Synthesis of 6*R*(β)-Tritylaminopenicillanic-3*R*(β)-alcohol, a Versatile Stereoisomer of Natural β-Lactams

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In an attempt to rationalize a synthesis of penicillin analogs modified at C(3), we have isolated the 3*R*(β)-carbinolamide derivative **4a**. The trityl substituent on N(6') seems to be responsible for the inversion of configuration which occurs at C(3) during the acid hydrolysis of the isocyanate intermediate. An hydrogen bond is formed on the β-face of the bell-shaped bicyclic skeleton between the N(6')-nitrogen lone pair and the C(3) hydroxyl group. On standing, the carbinolamide analog slowly isomerizes to its expanded bicyclic isomer **4b**, but the starting material may be easily recovered by treatment with acid. The postulated intermediate during isomerization, *i.e.*, the open aldehyde form, does not accumulate. Substitutions of the hydroxyl group at C(3) lead to a variety of compounds with the biologically active 3*S*(α) configuration. These may be used to study the importance of the carboxyl group of penicillins in their interaction with β-lactamases at the molecular level.

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Introduction.

During the seventies, major efforts were made in order to design new semi-synthetic analogs of penicillins and cephalosporins [1]. Modifications of the side-chain at C(6) or C(7) and of the carboxyl group at C(3) or C(4) have been the main topics of exhaustive reviews [2-5]. Unfortunately, many of these compounds have been tested solely for their biological activity. Most compounds modified at the carboxyl group have proven inactive and this supports the contemporary consensus that a free acidic residue is necessary to the target recognition of penicillin-sensitive enzymes [6]. For example, esterification of the C(3) carboxylate of penicillins greatly decreases antibacterial activity [3]. Among the exceptions to the carboxyl rule, we can cite the C(4)-phosphoryl analogs of cephalosporins [7], the 3-(5-tetrazolyl) analogs of penicillins [8] and the more recent monobactams [9] with a sulfonyl residue attached to the β-lactam nitrogen. It seems that bulky substituents are not necessarily prohibited, provided they develop some appropriate electrostatic features. However, on the basis of the available data, it is rather speculative to draw conclusions at the molecular level. Few studies have been reported which analyzed the importance of the carboxyl residue in terms of kinetic constants [10,11]. Today, the structure of model enzymes have been solved at high resolution [12-16], and it becomes possible and quite appealing to study the structure-reactivity relationships of β-lactam compounds at the kinetic level (leading to activation parameters) as well as at the thermodynamic level (leading to binding parameters).

This investigation is aimed at a better understanding of the molecular details of the mechanism of action of β-lactam antibiotics. Ultimately, this should lead to the de novo design of better therapeutic agents.

In the course of this project, we have synthesized a new and versatile C(3)-modified penicillin analog which can be used as a starting material to the synthesis of a variety of compounds useful for enzymatic studies. This compound, 6*R*(β)-tritylaminopenicillanic-3*R*(β) alcohol is very stable and may be kept for months at room temperature. Slow isomerization to a more expanded bicyclic isomer occurs, but it is completely reversible. The protective trityl side chain can be easily removed and replaced by any acyl side chain (benzylcarbonyl for penicillin G, benzyloxycarbonyl for penicillin V,...) without racemization at C(3). Activation and substitution of the C(3)-hydroxyl group yield penicillin analogs with the C(3)*S*(α) biologically active configuration. Hydrolysis of the β-lactam amide bond generally leads to the expulsion of the C(3) leaving group.

Results and Discussion.

The starting material, 6*R*(β)-aminopenicillanic acid (**1**) (6-APA) (Figure 1), a zwitterionic species, is very insoluble in organic solvents. Previously described methods of protection of the amino group by the bulky trityl substituent made use of mixtures of miscible organic and aqueous solvents [19,27]. In our hands, these methods gave substantial hydrolysis of the reactive trityl chloride. We realized that treating a suspension of 6-APA with two equivalents of HMDS in refluxing chloroforom led to rapid and complete solubilization of the starting material. The reason for the requirement of a second equivalent of HMDS is not clearly understood, but it seems to be of kinetic importance [17]. The soluble intermediate is then treated with trityl chloride in pure organic solvents. Activation of the carboxyl group gave the mixed anhydride intermediate **2a** (R = trityl) on which three different treatments were performed: (a) addition of a base catalyzed the rearrangement [18] in-

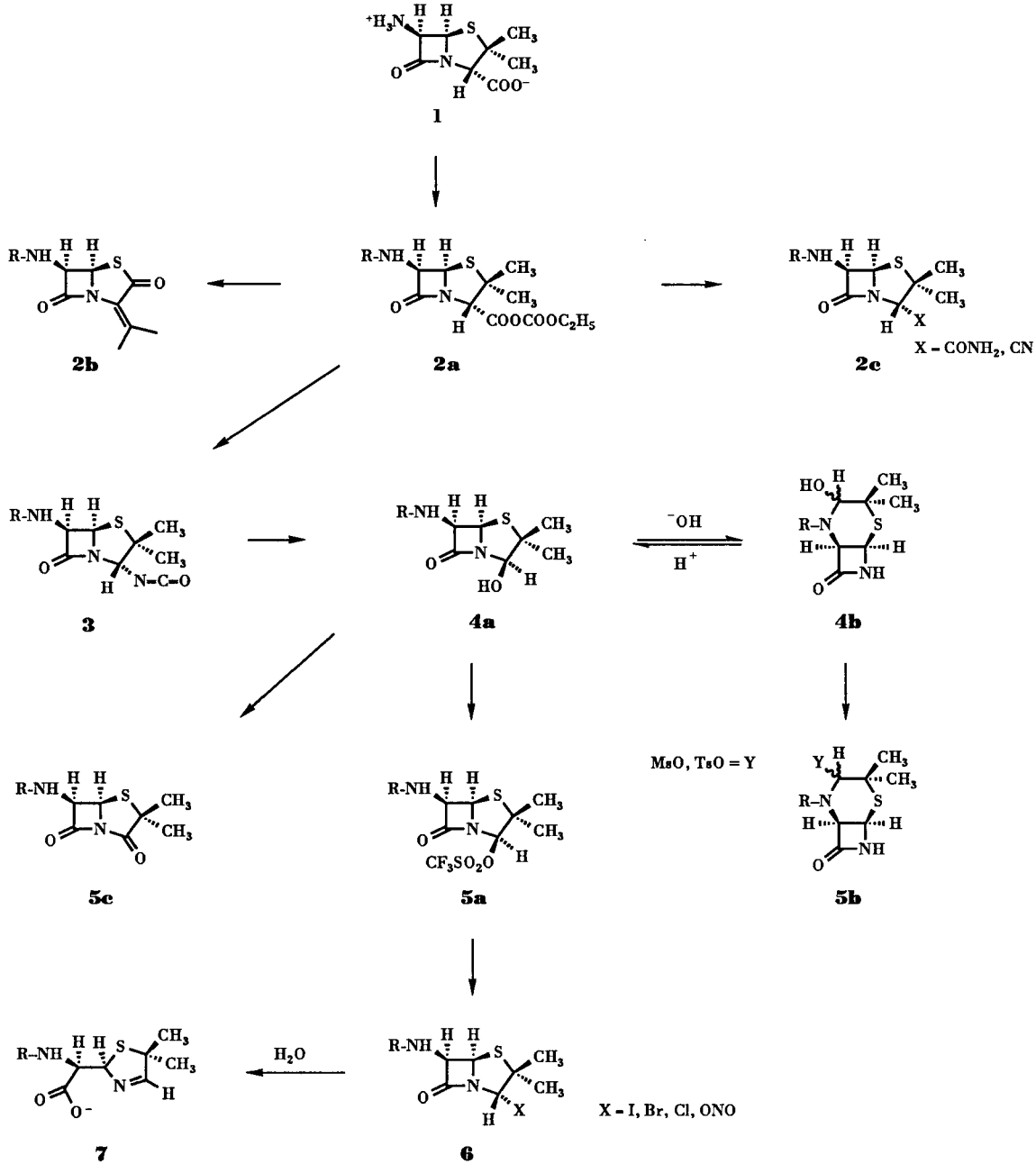

Figure 1. Synthesis strategy.

to the thiol ester **2b** (R = trityl), the reaction occuring with the formal loss of 1 mole of water from the starting tritylaminopenicillanic acid. The rearrangement has been explained in terms of a thiolate intermediate which can react intramolecularly with the activated acyl function. Although this reaction is fairly general, it is well known [19] that better yields are attained when using 6*R*(β)-amino derivatives instead of 6*R*(β)-acylamino derivatives because of interfering reactions involving the side-chain acylamino group.

As the anhydro derivative of 6*R*(β)-tritylaminopenicillanic acid is known to rearrange in water, the solvent and base were removed under reduced pressure as a toluene azeotropic mixture and the residue was treated with isopropyl alcohol, (b) addition of diammonium hydrogenophosphate gave the amide **2c** (R = trityl, X = $CONH_2$) which, under dehydration conditions, led to the nitrile (X = CN). In all cases, the trityl protective group could be easily removed by treatment with one equivalent of *p*-toluenesulfonic acid, (c) addition of sodium azide gave a mixture of the acyl azide and isonitrile derivatives. Sheehan and Brandt [20] showed that acid hydrolysis of the resulting isocyanate did not afford the amine, but the corresponding alcohol. In the phthalimido series, this was shown to be in equilibrium with the corresponding aldehyde and hydrolysis also afforded considerable amounts of the penicillin urea dimer. Although high dilution helped to increase the yield of alcohol, Heusler and Woodward [21] found it expedient to first add trichloroethanol to give the carbamate [22], followed by reduction with zinc in acetic acid to form the corresponding aldehyde. The equilibrium between open and closed forms has been shown to depend on the bulk of the 6β-amino substituent as well as on electronic effects, the proportion of aldehyde increasing in the series $PhCH_2CONH$, $PhOCH_2CONH$, $CCl_3CH_2OCONH < (CH_3)_3COCONH <$ Phthalimido [23,24] (Table 1). In this study, the trityl substituent has proved to favor the carbinolamide structure. Moreover, no penicillin urea dimer was isolated as by-product during the acid hydrolysis step.

Table 1

Aldehyde:Carbinolamide Equilibrium for Variously N(6')-Substituted Penicillanic-3*R*(β)-alcohol

N(6')-substituted	aldehyde: carbinolamide
N-acetylphenylglycylactonide	85:15
phthaloyl	70:30
t-butoxycarbonyl	15:85
1,1,1-trichloroethoxycarbonyl	10:90
phenylacetyl	10:90
phenoxyacetyl	10:90
trityl	0:100

We believe that the trityl substituent is excluded from the interior of the β face of the penam skeleton and that an hydrogen bond between the nitrogen lone pair of the C(6) amino side-chain and the OH group at C(3) stabilizes the carbinolamide structure and is responsible for the observed inversion of configuration at C(3) (Figure 2). The ^1H nmr studies on the benzylpenicillin analog **4a** (R = $PhCH_2CO$-) showed that, indeed, the H(3) is on the α-side of the molecule and is in interaction with the α-CH_3 which is itself in interaction with H(5). Compound **4a** (R = trityl) is very stable, but it was shown that it spontaneously isomerizes to its expanded isomer **4b**. This isomerization was greatly accelerated when passing the compound through a silica gel column or upon exposure to slightly basic conditions. Removal of the trityl side-chain upon treatment with *p*-toluenesulfonic acid led to complete recovery of the penam skeleton **4a** (R = H). The isomer **4b** was further identified through substitution of its hydroxyl group by activating agents (mesyl- and tosyl-chloride) to give mixtures of the **5b** diastereoisomers (R = trityl, Y = OMs, OTs). It is also remarkable that deprotection of **4a** (R = trityl) followed by acylation of the free amine occured without racemisation at C(3) and that no open aldehyde isomer was detectable. Indeed, the reaction mixture was analyzed by hplc in order to detect a possible isomerization occuring during the substitution of the side chain at C(6). Out of seven major peaks separated by the gradient described in Figure 3, only one (which eluted at 50% of eluent B) contained the β-lactam ring and this was shown to be identical with pure **4a** (R = $PhCH_2CO$-).

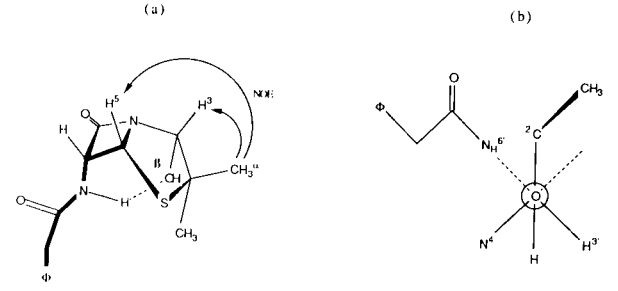

Figure 2. 6*R*(β)-Phenylacetylaminopenicillanic-3*R*(β)-alcohol as represented in space. The bulky acyl group is excluded from the interior of the bell-shaped skeleton and an hydrogen bond may be formed between NH(6') and O(3'), stabilizing both the C(3)*R* configuration and the carbinolamide structure: (a) NOE difference spectra showed a positive NOE effect on protons 3 and 5 when the (α)-methyl group at low field was irradiated. Irradiation of the (β)-methyl group at high field remained without effects on these protons. NOE difference spectra were taken in dimethylsulphoxide, in which solvent the two methyl groups are separated (Δδ = 0.06 ppm). (b) drawing to show how the hydrogen bond between the lone pair of the alcohol oxygen at C(3) and the NH acylamino group occurs when the staggered conformation along C(3)-OH is reached. The OH group is represented in the most stable conformation, antiperiplanar to the C(3)-C(2) bond. As estimated from nmr, only 5-10% could be in the gauche conformation, presumably because of unfavorable interactions with the endo methyl at C(2).

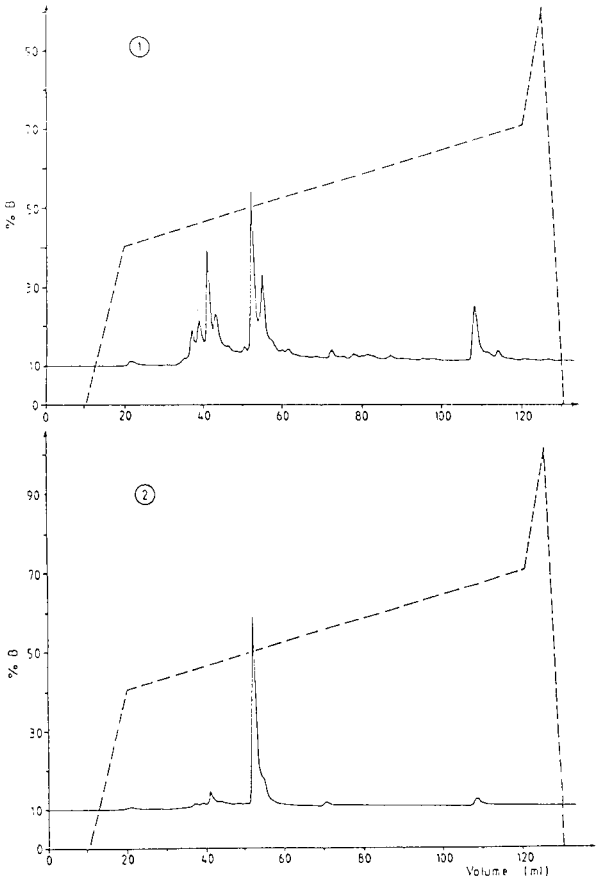

Figure 3. Purification of 6*R*(β)-phenylacetylaminopenicillanic-3*R*(β)-alcohol. One ml of dilute material in eluent A was injected. The figure shows the absorbance profile at 215 nm (-) and the gradient reaching 40% after 20 ml, 70% after 120 ml and 100% of eluent B after 130 ml. Each peak in (1) was tested in ir for the β-lactam structure. The pure compound **4a** (R = $PhCH_2CO$) eluted at 50% of eluent B. For further details, see experimental.

Thus, it seems that, when the OH group is on the β face of the bell-shaped penam skeleton, the carbinolamide structure is strongly stabilized through favorable contacts (Van der Waals and/or hydrogen bond). This led us to examine the possibility of substitution and oxydation at C(3).

Oxidation of secondary alcohols with acetic anhydride and DMSO is a mild oxidative method [25,26], but it is well known that it fails in cases where the alcohol is rapidly acetylated under the reaction conditions. Oxidation of **4a** (R = $PhCH_2CO$-) under very mild conditions yielded the imide **5c**. On the other hand, upon reaction with a more reactive anhydride, it was possible to isolate the 3*R*(β)-trifluoromethanesulfonyl analog **5a**. By substitution under very mild conditions, we have been able to obtain trace amounts of variously substituted penicillin analogs **6** (X = I, Br, Cl, ONO) with the substituents in the natural *S*(α) configuration. However, it has to be noted that all compounds substituted at C(3) with electron-withdrawing groups (except the alcohol) that we have isolated during this study are very sensitive to nucleophilic attack on the β-lactam carbon carbonyl. All these compounds are moisture sensitive and reaction with nucleophiles is always accompanied by the expulsion of the leaving group at C(3). This explains the rather poor yield reported for substitution at C(3) from **5a**. Nevertheless, reaction with water is slow and these compounds could be tried in enzymatic reactions.

In conclusion, this study led to the synthesis of a stereoisomer of natural β-lactams, 6*R*(β)-tritylaminopenicillanic-3*R*(β)-alcohol (**4a**), with a 100% stereochemical purity. The stereochemistry of this compound was assessed by NOE nmr experiments and it is believed that this configuration is maintained through an hydrogen bond between the NH group of the amino- or acylamino- side chain at C(6) and the alcohol oxygen at C(3). This interaction occurs in a minimum of conformational energy.

It was also shown that activation of the alcohol at C(3) with good leaving groups allowed nucleophilic substitutions to be performed at this position with a large variety of nucleophiles. These also restored the natural *S*(α) configuration.

Substituted compounds **6** were sensitive to nucleophilic attack at the β-lactam carbon carbonyl. This was presumably due to the inductive effect from the C(3) substituent.

Although hydrolysis of the β-lactam bond leads to expulsion of the C(3) substituent to give the dihydrothiazoline derivative **7**, it is rather unprobable that the two reactions be concomitent. Indeed, it has been shown that the rate-limiting step in the hydrolysis of the β-lactam ring is the formation of the tetrahedral intermediate. Moreover, even if the hydrolysis of the β-lactam bond would follow a concerted mechanism for these particular compounds, the expulsion of the leaving group at C(3) could not help the reaction because it is synperiplanar to the nitrogen lone pair. So, it is believed that the expulsion of the C(3) substituent occurs rapidly after opening of the β-lactam ring. Further chemical studies will be necessary in order to elucidate the mechanism.

For enzymatic studies, it will be necessary to synthesize more soluble compounds than those obtained in this study. Indeed, the loss of the acidic group at C(3) drastically decreases the solubility in water. It might prove convenient to have a more hydrophilic acylamido side chain at C(6). The intermediate compound **4a** has proven to be very versatile is this respect.

EXPERIMENTAL

General.

Melting points were determined on a Reichert (Austria) melting point apparatus. Elemental analyses were performed on a Carlo Erba EA 1108 analyzer. The ir spectra were recorded on

a Perkin Elmer 1320 spectrophotometer. The ^{1}H nmr spectra were taken on a 60 MHz Varian EM 360 L and on a 400 MHz Bruker AM and were reported in parts per million downfield from tetramethylsilane. Mass spectra were recorded on a Varian Mat 112 spectrometer. The hplc analyses were performed on a fplc apparatus (Pharmacia, Sweden) equipped with a P-3500 pump and a SS 250 ½" 10 Nucleosil 7 C18 column (Macherey-Nagel, Germany). The eluents and gradient were as follows: eluents A and B contained 0.1% TFA, respectively in water/acetonitrile (70/30, v/v) and in 100% acetonitrile; the gradient started with 10 ml of eluent A and went from 0 to 40% of eluent B over 10 ml, from 40 to 70% over 100 ml and from 70 to 100% of eluent B over 10 ml. The flow rate was 3.5 ml/min. Detection was at 215 nm.

6R(β)-Aminopenicillanic acid was a gift from Dr. M. Januszewski, Beecham S.A., Belgium. All other chemicals were from Janssen Chimica, Belgium.

6R(β)-Tritylaminopenicillanic Acid.

The following preparation represents a net improvement on the previously described methods [19,27].

To a solution of 5.0 g (23 mmoles) of 6β-aminopenicillanic acid in chloroform (150 ml) was added 10 ml (d = 0.765, 47 mmoles) of hexamethyldisilazane (HMDS). The mixture was stirred under reflux (60°) until complete dissolution. The ammonia formed was trapped in 1*M* hydrochloric acid. Solvent and excess HMDS were evaporated *in vacuo* and fresh chloroform (150 ml) was added. The solution was cooled on ice and 2.57 ml (d = 0.92, 23 mmoles) of *N*-methylmorpholine was added. Trityl chloride (8.5 g, 23 mmoles) in chloroform (50 ml) was added dropwise (0°) with stirring. The reaction was allowed to proceed for 2 hours at room temperature. The mixture was poured into an equal volume of crushed-ice/water at *p*H 1.5, washed 3 times with cold acidic brine and dried over magnesium sulphate. The solvent was evaporated (30°) *in vacuo* to yield a solid oil which crystallized in dichloromethane on addition of small amounts of *n*-hexane. Various problems may occur during the crystallization step if, for some reason, large amounts of triphenylcarbinol are present. When this was the case, the solid oil was dissolved in acetone/water (50/50) at 0° and the *p*H adjusted to 6.8 with 1*M* potassium hydroxide. The acetone was evaporated and the white precipitate of triphenylcarbinol filtered off. The aqueous phase was freeze-dried. The residual solid was suspended and stirred in acetone for 2 hours. The fine precipitate was collected and dried. The average yield, as calculated on more than 10 preparations, was around 90% (9.5 g, 20.7 mmoles); ir (chloroform): 1790, 1740 and 1660 cm^{-1}; ^{1}H nmr (deuteriochloroform):δ 1.23 (s, 3H), 1.26 (s, 3H), 4.17 (s, 1H), 4.27 (s, 2H), 5.63 (1H, labile), 7.13 (15H); ms: (m/z) 458, 412, 325, 259, 243, 182, 166, 154, 77.

Anal. Calcd. for $C_{27}H_{26}O_3N_2S$: C, 70.7; H, 5.7. Found: C, 70.5; H, 5.65.

6R(β)-Tritylaminopenicillanic Acid Ethyl Carbonate (**2a**).

The preparation of **2a** was adapted from a procedure described in ref [29]. 6β-Tritylaminopenicillanic acid [29] (5 g, 11 mmoles) was dissolved in dry tetrahydrofuran (200 ml) and cooled to −20°. *N*-Methylmorpholine (1.4 ml, d = 0.92, 12 mmoles) and ethyl chloroformate (1.2 g, d = 1.135, 12 mmoles) were added and the mixture was stirred at −10° for 90 minutes. Intermediate **2a** was not characterized, but derivatized *in situ* following three different routes leading to compounds **2b**, **2c** and **3**.

6R(β)-Tritylanhydropenicillin (**2b**).

This procedure was adapted from a previously described method published in ref [19]. While keeping the temperature at −10°, one more equivalent (1.4 ml, 12 mmoles) of *N*-methylmorpholine was added and the mixture was stirred for 90 minutes at room temperature. The reaction mixture was concentrated *in vacuo* and extracted with ether. The organic fraction was diluted in toluene and dry-evaporated. The residual oil was dissolved in isopropyl alcohol (30 ml) and kept at 4° overnight. The alcohol solution was discarded and the solid triturated in glacial acetic acid, yielding 0.9 g (2 mmoles, 18%) of 6R(β)-tritylanhydropenicillin, mp 164° (lit [19] 165-166°); ir (chloroform): 1820, 1700 and 1640 cm^{-1}; ^{1}H nmr (deuteriochloroform): δ 2.08 (d, 3H, J = 5.3 Hz), 2.17 (d, 3H, J = 5.3 Hz), 4.82 (d, 1H, J = 4.5 Hz), 5.05 (d, 1H, J = 4.5 Hz), 7.13 (15H).

Anal. Calcd. for $C_{27}H_{24}O_2N_2S$: C, 73.6; H, 5.45. Found: C, 73.4; H, 5.43.

6R(β)-Tritylpenicillamide (**2c**).

The procedure described here is an adaptation of a method found in ref [28,29]. Di-ammonium hydrogen phosphate *p*H 8.0 (1.45 g, 11 mmoles) in water (12.5 ml) was added while the temperature was kept at 0°. The mixture was stirred at 5° for 1 hour, poured into ice-cold water, extracted with dichloromethane, washed with water and dried over magnesium sulphate. After evaporation, the residue was crystallized in acetone-benzene, yielding 4.2 g (9.2 mmoles) of 6R(β)-tritylpenicillamide; ir (chloroform): 1790 and 1675 cm^{-1}; ^{1}H nmr (deuteriochloroform): δ 1.31 (s, 3H), 1.35 (s, 3H), 4.0 (s, 1H), 4.27 (d, 1H, J = 4.6 Hz), 4.35 (d, 1H, J = 4.6 Hz), 4.3-6.2-6.45 (3H, labile), 7.27 (15H).

Anal. Calcd. for $C_{27}H_{27}O_2N_3S$: C, 70.9; H, 5.91. Found: C, 71.0; H, 5.97.

Dehydration to the nitrile was performed as follows 0.5 g (1.1 mmole) of 6R(β)-tritylpenicillamide was dissolved in dry chloroform (50 ml) and cooled on ice. Pyridine (0.1 ml, 1.23 mmoles) in chloroform (2 ml) was added and a dropwise addition of methanesulfonyl chloride (0.1 ml, 1.29 mmoles) in chloroform (10 ml) was performed. The mixture was stirred at 0° for 1 hour and 0.1 ml (1.23 mmole) of pyridine in chloroform (2 ml) was added. The reaction was allowed to proceed for another 4 hours at room temperature. The organic mixture was poured into ice-cold water, washed and dried over magnesium sulphate. The residual oil obtained after evaporation could be crystallized in ether; ir (chloroform): 2400 and 1790 cm^{-1}; ^{1}H nmr (deuteriochloroform): δ 1.41 (s, 3H), 1.55 (s, 3H), 4.35 (d, 1H, J = 4.5 Hz), 4.45 (d, 1H, J = 4.5 Hz), 5.4 (s, 1H), 7.27 (15H).

Anal. Calcd. for $C_{27}H_{25}ON_3S$: C, 73.8; H, 5.70. Found: C, 74.0; H, 5.70.

6R(β)-Tritylaminopenicillanic-3S(α)-Isocyanate (**3**).

Sodium azide (0.8 g, 12 mmoles) in water (30 ml) was added. The mixture was stirred for 30 minutes at −5/0° and poured into crushed ice/water (300 ml). The aqueous phase was extracted 3 times with dichloromethane and the combined organic phases washed 3 times with water and dried over magnesium sulphate. After evaporation *in vacuo*, the crude product crystallized in petroleum ether 40:60 yielding a mixture (50/50) of 6β-tritylaminopenicillanic-3*S*-azide and -3*S*-isocyanate (2.5 g, 5 mmoles, yield 50%). No effort was made to completely convert the azide to the

isocyanate derivative as this seems to occur automatically when the mixture is hydrolyzed as described in the following step. Nevertheless, complete conversion to the isocyanate can be achieved by refluxing the mixture in chloroform for 3 hours, mp, 75-78°; ir (chloroform): 2260, 1790 and 1660 cm^{-1}; ^1H nmr (deuteriochloroform): δ 1.27 (s, 3H), 1.32 (s, 3H), 4.1 (s, 1H), 4.2 (d, 1H, J = 4.5 Hz), 4.35 (d, 1H, J = 4.5 Hz), 7.27 (15H). However, this complete conversion was accompanied by some degradation of the product, so that no meaningful elemental analysis could be performed.

6*R*(β)-Tritylaminopenicillanic-3*R*(β)-Alcohol (**4a**).

6*R*(β)-Tritylaminopenicillanic-3*S*(α)-isocyanate (5 g, 11 mmoles) was dissolved in tetrahydrofuran (250 ml) and added dropwise at room temperature to a solution made of 11 ml (11 mmoles) of 1*N* chlorhydric acid dispersed in 200 ml of water/tetrahydrofuran 50/50. After complete addition of the isocyanate, the reaction was allowed to process at room temperature for another 2 hours. The product of hydrolysis was poured into crushed ice/water (300 ml) and extracted with dichloromethane (300 ml). The organic phase was washed with water, dried over magnesium sulphate and evaporated *in vacuo*. The product, 6*R*(β)-trityl-aminopenicillanic-3*R*(β)-alcohol (3.8 g, 9 mmoles, yield 80%), crystallized in a mixture of diisopropyl ether/*n*-hexane, mp, 123-124°; ir (chloroform): 1770 and 1660 cm^{-1}; ^1H nmr (deuteriochloroform): δ 1.23 (s, 3H), 1.43 (s, 3H), 2.95 (1H, labile), 3.15 (1H, labile), 5.35 (d, 1H, J = 4.5 Hz), 5.45 (d, 1H, J = 4.5 Hz), 6.15 (s, 1H), 7.27 (15H); ms: (m/z) 430, 287, 259, 243, 182, 166, 154, 77.

Anal. Calcd. for $C_{26}H_{26}O_2N_2S$: C, 72.6; H, 6.05. Found: C, 72.1; H, 5.98.

On standing, this product slowly isomerizes to the other bicyclic compound; ^1H nmr (deuteriochloroform): δ 1.25 (s, 3H), 1.5 (s, 3H), 3.2 (1H, labile), 4.15 (d, 1H, J = 3.9 Hz), 4.27 (1H, labile), 4.3 (d, 1H, J = 3.9 Hz), 5.43 (s, 1H), 7.27 (15H).

6*R*(β)-Aminopenicillanic-3*R*(β)-Alcohol, *p*-Toluenesulfonate Salt.

The preparation of this salt was adapted from a procedure described in ref [30]. 6*R*(β)-Tritylaminopenicillanic-3*R*(β)-alcohol (2 g, 4.65 mmoles) was dissolved in acetone (200 ml). *p*-Toluenesulfonic acid monohydrate (0.8 g, 4.21 mmoles) was added and the mixture was stirred at room temperature for 10 minutes. The product of hydrolysis was precipitated in ether (100 ml) and kept cold overnight. The *p*-toluenesulfonate salt of 6*R*(β)-aminopenicillanic-3*R*(β)-alcohol (1.4 g, 3.72 mmoles, yield 80%) was collected by filtration; ir (potassium bromide): 1780, 1660 and 1190 cm^{-1}; ^1H nmr (dimethyl sulphoxide): δ 1.23 (s, 3H), 1.3 (s, 3H), 2.1 (s, 3H), 2.85 (3H, labile), 4.65 (d, 1H, J = 4.5 Hz), 4.7 (d, 1H, J = 4.5 Hz), 4.86 (s, 1H), 6.2 (d, 2H, J = 8.8 Hz), 6.86 (d, 2H, J = 8.8 Hz); pk_a (NH_3^+): 7.0 +/− 0.2 at 0° in acetone/water (40/10).

6*R*(β)-Phenylacetylaminopenicillanic-3*R*(β)-Alcohol (**4a**).

6*R*(β)-Aminopenicillanic-3*R*(β)-alcohol *p*-toluenesulfonate salt (1 g, 2.6 mmoles) was dissolved in a cold mixture of water/acetone 10/40 (250 ml). The *p*H was brought to 7.0 with a 0.1*N* sodium hydroxide solution and 0.35 ml (d = 1.17, 2.6 mmoles) of phenylacetyl chloride were rapidly added dropwise, while the *p*H was kept constant at 7.0. The solution was stirred for 10 minutes at 0° and poured into a crushed ice/water mixture. The aqueous phase was extracted with chloroform (3 x 100 ml) and the combined organic phases washed with water, dried over magnesium sulphate and evaporated *in vacuo*. 6β-Phenylacetylaminopenicillanic-3*R*(β)-alcohol crystallized in ether on addition of diisopropyl ether. The crude yellowish solid was recrystallized in acetonitrile, yielding a pure white product (0.65 g, 2.1 mmoles, yield 80%); ir (potassium bromide): 1790, 1680 and 1550 cm^{-1}; ^1H nmr (acetone): δ 1.41 (s, 3H), 1.42 (s, 3H), 3.64 (d, 2H), 5.32 (d, 1H, J = 4.5 Hz), 5.43 (d, 1H, J = 4.5 Hz), 5.64 (s, 1H), 6.39 (1H, labile), 7.3 (5H), 7.8 (1H, labile).

6*R*(β)-Phenylacetylaminopenicillanic-3*R*(β)-Triflate (**5a**).

6*R*(β)-Phenylacetylaminopenicillanic-3*R*(β)-alcohol (1 g, 3.2 mmoles) was dissolved in chloroform (50 ml) and the solution was cooled to −15°. *N*-Methylmorpholine (0.37 ml, 3.3 mmoles) was added and the mixture was stirred for 5 minutes; 0.56 ml (3.3 mmoles) of trifluoromethanesulfonic anhydride was added dropwise and the reaction left to proceed for 30 minutes at −15°. The cooling bath was removed and the temperature was allowed to return to 0°. The organic phase was washed with hydrochloric acid acidified ice-cold water, dried over magnesium sulphate and evaporated *in vacuo*. The solid residue (1 g, 2.3 mmoles, yield 72%) crystallized in ether in the presence of small amounts of diisopropyl ether, mp, 127-129°; ir (chloroform): 1780, 1660, 1550 and 1035 cm^{-1}; ^1H nmr (deuteriochloroform): δ 1.38 (s, 3H), 1.42 (s, 3H), 3.64 (d, 2H), 5.17 (d, 1H, J = 4.5 Hz), 5.35 (d, 1H, J = 4.5 Hz), 7.3 (5H), 7.95 (s, 1H).

6*R*(β)-Phenylacetylaminopenicillanic-3*R*(β)-*p*-toluenesulfonate (**5a**, R = H).

The same procedure was applied to the synthesis of **5b** from **4b**. 6*R*(β)-Phenylacetylaminopenicillanic-3*R*(β)-alcohol (1.4 g, 4.6 mmoles) was dissolved in dry tetrahydrofuran (150 ml) and cooled on ice. Pyridine (0.38 ml, 4.69 mmoles) was added. Tosyl chloride (0.9 g, 4.72 mmoles) in dry tetrahydrofuran (20 ml) was added dropwise and the mixture was stirred at 0° for 2 hours. The mixture was poured into crushed ice/water, extracted with chloroform, washed with water and dried over magnesium sulphate. The yield after evaporation was 99% (2.1 g, 4.6 mmoles); ir (chloroform): 1780, 1685 and 1375-1180 cm^{-1}; ^1H nmr (deuteriochloroform): δ 1.23 (s, 3H), 1.33 (s, 3H), 2.42 (s, 3H), 3.65 (d, 2H), 4.2 (1H, labile), 5.35 (d, 1H, J = 4.5 Hz), 5.56 (d, 1H, J = 4.5 Hz), 6.9 (5H), 7.1 (d, 2H, J = 8.8 Hz), 7.8 (d, 2H, J = 8.8 Hz), 8.8 (s, 1H).

6*R*(β)-Phenylacetylaminopenicillimide (**5c**).

One g (3.25 mmoles) of 6*R*(β)-phenylacetylaminopenicillanic-3*R*(β)-alcohol was dissolved in dimethylsulphoxide (20 ml) and 0.32 ml (3.2 mmoles) of acetic anhydride was added. The mixture was stirred for 96 hours at room temperature and poured into ice-cold water. Extraction was done with chloroform and the organic phase was washed with water and dried over magnesium sulphate. Evaporation *in vacuo* yielded 0.4 g (1.3 mmoles, 40%) of 6*R*(β)-phenylacetylaminopenicillimide; ir (chloroform): 1820, 1740 and 1680 cm^{-1}; ^1H nmr (deuteriochloroform): δ 2.31 (s, 3H), 2.48 (s, 3H), 3.65 (d, 2H), 5.13 (d, 1H, J = 4.5 Hz), 5.37 (d, 1H, J = 4.5 Hz), 7.13 (5H).

Anal. Calcd. for $C_{15}H_{16}O_3N_2S$: C, 59.2; H, 5.26. Found: C, 58.9; H, 5.18.

6*R*(β)-Phenylacetylaminopenicillanic-3*S*(α)-Chloride (**6**).

6*R*(β)-Phenylacetylaminopenicillanic-3*R*(β)-*p*-toluenesulfonate (2 g, 4.3 mmoles) was dissolved in 100 ml dry acetone together

with 0.5 g (4.6 mmoles) of tetramethylammonium chloride. The mixture was stirred for 2 days at room temperature. The acetone was evaporated, the residue was dissolved in chloroform, washed with water and the organic phase was dried over magnesium sulphate. After evaporation, the residue (1.5 g, 4.6 mmoles, 77%) was triturated in diisopropylether and collected by filtration; ir (chloroform): 1795 and 1685 cm^{-1}; ^1H nmr (deuteriochloroform): δ 1.78 (s, 3H), 1.87 (s, 3H), 3.65 (d, 2H), 5.35 (d, 1H, J = 4.5 Hz), 5.78 (d, 1H, J = 4.5 Hz), 6.92 (5H), 8.55 (s, 1H); ms (m/e): 324, 326.

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REFERENCES AND NOTES

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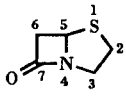
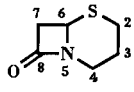
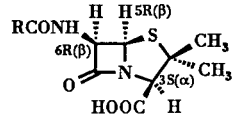
[1] The nomenclature generally employed in this area is as follows. The penam **1** and cepham **2** skeletons are the basic structures commonly encountered among the β-lactam antibiotics, the latter one bearing a double bond at position 3 as the ceph-3-em system. The penicillanic acid structure **3** generally bears an acylamino group at position 6 and has the natural configuration 3*S*, 5*R*, 6*R*. The chirality about the β-lactam (azetidinone) ring in the cephalosporins is the same as for the penicillins:



1



2



3

Reference is often made to stereochemical centers in terms of the trivial α,β convention, the α-face being the less hindered side of the folded, bicyclic penam of cepham skeletons. For more details, see P. G. Sammes [4].

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