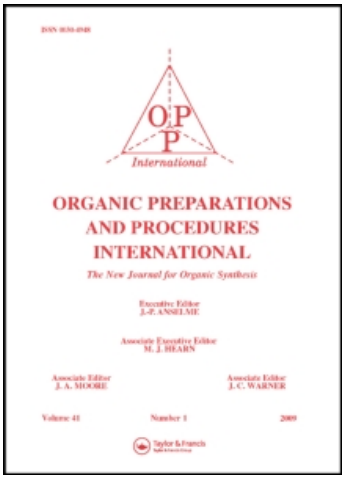

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IMPROVED PREPARATION OF PENICILLIN N AND ISOPENICILLIN N

George W. Huffman[a]
[a] The Lilly Research Laboratories, Eli Lilly and Company, Indianapolis, IN

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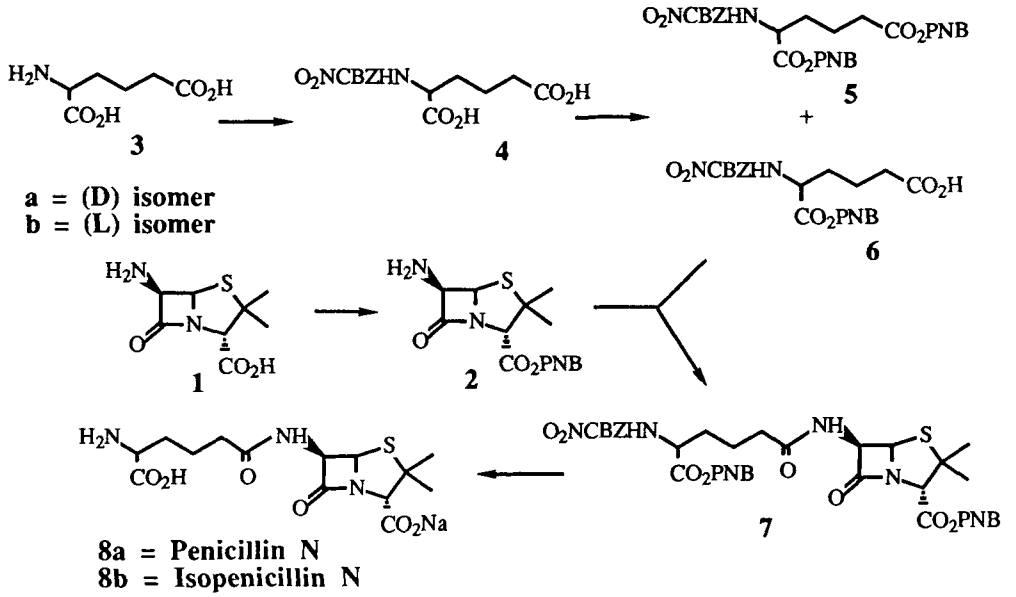
IMPROVED PREPARATION OF PENICILLIN N AND ISOPENICILLIN N

George W. Huffman

The Lilly Research Laboratories
Eli Lilly and Company
Indianapolis, IN 46285

During the study of the biosynthetic path leading to penicillins and cephalosporins, we had need of high purity isopenicillin N and penicillin N. We have found historically that isolation of the biosynthetically prepared penicillins has not proven feasible due to inherent instability of the compounds during the purification procedure. A synthetic approach seemed the most reasonable solution to the problem of obtaining the desired products of the purity required. In our hands previously published synthetic approaches[1] suffered from decomposition of the final products during prolonged hydrogenation of



protecting groups. Analysis of these routes, coupled with knowledge of the inherent

instability of the desired products led us to consider the synthesis of isopenicillin N or penicillin N as a tri-protected derivative in which all blocking groups could be removed quickly and simultaneously under neutral conditions. This tri-protected derivative should also have the characteristics of stability and ease of purification. By purifying the protected derivative at the penultimate step, we hoped to avoid any prolonged final chromatography that had previously proven to be the source of decomposition of our final products. The 4-nitrocarbobenzyloxycarbonyl amine protecting group[2] and 4-nitrobenzyl ester[3] seemed to be likely candidates for the desired protecting groups to satisfy our restrictive needs.

Utilizing either (D) or (L)-α-aminoadipic acid as starting material, depending upon which penicillin was desired, penicillin N (**8a**) or isopenicillin N (**8b**), the two products differing only in the configuration of the aminoadipyl side chain, the following reaction sequence was utilized. Reaction of the chiral α-aminoadipic acid (**3**) with 4-nitrocarbobenzyloxychloride afforded the desired nitro-Cbz protected derivative (**4**) in 81% yield. Esterification of the protected aminoadipic acid with 4-nitrobenzyl bromide resulted in isolation of the desired α-ester (**6**) and the α,δ-diester (**5**). Another compound was observed by TLC but not isolated (presumably the δ-ester). The desired mono-ester was separated from the δ-ester in 23% yield by flash column chromatography[4] utilizing a toluene/ethyl acetate gradient. The aminoadipic acid derivative was subsequently coupled to the penicillin ester (**2**) utilizing EEDQ[5] in a standard peptide coupling reaction. The resulting tri-protected derivative (**7**) was then isolated pure (58%) by flash chromatography. Storage of the purified penicillin as the stable tri-protected derivative thus enabled us to keep on hand a quantity of the desired masked penicillins from which the final products could easily be obtained. Hydrogenation of the tri-protected derivative (**7**) in THF/water with one equivalent of sodium bicarbonate and utilizing 10% Pd/C as catalyst for 15 minutes, followed immediately by lyophilization of the aqueous portion resulted in isolation (95+%) of the desired penicillins (**8a**) or (**8b**) as the mono sodium salts.

EXPERIMENTAL SECTION

Melting points were determined in open glass capillaries on a Thomas Hoover melting point apparatus and are uncorrected. NMR spectra were recorded on a General Electric QE 300

spectrometer. IR spectra were recorded with a Nicolet DX 10 spectrophotometer and optical rotations on a Perkin-Elmer model 241 polarimeter. Mass spectra were taken on VG ZAB-3 or Varian-MAT model 731 mass spectrometers. Elemental analysis were performed by the physical chemistry department of The Lilly Research Laboratories.

4-Nitrobenzyl-6-aminopenicillinate p-Toluenesulfonic Acid Salt (2) (Prepared according to the method of Manhas et al.[6]). 6-Aminopenicillanic acid (**1**; 21.6 g, 0.1 mol), was converted to 46.2 g (88%) of 4-nitrobenzyl-6-aminopenicillanate p-toluenesulfonic acid salt as described in the literature. ^{1}H NMR (300 MHz; $CDCl_3$): δ 1.28 (s, 3H); 1.42 (s, 3H); 2.28 (s, 3H); 4.48 (s, 1H); 5.00 (d, J=2, 1H); 5.21 (d, J=2, 2H); 5.43 (d, J=2, 1H); 7.04-8.20 (AB systems, 8+H).

N-4-Nitrobenzyloxycarbonyl-(D)-α-aminoadipic Acid (4a). (D)-α-aminoadipic acid (**3a**; 10.0 g, 62 mmol) was charged into a 500 ml 3 necked round bottom flask equipped with a magnetic stirring bar and two addition funnels. p-Dioxane (26.6 ml) and 2N NaOH (66.5 ml, 133 mmol) were added and the mixture stirred. Upon solution, 66.5 ml 2N NaOH (66.5 ml, 133 mmol) and 4-nitrobenzylchlorofomate (20.0 g, 92.7 mmol) dissolved in dioxane (102 ml) were added simultaneously from the two separate addition funnels over a 30 minute period maintaining the temperature between 20-25°. The reaction mixture was subsequently stirred for 18 hrs at 22.5°. The mixture was diluted with water (200 ml), filtered if necessary, and the pH adjusted to 3.0 with 1N HCl. The aqueous solution was extracted 4 times with 150 ml portions of ethyl acetate. The combined organic extracts were washed with 200 ml saline, dried over $MgSO_4$, filtered and reduced *in vacuo* to provide a yellow gum. The gum was stirred under hexanes overnight whereupon crystallization occurred. The product was removed by filtration; the filter cake washed with hexanes and ether, and dried *in vacuo* to provide 17.3 g (82%) of the desired product, mp. 137-137.2°.

^{1}H NMR (300 MHz; DMSO-d_6): δ 1.5-1.8 (m, 4H); 2.2-2.3 (m, 2H); 3.9-4.0 (s, 1H); 5.2 (s, 2H); 7.78 (d, J = 3, 1H); 7.6-8.3 (AB system, 4H). MS (FD) 341 (M+H); $[\alpha]_{589}$ +7.51° (c 1, EtOH).

Anal. Calcd for $C_{14}H_{16}N_2O_8$: C, 49.42; H, 4.74; N, 8.23.

Found: C, 49.38; H, 4.74; N, 8.08.

N-4-Nitrobenzyloxycarbonyl-(L)-α-aminoadipic Acid (4b). In a manner similar to that described above, (L)-α-aminoadipic acid was converted to the titled compound in 73% yield, mp. 136.5-137°.

^{1}H NMR (300 MHz; DMSO-d_6): δ 1.5-1.8 (m, 4H); 2.2-2.3 (m, 2H); 3.9-4.0 (s, 1H); 5.2 (s, 2H); 7.78 (d, J = 3, 1H); 7.6-8.3 (AB system, 4H). MS (FD) 341 (M+H).

Anal. Calcd for $C_{14}H_{16}N_2O_8$: C, 49.42; H, 4.74; N, 8.23.

Found: C, 49.30; H, 4.68; N, 8.20.

N-4-Nitrobenzyloxycarbonyl-(D)-α-aminoadipic Acid α-4-Nitrobenzyl Ester (6a). 4-Nitrobenzyloxycarbonyl-(D)-α-aminoadipic acid (**4a,** 17.3 g, 50 mmol) was dissolved in DMF (125 ml) at 75-80°. Upon solution, dicyclohexylamine (10.12 ml, 50.8 mmol) was added and the temperature maintained at 75-80° while 4-nitrobenzyl bromide (10.80 g, 50 mmol) dissolved in DMF (30 ml) was added in one portion. The mixture was stirred for 1 hr and permitted to come to ambient temperature (22.5°). Ethyl acetate (250 ml) was added and the resulting precipitate was removed by filtration and washed with ethyl acetate (100 ml). The filtrate and washings were combined, washed 6 times with 100 ml portions of 1N HCl, 3 times with 100 ml portions of water, and 2 times with saline. The organic layer was dried with $MgSO_4$, filtered, and reduced *in vacuo* to provide about 22.1 g of a yellow gum. The gum was dissolved in the minimal amount of ethyl ether, crystallization induced, and the precipitate removed by filtration to provide 12.3 g of off-white crystals. The crystals were dissolved in 200 ml CH_2Cl_2 and deposited on 36 g silica gel. The solvent was carefully removed by evaporation, and the silica gel deposited carefully on top of a 12 cm OD column containing 360 g hexane packed silica gel. The products were flash chromatographed[4] utilizing a toluene/ethyl acetate (0-100%) gradient. 3.5 L Eluent was collected in 130 20 ml fractions; the different compounds were identified and pooled to provide 2.81 g (9%) diester and 5.43 g (23%) titled product, mp. 110°.

^{1}H NMR (300 MHz; $CDCl_3$): δ 1.6-2.1 (m, 4H); 2.4 (m, 2H); 4.45 (m, 1H); 5.20 (s, 2H); 5.30 (d, 2H); 5.48 (d, 1H); 7.4-8.3 (AB systems, 8H). MS (FD) 476 (M+H). $[\alpha]_{589}$ +4.95° (c 1, EtOH).

Anal. Calcd for $C_{21}H_{21}N_3O_{10}$: C, 53.06; H, 4.45; N, 8.84.

Found: C, 52.90; H, 4.34; N, 8.87.

N-4-Nitrobenzyloxycarbonyl-(L)-α-aminoadipic Acid α-4-Nitrobenzyl Ester (6b). In a manner similar to that described above, 4-nitrobenzyloxycarbonyl-(D)-α-aminoadipic acid (**4b**) was converted to the titled compound in 19% yield, mp. 109-110°. ^{1}H NMR (300 MHz; $CDCl_3$): δ 1.6-2.1 (m, 4H); 2.4 (m, 2H); 4.47 (m, 1H); 5.22 (s, 2H); 5.30 (d, 2H); 5.52 (d, 1H); 7.4-8.3 (AB systems, 8H). MS (FD) 476 (M+H). $[\alpha]_{589}$ -6.34° (c 0.769, EtOH).

Anal. Calcd for $C_{21}H_{21}N_3O_{10}$: C, 53.06; H, 4.45; N, 8.84.

Found: C, 53.01; H, 4.30; N, 8.90.

N-4-Nitrobenzyloxycarbonyl Penicillin N Di-4-nitrobenzyl Ester (7a). 4-Nitrobenzyl-6-aminopenicillanate HOTs salt (**2,** 6.8 g, 13 mmol) was sprung to the free base in CH_2Cl_2 with saturated $NaHCO_3$ solution. After washing with water and drying over $MgSO_4$, a clear gum (3.76 g, 10.7 mmol) was obtained. This was dissolved in CH_2Cl_2 (84 ml) along with 4-nitrobenzyloxycarbonyl-(D)-α-aminoadipic acid α-4-nitrobenzyl ester (**6a,** 4.75 g, 10.0 mmol). To this solution was added EEDQ (2.47 g, 10 mmol) and the resulting solution was stirred 18 hrs at 22.5°. TLC (toluene/ethyl acetate: 7/3, silica gel plates) revealed the appearance of a new spot (Rf = 0.40). The reaction mixture was reduced *in vacuo* to low volume, taken up in ethyl acetate (150 ml), washed with cold 1N HCl, saturated $NaHCO_3$ solution, and saline; dried over $MgSO_4$, filtered and reduced *in vacuo* to provide 8.20 g of a white foam. 7.4 g Of this material was deposited on 21 g silica gel (CH_2Cl_2) and chromatographed (flash column) over 210 g hexane packed silica gel utilizing a toluene/ethyl acetate gradient (0-100%) collecting 3.0 L eluent in 150 20 ml fractions. The major component was identified and pooled, solvent removed *in vacuo* to provide 4.738 g (59%) of a white foam. NMR and MS confirmed the product.

IR (KBr) 3324, 2929, 1746, 1684, 1627 cm^{-1}.

^1H NMR (300 MHz, $CDCl_3$) δ 1.43 (s, 3H); 1.63 (s, 3H); 1.5-2.0 (m, 4H); 2.2-2.4 (m, 2H); 4.42 (m, 1H); 5.20-5.33 (m, 6H); 5.52 (d, J = 1, 1H); 5.58 (d, J-2, 1H) 5.69 (dd, J = 1, 2, 1H); 6.19 (d, J = 3, 1H); 7.18 (d, J = 2, 1H); 7.25 (d, J = ?, 1 H); 7.42-8.30 (AB systems, 12H); MS (FD) 809 (M+H); $[\alpha]_{589}$ +174.6° (c 1, EtOH).

N-4-Nitrobenzyloxycarbonyl Isopenicillin N Di-4-nitrobenzyl Ester (7b). In a manner similar to that described above, 4-nitrobenzyloxycarbonyl-(L)-α-aminoadipic acid α-4-nitrobenzyl ester was utilized to acylate 4-nitrobenzyl-6-aminopenicillanate. The desired product was obtained in 64% yield as a white foam after chromatography.

IR (KBr) 3324, 2929, 1746, 1684, 1677, 1654, 1627, 1523 cm^{-1}.

^1H NMR (300 MHz, $CDCl_3$) δ 1.43 (s, 3H); 1.61 (s, 3H); 1.5-2.1 (m, 4H); 2.2-2.4 (m, 2H); 4.47 (m, 1H); 5.20-5.33 (m, 6H); 5.53 (d, J = 1, 1H); 5.58 (d, J-2, 1H) 5.69 (dd, J = 1, 2, 1H); 6.19 (d, J = 3, 1H); 7.18 (d, J = 2, 1H); 7.25 (d, J =1, 1 H); 7.49-8.27 (AB systems, 12H); MS (FD) 808, 809 (M+H); $[\alpha]_{589}$ + 89.76° (c 1, EtOH).

Penicillin N Mono Sodium Salt (8a). N-4-nitrobenzyloxycarbonyl penicillin N di-4-nitrobenzyl ester (**7a,** 808 mg, 1.0 mmol) was added to a mixture of $NaHCO_3$ (84 mg, 1 mmol) in H_2O (50 ml) and freshly distilled THF (50 ml) containing 1.6 g 10% Pd on carbon in a 500 ml pressure bottle. The resulting mixture was flushed with hydrogen and hydrogenated at 60 PSI for 15 minutes at 22.5°. The mixture was quickly filtered through a layer of decolorizing carbon and a talc pad into a cold receiver (Note: catalyst and pad were not washed!) The filtrate was extracted with cold ethyl acetate and the aqueous layer was shell frozen and lyophilized to provide 419 mg (100% ?) of the mono sodium salt **8a** as a fluffy, light cream colored product.

^1H NMR (300 MHz; D_2O): δ 1.52 (s, 3H); 1.64 (s, 3H); 1.5-1.8 (m, 2H); 1.8-2.0 (m, 2H); 2.40 (t, J = 1 Hz, 2H); 3.68 (t, J = 1, 1H); 4.25 (s, 1H); 5.47 (d, J = 1, 1H); 5.55 (d, J = 1, 1H); MS(FAB) 382 (M+H), 404 (M+Na); $[\alpha]_{589}$ -0.2° (c 1, H_2O).

Isopenicillin N Mono Sodium Salt (8b). N-4-nitrobenzyloxycarbonyl isopenicillin N di-4-nitrobenzyl ester (**7b,** 553 mg, 0.68 mmol) was added to a mixture of $NaHCO_3$ (57

mg, 0.68 mmol) in H_2O (50 ml) and freshly distilled THF (50 ml) containing 1.0 g 10% Pd on carbon in a 500 ml pressure bottle. The resulting mixture was flushed with hydrogen and hydrogenated at 60 PSI for 20 minutes at 22.5°. The mixture was filtered through a layer of decolorizing carbon and a talc pad into a cold receiver (Note: catalyst and pad were not washed!). The filtrate was extracted with cold ethyl acetate, and the aqueous layer was shell frozen and lyophilized to provide 278.2 mg (100% ?) of the mono sodium salt **8b** as an electrostatic yellow product.

^1H NMR (300 MHz; D_2O): δ 1.52 (s, 3H); 1.64 (s, 3H); 1.5-1.8 (m, 2H); 1.8-2.0 (m, 2H); 2.40 (t, J = 1 Hz, 2H); 3.75 (t, J = 1, 1H); 4.25 (s, 1H); 5.47 (d, J = 1, 1H); 5.55 (d, J = 1, 1H); MS(FAB) 382 (M+H), 404 (M+Na); $[\alpha]_{589}$-0.4° (c 1, H_2O).

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