Pergamon

Tetrahedron 56 (2000) 7601–7606

TETRAHEDRON

Synthesis of Penicillin N and Isopenicillin N

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Received 7 April 2000; revised 12 July 2000; accepted 27 July 2000

Abstract—Enantiomeric 2-(*N*-allyloxycarbonyl)aminoadipic acid 1-allyl esters were obtained from the corresponding 2-(*N*-trityl)aminoadipic acid diallyl esters following selective hydrolysis of the allyl 6-ester group and subsequent exchange of the trityl group by the allyloxycarbonyl function. The resulting monoacids were used to acylate 6-aminopenicillanic acid allyl ester using a carbodiimide-mediated coupling. The products, the L- and D-isomers of 6-[6-(2-(*N*-allyloxycarbonyl) aminoadipyl)]aminopenicillanic acid diallyl ester were deprotected in one step by catalytic allyl transfer using tetrakis-(triphenylphosphine)palladium(0), to afford isopenicillin N and penicillin N, respectively. The presented straightforward route to penicillin N and isopenicillin N is uniquely compatible with the sensitive nature of the condensation products and gives entry to a new and high yielding procedure that is superior to existing approaches. © 2000 Elsevier Science Ltd. All rights reserved.

Introduction

Isopenicillin N (**1b**, Fig. 1) is an intermediate common to the biosynthesis of penicillins and cephalosporins.[1] In cephalosporin producing organisms isopenicillin N is converted to penicillin N (**1a**) by an enzyme referred to as isopenicillin N/penicillin N epimerase. Small amounts of **1a** and **1b** are of pivotal importance in laboratory studies related to the biosynthesis of penicillins and cephalosporins and play a central role in molecular pathway engineering studies.

Several syntheses of these compounds have been published during the last two decades.[2–7] The choice of the protecting groups in these syntheses is restricted due to the fact that **1a** and **1b** are highly unstable in both acidic and alkaline media.[2] The method of choice seemed to be the application of protecting groups of the benzyl-type which are removable under neutral conditions, i.e. catalytic hydrogenolysis, and is used in all previously described procedures. However, a severe disadvantage of catalytic hydrogenolysis is the very large amount of catalyst that is required to obtain complete hydrogenolysis, which is due to catalyst poisoning effects of the sulfur atom present in the penicillin nucleus.[8] Moreover, this phenomenon leads to a drastic reduction in yield due to degradation of the penicillin nucleus. We reasoned that this problem could be avoided by employing allyl-type protecting groups[9] that can be easily removed under neutral conditions. A well-known procedure is catalytic allyl-transfer to a suitable allyl acceptor, e.g. 5,5-dimethyl-1,3-cyclohexanedione (dimedone), offering an essentially neutral reaction medium. The usual catalyst is tetrakis-(triphenylphosphine)palladium(0), which is readily soluble in the majority of organic solvents. Since the central palladium atom is protected by its ligands against complexation with thiocompounds, it can catalyze allyl transfer even in sulfur-containing solvents (e.g. dimethylsulfoxide).

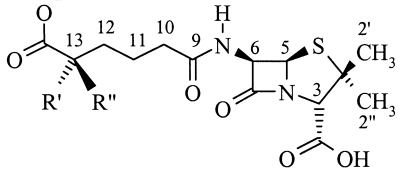

Figure 1. Penicillin N (**1a**: R′=H, R″=NH_3^+) and isopenicillin N (**1b**: R′=NH_3^+, R″=H).

A further drawback of the currently recommended routes is the absence of a general and simple route to 2-aminoadipic acid 1-esters, suitable for condensation with the aminopenicillanic acid moiety. The appropriately protected aminoadipic acid derivatives are obtained from multi-step sequences frequently including laborious chromatography isolation procedures. Itoh[10] developed a procedure in which the 2-benzyloxycarbonylamino- and the 1-carboxylic functions in benzyloxycarbonylaspartic and glutamic acids

Keywords: allyl protection; 2-aminoadipic acid; catalytic allyl transfer; isopenicillin N; penicillin N.
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PII: S0040-4020(00)00673-6

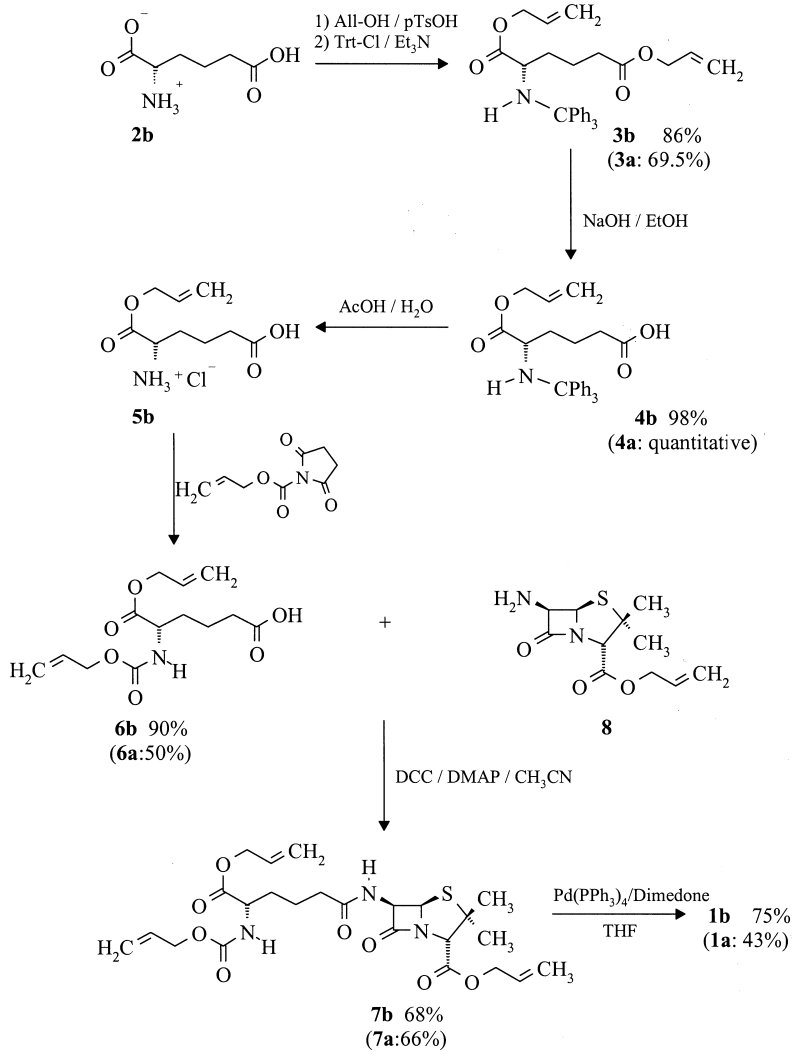
are protected simultaneously by formation of oxazolidine derivatives, by reaction with paraformaldehyde under acidic conditions, thereby leaving the 6-carboxylic group free for further reaction. Lal and Kulkarni[7] applied this method successfully in the partial protection of 2-aminoadipic acid, but they also had to face the slow rate of the final hydrogenolysis. Allyl protection of the β-lactam nucleus of our target molecules has already been reported by Manhas et al., who published a convenient method for the preparation of 6-aminopenicillanic acid (6-APA) allyl ester 4-toluenesulfonate.[11] The required 2-aminoadipic acid derivatives, however, have not yet been described.

An alternative high yielding procedure for the preparation of **1a** and **1b**, using allyl protection of both the 1-carboxyl group in the side chain and the carboxyl group of the penicillin nucleus, and allyloxycarbonyl for protection of the amino group, is presented in this paper (Scheme 1).

Results and Discussion

Discrimination between the two carboxylic acid functions in 2-aminoadipic acid was realized using transient protection[12–14] of the amino group with the triphenylmethyl (trityl) function. It is well known that the trityl group deactivates the 1-carboxylic function towards both hydrolysis and transesterification.[13] Thus, for the preparation of the desired 2-aminoadipic acid derivatives, the appropriate enantiomer of the amino dicarboxylic acid **2** was esterified with allyl alcohol and then tritylated using standard procedures. Attempts to replace benzene by toluene in the esterification reaction failed. It was noted that at the higher temperature of the allyl alcohol–toluene azeotrope (92°C) some starting material was converted into a cyclic six-membered amide ring, a phenomenon that was not observed at the boiling point of the allyl alcohol–benzene azeotrope (77°C). Furthermore, a significant amount of allyl alcohol is withdrawn from the reaction mixture in the allyl alcohol–toluene azeotrope (50:50) compared to that of allyl alcohol–benzene (17:83).

2-(*N*-Trityl)aminoadipic acid 1-allyl ester (**4**) was readily obtained by alkaline hydrolysis in aqueous ethanol. It was noted that unwanted formation of the 6-ethyl ester took place as a function of the amount of water present in the



Scheme 1. Synthesis of isopenicillin N (**1b**, Fig. 1) from L-2-aminoadipic acid and 6-aminopenicillanic acid using allyl type protective groups (the synthesis of penicillin N encompasses the same steps, only us using D-2-aminoadipic acid as starting material).

hydrolysis reaction. Addition of 10% of water appeared to be the correct amount to suppress ethyl ester formation on the one hand whilst maintaining solubility on the other. This selective hydrolysis of N^{α}-tritylaminodicarboxylic acid diesters was further generalized with experiments to obtain 1-esters of aspartic and glutamic acid. In these experiments (experimental details not given), it appeared that the α-ester function of *N*-tritylaspartic acid diallyl ester was considerably more susceptible towards base-catalyzed hydolysis than those of *N*-tritylaminoadipic acid diallyl ester and *N*-tritylglutamic acid diallyl ester. In our view, this phenomenon is due to anchimeric assistance by the β-carboxylate function of aspartic acid, once it is liberated.

Subsequent acid-catalyzed hydrolysis of the trityl group in **4** using acetic acid readily afforded 2-aminoadipic acid 1-allyl ester (**5**).

Acylation with allyl succinimidyl carbonate gives the required 2-(*N*-allyloxycarbonyl)aminoadipic acid 1-allyl ester (**6**). For the L-isomer the sequence of steps from **4b** was performed in an excellent 76% yield. During the synthesis of the D-isomer an unexpected hydrolysis problem forced us to purify extensively crude valuable **5a** thereby making a fair comparison of yields impossible. We noted that this intermediate, upon prolonged storage at low temperatures, slowly hydrolyzes to give aminoadipic acid (approx. 0.05% h^{-1} at 0–5°C), a phenomenon which can be easily circumvented by avoiding prolonged storage of monoester **5a**.

Activation with dicyclohexylcarbodiimide, DCC, was found to be effective for acylation of the free amino function in 6-APA-allyl ester (**8**). The 4-nitrophenyl and 1-succinimidyl esters of **6** were ineffective: even after prolonged reaction times, only starting material was recovered. Thus, DCC mediated coupling of **8** with the appropriate isomer of aminoadipic acid derivative **6** afforded the fully protected penicillin N (**7a**) and isopenicillin N (**7b**) in quantitative yield as judged by TLC. Upon purification by flash chromatography, a necessary step prior to the deprotection sequence, the isolated yields of **7a** and **7b** were 66% and 68%, respectively. These yields may be considered good since it is well known that silica gel chromatography of hydrophobic β-lactam derivatives is a low yield purification process (due to product degradation and irreversible attachment to the column material).

Removal of the three protective functions by allyl transfer to dimedone was performed satisfactorily in THF. Although the deprotection also occurs smoothly in dichloromethane, this solvent should not be used in conjunction with penicillin derivatives. It has been shown that minute amounts of acid are formed during the deprotection reaction in dichloromethane, which catalyze the complete hydrolysis of the β-lactam ring if trace amounts of water are present in the solvent.[15] Dimedone (pK_a=5.2) has been recommended as a neutral nucleophile in allyl transfer reactions[9] and has been applied here successfully. However, dimedone can also react as a diketone to give a Schiff-base. Indeed, the slow formation of this compound was, in some cases, observed during our experiments. Since the Schiff base is a stable by-product, which can only be hydrolyzed using conditions that are incompatible with the maintenance of the penicillin skeleton, its formation should be suppressed. A good alternative to dimedone as a neutral allyl acceptor may be *N*,*N*′-dimethylbarbituric acid[16–18] (DMBS, pK_a=4.7), a compound that hardly possesses diketone character. The use of DMBS for this specific application is currently under investigation.

Isolation of the end products **1** as lyophilized disodium salts needs to be performed carefully by keeping temperatures low and pH-values close to neutral or slightly acidic. By nature, these penicillins are extremely susceptible towards degradation, a process that can readily occur, e.g. during NMR analysis.

Conclusion

N-Tritylamino acid 1-esters behave as deactivated esters while, simultaneously, remote ester functions in bivalent ester molecules retain their normal reactivity[13] (i.e. they can selectively undergo hydrolysis or base-catalyzed transesterification). Furthermore they resist base-induced racemization.[14] These features provide a general route to selectively protected 2-aminodicarboxylic acid esters and are applied here successfully in a straightforward synthesis of L- and D-2-(*N*-allyloxycarbonyl)aminoadipic acid 1-allyl esters (**6**). The latter are used to acylate 6-aminopenicillanic acid allyl ester resulting in the efficient formation of the two fully protected penicillin N diastereoisomers. Removal of the two allyl- and allyloxycarbonyl protecting groups can be performed smoothly in one operation using catalytic allyl transfer. During this process, the catalyst, tetrakis-(triphenylphosphine)palladium(0), is not poisoned by thioethers, a phenomenon that often occurs in β-lactam chemistry.

Application of dimedone as the recommended allyl receptor is to be further investigated, since this diketone can slowly produce a very stable Schiff-base with the 2-amino group of the aminoadipic moiety once it is released. Preliminary experiments employing *N*,*N*′-dimethylbarbituric acid as allyl acceptor have shown that this alternative for dimedone leads to improved deprotection results.

The present route to penicillin N and isopenicillin N exploiting allyl-type protecting groups combines an efficient derivatization of the rare and expensive 2-aminoadipic acid enantiomers to partially protected acids, ready for acylation of the amino group of 6-aminopenicillanic acid allyl ester, with effective deprotection compatible with the sensitive nature of the two diastereoisomeric condensation products affording the two scientifically highly interesting and hitherto laboriously accessible compounds.

Experimental

General

Allyl alcohol, L- and D-2-aminoadipic acid, dimedone, potassium hydrogen sulfate, tetrakis-(triphenylphosphine)-palladium(0), triethyl amine (stored on 3 Å molecular sieve), and trityl chloride were purchased from Aldrich.

4-Dimethylaminopyridine (DMAP) was purchased from Merck. Benzene, *N,N′*dicyclohexylcarbodiimide (DCC), 4-toluenesulfonic acid monohydrate, and the extra dry quality solvents acetonitrile, CH_2Cl_2 and THF were purchased from Acros Organics. Allylsuccinimidyl carbonate was obtained as a low melting solid (mp 12°C) by reacting 1-hydroxysuccinimide with allyl chloroformate[19] and triethylamine (1 equiv.) in acetonitrile (yield 92%). 6-APA-allyl ester 4-toluenesulfonate was prepared according to a literature procedure.[11]

In extraction procedures, organic phases were dried solely with Na_2SO_4 since magnesium ions derived from $MgSO_4$, often cause complications due to the formation of intractable complex salts.

Thin layer chromatography was performed on pre-coated silica plates (Merck Silicagel 60 F_{254}); column chromatography was on silica (Lichroprep silica 60). Detection was by UV-fluorescence and by spraying with $MeOH/H_2SO_4$ (4:1, v/v) for trityl derivatives, with ninhydrin for free amines, or with chlorine–TDM[20] for amines and *N*-acylated compounds. Optical rotations were measured at room temperature using a Perkin–Elmer 241 polarimeter. ^1H NMR and ^{13}C NMR spectra were obtained using a Bruker AM 360 spectrometer (TMS as internal standard) for solutions in $CDCl_3$ as the solvent. The ^{13}C NMR spectra of **1** were recorded on a Bruker AM 300 NMR spectrometer (in $CDCl_3$).

Quantitative ^1H NMR experiments were performed in duplicate at 600 MHz on a Bruker AMX 600 spectrometer. To an accurately weighed quantity of **1** was added a known quantity of internal standard (maleic acid, in phosphate buffer pH 6.4); the mixture was lyophilized and then dissolved in D_2O. Spectra were determined using 90° pulses (8.5 μs); the FID was multiplied with an exponential function causing line-broadening of 0.2 Hz. The integral of the H^5 proton and the internal standard were measured and the purity of **1** was calculated. The accuracy of this absolute method is considered to be better than 0.5% according to validation studies on similar compounds. Similar quantifications were used for intermediate products.

Low resolution molecular masses were obtained using Liquid Secondary Ion Mass Spectroscopy (LSIMS), a method that is particularly suitable for the measurement of highly labile products, using an AMD 604 high field mass spectrometer and were recorded on a Kratos Mach3 data system.

2-(*N*-Trityl)aminoadipic acid diallyl ester (3). 2-Aminoadipic acid (**2**, 10 g, 62 mmol) was heated under reflux in freshly distilled allyl alcohol (100 ml), benzene (250 ml) and 4-toluenesulfonic acid monohydrate (13 g, 68 mmol). The distillate was collected in a Dean–Stark trap and heating was continued until formation of water ceased and TLC indicated the absence of free aminoadipic acid (approx. 48 h). Volatile constituents were removed by concentration in vacuo; the residue crystallized very slowly to give waxy, low melting material of 2-aminoadipic acid diallyl ester 4-toluenesulfonate. A stirred,cooled (0°C) solution of this material (25.2 g, 61 mmol) in CH_2Cl_2 extra-dry (200 ml) and triethylamine (18.5 ml, 132 mmol) was treated with trityl chloride (17.0 g, 61 mmol). The reaction mixture was left at room temperature for 4 h and concentrated in vacuo. The residue was diluted with EtOAc (200 ml), filtered and the filtrate washed with water, dried (Na_2SO_4) and concentrated in vacuo. Column chromatography (silica-60, 600 g, column ∅ 50 mm, elution with EtOAc–hexane 1:4) gave a colorless syrup.

L-Isomer (**3b**): TLC, one spot R_f=0.35, EtOAc–hexane (1:4); yield 26 g (86%); purity 83%; $[\alpha]_D^{22}$=+39.6 (*c*=1, $CHCl_3$). ^1H NMR ($CDCl_3$): δ=1.5–1.9 (m, 4H, H^3+H^4); 2.34 (t, *J*=6.5 Hz, 2H, H^5); 3.34 (m, 1H, H^2); 3.9 (m, 2H, CH_2=CHCH_2–); 4.7 (d, *J*=5.8 Hz, 2H, CH_2=CHCH_2–); 5.0–5.5 (m, 4H, CH_2=CHCH_2–); 5.5–6.3 (m, 2H, CH_2=C*H*CH_2–); 7.0–7.6 (m, 15H, Ph). ^{13}C NMR ($CDCl_3$): δ=20.7 (C-3); 33.9 (C-5); 35.1 (C-4); 55.6 (C-2); 64.8 (CH_2=CH*C*H_2–); 118.0 (*C*H_2=CHCH_2–); 118.2 (*C*H_2=CHCH_2–); 126.3, 127.7, 128.7 (Ph (*tert*)); 131.7 (CH_2=*C*HCH_2–); 132.1 (CH_2=*C*HCH_2–); 145.8 (Ph (*quat*)); 172.6 and 174.3 (carboxyl carbons). *m/z* (%): 484.2 [M^+], calculated for $C_{31}H_{34}NO_4$: 484.2488.

D-Isomer (**3a**, using as starting material 5 g of **2**): TLC one spot, R_f=0.36, EtOAc–hexane (1:4); yield 10.4 g (69.5%); purity 83%; $[\alpha]_D^{22}$=−39.7 (*c*=1, $CHCl_3$). ^1H NMR ($CDCl_3$): δ=1.5–1.9 (m, 4H, H^3+H^4); 2.35 (t, *J*=6.5 Hz, 2H, H^5); 3.36 (m, 1H, H^2); 4.09 (m, 2H, CH_2=CHCH_2–); 4.6 (d, *J*=5.8 Hz, 2H, CH_2=CHCH_2–); 5.1–5.4 (m, 4H, CH_2=CHCH_2–); 5.5–6.0 (m, 2H, CH_2=C*H*CH_2–); 7.1–7.6 (m, 15H, Ph). ^{13}C NMR ($CDCl_3$): δ=20.65 (C-3); 33.90 (C-5); 35.1 (C-4); 55.6 (C-2); 64.87 (CH_2=CH*C*H_2–); 118.06 (*C*H_2=CHCH_2–); 118.24 (*C*H_2=CHCH_2–); 126.41, 127.79, 128.75 (Ph (*tert*)); 131.76 (CH_2=*C*HCH_2–); 132.11 (CH_2=*C*HCH_2–); 145.81 (Ph (*quat*)); 172.65 and 174.31 (carboxyl carbons). *m/z* (%): 484.3 [M^+], 506.4 [M+Na], calculated for $C_{31}H_{34}NO_4$: 484.2488.

2-(*N*-Trityl)aminoadipic acid 1-allyl ester (4). A stirred solution of compound **3** (25.5 g, 52.1 mmol) in 125 ml EtOH/water (9:1, v/v), maintained at 50°C, was treated slowly with aqueous NaOH (1 M, 58 ml, 1.1 equiv.); the mixture was left for 2 h at 50°C and was then concentrated in vacuo and diluted with water (50 ml). The aqueous concentrate was neutralized with acetic acid (1 M, 58 ml) and the precipitated oil was taken up in CH_2Cl_2 (100 ml); the aqueous layer was extracted with CH_2Cl_2 (2×25 ml) and the combined organic phases were washed successively with water (25 ml) and with a saturated NaCl solution (2×25 ml). The organic phase was dried (Na_2SO_4), concentrated and dried in vacuo to remove residual CH_2Cl_2, to give a sticky colorless syrup.

L-Isomer (**4b**): TLC one spot, R_f=0.14, EtOAc–hexane (1:4), R_f=0.7, EtOAc–hexane (1:1); yield 23 g (98%); purity 80.5%; $[\alpha]_D^{22}$=+35.05° (*c*=2, CH_2Cl_2). ^1H NMR ($CDCl_3$): δ=1.5–2.0 (4H, H^3+H^4); 2.36 (t, *J*=6.9 Hz, 2H, H^5); 3.37 (t, *J*=5.0 Hz,1H, H^2); 3.8–4.25 (m, 2H, CH_2=CHCH_2–); 5.10–5.25 (m, 3H, NH and CH_2=CHCH_2–); 5.55–5.8 (m, 1H, CH_2=C*H*CH_2–); 7.0–7.6 (m, 15H, Ph). *m/z* (%): 444.2 [M^+], calculated for $C_{28}H_{30}NO_4$: 444.2175.

D-Isomer: (**4a**, using as starting material 10.4 g of **3**): TLC one spot, R_f=0.15, EtOAc–hexane (1:4); yield 10.1 g as crude extract (quantitative); $[\alpha]_D^{22}=-30.2$ (c=2, CH_2Cl_2).

2-(*N*-Allyloxycarbonyl)aminoadipic acid 1-allyl ester (6). A stirred solution of compound **4** (22.2 g, 50 mmol) in a mixture of AcOH/water/2 M HCl (200 ml, 160:15:25 v/v/v) was heated to 60°C for 30 min and subsequently cooled down to room temperature. The precipitated triphenylmethanol was collected by filtration. Ether (150 ml) and water (20 ml) were added to the filtrate. The two phases were re-extracted, to remove triphenylmethanol completely; the combined aqueous phases were concentrated in vacuo, to yield pale yellow syrup of 2-aminoadipic acid 1-allyl ester hydrochloride (**5**) which solidified. This product, dissolved in a mixture of acetonitrile and water (60 ml, 4:1 v/v) was treated with triethylamine (6.2 ml, 44 mmol) to give pH 9.3. Subsequently, a solution of allyl succinimidyl carbonate (5.33 g, 26.8 mmol, 1.27 equiv.) in acetonitrile (10 ml) was added, the apparent pH being controlled with a pH-stat. The pH of the mixture decreased immediately and further triethylamine was added to restore the pH to 9. The almost clear solution was filtered and concentrated to remove organic solvent. Water (40 ml) was added and the product was precipitated by the addition of a small excess of 1.5 M hydrochloric acid (20 ml), to give pH <2. The decanted water phase was then extracted with ether (4×20 ml) and the ether phases were combined with the main product. The obtained extract of the crude product was washed with 10% aqueous NaCl (3 times), dried (Na_2SO_4) and concentrated in vacuo to leave clear syrup.

L-Isomer (**6b**): TLC R_f=0.8, BuOH–AcOH–water (4:1:1); yield 12.9 g (90%); purity 75%; $[\alpha]_D^{20}=+6.0$ (c=1, $CHCl_3$). ^{1}H NMR ($CDCl_3$): δ=1.6–2.0 (m, 4H, H^3+H^4); 2.40 (m, 2H, H^5); 4.40 (m, 1H, H^2); 4.57+4.65 (2×d, J=5.4 Hz, 4H, CH_2═CHCH_2–); 5.2–5.5 (m, 4H, CH_2═CHCH_2–); 5.85–6.0 (m, 2H, CH_2═CHCH_2–); 6.1 (bs, 1H, NH). The acid was crystallized as the dicyclohexylammonium salt by dissolving the syrup in ether and adding dicyclohexylamine. TLC two spots, R_f=0.8 (acid) and 0.57 (amine), BuOH–AcOH–Water (4:1:1); mp 117.5°C; $[\alpha]_D^{20}=-1.9$ (c=1, $CHCl_3$). Anal. calcd for $C_{25}H_{42}N_2O_6$ (466.61): C 64.35, H 9.07, N 6.00. Found: C 63.93, H 8.85, N 5.90.

D-Isomer (**6a**, using 3.63 g of crude **5a** as starting material; as explained in the text, this crude material contained D-2-aminoadipic acid and was extensively purified by silicagel chromatography using hexane–EtOAc (9:1) prior to further reaction): TLC R_f=0.75, BuOH–AcOH–Water (4:1:1); yield 2.6 g (approx. 50% based on crude **5a**); purity 70%; $[\alpha]_D^{20}=-1.7$ (c=1, CH_2Cl_2). ^{1}H NMR ($CDCl_3$): δ=1.6–2.0 (m, 4H, H^3+H^4); 2.40 (m, 2H, H^5); 4.37 (m, 1H, H^2); 4.57+4.65 (2×d, J=5.4 Hz, 4H, CH_2═CHCH_2–); 5.15–5.5 (m, 4H, CH_2═CHCH_2–); 5.68 (bs, 1H, NH); 5.85–6.0 (m, 2H, CH_2═CHCH_2–). ^{13}C NMR ($CDCl_3$): δ=21.0 (C-4); 31.6 (C-3); 33.7 (C-5); 54.1 (C-2); 66.3 (CH_2═CH*C*H_2–); 119.2 (*C*H_2═CHCH_2–); 131.9 (CH_2═*C*HCH_2–); 169.3 and 176.3 (carboxyl carbons).

6-[6-(2-(*N*-Allyloxycarbonyl)aminoadipyl)]aminopenicillanic acid diallyl ester (7). A stirred, cooled (−20°C) solution of compound **6** (4.6 g, 16.1 mmol) and 6-aminopenicillanic acid allyl ester 4-toluenesulfonate (7.24 g, 16.9 mmol) in dry acetonitrile (150 ml), containing triethylamine (2.48 ml, 17.7 mmol) and a catalytic amount of DMAP was treated with DCC (3.65 g, 17.7 mmol), set aside at 4°C for 24 h and was then allowed to attain room temperature gradually. The suspension was cooled again to −20°C, filtered and the filtrate was evaporated in vacuo. The solid residue was dissolved in pure anhydrous EtOAc (30 ml), washed with ice-water (2×10 ml), ice-cold aqueous 0.05 M $KHSO_4$ solution (5 ml), saturated $NaHCO_3$ solution (pH 7–8, 10 ml) and saturated NaCl solution (2×10 ml), then dried (Na_2SO_4) and concentrated in vacuo. Column chromatography (silica 60, EtOAc–hexane 2:1 v/v) of the residue followed by evaporation of solvent gave the expected compound as a white foam.

Protected isopenicillin N (**7b**): TLC R_f=0.65, EtOAc–hexane (2:1); yield 6.6 g (68%); purity 56%; $[\alpha]_D^{20}=+144$ (c=1.5, $CHCl_3$). IR (KBr): ν=1780 cm^{-1} (β-lactam carbonyl). ^{1}H NMR ($CDCl_3$): δ=1.52 (s, 3H, $H^{2'}$); 1.66 (s, 3H, $H^{2''}$); 1.74 (m, 2H, H^{11}); 1.74–1.90 (m, 2H, H^{12}); 2.29–2.34 (m, 2H, H^{10}); 4.38 (m, 1H, H^{13}); 4.44 (s, 1H, H^3); 4.58–4.67 (m, 6H, CH_2═CHCH_2–); 5.22–5.39 (m, 6H, CH_2═CHCH_2–); 5.40 (s, 1H, NH); 5.55 (d, 1H,, J=4.0 Hz, H^5); 5.69 (d, 1H,, J=4.0 Hz, H^6); 5.91–5.92 (m, 3H, CH_2═CHCH_2–); 6.26 (s, 1H, NH). ^{13}C NMR ($CDCl_3$): δ=21.5 (C-11); 27.5 (C-2′); 31.9 (C-2″); 32.4 (C-12); 35.5 (C-10); 53.8 (C-13); 58.9 (C-6); 65.3 (C-2); 66.4, 66.5, 66.7 (CH_2═CH*C*H_2–); 68.4 (C-5); 70.9 (C-3); 118.3, 119.5, 120.3 (*C*H_2═CHCH_2–); 131.4, 132.9, 133.0 (CH_2═*C*HCH_2); 156.2 (allyloxycarbonyl); 167.7, 172.0, 172.2 (carboxyl carbons); 174.2 (C-7).

Protected penicillin N (**7a**): starting from 2.5 g of **6a**: TLC R_f=0.69, EtOAc–Hexane (2:1); yield 3.5 g (66%); purity 50%; $[\alpha]_D^{20}=+87.8$ (c=1.5, CH_2Cl_2). IR (KBr): ν=1780 cm^{-1} (β-lactam carbonyl). ^{1}H NMR ($CDCl_3$): δ=1.52 (s, 3H, $H^{2'}$); 1.67 (s, 3H, $H^{2''}$); 1.72–1.90 (m, 2H, H^{12}); 1.76 (m, 2H, H^{11}); 2.27–2.38 (m, 2H, H^{10}); 4.39 (m, 1H, H^{13}); 4.44 (s, 1H, H^3); 4.57–4.67 (m, 6H, CH_2═CHCH_2–); 5.08–5.44 (m, 6H, CH_2═CHCH_2–); 5.41 (s, 1H, NH); 5.55 (d, 1H,, J=4.0 Hz, H^5); 5.70 (d, 1H, J=4.0 Hz, H^6); 5.91–5.92 (m, 3H, CH_2═CHCH_2–); 6.34 (s, 1H, NH). ^{13}C NMR ($CDCl_3$): δ=21.5 (C-11); 27.5 (C-2′); 31.9 (C-2″); 32.5 (C-12); 35.5 (C-10); 53.8 (C-13); 58.9 (C-6); 65.2 (C-2); 66.3, 66.6, 66.6 (CH_2═CH*C*H_2–); 68.5 (C-5); 70.9 (C-3); 118.3, 119.5, 120.3 (*C*H_2═CHCH_2–); 131.4, 131.8, 132.9 (CH_2═*C*HCH_2); 156.4 (allyloxycarbonyl); 167.7, 172.1, 172.2 (carboxyl carbons); 174.2 (C-7).

(Iso)penicillin N disodium salt (1). To a stirred solution of compound **7b** (2.45 g, 4.7 mmol) in peroxide-free THF (25 ml) and maintained under argon, dimedone (3.29 g, 23.5 mmol) and tetrakis-(triphenylphosphine)palladium(0) (0.36 g, 0.3 mmol) were added. The solution was kept for 16 h at room temperature, during which time a precipitate was gradually formed. The solid material was collected by filtration, washed with THF (2×5 ml), dissolved in ice-cold water (25 ml) and the pH was carefully adjusted to 7 by addition of an aqueous solution of 10% $NaHCO_3$ (w/w). The solution was extracted with EtOAc (3×5 ml), the

aqueous layer was decolorized with active charcoal and subjected to lyophilization.

Isopenicillin N sodium salt (1b). Yield 1.22 g (75%); purity 82%; $[\alpha]_D^{20}=+145$ (c=0.5, H_2O); IR (KBr) ν=1775 cm^{-1} (β-lactam carbonyl). ^1H NMR (600 MHz, D_2O): δ=1.52 (s, 3H, $H^{2'}$); 1.63 (s, 3H, $H^{2''}$); 1.73–1.92 (m, 4H, $H^{11}+H^{12}$); 2.40 (t, 2H, J=6.4 Hz, H^{10}); 3.73 (t, 1H, J=6.0 Hz, H^{13}); 4.24 (s, 1H, H^3); 5.46 (d, 1H, J=4.0 Hz, H^6); 5.56 (d, 1H, J=4.0 Hz, H^5). NMR chemical shifts are in accordance with those published by Huffman.[6] Chemical shifts, *J*-, and IR-values are in accordance with those published by Vanderhaeghe et al.[2]

Penicillin N sodium salt (**1a**): starting from 0.46 g of **7a**: yield 0.16 g (43%); purity 72%; $[\alpha]_D^{20}=+254$ (c=0.5, H_2O); IR (KBr) ν=1780 cm^{-1} (β-lactam carbonyl). ^1H NMR (600 MHz, D_2O): δ=1.52 (s, 3H, $H^{2'}$); 1.63 (s, 3H, $H^{2''}$); 1.73–1.92 (m, 4H, $H^{11}+H^{12}$); 2.40 (t, 2H, J=6.4 Hz, H^{10}); 3.73 (t, 1H, J=6.0 Hz, H^{13}); 4.24 (s, 1H, H^3); 5.46 (d, 1H, J=4.0 Hz, H^6); 5.56 (d, 1H, J=4.0 Hz, H^5). NMR chemical shifts are in accordance with those reported by Huffman.[6] Chemical shifts, *J*-, and IR-values are in complete agreement with those published by Vanderhaeghe et al.,[2] Baldwin et al.[4] and Lal and Kulkarni.[7]

References

1. Baldwin, J. E.; Schofield, C. *The Chemistry of β-Lactams*; Page, M. I., Ed.; Blackie Academic and Professionals: Glasgow, 1992, pp 1–78.
2. Vanderhaege, H.; Vlietinck, A.; Claesen, M.; Parmentier, G. *J. Antibiot.* **1974**, *27*, 169–177.
3. Usher, J. J.; Loder, B.; Abraham, E. P. *Biochem. J.* **1975**, *151*, 729–739.
4. Baldwin, J. E.; Herchen, S. R.; Singh, P. D. *Biochem. J.* **1980**, *186*, 881–887.
5. Jensen, S. E.; Westlake, D. W. S.; Bowers, R. J.; Wolfe, S. *J. Antibiot.* **1982**, *35*, 1351–1360.
6. Huffman, G. W. *Org. Prep. Proc.* **1988**, *20*, 497–503.
7. Lal, B.; Kulkarni, B. K. Indian . *J. Chem. B* **1991**, *30*, 230–232.
8. Stedman, R. J.; Swered, K.; Hoover, J. R. E. *J. Med. Chem.* **1964**, *7*, 117–119.
9. Kunz, H.; Unverzagt, C. *Angew. Chem., Int. Ed. Engl.* **1984**, *96*, 426–427.
10. Itoh, Z. *Chem. Pharm. Bull. (Japan)* **1969**, *17*, 1679–1684.
11. Manhas, M. S.; Gala, K.; Bari, S. S.; Bose, A. K. *Synthesis* **1983**, 549–552.
12. Helferich, B.; Moog, L.; Jünger, A. *Ber. Dtsch. Chem. Ges.* **1925**, *58*, 872–886.
13. Amiard, G.; Heymes, R. *Bull. Soc. Chim. Fr.* **1956**, 97–101.
14. Barlos, K.; Papaioannou, D.; Patrianakou, S.; Tsegenidis, T. *Liebigs Ann. Chem.* **1986**, 1950–1955.
15. Personal communication of Professor Dr F. Albericio, Barcelona, Spain.
16. Kunz, H.; März, J. *Angew. Chem., Int. Ed. Engl.* **1988**, *100*, 1424–1425.
17. Kimbonguila, M.; Merzouk, A.; Guibé, F.; Loffet, A. In: *Peptides 1994*, Proceedings of the 23rd European Peptide Symposium; Maia, H.L.S., Ed.; Escom: Leiden, 1995, pp 159–160.
18. Clark-Lewis, J. W.; Thompson, M. J. *J. Chem. Soc.* **1959**, 1628–1629.
19. Stevens, C. M.; Watanabe, R. *J. Am. Chem. Soc.* **1950**, *72*, 725–727.
20. von Arx, E.; Brugger, M.; Faupel, M. *J. Chromatogr.* **1976**, *120*, 224–228.