JOURNAL OF LABELLED COMPOUNDS AND RADIOPHARMACEUTICALS
J Label Compd Radiopharm 2003; **46**: 773–779.
Published online in Wiley InterScience (www.interscience.wiley.com). DOI: 10.1002/jlcr.717

Research Article

Synthesis of tritium labelled mecillinam

Søren Melsing Frederiksen and Gunnar Grue-Sørensen*
LEO Pharma, Medicinal Chemistry Research, Industriparken 55, DK-2750 Ballerup, Denmark

Summary

The potent β-lactam antibiotic mecillinam has successfully been labelled with tritium. 2,3,4,7-Tetrahydro-1H-azepin-1-carbaldehyde was formed in a ring-closing metathesis reaction and then condensed with trimethylsilyl protected penicillanic acid to give dehydro-mecillinam. Pd/C catalysed tritiation then gave [$^3H_2$]-mecillinam (6-β (([3,4-$^3H_2$]hexahydro-1H-azepin-1-yl)methyleneamino)penicillanic acid) with a radiochemical purity of 96% and a specific activity of 24 Ci/mmol.

Key Words: mecillinam; tritium; ring-closing metathesis

Introduction

Mecillinam[1,2] (**10a**) is a narrow-spectrum penicillin, selectively active against Gram-negative organisms and currently primarily used for treatment of cystitis. For biological studies we needed labelled mecillinam with a specific activity >10 Ci/mmol. The penicillanic acid moiety of other penicillins has been labelled with tritium via an exchange reaction with tritiated water.[3,4] This method requires a large excess of tritium and the specific activities obtained were ≤ 0.4 Ci/mmol. Pivmecillinam (pivaloyloxymethyl ester of mecillinam) has been labelled with carbon-14 in the amidine group[5] but the maximal specific

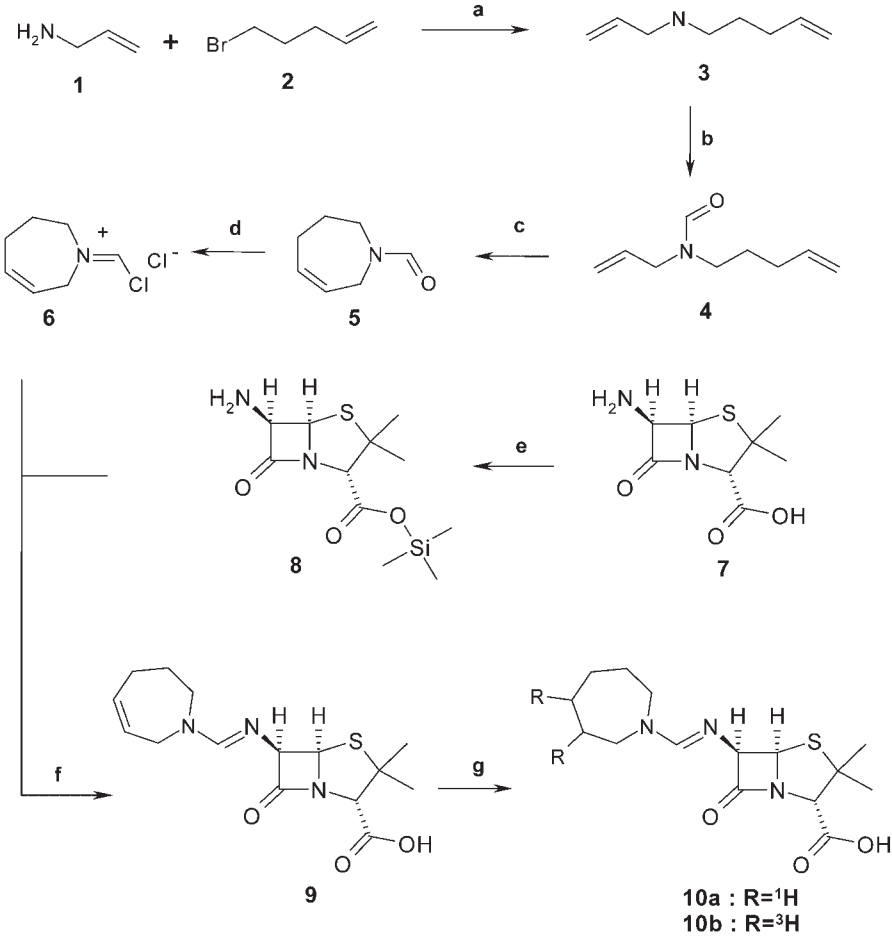
*Correspondence to: Gunnar Grue-Sørensen, LEO Pharma, Medicinal Chemistry Research, 55 Industriparken, DK-2750 Ballerup, Denmark. E-mail: grs@leo-pharma.com

Received 6 March 2003
Accepted 20 March 2003

activity of carbon-14 is 0.06 Ci/mmol. To obtain the desired level of specific activity we decided to introduce tritium via a catalytic tritiation of a double bond in a tetrahydro-1H-azepin precursor to be synthesized via a ring-closing metathesis (RCM) reaction.[6] Here we report our synthesis.

Results and discussion

The secondary amine **3** was prepared in 61% yield by alkylation of allylamine (**1**) with 5-bromopent-1-ene (**2**) using sodium iodide as a catalyst (see Scheme 1). The RCM reaction was unsuccessful when performed on the amine **3**. Formylation of the amine **3** with formyl acetic anhydride gave the amide **4** in 65% yield (*cf*. Jahngen and Rossomando[7]) and this compound smoothly underwent the RCM reaction using a Grubbs' ruthenium-based catalyst in a very dilute dichloromethane solution (*cf*. Visser *et al*.[8]). Similar observations, that increased bulkiness around the nitrogen atom will lead to more favourable transition states in the RCM reaction, have been reported.[6,9] Extensive purification to remove the catalyst finally gave the *N*-formyl tetrahydro-1H-azepin **5** in a yield of 24%. The amide **5** was activated by reaction with oxalyl chloride in diethylether.[2] The Vilsmeier-type activated amide **6** was not isolated, but, after removal of the solvent and dissolution in dichloromethane, was used directly in the following coupling reaction. 6-Aminopenicillanic acid (**7**) was protected using 2 equivalents chlorotrimethylsilane and triethylamine (*cf*. Grossmann and Hardcastle[10]) and immediately coupled with the chloroiminium chloride **6**. The coupling was initiated in dichloromethane at -78°C and the temperature was then slowly raised to ambient level (*cf*. Lund[2]). Compounds containing β-lactam rings are labile when chromatographed on silica gel and dehydro-mecillinam **9** was therefore purified by preparative reversed phase HPLC by using an unbuffered eluent of water and acetonitrile to avoid a salt residue after evaporation of the solvents. The tritiation was performed by treating dehydro-mecillinam **9** and 10% Pd/C in water with tritium-gas at 21°C/1 atm for 2 h. Both the imino-functionality and the β-lactam ring were stable under those conditions.[11] Purification by reversed phase HPLC (*cf*. Strojny and Silva[12]) afforded the desired product **10b** with 96% radiochemical purity and a specific activity of 24 Ci/mmol.



Scheme 1. Synthesis of [^{3}H]-Mecillinam. Reagents and conditions: (a) NaI, 50°C, 90 min; (b) HCOOH, $(CH_3CO)_2O$, 55°C, 1 h; (c) Bis(tricyclohexylphosphine)benzylidene ruthenium(IV) dichloride, CH_2Cl_2, reflux, 4 h; (d) $(COCl)_2$, Et_2O, 0°C, 40 min; (e) TMS-Cl, Et_3N, CH_2Cl_2, room temperature, 75 min; (f) mixing contents of (d) and (e) in CH_2Cl_2, −78°C to room temperature, 3.5 h; (g) [$^3H_2$], 10% Pd/C, H_2O, room temperature, 2 h.

Experimental

General

Preparative HPLC of dehydro-mecillinam was performed using a Gilson apparatus with a Gilson UV/VIS-155 UV-detector (simultaneous detection at 220 and 254 nm). Preparative HPLC of [$^3H_2$]-

mecillinam was performed using a Merck Hitachi apparatus with a L4250 UV-VIS detector (254 nm). Chemical and radiochemical purity was determined by HPLC on a Merck Hitachi apparatus (L-6200 pump) on a Spherisorb ODS1 column (150 mm × 4.6 mm) with 0.01 M phosphate buffer (pH 5)/acetonitrile 9:1 as eluent (1 ml/min) and UV-detection (254 nm) (L-4250 UV-VIS detector) or radioactivity detection (Packard Flow System Analyzer Model D525F1 with Ultima-Flo M (Packard) as scintillation liquid). Concentrations and specific activities were determined by HPLC by comparison of peak areas of radio-inactive reference compounds. A Packard Tri-Carb 2900TR Liquid Scintillation Analyzer was used to determine activity in liquid samples using Pico-Fluor 40 (Packard) as scintillation cocktail. ^{1}H and ^{13}C NMR spectra were obtained on a Bruker ARX300 spectrometer. Chemical shifts are reported in ppm with tetramethylsilane (TMS) in $CDCl_3$ or sodium 3-(trimethylsilyl) propionate-2,2,3,3-d_4 (TMSP-d_4) in D_2O as internal reference. ES Mass spectra were obtained on a VG Quattro II mass spectrometer in positive ion electrospray mode. IR spectrum was obtained on a Bruker EQVINOX 55 FT IR spectrometer.

*Allyl-pent-4-enyl-amine (**3**)*

5-Bromopent-1-ene (**2**) (7.55 g, 50.6 mmol) was slowly added to a vigorously stirred suspension of sodium iodide (534 mg, 3.6 mmol) in allylamine (**1**) (14.45 g, 253.1 mmol). The mixture was stirred at 50°C for 1.5 h. After cooling potassium carbonate (14 g) was added to the mixture. After standing for 48 h at 5°C water (30 ml) was added and the potassium carbonate dissolved. After ensuring a pH-level >10 the solution was extracted with diethyl ether (2 × 15 ml). The combined organic layers were washed with brine, dried over sodium sulphate, filtered and concentrated *in vacuo* (30°C, 800 mbar). The residue was purified by distillation (41–44°C, 13 mbar) which afforded the product (**3**) in 61% yield (3.84 g). ^{1}H NMR ($CDCl_3$) δ 5.98–5.74 (m, 2H), 5.17 (dq, 1H), 5.08 (dq, 1H), 5.02 (dq, 1H), 4.95 (m, 1H), 3.25 (dt, 2H), 2.62 (bt, 2H), 2.10 (m, 2H), 1.60 (m, 2H), 1.07 (bs, NH). ^{13}C NMR ($CDCl_3$) δ 138.5, 137.1, 115.7, 114.6, 52.5, 48.9, 31.6, 29.4. MS: m/z 126.06 [M^+].

*N-Allyl-N-pent-4-enyl-formamide (**4**)*

Acetic anhydride (5 ml, 53 mmol) was slowly added to a stirred mixture of formic acid (20 ml, 530 mmol) and allyl-pent-4-enyl-amine (**3**) (1.0 g,

7.99 mmol) under argon at room temperature. The reaction was stirred at 55°C for 1 hour. After cooling to room temperature the mixture was concentrated *in vacuo*. Water (30 ml) was added to the residue and the pH level was adjusted to 11–12 by addition of 2N sodium hydroxide. The mixture was extracted with ethyl acetate (2 × 30 ml). The combined organic phases were acidified with 2 N hydrochloric acid until pH 5. The solution was then washed with water (2 × 20 ml) and with brine (20 ml). The solution was dried over sodium sulphate, filtered and concentrated *in vacuo* to give the desired product (**4**) in 65% yield (2.39 g). ^{1}H NMR ($CDCl_3$) δ (two conformers, 1:1) 8.09 (s, 1H), 5.88–5.66 (m, 2H), 5.24–5.19 (m, 2H), 5.10–4.94 (m, 2H), 3.95 (m) and 3.83 (m) (2H), 3.31 (m) and 3.23 (bt) (2H), 2.12–2.00 (m, 2H), 1.70–1.56 (m, 2H). ^{13}C NMR ($CDCl_3$) δ (two conformers, 1:1) 162.9, 162.7, 137.6, 137.0, 133.5, 132.5, 118.4, 117.9, 115.8, 115.16, 50.2, 46.3, 44.6, 41.9, 31.0, 30.4, 27.3, 26.3. MS: m/z 154.04 [M^+].

2,3,4,7-Tetrahydro-azepin-1-carbaldehyde (**5**)

Under an argon atmosphere *N*-allyl-*N*-pent-4-enyl-formamide (**4**) (1.0 g, 6.52 mmol) was diluted with dichloromethane (650 ml) and while stirring bis(tricyclohexylphosphine)benzylidene ruthenium(IV) dichloride (107 mg, 0.13 mmol) was added. The solution was refluxed 4 h and then concentrated *in vacuo*. The residue was filtered through silica gel (43–60 μ) to remove the catalyst. Ethyl acetate/petroleum ether (b.p. 35–50°C) 4:1 was used as eluent. This filtration was repeated. Presence of catalyst was visible due to strong colourization of the silica gel. The resulting filtrate was concentrated, redissolved in ethyl acetate (20 ml) and filtered through a layer of activated carbon. After concentration *in vacuo* the residue was purified by flash chromatography using ethyl acetate/petroleum ether (b.p. 35–50°C) 4:1. This gave the desired product (**5**) in 24% yield (199 mg). ^{1}H NMR ($CDCl_3$) δ (two conformers, 1:1) 8.08 (s) and 8.04 (s) (1H), 5.87–5.61 (m, 2H), 4.00 (m) and 3.91 (m) (2H), 3.63 (bt) and 3.52 (bt) (2H), 2.30–2.20 (m, 2H), 1.94–1.82 (m, 2H). ^{13}C NMR ($CDCl_3$) δ (two conformers, 1:1) 162.6, 161.9, 132.7, 132.2, 127.4, 126.8, 49.7, 47.1, 44.0, 41.2, 27.7, 27.2, 26.6, 26.0. MS: m/z 126.03 [M^+].

1-Chloromethylene-2,3,4,7-tetrahydro-1H-azepin chloride (**6**)

2,3,4,7-Tetrahydro-azepin-1-carbaldehyde (**5**) (494 mg, 3.94 mmol) was diluted in diethylether (10 ml) under argon in a dried flask. The solution was cooled to 0°C and oxalyl chloride (341 μl, 3.94 mmol) diluted in

diethylether (2 ml) was slowly added. Precipitation was immediately observed. After 40 min most of the diethylether was removed via a canula. The residue was then dissolved in dichloromethane (6 ml) and used immediately as described below.

6-*Aminopenicillanic acid trimethylsilyl ester (***8***)*

6-Aminopenicillanic acid (**7**) (852 mg, 3.94 mmol) was dissolved in dichloromethane (17 ml) and triethylamine (797 mg, 7.88 mmol) under argon in a dried flask. After 25 min of stirring 6-aminopenicillanic acid had dissolved and trimethylsilyl chloride (856 mg, 7.88 mmol) was added. Some precipitation was observed. The mixture was stirred for 50 min and used immediately as described below.

6-*β((2,3,4,7-Tetrahydro-1H-azepin-1-yl)methyleneamino)penicillanic acid (***9***)*

The 6-aminopenicillanic acid trimethylsilyl ester (**8**) solution (see above) was cooled to −78°C and while vigorously stirred under argon the solution of the chloroiminium chloride **6** (see above) (3.94 mmol in 6 ml dichloromethane) was added via syringe. The reaction slowly became orange and was stirred for 1.5 h at −78°C and then for 2 h at room temperature. The solution was concentrated to dryness *in vacuo*. The residue was purified in portions each containing roughly 50 mg using a Merck Lichrosper RP-18 250-10 column with water/acetonitrile 85:15 as eluent (2 ml/min, UV-detection at 220 and 254 nm). Compound **9** eluted after 17.01 min. Of the total crude product (2.54 g) only a fraction (357 mg) was purified to give 75 mg (0.23 mmol) of the desired product (**9**). ^{1}H NMR (D_2O) δ (two conformers, 1:1) 8.04 (bs) and 7.99 (bs) (1H), 6.04–5.88 (m, 1H), 5.81–5.67 (m, 1H), 5.58 (d) and 5.57 (d) (1H), 5.43 (d) and 5.41 (d) (1H), 4.31 (s, 1H), 4.29 (m, 1H), 4.15 (m, 1H), 3.84 (bt, 1H), 3.71 (m, 1H), 2.44–2.26 (m, 2H), 2.11–1.98 (m, 2H), 1.70 (s, 3H), 1.55 (s, 3H). ^{13}C NMR (D_2O) δ (two conformers, 1:1) 177.1, 175.0, 174.9, 158.7, 157.6, 138.5, 136.1, 126.2, 125.1, 76.4, 69.5, 69.4, 67.8, 67.8, 65.6, 65.2, 59.8, 55.5, 52.3, 48.1, 31.9, 30.0, 29.8, 28.4, 27.5, 25.8. MS: *m/z* 324.09 [M^+]. IR (0.3% in KBr): 1773 cm^{-1} (C=O of β-lactam-ring).

6-*β((3,4-* $^{3}H_2$*]-Hexahydro-1H-azepin-1-yl)methyleneamino)penicillanic acid (***10*b***)*

6-β ((2,3,4,7-Tetrahydro-1H-azepin-1-yl)methyleneamino)penicillanic acid (**9**) (3.6 mg) was dissolved in water (0.5 ml) and 10% Pd/C-catalyst

(4 mg) was added. The solution was stirred under a tritium atmosphere for 2.5 h and the catalyst was removed by filtration. The residue was initially purified by HPLC using a Merck Lichrosper RP-18 250-10 column using water/acetonitrile 75:25 as eluent (Retention time was 9.9 min) (4 ml/min, UV-detection at 220 nm). The solvent was evaporated *in vacuo*. The product obtained was 74% pure and was further purified by HPLC using 0.01 M phosphate buffer (pH 5)/acetonitrile 90:10 as eluent (Retention time was 27.6 min). The solvent was evaporated *in vacuo* and the residue redissolved in water (5 ml) to give the desired product (**10b**) (13.4 mCi) with 96% radiochemical purity and a specific activity of 24 Ci/mmol.

Acknowledgements

We are indebted to Grethe Aagaard (NMR), Malene Mohr (MS), Dr. Karen Margrethe Engell and Peter Anker Jørgensen (HPLC) (Leo Pharma A/S) and to Dr. Ewald Bannwart (catalytic tritiation) (RC TRITEC AG, Teufen, Switzerland) for invaluable assistance.

References

1. Neu HC. *Pharmacotherapy* 1985; **5**: 1–10.
2. Lund FJ., Lovens Kemiske Fabrik Produktionsaktieselskab, Patent appl., US3957764, 1976.
3. Usher JJ, Loder B, Abraham EP. *Biochem J* 1975; **151**: 729–739.
4. von Daehne W, Hansen ET, Rastrup-Andersen N. *Spec Publ R Soc Chem* 1985; **52**: 375–380.
5. Uemura I, Tokiwa T, Kadowaki H; Koike M. *Chemotherapy* 1977; **25**: 115–122
6. Phillips AJ, Abell AD. *Aldrichimica Acta* 1999; **32**: 75–89.
7. Jahngen GE, Rossomando EF. *Synth Commun* 1982; **12**: 601–606.
8. Visser MS, Heron NM, Didiuk MT, Sagal JF, Hoveyda AH. *J Am Chem Soc* 1996; **118**: 4291–4298.
9. Rutjes FPJT, Schoemaker HE. *Tetrahedron Lett* 1997; **38**: 677–680.
10. Grossmann JH, Hardcastle GA. Bristol-Myers Co., Patent appl., DE2611286, 1976.
11. Baltzer B, Lund F, Rastrup-Andersen N. *J Pharm Sci* 1979; **68**: 1207–1215.
12. Strojny N, De Silva JAF. *J Chromatogr* 1980; **181**: 272–281.

