SYNTHESIS OF DEUTERIUM- AND TRITIUM-LABELED 6β-BROMOPENICILLANIC ACID

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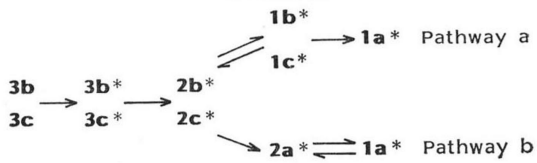
The synthesis of 6β-bromopenicillanic acid labeled with deuterium and tritium in the β-methyl group is described. The *S*-sulfoxide of benzyl- or *p*-methoxybenzyl 6α-bromopenicillanate is refluxed in benzene containing an excess of *tert*-BuOD, D_2O or HTO. After deoxygenation and deprotection of the ester, the labeled 6α-bromopenicillanic acid is epimerized (*N*,*O*-bis(trimethylsilyl)acetamide/1,5-diazabicyclo[4.3.0]non-5-ene in CH_2Cl_2). The two epimers are separated by column chromatography.

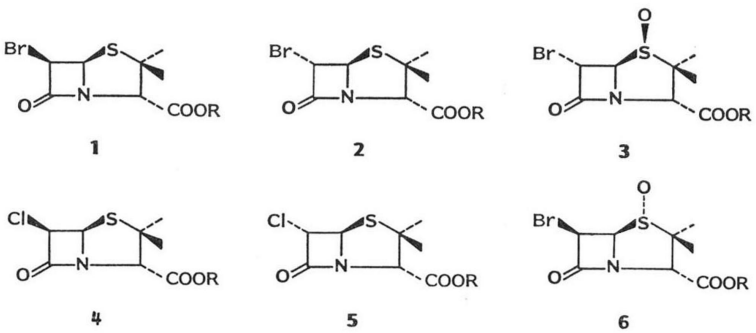
6β-Halopenicillanic acids are irreversible inhibitors of β-lactamases[1,2]. 6β-Bromopenicillanic acid (**1a**), ^3H-labeled in the 2β-methyl group, has been used to elucidate the mechanism of β-lactamase-inactivation by this class of inhibitors[2]. Preparations containing labeled **1a** have been prepared[2] by epimerization of the 6α-isomer **2a** or by stereo-selective reduction of 6,6-dibromopenicillanic acid. Both labeled components were prepared from tritiated 6β-aminopenicillanic acid, which can be obtained by a six-step reaction sequence from phenoxymethylpenicillin[3] or benzylpenicillin[4]. The label was introduced in the 2β-methyl group by heating the *S*-sulfoxide of the penicillin ester in benzene containing tritiated water. Labeling occurs specifically into the 2β-position by ^1H-^3H or ^1H-^2H exchange during sulfoxide-sulfenic acid equilibrium induced by heating[5~7].

Scheme 1.



* Refers to a labeled compound.

The present report deals with a more direct method for the preparation of labeled **1a**, in which the label (deuterium or tritium) is introduced in 6α-bromopenicillanic ester *S*-sulfoxide. Reaction sequences investigated during this work are summarized in Scheme 1 (labeled compounds are marked



a R = H
b R = Benzyl
c R = *p*-Methoxybenzyl
d R = Trimethylsilyl

with an asterisk). The reaction sequence starting with the benzyl ester **3b** will be discussed first.

m-Chloroperbenzoic acid oxidation of **2b** gave the crystalline *S*-sulfoxide **3b** in a 90% yield. The *S*-configuration was assigned in analogy with the work of HARRISON and HODGE[8]. These authors demonstrated that *m*-chloroperbenzoic acid converts 6α-halopenicillanates into a mixture of sulfoxides in which the *S*-isomer predominates. A *S*/*R* ratio of 88: 12 was reported for methyl 6α-chloropenicillanate and 92: 8 for the corresponding bromopenicillanate. Their assignment of *R* and *S* configuration was based on ^{13}C NMR spectroscopy. Comparison of ^{1}H NMR spectra of **2b** and **3b** (taken in $CDCl_3$) showed that conversion of **2b** into its *S*-sulfoxide **3b** is characterized by a marked upfield shift of the 2α-methyl signal (0.27 ppm) and of the H-5 signal (0.30 ppm) and by a downfield shift of the 2β-methyl signal (0.09 ppm) and of H-5 (0.18 ppm). These shifts are similar to those reported earlier[8].

When compound **3b** was heated in benzene containing an excess of D_2O or *tert*-BuOD, ^{1}H NMR of the reaction product showed deuterium incorporation in the 2β-methyl group (80~85% in the presence of D_2O and 30~35% in the presence of *tert*-BuOD). Deoxygenation (PBr_3/dimethylformamide)[9] of **3b** gave the deuteriated benzyl ester **2b***. Deuterium incorporation determined by ^{1}H NMR was confirmed by mass spectrometry of **2b***.

The next step in the proposed scheme (pathway a) is the epimerization of **2b*** at C-6. Epimerization of 6-halopenicillanic esters has been studied several years ago in our laboratory (J. THOMIS, Thesis, 1974). It was found that these esters epimerize in the presence of 1,5-diazabicyclo[4.3.0]non-5-ene (DBN). Progress of epimerization of the benzylesters **2b** and **5b** was followed by gas liquid chromatography. Part of these results are summarized in Table 1. It can be seen that the equilibrium ratio is in favor of the 6α-epimer for both halopenicillanates. The α/β ratio of 83: 17 observed for the chloropenicillanates is almost identical to the 80: 20 value obtained for DBN-catalyzed epimerization of silylated acylamino-[10] or 6-(*p*-nitrobenzylidene amino)penicillanates[11]. The ratio 88: 12 observed for the bromopenicillanates is somewhat lower and resembles the 90: 10 ratio reported[12] for epimerization of penicillanates with imino chloride side chains. Decomposition of the 6β- and eventually of the 6α-halopenicillanates occurs during epimerization, even at −20°C. 6-Chloropenicillanates showed a recovery of 81% after 35 minutes. The recovery of 6-bromopenicillanates observed for identical reaction condition was only 50%. The use of toluene as a solvent instead of CH_2Cl_2 offers no specific advantage, since decomposition proceeds almost at the same rate in both solvents. Epimerization however is somewhat slower in toluene. Epimerization does not occur in the presence of triethylamine.

Table 1. Base-catalyzed epimerization[a] of **2b** and **5b**.

Time (minutes)	Ratio **2b**/**1b** (recovery)[b]		Ratio **5b**/**4b** (recovery)
	$CH_3C_6H_5$[c]	CH_2Cl_2	CH_2Cl_2
5	92: 8 (93%)	94: 6 (94%)	90: 10 (99%)
15	90: 10 (87%)	91: 9 (84%)	87: 13 (97%)
20	89: 11 (79%)	—	—
25	—	90: 10 (76%)	85: 15 (97%)
30	89: 11 (78%)	—	—
35	—	88: 12 (50%)	83: 17 (81%)

[a] Epimerization at −20°C in the presence of 1 equiv DBN and a 0.4 M concentration of the **2b** or **5b**.
[b] The recovery is the sum of **2b** and **1b** (or **5b** and **4b**).
[c] Solvent.

When 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) is used as a catalyst both epimerization and degradation are faster than in the presence of DBN.

It has been reported[13] for that the α/β ratio of 6-acylaminopenicillanates is lower when the reaction is carried out on the sulfoxides. Thus **3b** was treated with 1 equivalent of DBN in CH_2Cl_2 at temperatures ranging from $-80°C$ to $25°C$. Epimerization was not observed under these conditions. Epimerization of 6α-iodopenicillanates was not studied in detail. However it should be noted that methyl 6β-iodopenicillanate was isolated in a 3 to 4% yield upon treatment of the 6α-isomer[14] with DBN under the conditions mentioned for **2b** and **5b**.

The mixture of **1b*** and **2b*** obtained by epimerization of **2b*** (20 minutes at $-20°C$ in CH_2Cl_2 containing 1 equivalent of DBN) was separated by chromatography on a silica gel column yielding about 7.8% **1b*** and 64% **2b***. The 6β-chloropenicillanate **4b** was isolated in a similar way. ^1H NMR spectra of both **1b** and **4b** showed a coupling constant of 4 Hz for H-5 and H-6, which is in agreement with a *cis* relationship of these protons. Another feature observed in the spectra of the 6β-isomers is a downfield shift of H-6 (0.4 to 0.5 ppm) and of H-5 (0.2 to 0.3 ppm) relative to the α-isomers.

Introduction of deuterium was also investigated at the stage of the sulfoxide of the β-bromoester **1b**. Thus **1b** was oxidized with *m*-chloroperbenzoic acid yielding the *R*-sulfoxide **6b**. The *R*-configuration of the sulfoxide was derived from a comparison of ^1H NMR spectra of **1b** and **6b** recorded in $CDCl_3$ and C_6D_6. Aromatic solvent induced shifts were similar to those reported for the *R*-sulfoxide of methyl 6β-phthalimidopenicillanate[15]. The sulfoxide **5b** however is rather unstable and decomposed completely upon the reaction conditions used for deuterium exchange.

The last step in the reaction sequence (pathway a) is the debenzylation of **1b***. We investigated the method of TSUJI *et al.*[16], which is known to remove benzyl esters under mild conditions in the cephalosporin series. Preliminary experiments were conducted with the more stable 6α-isomer **2b**. When **2b** was reacted with $AlCl_3$ and anisole in CH_2Cl_2-$MeNO_2$ at 0°C in the conditions described by TSUJI, the free acid **2a** was not obtained. When the original procedure was somewhat modified (decreasing of the temperature to $-20°C$ and using CCl_4 as a solvent) the free acid **2a** was found to be present in the reaction mixture, but decomposed when isolation was carried out according to TSUJI. However, when the reaction mixture was freed from $AlCl_3$, anisole and **2b** (eventually present) by chromatography on a silica gel column, the free acid **2a** was isolated in a 68% yield. Unfortunately the modified procedure did not work for the less stable β-isomer **1b**. Replacement of $AlCl_3$ by other Lewis acids *e.g.* $AlBr_3$, BCl_3, $SbCl_3$ was also unsuccessful.

Therefore the reaction sequence was repeated starting with the *p*-methoxybenzyl ester **2c**. According to KEMP *et al.*[17] the *p*-methoxybenzyl ester of 6β-iodopenicillanic acid can be removed (28% yield) by treatment of the ester with CF_3COOH in CH_2Cl_2. In our hands the procedure gave highly impure **1a** in a low yield (about 10% or less). A somewhat modified procedure deprotected the more stable 6α-isomer **2c** with a yield of 76%, but was also unsuccessful for **1c**. For this reason we switched to the trimethylsilyl ester (pathway b). Thus the free acid **2a***, obtained by deprotection of **2b*** or **2c***, was converted into its trimethylsilyl ester **2d*** by treatment with *N,O*-bis(trimethylsilyl)acetamide (BSA) and was epimerized in the presence of DBN. Hydrolysis of the trimethylsilyl esters afforded a mixture of **2a*** and **1a*** in a ratio of about 92: 8. This procedure was found to be more convenient than epimerization of the potassium salt of **2a** in aqueous solution as described by VON DAEHNE[18] and by LOOSEMORE and PRATT[1]. Separation of the two epimers was obtained by conventional silica gel column chromatography. The mobile phase used was the same as that reported by VON DAEHNE for dry column chro-

matography of **1a** and **2a**. The yield of **1a*** was 5% while 76% of **2a*** was recovered. The latter can be recycled to improve the yield of **1a***.

Tritiated **1a*** with a specific activity of 2.5 mCi/mmol was prepared according to pathway b. The tritiated starting material **3c*** (2.5 mCi/mmol) was obtained by heating **3c** in benzene containing HTO.

Experimental

Melting points were determined with a Büchi-Tottoli apparatus and are uncorrected. Solutions were evaporated under reduced pressure at a bath temperature of 30°C. Precoated Merck silic gel F254 plates were used for TLC. Column chromatography was performed on silica gel (Merck 0.040~0.063 mm). IR spectra were recorded on a Perkin-Elmer 257 spectrometer. ^{1}H NMR spectra were recorded on a 60 MHz Hitachi Perkin-Elmer R-24 and a 90 MHz Jeol FX-90Q instrument in $CDCl_3$ with tetramethylsilane as internal standard unless stated otherwise. Mass spectra were determined with an AEI MS-12 apparatus. Radioactivity measurements were performed by liquid scintillation using a Packard Tri-Carb spectrometer.

Benzyl 6α-Bromopenicillanate (**2b**)

Benzylbromide (3.9 ml, 33 mmol) was added to a solution of potassium 6α-bromopenicillanate[19] (9.54 g, 30 mmol) in 200 ml DMF. The reaction mixture was stirred overnight at room temperature, poured into 500 ml ice-water and extracted with EtOAc (3×75 ml). The EtOAc layer was washed with water, dried and evaporated. Chromatography of the residue on a silica gel column (CH_2Cl_2 as mobile phase) afforded 9.4 g (25.5 mmol, 85% yield) of **2b** as a pale yellow oil; $[\alpha]_D^{25}$ +144° (*c* 1, acetone); IR (CH_2Cl_2) 1784 (β-lactam), 1745 (ester) cm^{-1}; ^{1}H NMR ($CDCl_3$, 60 MHz) δ 1.36 (s, α-CH_3), 1.55 (s, β-CH_3), 4.50 (s, H-3), 4.71 (d, J=1.5 Hz, H-6), 5.14 (s, $CH_2C_6H_5$), 5.35 (d, J=1.5 Hz, H-5), 7.3 (s, C_6H_5) ppm; MS *m/z* 385 (M^+); TLC Rf 0.63 (CH_2Cl_2).

p-Methoxybenzyl 6α-Bromopenicillanate (**2c**)

Freshly prepared *p*-methoxybenzyl chloride[20] (17 g, 109 mmol) was reacted with potassium 6α-bromopenicillanate (34.66 g, 109 mmol) as described in the previous section yielding 34.9 g (87.2 mmol, 80% yield) of **2c** as a pale yellow oil. $[\alpha]_D^{25}$ +154° (*c* 1, acetone); IR (KBr) 1790 (β-lactam), 1740 (ester) cm^{-1}; ^{1}H NMR ($CDCl_3$, 90 MHz) δ 1.36 (s, α-CH_3), 1.57 (s, β-CH_3), 3.80 (s, OCH_3), 4.51 (s, H-3), 4.76 (d, J=1.5 Hz, H-6), 5.1 (s, $CH_2C_6H_5$), 5.36 (d, J=1.5, H-5), 6.8~7.4 (m, C_6H_5) ppm; MS *m/z* 399 (M^+); TLC Rf 0.42 (CH_2Cl_2).

Benzyl 6α-Bromopenicillanate *S*-Sulfoxide (**3b**)

A solution of 85% *m*-chloroperbenzoic acid (1.19 g, 6 mmol) in CH_2Cl_2 (100 ml) was added over a period of 30 minutes to a cooled (0°C) solution of **2b** (2.21 g, 6 mmol) in CH_2Cl_2 (100 ml). The solution was stirred for 30 minutes at 0°C and washed with an aqueous solution of $NaHCO_3$ (5%). The organic layer was dried and evaporated. Crystallization of the residue from benzene afforded 2.1 g (5.44 mmol, 90% yield) of **3b**; mp 109~111°C; $[\alpha]_D^{25}$ +179.5° (*c* 1, acetone); IR (KBr) 1793 (β-lactam), 1720 (ester), 1055 (sulfoxide) cm^{-1}; ^{1}H NMR ($CDCl_3$, 90 MHz) δ 1.09 (s, α-CH_3), 1.64 (s, β-CH_3), 4.54 (s, H-3), 4.99 (d, J=1.5 Hz, H-5 or H-6), 5.05 (d, J=1.5 Hz, H-6 or H-5), 5.22 (AB pattern, J=12 Hz, $CH_2C_6H_5$), 7.35 (s, C_6H_5) ppm; (C_6D_6, 90 MHz) δ 0.42 (s, α-CH_3), 1.18 (s, β-CH_3), 4.24 (d, J=1.2, H-6), 4.51 (s, H-3), 4.70 (AB pattern, J=12 Hz, $CH_2C_6H_5$), 4.92 (d, J=1.2, H-5), 7.16 (s, C_6H_5) ppm; TLC Rf 0.11 (CH_2Cl_2), Rf 0.52 (CH_2Cl_2 - acetone, 95: 5).

p-Methoxybenzyl 6α-Bromopenicillanate *S*-Sulfoxide (**3c**)

p-Methoxybenzyl 6α-bromopenicillinate (**2c**) (4 g, 10 mmol) was oxidized with *m*-chloroperbenzoic acid as described for **2b**. The residue obtained upon evaporation of the CH_2Cl_2 layer was triturated with ether. The precipitate was isolated and recrystallized from CH_2Cl_2 - Et_2O yielding 2.5 g (5 mmol, 60% yield) of **3c**, mp 111~113°C (dec); $[\alpha]_D^{25}$ +171° (*c* 1, acetone); IR (KBr) 1790 (β-lactam), 1720 (ester), 1055 (sulfoxide) cm^{-1}; ^{1}H NMR ($CDCl_3$, 90 MHz) δ 1.07 (s, α-CH_3), 1.63 (s, β-CH_3), 3.82 (s, OCH_3), 4.52 (s, H-3), 5.00 (d, J=1.5 Hz, H-5), 5.05 (d, J=1.5 Hz, H-6), 5.16 (AB-pattern, J=12 Hz, $CH_2C_6H_5$),

6.8~7.4 (m, C_6H_5) ppm; TLC Rf 0.05 (CH_2Cl_2), Rf 0.5 (CH_2Cl_2 - acetone, 95: 5).

Labeling of the *S*-Sulfoxide of Benzyl- and *p*-Methoxybenzyl 6α-Bromopenicillanate

With Me_3COD: A solution of the *S*-sulfoxide ester **3b** or **3c** (1 mmol) in anhydrous benzene (30 ml) containing Me_3COD (10 ml) was refluxed for 8 hours (the reflux cooler was provided with a $CaCl_2$ guard tube). Evaporation of the reaction mixture and crystallization afforded **3b*** and **2c*** in a nearly quantitative yield. ^1H NMR spectra of **3b*** and **2c*** showed a deuterium incorporation of 30 to 50% in the 2β-methyl group.

With D_2O: A solution of the sulfoxide ester **3b** or **3c** (1 mmol) in anhydrous benzene (120 ml) containing D_2O (1.2 ml) was refluxed for 16 hours. Evaporation and crystallization afforded **3b*** and **3c*** (nearly quantitative yield). The ^1H NMR spectra showed a deuterium incorporation of 80 to 85% in the 2β-methyl group.

With HTO: A solution of **3c** (8.32 g, 20 mmol) in anhydrous benzene (500 ml) containing 0.4 ml HTO (9 mCi/mmol) was refluxed for 24 hours. Evaporation and repeated crystallization afforded 7.5 g of tritiated **3c*** (2.5 mCi/mmol).

[β-*Methyl*-^{2}H]benzyl and *p*-Methoxybenzyl 6α-Bromopenicillanate (**2b*** and **2c***)

PBr_3 (4 mmol) was added to a cooled (0°C) solution of **3b*** or **3c*** (1 mmol) in DMF (50 ml). The mixture was kept at 0°C for 20 minutes and poured into 100 ml aqueous $NaHCO_3$ (5%). The aqueous layer was extracted with EtOAc (3×50 ml), the combined EtOAc layer was washed with H_2O, dried and evaporated affording **2b*** or **2c*** in a yield which ranged from 90 to 95%. The deuterium content (as determined by ^1H NMR) was confirmed by mass spectrometry.

Benzyl 6α-Chloropenicillanate (**5b**)

Potassium 6α-chloropenicillanate[10] (617 mg, 2.25 mmol) was reacted with benzyl bromide (2.55 mmol) in DMF (45 ml) as described for **2a**, yielding 619 mg (75% yield) of crystalline **5b** (crystallized from Et_2O - hexane); mp 49.5~50°C; $[\alpha]_D^{25}$ +165° (*c* 0.5, acetone); IR (KBr) 1783 (β-lactam), 1739 (ester) cm^{-1}; ^1H NMR ($CDCl_3$, 60 MHz) δ 1.35 (s, α-CH_3), 1.53 (s, β-CH_3), 4.54 (s, H-3), 4.73 (d, J= 1.5 Hz, H-6), 5.19 (s, $CH_2C_6H_5$), 5.29 (d, J=1.5 Hz, H-5), 7.3 (s, C_6H_5) ppm; MS *m/z* 325 (M^+); TLC Rf 0.65 (CH_2Cl_2).

Kinetics of Base-catalyzed Epimerization of **2b** and **5b**

A solution of **2b** or **5b** (0.4 mmol) in CH_2Cl_2 or toluene (1 ml) containing 5 mg eicosane as internal standard was cooled to −20°C and the epimerization catalyst (0.4 mmol) was added. Samples (0.1 ml) were taken at regular intervals, diluted with 1 ml of the solvent and extracted with 5% aqueous H_3PO_4. The organic layer was analyzed by gas liquid chromatography using a 3% OV-1 column (on Gas-Chrom Q, 152 cm×4 mm ID) at 180°C, an FID detector (250°C) and N_2 as carrier gas (60 ml/minute). Retention times obtained under these conditions are; internal standard 8 minutes; **2b** 28 minutes; **1b** 39.4 minutes; **5b** 19.6 minutes; **4b** 26.8 minutes.

[β-*Methyl*-^{2}H]benzyl 6β-Bromopenicillanate (**1b***)—Epimerization of **2b***

A solution of **2b*** (10 g, 27.1 mmol) in CH_2Cl_2 (50 ml) was cooled (−20°C) and a cooled solution of DBN (3.25 ml, 27 mmol) in CH_2Cl_2 (10 ml) was added. The stirred reaction mixture was kept for 15 minutes at −20°C, diluted with CH_2Cl_2 (4 ml) and washed with 5% aqueous H_3PO_4 (100 ml). The organic layer was dried, evaporated to dryness and the residue was chromatographed on a 200 g silica gel column, using CH_2Cl_2 - cyclohexane (80: 20) as a mobile phase. This afforded 6.42 g (64% yield) of the α-epimer **2b***, 780 mg (7.8%) mixture of both epimers (mainly α) and 1.4 g (14% yield) of the β-epimer **1b*** (oil); $[\alpha]_D^{25}$ +172° (*c* 1, acetone); IR (CH_2Cl_2) 1785 (β-lactam), 1750 (ester) cm^{-1}; ^1H NMR ($CDCl_3$, 90 MHz) δ 1.4 (s, α-CH_3), 1.64 (s, β-CH_3), 4.52 (s, H-3), 5.16 (s, $CH_2C_6H_5$), 5.26 (d, J=4 Hz, H-6), 5.54 (d, J=4 Hz, H-5), 7.34 (s, C_6H_5) ppm; (C_6D_6, 90 MHz) δ 1.07 (s, α-CH_3), 1.35 (s, β-CH_3), 4.28 (s, H-3), 4.74 (d, J=4 Hz, H-6), 5.14 (d, J=4 Hz, H-5) ppm; TLC Rf 0.52 (CH_2Cl_2).

[β-*Methyl*-^{2}H]-*p*-methoxybenzyl 6β-Bromopenicillanate (**1c***)—Epimerization of **2c***

Epimerization of **2c*** (2.4 g, 6 mmol) with DBN (6 mmol) using the condition described for the

preparation of **1b*** afforded after chromatography on silica gel (250 g) column, using CH_2Cl_2 - cyclohexane, 97: 3 as a mobile phase, 1.32 g (55%) of the α-epimer **2c*** and 180 mg (7.5%) of the β-isomer **1c*** as an oil; $[\alpha]_D^{25}$ +159° (*c* 1, acetone); IR (KBr) 1790 (β-lactam), 1740 (ester) cm^{-1}; NMR ($CDCl_3$, 90 MHz) δ 1.38 (s, α-CH_3), 1.63 (s, β-CH_3), 3.81 (s, OCH_3), 4.51 (s, H-3), 5.12 (s, $CH_2C_6H_5$), 5.29 (d, J= 4 Hz, H-6), 5.55 (s, J=4 Hz, H-5), 6.8~7.4 (m, C_6H_5) ppm; TLC Rf 0.27 (CH_2Cl_2).

When the epimerization was carried out in the presence of 0.5 equiv DBN (instead of 1 equiv) for 45 minutes at −20°C degradation was less pronounced (68% yield for the α- and 12% yield for the β-epimer).

Benzyl 6β-Chloropenicillanate (**4b**)—Epimerization of **5b**

Epimerization of **5b** (1.3 g, 4 mmol) with DBN (4 mmol) using the conditions described for **2b*** afforded after chromatography on a silica gel (40 g) column with CH_2Cl_2 as a mobile phase 750 mg (54%) of **5b**, 110 mg (8.3%) of a mixture of α- and β-epimers and 90 mg (6.9%) of pure **4b** (oil); $[\alpha]_D^{20}$ +117° (*c* 0.5, acetone); IR (KBr) 1789 (β-lactam), 1743 (ester) cm^{-1}; ^1H NMR ($CDCl_3$, 60 MHz) δ 1.36 (s, α-CH_3), 1.57 (s, β-CH_3), 4.45 (s, H-3), 5.19 (s, $CH_2C_6H_5$), 5.22 (d, J=4 Hz, H-6), 5.50 (d, H=4 Hz, H-5), 7.28 (s, C_6H_5) ppm; MS *m/z* 325 (M^+); TLC Rf 0.50 (CH_2Cl_2).

Methyl 6β-Iodopenicillanate

A solution of methyl 6α-iodopenicillanate[14] (1 g, 2.94 mmol) in CH_2Cl_2 (15 ml) was cooled (0°C) and DBN (0.36 ml, 2.94 mmol) was added. The reaction mixture was kept at 0°C for 5 minutes and washed with dilute aqueous H_3PO_4. The organic layer containing the α- and β-epimers in a ratio of about 90: 10 (^1H NMR) was separated into its components by repeated silica gel column chromatography using C_6H_6 - Me_2CO (99: 1) as a mobile phase affording 30 mg of the title compound as a colorless oil; $[\alpha]_D^{25}$ +240° (*c* 0.2, $CHCl_3$); IR (CH_2Cl_2) 1790 (β-lactam), 1750 (ester) cm^{-1}; ^1H NMR ($CDCl_3$, 60 MHz) δ 1.45 (s, α-CH_3), 1.69 (s, β-CH_3), 3.77 (s, OCH_3), 4.55 (s, H-3), 5.38 (d, J=4 Hz, H-6), 5.63 (d, J=4 Hz, H-5) ppm; MS *m/z* 341 (M^+).

Benzyl 6β-Bromopenicillanate *R*-Sulfoxide (**6b**)

1b (185 mg, 0.5 mmol) was oxidized with *m*-chloroperbenzoic acid as described for **2b** yielding 155 mg (0.4 mmol) of the title compound as an oil; IR (KBr) 1800 (β-lactam), 1740 (ester), 1055 (sulfoxide) cm^{-1}; ^1H NMR ($CDCl_3$, 90 MHz) δ 1.33 (s, α-CH_3), 1.54 (s, β-CH_3), 4.45 (s, H-3), 4.84 (d, J=4 Hz, H-5), 5.29 (d, J=4 Hz, H-6) ppm; (C_6D_6, 90 MHz) δ 1.01 (s, α-CH_3), 1.06 (s, β-CH_3), 4.32 (s, H-3), 4.72 (d, J=4 Hz, H-5), 4.55 (d, J=4 Hz, H-6) ppm.

Debenzylation of **2b*** with $AlCl_3$

A solution of deuteriated benzyl 6α-bromopenicillanate (370 mg, 1 mmol) in CCl_4 (10 ml) containing anisole (0.61 ml, 6 mmol) was cooled to −20°C. A suspension of $AlCl_3$ (4.16 mg, 3.12 mmol) in 20 ml CCl_4 was added. The reaction mixture was kept for 4 hours at −20°C and percolated through a 30 g silica gel column (ID 3 cm) at a flow rate of 5 ml/minute. The column was eluted with Me_2CO - AcOH (99: 1) at the same flow rate. Fractions containing the free acid were pooled and evaporated to dryness. After removal of the residual AcOH by codistillation with toluene under reduced pressure, the residue was taken up in 40 ml $CHCl_3$ - H_2O (1: 1) and adjusted to pH 6.8 with KOH. The aqueous layer was separated, washed with $CHCl_3$ and evaporated to dryness. Trituration with Me_2CO afforded 220 mg (68% yield) of the crystalline potassium salt of **2a***. Physical constants and spectral data were in agreement with those reported in the literature.

Cleavage of the *p*-Methoxybenzyl Ester for **2c***

Trifluoroacetic acid (1.6 ml, 20 mmol) was added to a solution of **2c*** (400 mg, 1 mmol) in CCl_4 (20 ml). The reaction mixture was stirred at room temperature for 1 hour, diluted with benzene (100 ml) and evaporated. The residue was taken up in a mixture of CH_2Cl_2 (10 ml) and H_2O (10 ml) and immediately neutralized to pH 6.8 with KOH. The aqueous layer was separated, washed with CH_2Cl_2 and evaporated. Trituration of the residue with acetone gave 240 mg (76% yield) of the crystalline potassium salt of **2a***. Physical constants and spectral data were in agreement with those reported in the literature. Cleavage of 1 mmol of the ester for 1 hour in the presence of 10 mmol CF_3COOH was

incomplete and gave a 31% yield of the potassium salt of **2a***.

[*β-Methyl*-^{2}H]-6β-bromopenicillanic Acid (**1a***)—Epimerization of **2a***

BSA (2.47 ml, 10 mmol) was added to a solution of **2a*** (2.8 g, 10 mmol) in CH_2Cl_2 (50 ml). The reaction mixture was stirred for 20 minutes at room temperature and cooled to −20°C. DBN (1.24 ml, 10 mmol) was added, the solution was kept at −20°C for 20 minutes, diluted with CH_2Cl_2 (20 ml) and washed with a 5% aqueous solution of H_3PO_4 (20 ml). The CH_2Cl_2 layer was dried and evaporated. TLC (Et_2O - petroleum ether - HCOOH, 70: 30: 0.1) showed the presence of the α-epimer (Rf 0.27) and the β-epimer (Rf 0.21). The mixture was separated on a 100 g silica gel column (using EtOH - petroleum ether - HCOOH, 70: 30: 0.1) as a mobile phase, yielding 2.12 g (76%) of the **2a*** and 125 mg (5%) of the **1a***, which crystallized from ether. Physical constants were in agreement with those reported[15]. IR (KBr) 1800 (β-lactam), 1760, 1710 (COOH) cm^{-1}; ^{1}H NMR (C_6D_6, 90 MHz) δ 1.6 (s, α-CH_3), 1.73 (s, β-CH_3), 4.53 (s, H-3), 5.34 (d, J=4 Hz, H-5), 5.56 (d, J=4 Hz, H-6) ppm.

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