 %); ^{1}H-nmr (60 MHz) δ 0.02 (6H, s), 0.83 (9H, s), 1.03 (3H, d, J = 6.7 Hz), 1.10 (3H, d, J = 6.0 Hz), 1.40 (9H, s), 2.60 (1H, qd, J = 6.2 and 6.7 Hz), 4.07 (1H, qd, J = 6.2 and 6.0 Hz).

(*syn*)-3-(t-Butyldimethyl)silyloxy-2-methylbutan-1-ol (**21**) : **21** was prepared according to reference 6; ir (film) 3360 (br), 2960, 2930, 2890, 2860, 1470, 1460, 1255, 1148, 1093, 1040, 835, 772 cm^{-1}; ^{1}H-nmr (200 MHz) δ 0.12 (6H, s), 0.92 (9H, s), 0.99 (3H, d, J = 7.0 Hz), 1.24 (3H, d, J = 6.2 Hz), 1.63 (1H, qddd, J = 3.6, 5.6, 6.0 and 7.0 Hz), 2.95 (1H, br s), 3.58 (1H, dd, J = 10.8 and 5.6 Hz), 3.79 (1H, dd, J = 10.8 and 3.6 Hz), 3.86 (1H, qd, J = 6.0 and 6.2 Hz); ^{13}C-nmr ppm - 5.14, - 4.57, 12.23,

17.89, 18.32, 25.73, 40.84, 65.52, 71.99.

(*anti*)-3-(t-Butyldimethyl)silyloxy-2-methylbutan-1-ol (**22**) : The thiol ester (**20b**) (1.121 g, 3.69 mmol) dissolved in THF (100 ml) under argon atmosphere was reacted with 2 ml of a solution of lithium borohydride (2M in THF) for 5 h under reflux. Then water (23 ml) was added dropwise with care, the resulting mixture was stirred for 15 min and then extracted with CH_2Cl_2 (3 x 30 ml). Drying over $MgSO_4$, evaporation and flash chromatography on silica gel (hexane-ether 9 : 1) gave the alcohol (**22**) as a pale yellow oil : yield 0.651 g (81 %); ir (film) 3360 (br), 2960, 2930, 2890, 2860, 1470, 1460, 1255, 1105, 1075, 1035, 835, 772 cm^{-1}; ^{1}H-nmr (200 MHz) δ 0.08 (6H, s), 0.79 (3H, d, J = 7.0 Hz), 0.89 (9H, s), 1.15 (3H, d, J = 6.5 Hz), 1.95 (1H, qddd, J = 3.5, 4.5, 8.8 and 7.0 Hz), 3.10 (1H, br s), 3.50 (1H, dd, J = 10.7 and 4.5 Hz), 3.70 (1H, dd, J = 10.7 and 8.8 Hz), 4.00 (1H, qd, J = 3.5 and 6.0 Hz); ^{13}C-nmr ppm - 4.95, - 4.23, 14.59, 17.93, 22.09, 25.83, 41.74, 65.81, 73.91.

(*syn*)-3-(t-Butyldimethyl)silyloxy-2-methylbutan-1-al (**10**) : **10** was prepared according to reference 6; ir (film) 1740 cm^{-1}; ^{1}H-nmr (200 MHz) δ 0.02 (6H, s), 0.82 (9H, s), 1.02 (3H, d, J = 6.8 Hz), 1.13 (3H, d, J = 6.3 Hz), 2.33 (1H, qdd, J = 0.4, 4.3 and 6.8 Hz), 4.23 (1H, qd, J = 4.3 and 6.3 Hz), 9.71 (1H, d, J = 0.4 Hz); ^{13}C-nmr ppm - 4.48, - 3.75, 8.66, 18.51, 21.73, 26.27, 54.00, 68.76, 205.52.

(*anti*)-3-(t-Butyldimethyl)silyloxy-2-methylbutan-1-al (**11**) : The alcohol (**22**) (2.18 g, 10 mmol) was dissolved in dry CH_2Cl_2 (10 ml) and added rapidly to a stirred suspension of pyridinium chlorochromate (4.3 g, 20 mmol) in CH_2Cl_2 (15 ml) under argon atmosphere at 20° C. After 6 h, dry ether (60 ml) was added and the reaction mixture was stirred for an additional 30 min. The ether solution was decanted and the residue was triturated with ether (2 x 20 ml). The combined ether solutions were passed through a filtration column of silica gel. The filtrate was then concentrated and passed through a second filtration column of silica gel using hexane - ether (10 : 1) for elution. The aldehyde (**11**) was isolated as a colourless oil : yield 1.787 g (82 %); ir (film) 1740 cm^{-1}; ^{1}H-nmr (200 MHz) δ 0.07 (6H, s), 0.80 (9H, s), 1.07 (3H, d, J = 7.0 Hz), 1.22 (3H, d, J = 6.3 Hz), 2.37 (1H, qdd, J = 2.6, 6.0 and 7.0 Hz), 4.03 (1H, qd, J = 6.0 and 6.3 Hz), 9.74 (1H, d, J = 2.6 Hz).

Both aldehydes (**10**) and (**11**) were unstable, even when stored at low temperature (- 60° C) and were prepared just before use in the Wittig coupling reactions.

(5,6 *syn*)-(3,4 *trans*)-6-(t-Butyldimethyl)silyloxy-5-methyl-1-trimethylsilylhept-3-en-1-yne (**23**) : To a suspension of trimethylsilylpropargyl(triphenyl)phosphonium bromide (**13**) (0.836 g, 1.85 mmol) in dry THF (5 ml), stirred at -78° C under argon atmosphere, was added dropwise a solution of butyllithium (0.8 ml, 2.3 M in hexane). The mixture was allowed to warm up -40° C over 2 h, stirred for 30 min at - 40° C, then recooled at - 78° C. A solution of aldehyde (**10**) (0.256 mg, 1.19 mmol) in THF (2 ml) was added dropwise. The mixture was stirred for 2 h at 0° C, then diluted with cold ether (20 ml) and filtered through celite together with further washings with ether (4 x 20 ml) of the residue in the flask. Concentration followed by two successive flash-chromatographies on silica gel (hexane-ether 1 : 1, then EtOAc-hexane 1 : 39; R_F = 0.55) gave pure **23** : yield 0.340 g (93 %, pale yellow oil); ^{1}H-nmr (200 MHz) δ 0.04 (6H, s), 0.18 (9H, s), 0.98 (9H, s), 0.98 (3H, d, J = 6.8 Hz), 1.07 (3H, d, J = 6.2 Hz), 2.20 (1H, qddd, J = 6.8, 1.2, 6.1 and 7.8 Hz), 3.63 (1H, qd, J = 6.2 and 6.1 Hz), 5.50 (1H, dd, J = 1.2 and 16.1 Hz), 6.18 (1H, dd, J = 7.8 and 16.1 Hz).

(5,6 *anti*)-(3,4 *trans*)-6-(t-Butyldimethyl)silyloxy-5-methyl-1-trimethylsilylhept-3-en-1-yne (**24**) : The olefine (**24**) was prepared according to the procedure described for **23** from 1.60 g (7.4 mmol) of aldehyde (**11**), 5.19 g (11.5 mmol) of phosphonium bromide (**13**) and 5 ml of butyllithium (2.3M in hexane). Work-up and flash chromatography gave 1.714 g of **24** (75 %, yellow oil); ^{1}H-nmr (200 MHz) δ 0.04 (6H, s), 0.18 (9H, s), 0.89 (9H, s), 0.89 (3H, d, J = 6.9 Hz), 1.04 (3H, d, J = 6.2 Hz), 2.19 (1H, qd, J = 6.9 and 6.0 Hz), 3.70 (1H, qd, J = 6.2 and 6.4 Hz), 5.49 (1H, d, J = 16.1 Hz), 6.17 (1H, dd, J = 8.3 and 16.1 Hz).

(5,6 *syn*)-(3,4 *trans*)-6-Hydroxy-5-methylhept-3-enoic acid (**8**) : Cyclohexene (2 ml, 20 mmol) in dry THF (1 ml) was smoothly treated at - 10° C, under argon atmosphere, with a solution of borane (1 M in THF, 10 ml, 10 mmol). After 2 h at 0° C under stirring, the mixture was cooled at - 10° C and a solution of the olefin (**23**) (0.872 g, 2.81 mmol) in THF (5 ml) was added dropwise. After 3 h at - 5° C, the mixture was successively treated with dry methanol (1.3 ml), with a 2N aqueous solution of NaOH (6.5 ml) and with a 30 % aq. solution of hydrogen peroxide (3.5 ml). The temperature of the reaction mixture could not exceed 10° C. After stirring overnight, the mixture was poured on water (50 ml) and sodium hydroxide (6 ml, 2 M solution) was added. After washing with ether (3 x 20 ml), the aqueous solution was carefully acidified to pH3 with 2N HCl and extracted with CH_2Cl_2 (3 x 30 ml). Drying and concentration gave the acid (**25**) as a colourless oil (yield 0.604 g; 79 %).

This material was dissolved in a 5 : 95 solution of 50 % aqueous HF in acetonitrile (5 ml) and stirred for 20 min. Water (8 ml) and $CHCl_3$ (13 ml) were added. Decantation, extraction with $CHCl_3$ (3 x 10 ml), drying ($MgSO_4$) and evaporation gave **8** as a pale yellow oil : yield 0.290 mg (84 %); ^{1}H-nmr (200 MHz) δ 1.02 (3H, d, J = 6.8 Hz), 1.14 (3H, d, J = 6.6 Hz), 2.27 (1H, qdd, J = 6.1, 6.3 and 6.8 Hz), 3.09 (2H, d, J = 5.4 Hz), 3.71 (1H, qd, J = 6.1 and 6.6 Hz), 5.57 (2H, m), 5.90 (2H, br s, OH).

(5,6 *anti*)-(3,4 *trans*)-6-Hydroxy-5-methylhept-3-enoic acid (**9**) : The acid (**26**) was prepared as above from 20.7 ml of borane solution (1M in THF), 4.12 ml of cyclohexene, 1.70 g (5.48 mmol) of the precursor (**24**) in 10 ml of THF, and for the work up, 2.5 ml of MeOH, 12.7 ml of 2N aqueous NaOH, 6.8 ml of 30 % aq. H_2O_2 (yield : 1.22 g; 82 %, colourless oil). Treatment of **26** with aq. HF in MeCN as above yielded 0.650 g (96 %) of the acid (**9**) as a pale yellow oil; ^{1}H-nmr (200 MHz) δ 1.01 (3H, d, J = 6.8 Hz), 1.16 (3H, d, J = 6.3 Hz), 2.16 (1H, qdd, J = 6.3, 7.7 and 6.8 Hz), 3.08 (2H, d, J = 6.1 Hz), 3.59 (1H, qd, J = 6.3 and 6.3 Hz), 5.48 (1H, dd, J = 15.5 and 7.7 Hz), 5.62 (1H, td, J = 15.5 and 6.1 Hz), 6.16 (2H, br s, OH).

(5,6 *syn*)-(3,4 *trans*)-6-Hydroxy-5-methyl-3,4-epoxyheptanoic acid (**6**) : m- Chloroperbenzoic acid (0.356 g, 1.90 mmol) was added to a stirred solution of **8** (0.300 g, 1.90 mmol) in dry CH_2Cl_2 (15 ml). After 5 h at room temperature, the solvent was removed under reduced pressure. The residue was triturated with water (3 ml) and the aqueous solution was carefully removed by filtration through a wad of cotton-wool. The remaining solid was washed with further aliquots of water (3 x 3 ml). The combined aqueous solutions were concentrated under vacuum, then washed with CCl_4 (1 ml), and finally evaporated to dryness at low pressure to afford the epoxide (**6**) as a colourless oil (the neat epoxide was unstable but could be stored as an aqueous solution in the dark at 4° C for several days); yield : 0.239 g (72 %); ^{1}H-nmr (D_2O, 200 MHz, two diastereoisomers in a 3 : 2 ratio) δ 0.74 and 0.78 (3H, two d, J = 7.1 Hz, CH_3-5), 0.98 and 0.97 (3H, two d, J = 6.4 Hz, CH_3-6), 1.15 (1H, m, H-5), 2.35 and 2.25 (1H, two dd_{AB}, J = 7.2 and 16.8 Hz, H-2), 2.61 and 2.59 (1H, two dd_{AB}, J = 9.5 and 16.8 Hz, H-2), 2.70 and 2.68 (1H, two dd, J = 2.5 or 2.7 and 8.0 or 7.6 Hz, H-4), 3.02 and 3.11 (1H, two ddd, J = 2.5 or 2.7, 7.2 and 9.5 Hz, H-3), 3.64 and 3.60 (1H, two qd, J = 6.5 and 6.4 Hz, H-6).

(5,6 *anti*)-(3,4 *trans*)-6-Hydroxy-5-methyl-3,4-epoxyheptanoic acid (**7**): From m-CPBA (0.491 g, 2.62 mmol) and **9** (0.414 g, 2.62 mmol) in CH_2Cl_2 (25 ml), according to the procedure described above, was obtained epoxide (**7**) as a colourless oil (unstable material); yield 0.389 g (85 %); ^{1}H-nmr (D_2O, 200 MHz, two diastereoisomers in a 1 : 1 ratio) δ 0.83 and 0.86 (3H, two d, J = 6.7 Hz, CH_3-5), 1.08 (3H, d, J = 6.4 Hz, CH_3-6), 1.30 and 1.38 (1H, two qdd, J = 5.2, 6.9 and 6.7 Hz, H-5), 2.42 (1H, dd_{AB}, J = 4.4 and 16.9 Hz, H-2), 2.72 and 2.74 (1H, two dd_{AB}, J = 7.0 and 16.9 Hz, H-2), 2.82 and 2.85 (1H, two dd, J = 2.5 and 6.9, H-4), 3.12 and 3.23 (1H, two ddd, J = 2.5, 4.4 and 7.0, H-3), 3.70 and 3.71 (1H, two qd, J = 5.2 and 6.4 Hz, H-6).

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30. Biological evaluation against bacteria was performed according to reference 33.

31. Soluble D,D-peptidases excreted by Streptomyces R61 and Actinomadura R39 were used for this study as described in reference 34.

32. Isolated β-lactamases from *Bacillus licheniformis* 749, *Bacillus cereus* 5/B/6 and *Enterobacter cloacae* P99 were used for this study as described in reference 34.

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