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Synthesis of (2RS,E)-3-Ethylidene-azetidine-2-carboxylic Acid (rac. Polyoximic Acid)

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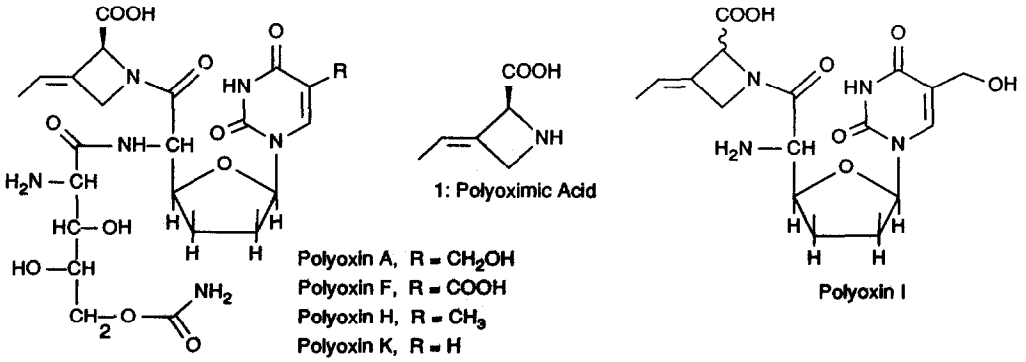
Abstract: *Treatment of α-diazo β-ketoester* ***4*** *with a catalytic amount of rhodium (II) acetate followed by Wittig reaction with phosphorane* ***5*** *gave the isomeric phenylthioester derivative* ***6a*** *and* ***6b****. Reduction of* ***6a*** *led to the corresponding allylic alcohol* ***7****, which was further converted into the bromide* ***8****. Dehalogenation of* ***8*** *with* $NaBH_4$*/ DMF resulted in compound* ***9****, which was deprotected with trifluoroacetic acid, giving racemic polyoximic acid (****1****).*

Polyoxins are peptidyl nucleoside antibiotics isolated from the culture broth of *Streptomyces cacaoi* var. *asoensis*[1]. They show a marked activity against phytopathogenic fungi. Their activity can be explained by the competitive inhibition of the enzyme chitin synthase[2,3] which catalyzes the final step of the biosynthesis of chitin, an essential component of the fungal cell wall structure. Polyoxins inhibit also the chitin synthase of the medically important human pathogen *Candida albicans* in cell-free systems[4] at low concentrations (K_i~10^{-6}M), but inhibition of the growth of intact cells can be only reached at rather high concentrations (K_i~10^{-3}M)[5]. These results stimulated us[6] and other groups[7] to synthesize structural analogues in order to overcome this drawback, but to our knowledge no completely satisfying solution to this problem was found as yet[8].

Whereas the majority of the polyoxins are dipeptides, there are also four polyoxins (polyoxin A, F, H, K) known, which are tripeptides. These four polyoxins, as well as polyoxin I contain in contrast to the other polyoxins the structurally unique (*2S,E*)-3-ethylidene-azetidine-2-carboxylic acid (1), named polyoximic acid, which is linked by a peptide bond to the C-terminal of the central nucleoside-amino acid (Scheme 1).



Scheme 1.

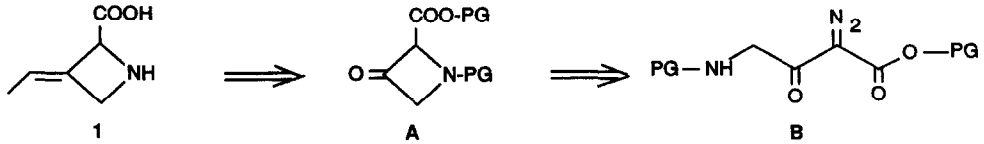
Whereas for the basic nucleoside skeleton (indicated as polyoxin C in the literature)[9] as well as for the second amino acid, commonly named 5-O-carbamoylpolyoxamic acid[10] several syntheses are known, no synthesis of the third constituent, polyoximic acid (1) has been reported until now. Only some attempts to synthesize 1 were published in the literature[11,12]. The closest approach to the final structure is based on a [2+2] cycloaddition of ethyl (tosylimino)acetate to methylallene giving racemic ethyl (*E*)-3-ethylidene-1-tosyl-azetidine-2-carboxylate as a side product[11]. Besides the low yield (3%) and the tedious separation from isomeric products in the cycloaddition step, it seems to be very unlikely to remove the protecting tosyl group from the nitrogen of the compound mentioned above, without destroying the molecule.

Beside the need of a suitable synthesis for 1, in order to fill the gap in the total synthesis of polyoxins, polyoximic acid represents a challenge for the synthetic chemist, despite it is a small, simple molecule. In this paper an efficient synthesis of racemic polyoximic acid (1) in 7 steps starting from commercially available Boc-glycine (2) will be reported.

In order to elaborate a short and economic synthesis, minimal functional group transformation was anticipated. For this reason a strategy has to be worked out, in which both, the amino and the carboxylic acid group would be already present in the starting material or should be introduced in an early step of the synthesis. Furthermore, in case of an acyclic starting material, a powerful method for efficient cyclization is necessary. Otherwise azetidine formation could fail or perform in low yield, because the ring closure to azetidines is a rather unfavorable process, due to strain and entropic factors.

It is known from literature[1a] that the α-hydrogen (H-C_2) of polyoximic acid, obtained from natural sources, is rather acidic. This mobile hydrogen is responsible for a high tendency to racemization which thwarts the isolation of optically active polyoximic acid from polyoxin A, even when mild acidic or basic conditions for peptide bond cleavage were applied. That polyoximic acid actually exists in optically active form (*S*-enantiomer) in the polyoxins, was proved by hydrogenation of the double bond, previous to peptide bond cleavage. The corresponding dihydro-polyoximic acid was more stable to racemization and could thus be isolated in enantiomerically pure form[1a]. In synthesis planning, it has to be taken into account that a possible consequence of this easy removable hydrogen could cause a double bond shift from the exocyclic position into the endocyclic postion.

Finally, suitable protecting groups have to be chosen, which allow their selective removal without effecting the desired product. A retrosynthetic plan in order to reach this goal is depicted in Scheme 2.



PG = suitable protecting group

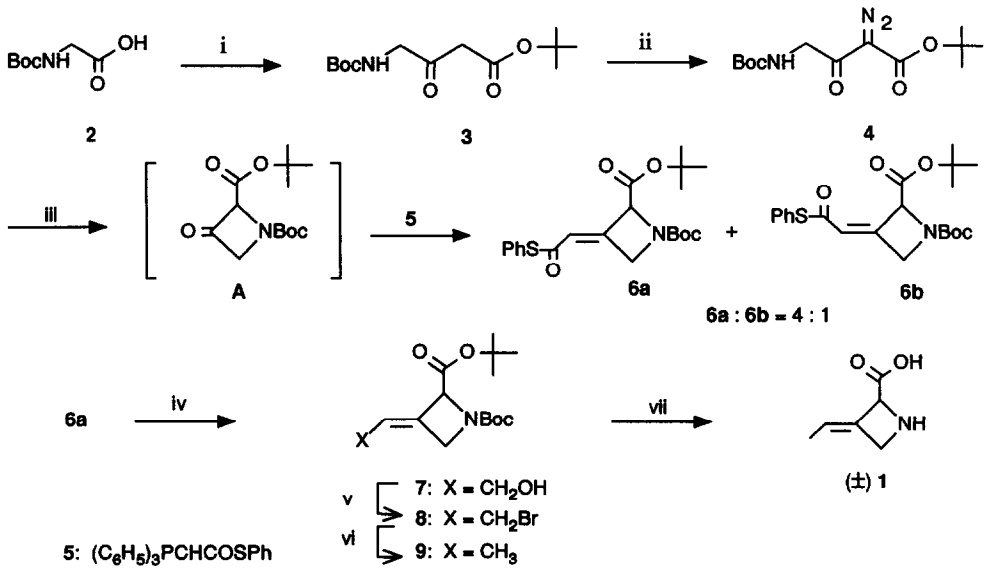
Scheme 2.

For the formation of azetidine derivative A, cyclization of an acyclic precursor B, based on a rhodium carbenoid intramolecular N-H insertion reaction, was anticipated. This type of reaction was successfully applied in the synthesis of carbapenems[13], a group of highly strained ß-lactams and seemed therefore to be applicable in the present case. Checking the literature for compounds representing structure A, a publication was found which reported the synthesis of heterocycles and among them of a 3-oxo-azetidine-2-carboxylate derivative, in which the carboxylic group was protected in form of a methyl ester and the amino function by a benzyloxycarbonyl group[14]. In this paper it was also mentioned that this compound was extremly labile against nucleophilic ring opening and did not even survive chromatography on silica gel, using methanol as eluent. From theoretical point of view this finding is not surprising. Because of the ring strain, structure A should be prone to ring opening as a result of a retro-Dieckmann reaction. In any case, further transformation of A by a Wittig or Wittig-type reaction seemed to be crucial with respect to the lability of structure A and the position and geometry of the double bond, which has to be formed. A further open question was the correct

choice of the protecting groups. A benzyloxycarbonyl group as mentioned in the above case has to be ruled out, because selective hydrogenation in presence of the double bond was expected to be accomplished only with difficulty. On the other hand it was estimated in the literature[15] in order to explain an unpredicted result, that a Boc-protected nitrogen may not be sufficiently nucleophilic to intercept the electrophilic rhodium carbenoid intermediate.

Despite this possible contra against a Boc-protected nitrogen and being aware that the Wittig reaction with the labile structure A could become very crucial, especially in case of bulky protecting groups, Boc-glycine (2) was chosen as starting material. Following this strategy, the synthesis of polyoximic acid could be performed, as depicted in Scheme 3.

Due to the high tendency of 1 to racemization, an asymmetric synthesis was not attempted.

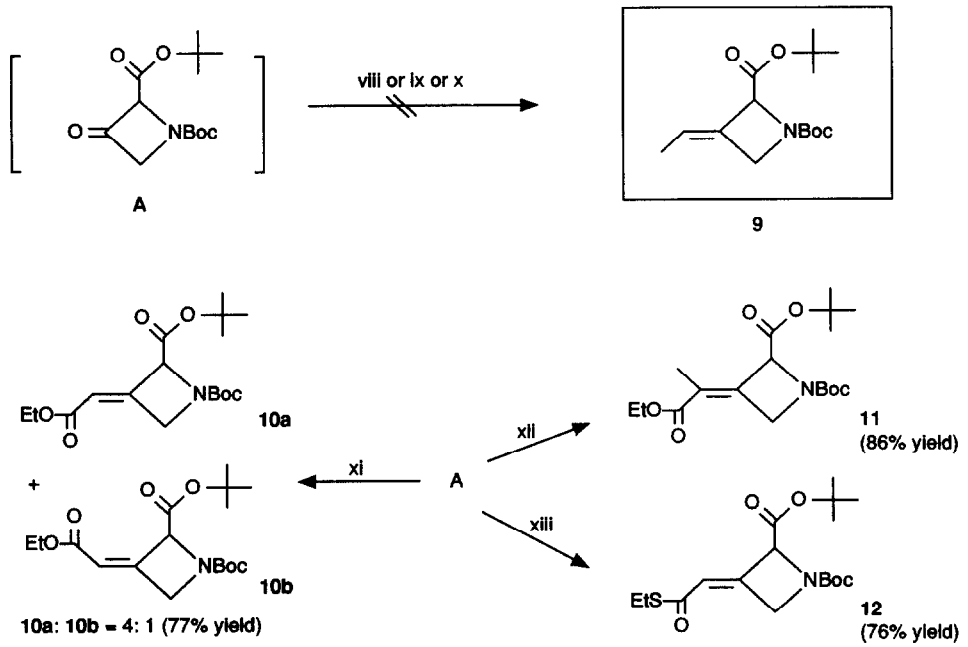


i: $CO(Im)_2$; $HOOCCH_2COOtBu$, $Mg(OEt)_2$; ii: $HOOC(C_6H_4)SO_2N_3$ or $CH_3SO_2N_3$, Et_3N;
iii: $Rh_2(OAc)_4$, CH_2Cl_2; $(C_6H_5)_3PCHCOSC_6H_5$ (5); iv: $NaBH_4$, $CeCl_3.6H_2O$, EtOH;
v: $(C_6H_5)_2PCH_2CH_2P(C_6H_5)_2$, CBr_4; vi: $NaBH_4$, DMF; vii: CF_3COOH.

Scheme 3.

The β-ketoester 3 was prepared from Boc-glycine analogous to existing procedures[16] in good yield. For this purpose, the carboxylic group of Boc-glycine was activated by reaction with N,N'-carbonyldiimidazole and treated further with the magnesium enolate of hydrogen tert-butyl malonate (prepared from hydrogen tert-butyl malonate and magnesium ethoxide)[17]. The diazo transfer to the β-ketoester 3 was accomplished by using either (p-carboxyphenyl)sulfonyl azide or methanesulfonyl azide and triethylamine as base, resulting in compound 4. Cyclization of α-diazo β-ketoester 4 proceeded without any problems, despite the bulky tert-butyl ester and Boc group, by refluxing compound 4 with a catalytical amount of rhodium (II) acetate in dichloromethane. To avoid any ring opening during work up and purification, the reaction solution was used directly for the Wittig reaction. Classical Wittig reaction with ethylidenetriphenylphosphorane failed as expected due to the strong basic conditions. Other methods, proceeding with less basic reagents, like chromium dichloride/ 1,1-diiodoethane[18] or Tebbe reagent[19] failed as well. On the other hand, stabilized phosphonium ylides like carbethoxyethylidene-triphenylphosphorane gave promising results (Scheme 4). In this and the other cases, side products, which occurred only in small amounts, were neglected. Taking into account this small inaccuracy, no compound with an endocyclic double bond could be found.

Another problem encountered in this reaction was the selective reduction of the newly created ester group. In order to avoid drastic conditions for this reduction, the introduction of a latent ester group was anticipated. Thiol esters are known to be reduced rather easily[20] and therefore a search for appropriate Wittig reagents was done. In the literature the only suitable reagent which was found was ethylthiocarboxymethylene-triphenylphosphorane[21]. This reagent could successfully be applied in the present case, resulting mainly in compound **12** (Scheme 4). The reduction of **12** turned out to be rather sluggish and therefore the preparation of the more reactive phenylthio ester was anticipated. The corresponding Wittig reagent **5** could be prepared analogous to the above mentioned Wittig reagent. Reaction of **A** with **5** gave a 4:1 mixture of **6a** and **6b** in 74% yield. The geometric (*cis/trans*) assignment to **6a** and **6b** as well as to **10a, 10b, 11** and **12** was made by NOE measurements. A NOE (~4%) between the α-H (H-C_2) and the olefinic H (H-C_5) and a NOE (~2%) between the H-C_5 and H-C_4 were found for the compounds **6a, 10a** and **12**. For the compound **11** a NOE (~10%) between H-C_2 and the methyl group at C_5 was measured.



Scheme 4.

After the unsuccessful trying of several borohydride reagents, the combination of $NaBH_4$ and cerium trichloride (Luche reagent) in ethanol was used for the reduction of thioester **6a**, resulting in compound **7** in 84% yield. Conversion of the hydroxymethyl to the methyl group was accomplished in a relatively straightforward manner. The bromo compound **8**, prepared in 94% yield from **7** by treatment with ethylene-bis-(diphenylphosphine) and tetrabromomethane in dichloromethane between -50°C and -15°C, was reduced with sodium borohydride in DMF[22] to give **9** in 81% yield. No compound with endocyclic double bond could be detected during this reaction. NOE measurements for compound **9** proved, that *E*-geometry was retained. The protecting groups of compound **9** were removed by treating with triflouroacetic acid, resulting in (±)**1** in 82% yield. The ^{1}H-NMR spectrum is comparable to the spectrum shown in the literature[1a] and the other analytical data are in good agreement with the data given there.

EXPERIMENTAL SECTION

Melting points were taken on a Kofler hot stage melting point apparatus (Reichert) and are uncorrected. IR spectra were obtained with a Perkin-Elmer 198 photometer. ^1H and ^{13}C NMR spectra were recorded in solution at 250 MHz (Bruker WM 250) and at 500 MHz (Bruker AMX 500). Mass spectra were measured on a VG 70-SE instrument (VG Analytical) operating at 8 kV accelerating voltage. Column chromatography was accomplished on silica gel 60 (0.063 - 0.2 mm, Merck) under hydrostatic pressure or on commercially available columns (Lobar Fertigsäule, filled with LiChroprep Si 60, 0.04 - 0.063 mm, Merck) using pressures up to 5 bars. Thin layer chromatography was performed on silica gel 60 F_{254} (Merck) or on dodecyl - silane-coated silica gel (reversed phase chromatography) OPTI-UP C_{12} (Anteg AG). The spots were visualized by either quenching of UV fluorescence (λ_{max}= 254 nm) or by staining with ninhydrin, followed by heat, or with potassium permanganate. The purity of the products was checked by high-performance liquid chromatography (Beckman 114M) on a column (250x4.6 mm) of Nucleosil $5C_8$ (Merck) with a water/ acetonitrile gradient and a Beckman 165 UV detector.

Tetrahydrofuran (THF) was obtained dry and oxygen free by distillation from $LiAlH_4$ under argon atmosphere. All other solvents were reagent grade quality and if necessary were dried by storing over molecular sieves (0.4 nm). Hydrogen tert-butyl malonate[17], (ethylthiocarbonyl-methylene)-triphenyl-phosphorane[21], (*p*-Carboxyphenyl)sulfonyl azide[23] and methanesulfonyl azide[24] were prepared according to literature procedures. All other reagents were from commercial sources and used as obtained.

(2*RS*,*E*)-3-Ethylidene-azetidine-2-carboxylic acid (1): 1g (3.53 mmol) of 9 was dissolved at 0°C in 30 ml of trifluoroacetic acid and kept at this temperature for one hour. Afterwards the solution was brought to room temperature and was allowed to stay for further 3 h. The trifluoroacetic acid was removed in vacuo and the residue was dissolved in 20 ml of water. This solution was passed through a column of Dowex 50W (H^+). After washing with water the absorbed product was eluted with 3% aqueous NH_3. Freeze drying afforded 365 mg (82% yield) of (±)**1** in form of a colourless powder, which was crystallized from methanol: m.p. 154-156°C, dec. (lit.[1a]: 158-160°C, dec.). IR (KBr): 3440, 3100, 2960, 2920, 1610, 1385, 810 cm^{-1}. ^1H-NMR (500 MHz, D_2O) δ 1.51 (dq, J_1=2Hz, J_2=7.1Hz, 3H-6), 4.65 + 4.71 (AB system, J=12.5Hz, further split by small couplings, 2H-4), 5.47 (m, H-2), 5.76 (qq, J_1=2.6Hz, J_2=7.1Hz). ^{13}C-NMR (D_2O) δ 15.4 (C_6), 56.3 (C_4), 70.6 (C_2), 126.0 (C_5), 127.5 (C_3), 172.3 (-COOH). MS: 128 $(MH)^+$, 82 $(M\text{-}COOH)^+$, 44 $(CO_2)^+$.

***tert*-Butyl [(*tert*-butoxycarbonyl)amino]-3-oxo-butanoate (3)**: A solution of magnesium *tert*-butyl malonate was prepared by dissolving 4.95 g (31 mmol) of hydrogen *tert*-butyl malonate in 150 ml of anhydrous THF, followed by cooling to -30°C and portionwise treatment with 3.55 g (31 mmol) of magnesium ethoxide. Stirring was continued for 5 h, in which time the reaction solution was allowed to warm to room temperature. In a separate flask, 5.25 g (30 mmol) of Boc-glycine were dissolved in 100 ml anhydrous THF and treated portionwise with 5.35 g (33 mmol) of *N*,*N'*-carbonyldiimidazole at -30°C. After stirring for half an hour at -30°C and two hours at room temperature, the reaction mixture was added dropwise to the solution of magnesium *tert*-butyl malonate, which was cooled to -30°C. Stirring was continued for half an hour at -30°C. Afterwards the cooling bath was removed and the mixture was stirred for further 16 h. Work up was performed by diluting the reaction mixture with 500 ml of diethyl ether and 500 ml of ethyl acetate followed by washing with 1N HCl, a saturated solution of $NaHCO_3$ and water (3x). The organic phase was dried over Na_2SO_4 and the solvents evaporated in vacuo giving 8 g of raw material, which was purified by column chromatography on silica gel with hexane/ ethyl acetate (4/1), leading to 6.71 g (82% yield) of a colourless oil, which solidified after standing overnight at -20°C: m.p. 37-39°C. ^1H-NMR (250 MHz, $CDCl_3$) δ 1.44 (s, 9H), 1.46 (s, 9H), 3.39 (s, 2H, -CH_2COO-), 4.10 (d, J=5Hz, 2H, -NCH_2CO-), 5.20 (s, broad, 1H, -CONH-).

***tert*-Butyl 4-[(*tert*-butoxycarbonyl)amino]-2-diazo-3-oxo-butanoate (4)**: 3.28 g (12 mmol) of β-keto ester 3 and 2.72 g (12 mmol) of (*p*-carboxyphenyl)sulfonyl azide were dissolved in 100 ml of anhydrous acetonitrile and cooled to 0°C; 3.33 ml (24 mmol) of triethylamine were added dropwise under stirring. After 5 min, the ice bath was removed and stirring continued for one and a half hours. The precipitate, which had

formed was filtered off and the solvent of the filtrate was evaporated in vacuo. The residue was taken up in ethyl acetate and the solution washed with saturated $NaHCO_3$ solution, with 1N HCl and 3 times with brine. After drying over Na_2SO_4 the solvent was evaporated in vacuo. The resulting yellow oil was purified by chromatography on silica gel with hexane/ ethyl acetate (4/1) leading to 3.43 g (96% yield) of a slightly yellow oil. IR (CH_2Cl_2) 3430, 2970, 2930, 2140, 1710, 1665 cm^{-1}. ^{1}H-NMR (250 MHz, $CDCl_3$) δ 1.44 (s, 9H), 1.46 (s, 9H), 4.38 (d, J=5.5Hz, 2H, -CH_2COO-), 5.23 (s, broad, 1H, -CONH-).

Phenylthio-carbonyl-methylenetriphenylphosphorane (5): 16.67 g (120 mmol) of bromoacetic acid were dissolved in 500 ml of dry CH_2Cl_2 and 13.5 ml (132 mmol) of thiophenol and 1.46 g (12 mmol) of dimethylaminopyridine were added. The reaction mixture was cooled to 0°C and 26 g (126 mmol) of dicyclohexylcarbodiimide were added portionwise. After standing for 18 h at room temperature the precipitate which formed during this time, was removed by filtration through Celite, followed by rinsing the filter cake with small portions of CH_2Cl_2. The filtrate was washed with saturated $NaHCO_3$ solution, water and brine, then was dried over Na_2SO_4 and concentrated in vacuo to give a yellow oil. 180 ml of dry benzene were added to the oil and the unsoluble solid residue was filtered off again. 31.56 g (120 mmol) of triphenylphosphine were added to the filtrate and the reaction mixture was allowed to stand at room temperature for 3 days to yield 42.76 g (72%) of colourless crystals after filtering and washing with benzene. The crystals were dissolved in 150 ml of CH_2Cl_2 and vigorously stirred with 100 ml of 10% aqueous Na_2CO_3 solution for 30 min. The layers were separated and the aqueous layer was extracted twice with CH_2Cl_2. The combined organic phases were partially concentrated in vacuo and then diluted with pentane. After standing two days at -20°C, a crystalline precipitate was formed, which was filtered off and dried. 35.4 g (99% yield, calculated from phosphonium salt or 71% total yield) of colourless crystals were obtained: m.p. 157-158°C.

***tert*-Butyl (*2RS,E*)-1-*tert*-butoxycarbonyl-3-(phenylthiocarbonyl-methylidene)-azetidine-2-carboxylate (6a) and *tert*-butyl (*2RS,Z*)-1-*tert*-butoxycarbonyl-3-(phenylthiocarbonyl-methylidene)-azetidine-2-carboxylate (6b)**: To a solution of 5.84 g (19.53 mmol) of 4 in 200 ml of anhydrous dichloromethane, 20 mg (~0.5 mol%) of rhodium acetate were added under an atmosphere of argon. The reaction mixture was heated to reflux and boiled for two hours. Afterwards the reaction was cooled to -30°C and a solution of 8.85 g (21.48 mmol) of phosphorane 5 in 100 ml of anhydrous dichloromethane was added dropwise under stirring. The reaction mixture was kept at -20°C for 18 h, warmed to room temperature and the solvent evaporated under vacuo. Chromatography on silica gel using hexane/ ethyl acetate (4/1) gave 5.06 g (52% yield) of **6a** and 1.3 g (13% yield) of **6b**. **6a**: m.p. 75-78°C (hexane/ ether), IR (CH_2Cl_2) 3050, 2975, 2830, 1745, 1705, 1665 cm^{-1}.^{1}H-NMR (500 MHz, $CDCl_3$) δ 1.45 (s,9H, -tBu), 1.52 (s, 9H, -tBu), 4.78 (dt, $J_{4,4'}$=19 Hz, $J_{2,4}$=2.3Hz, $J_{4,5}$=2.4Hz, H-4), 4.85 (dq, $J_{4,4'}$=19Hz, $J_{2,4'}$=4.4Hz, $J_{4',5}$=2.5Hz, H-4'), 5.09 (quintett, $J_{2,4}$=2.3Hz, $J_{4,4'}$=4.4Hz, $J_{2,5}$=2.2Hz), 6.31 (q, $J_{2,5}$=2.2Hz, $J_{4,5}$=2.4Hz, $J_{4',5}$=2.5Hz H-5), 7.44 (m, 5H, arom.).^{13}C-NMR ($CDCl_3$) δ 28.0, 28.2 (2xtBu), 59.5 (C_4) 69.7 (C_2), 80.5, 82.5 (2xq.C), 119.3 (C_5), 127.1 (C_3), 129,17 ($C_{3'}$, $C_{5'}$), 129.55 ($C_{4'}$), 134.3 ($C_{2'}$, $C_{6'}$), 148.4 ($C_{1'}$), 154.9 (-NCOO-), 165.7 (-COO-), 186.0 (-COS-). **6b**: colourless oil. ^{1}H-NMR (250 MHz, $CDCl_3$) δ 1.44 (s, 2tBu), 4.52 (dt, J_1=15.2Hz, J_2=1.8Hz, H-4), 4.74 (dq, J_1=15.2Hz, J_2=4.2Hz, H-4'), 5.28 (ddd, J_1=1.8Hz, J_2=2.1Hz, J_3=4.2Hz, H-2), 6.22 (ddd, J_1=2.1Hz, J_2=2.1Hz, J_3=2.1Hz, H-5). ^{13}C-NMR ($CDCl_3$) δ 27.8, 28.2 (2xtBu), 57.5 (C_4), 73.0 (C_2), 80.7, 82.1 (2xq.C), 119.6 (C_5), 127.1 (C_3), 129.1 ($C_{3'}$, $C_{5'}$), 129.5 ($C_{4'}$), 134.3 ($C_{2'}$, $C_{6'}$), 146.5 ($C_{1'}$), 154.9 (-NCOO-), 166.1 (-COO-), 185.3 (-COS-).

***tert*-Butyl (*2RS,E*)-1-*tert*-butoxycarbonyl-3-(2'-hydroxyethylidene)-azetidine-2-carboxylate** (7): 4.5 g (10.39 mmol) of thioester **6a** were dissolved in 200 ml of ethanol and cooled to -20°C. 130 ml of a 0.4 N solution of $CeCl_3.6H_2O$ in ethanol were dropped into the solution and simultaneously 2.34 g (62.33 mmol) of $NaBH_4$ were added portionwise. After addition, the reaction mixture was allowed to reach 10°C in 3 hours. Most of the solvent was carefully removed in vacuo and the remaining concentrated solution was poured on a mixture of cracked ice and 1N HCl. The organic material was extracted 3x with ethyl acetate. After washing with brine (3x) and drying over Na_2SO_4, the solvent was removed in vacuo. 3.7 g of residue were purified by chromatography on silica gel using hexane/ ethyl acetate = 4/ 1 as eluent. 2.6 g (84% yield) of 7 in form of a colourless oil were obtained. IR (CH_2Cl_2) 3600, 2970, 2930, 2870, 1740, 1725, 1700 cm^{-1}. ^{1}H-NMR (250 MHz, $CDCl_3$) δ 1.44 (s, tBu), 1.49 (s, tBu), 1.171 (s, OH), 4.14 (s, br. 2H-6), 4.51 (dq, J_1=13Hz, J_2~2Hz, H-4), 4.62 (m, H-4'), 4.99 (s, br. H-2), 5.69 (m, H-5).

***tert*-Butyl (2*RS*,*E*)-3-(2'-bromoethylidene)-1-*tert*-butoxycarbonyl-azetidine-2-carboxylate (8)**: 1.53 g (5.12 mmol) of 7 and 2.245 g (5.64 mmol) of ethylene-bis-(diphenylphosphine) were dissolved in 100 ml anhydrous dichloromethane and coold to -50°C. A solution of 1.87 g (5.64 mmol) tetrabromomethane in 50 ml dichloromethane was added dropwise at this temperature. The reaction mixture was allowed to reach -15°C in two and a half hours. After warming to room temperature the solvent was evaporated in vacuo and the residue chromatographed on silica gel with hexane/ ethyl acetate (1/1). 1.74 g (94% yield) of a colourless oil, which crystallized on standing, were obtained. An analytical sample was recrystallized from pentane/ diethyl ether: m.p. 76-77°C. IR (CH_2Cl_2) 2970, 2930, 1745, 1720, 1700 cm^{-1}. ^{1}H-NMR (250 MHz, $CDCl_3$) δ 1.45 (s, tBu), 1.49 (s, tBu), 3.81 (dd, J_1=1Hz, J_2= 8Hz, 2H-6), 4.50 (m, H-4), 4.60 (m, H-4'), 5.02 (s, br. H-2), 5.85 (m, H-5).

***tert*-Butyl (2*RS*,*E*)-1-*tert*-butoxycarbonyl-3-ethylidene-azetidine-2-carboxylate (9)**: To a solution of 1.74 g (4.8 mmol) of the bromide **8** in 40 ml of anhydrous DMF, 182 mg of $NaBH_4$ were added in small portions. After addition, the solution was stirred for further 3 h. Work up was performed by pouring the reaction solution on a mixture of cracked ice and 1N HCl. The aqueous solution was extracted 3 times with a mixture of diethyl ether and ethyl acetate (1/1), then the organic phase was washed 5 times with small portions of water and dried over Na_2SO_4. Evaporation of the solvents led to a residue, which was purified by column chromatography on silica gel with hexane/ ethyl acetate (4/1). 1.1 g (81%) of **9** in form of a colourless oil were obtained. IR (CH_2Cl_2) 2970, 2930, 1740, 1700 cm^{-1}. ^{1}H-NMR (500 MHz, $CDCl_3$) δ 1.46 (s, tBu), 1.49 (s, tBu), 1.54 (dq, J_1=1.7Hz, J_2=6.9Hz, 3H-6), 4.40 + 4.51 (AB system, J=13.5Hz, further split by small couplings, 2H-4), 4.92 (m, H-2), 5.54 (qq, J_1=6.9Hz, J_2=2.4Hz, H-5). ^{13}C-NMR ($CHCl_3$) δ 13.1 (C_6), 28.0, 28.2 (2xtBu), 55.3 (C_4), 69.2 (C_2), 79.8, 81.4 (2xq.C), 118.2 (C_5), 127.6 (C_3), 155.2 (-NCOO-), 167.8 (-COO-).

***tert*-Butyl (2*RS*,*E*)-1-*tert*-butoxycarbonyl-3-(ethoxycarbonyl-methylidene)-azetidine-2-carboxylate (10a)** and ***tert*-butyl (2*RS*,*Z*)-1-*tert*-butoxycarbonyl-3-(ethoxycarbonyl-methylidene)-azetidine-2-carboxylate (10b)**: Following the method described for the preparation of **6a** and **6b**, 210 mg of **10a** (colourless oil, 62% yield) and 50 mg of **10b** (colourless oil, 15% yield) were obtained from 300 mg (1mmol) of α-diazo β-ketoester **5** and 522 mg (1.5 mmol) (carbethoxyethylidene)triphenylphosphorane. **10a**: ^{1}H-NMR (250 MHz, $CDCl_3$) δ 1.29 (t, J=7Hz, -CH_3), 1.46 (s, tBu), 1.50 (s, tBu), 4.19 (q, J=7Hz, -CH_2-), 4.76 (dt, $J_{4,4'}$=16Hz, $J_{2,4}$=2.5Hz, $J_{4,5}$=2.5Hz, H-4), 4.84 (ddd, $J_{4,4'}$=16Hz, $J_{2,4'}$=4.25Hz, $J_{4',5}$=2.5Hz, H-4'), 5.10 (ddd, $J_{2,4}$=2.5Hz, $J_{2,5}$=2.5Hz, $J_{2,4'}$=4.5Hz, H-2), 5.92 (ddd, $J_{2,5}$=2.5Hz, $J_{4,5}$=2.5Hz, $J_{4',5}$=2.5Hz, H-5). ^{13}C-NMR ($CDCl_3$) δ 14.1 (-CH_3), 15.0, 15.2 (2xtBu), 58.7 (C_4), 60.6 (-CH_2-), 69.4 (C_2), 80.5, 82.7 (2xq.C), 114.4 (C_5), 150.0 (C_3), 155.0 (-NCOO-), 164.8 (-COO-), 165.9 (-COO-). **10b**: ^{1}H-NMR (250 MHz, $CDCl_3$) δ 1.28 (t, J=7.1Hz, -CH_3), 1.47 (s, tBu), 1.49 (s, tBu), 4.14 (m, -CH_2-), 4.51 (dt, J_1=14.5Hz, J_2=1.7Hz, H-4), 4.73 (dq, J_1=14.5Hz, J_2=4.2Hz, H-4'), 5.27 (m, H-2), 5.82 (m, H-5).

***tert*-Butyl (2*RS*,*E*)-1-*tert*-butoxycarbonyl-3-(1'-ethoxycarbonyl-ethylidene)-azetidine-2-carboxylate (11)**: Following the method described for the preparation of **6a** and **6b**, compound **11** (980 mg, colourless oil, 86% yield) was obtained from 1g (3.35 mmol) of **4** and 1.34 g (1.47 mmol) (carbethoxymethylene)-triphenylphosphorane. ^{1}H-NMR (250 MHz, $CDCl_3$) δ 1.30 (t, J=7Hz, -CH_3), 1.48 (s, tBu), 1.52 (s, tBu), 1.86 (q, J=2Hz), 4.22 (q, J=7Hz, -CH_2-), 4.66 ($J_{4,4'}$=15Hz, $J_{2,4}$=2Hz, $J_{4,Me}$=2Hz, H-4), 4.82 ($J_{4,4'}$=15Hz, $J_{2,4'}$=4Hz, $J_{4',Me}$=2Hz, H-4'), 5.08 ($J_{2,4}$=2Hz, $J_{2,4'}$=4Hz, $J_{2,Me}$=2Hz, H-2).

***tert*-Butyl (2*RS*,*E*)-1-*tert*-butoxycarbonyl-3-(ethylthiocarbonyl-methylidene)-azetidine-2-carboxylate (12)**: Followed the method described for the preparation of **6a** and **6b**, the product (542 mg, 76% yield) was obtained from 600 mg (2 mmol) of **4** and 800 mg (2.2 mmol) of (ethylthiocarbonyl-methylene)-triphenylphosphorane. Colourless crystals: m.p. 60-65°C (ether, pentane). IR (CH_2Cl_2) 2970, 2930, 1745, 1710, 1650 cm^{-1}. ^{1}H-NMR (250 MHz, $CDCl_3$) δ 1.29 (t, J=7.4Hz, -CH_3), 1.46 (s, tBu), 1.50 (s, tBu), 2.96 (q, J=7.4Hz, -CH_2-), 4.78 (dt, J_1=16.3Hz, J_2=2.4Hz, H-4), 4.88 (ddd, J_1=16.3Hz, J_2=2.4Hz, J_3=4.4Hz, H-4'), 5.10 (m, H-2), 6.22 (q, J=2.4Hz, H-5).

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REFERENCES AND NOTES

1. a) Isono, K.; Asahi, K. and Suzuki, S. *J. Am. Chem. Soc.* **1969**, *91*, 7490. b) Isono, K. and Suzuki, S. *Heterocycles*, **1979**, *13*, 333. c) Isono, K. *J. Antibiotics*, **1988**, *41*, 1711.
2. Gooday, G. W., Abhandlungen der Deutschen Akademie der Wissenschaften zu Berlin (DDR), Abteilung Mathematik, Naturwissenschaften, Technik, Issue 2N, 1979, p 159.
3. Adams, D.J. and Gooday, G.W. Abhandlungen der Akademie der Wissenschaften der DDR, Abteilung Mathematik, Naturwissenschaften, Technik, Issue 1N, 1982, p 39.
4. Chiew, Y.Y.; Shepherd, M.G. and Sullivan, P.A. *Arch. Microbiol.* **1980**, *125*, 97.
5. Becker, J.M.; Covert, M.L.; Shenbagamurthi, P.; Steinfeld, A.S. and Naider, F. *Antimicrob. Agents Chemother.* **1983**, *23*, 926 and references cited therein.
6. Emmer, G.; Ryder, N.S. and Grassberger, M.A. *J. Med. Chem.* **1985**, *28*, 278.
7. a) Naider, F.; Shenbagamurthi, P.; Steinfeld, A.S.; Smith H.A.; Boney, C. and Becker, J.M. *Antimicrob. Agents Chemother.* **1983**, 787. b) Shenbagamurthi, P.; Smith, H.A.; Becker, J.M. and Naider, F. *J. Med. Chem.* **1986**, *29*, 802. c) Boehm, J.C. and Kingsbury, W.D. *J. Org. Chem.* **1986**, *51*, 2307.
8. Polyoxin L analogues containing α-amino fatty acids, prepared by Khare, R.K.; Becker, J.M. and Naider, F.R. (*J. Med. Chem.* **1988**, *31*, 650) showed a remarkable improvement in potency on growth inhibition of *C. albicans*, but were probably not good enough when tested *in vivo*.
9. a) Damodaran, N.P.; Jones, J.H. and Moffatt J.G. *J. Amer. Chem. Soc.* **1971**, *93*, 3812. b) Tabusa, F.; Yamada, T.; Suzuki, K. and Mukayama, T. *Chem. Letters* **1984**, 405. c) Boehm, J.C. and Kingsbury, W.D. *J. Org. Chem.* **1986**, *51*, 2307. d) Barrett, A.G. and Lebold, S.A. *J. Org. Chem.* **1990**, *55*, 3853.
10. a) Garner, P. and Park, J.M. *J. Org. Chem.* **1988**, *53*, 2979 and references cited therein. b) Duréault, A.; Carreaux, F. and Depezay, J.C. *Synthesis*, **1991**, 150.
11. Baumann, H. and Duthaler, R.O. *Helv. Chim. Acta* **1988**, *71*, 1025.
12. Baumann, H. and Duthaler, R.O. *Helv. Chim. Acta* **1988**, 71, 1035.
13. a) Ratcliffe, R.W.; Salzmann, T.N. and Christensen, B.G. *Tetrahedron Lett.* **1980**, *21*, 31-34. b) Salzmann, T.N.; Ratcliffe, R.W.; Christensen, B.G. and Bouffard, F.A. *J. Am. Chem. Soc.* **1980**, *102*, 6161.
14. Moyer, M.P.; Feldman, P.L. and Rapoport, H. *J. Org. Chem.* **1985**, *50*, 5223.
15. Moody, C.J. and Taylor, R.J. *Tetrahedron Lett.* **1987**, *28*, 5351.
16. Bram, G. and Vilkas, M. *Bull. Soc. Chim. France* **1964**, 945.
17. Brunwin, D.M.; Lowe, G. and Parker, J. *J. Chem. Soc. (C)* **1971**, 3756.
18. Okazoe, T.; Takai, K. and Utimoto, K. *J. Am. Chem. Soc.* **1987**, *109*, 951.
19. Cannizzo, L.F. and Grubbs, R.H. *J. Org. Chem.* **1985**, *50*, 2386.
20. a) Liu, H.-J.; Bukownik, R.R. and Pednekar, P.R. *Synthetic Commun.* **1981**, *11*, 599. b) Kotsuki, H.; Yoshimura, N.; Ushio, Y.; Ohtsuka, T. and Ochi, M. *Chem. Letters* **1986**, 1003. c) Liu, H.-J. and Luo, W. *Synthetic Commun.* **1989**, *19*, 387.
21. Keck, G.E.; Boden, E.P. and Mabury, S.A. *J. Org. Chem.* **1985**, *50*, 709.
22. Vol'pin, M.; Dvolaitzky, M. and Levitin, I. *Bull. Soc. Chim. France* **1970**, 1526.
23. Hendrickson, J.B. and Wolf, W.A. *J. Org. Chem.* **1968**, *33*, 3610.
24. Danheiser, R.L.; Miller, R.F.; Brisbois, R.G. and Park, S.Z. *J. Org. Chem.* **1990**, *55*, 1959.