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Synthesis of orthogonally protected (*3R,4S*)- and (*3S,4S*)-4,5-diamino-3-hydroxypentanoic acids

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Summary. The paper describes two methods of the synthesis of ethyl (*3R,4S*)- and (*3S,4S*)-4-[(benzyloxycarbonyl)amino]-5-[(*tert*-butyloxycarbonyl)amino]-3-hydroxypentanoates, useful for the syntheses of edeine analogs. Differently N-protected (*S*)-2,3-diaminopropanoic acid was used as a substrate in both procedures. The absolute configuration of newly generated asymmetric carbon atoms C-3 in β-hydroxy-γ,δ-diamino products was assigned by means of ^{1}H NMR spectroscopy after their transformation into corresponding piperidin-2-ones.

Keywords: Amino acids – Protecting groups – NMR spectroscopy

Introduction

In our previous studies on edeine antibiotics and their analogs it was demonstrated that the presence of the free ionizable carboxyl group in (*2R,6S,7R*)-2,6-diamino-7-hydroxyazelaic acid (A_2ha) moiety is not essential for biological activity of these antibiotics (Mazerski et al., 1981; Gumieniak et al., 1983). The A_2ha residue, rare, non-natural amino acid was detected only in edeine antibiotics (Hettinger et al., 1968). The synthesis of protected (*3R,4S*)-4,8-diamino-3-hydroxyoctanoic acid replacing A_2ha present in native edeine D was reported (Gumieniak et al., 1984). Continuing our research program we have undertaken the synthesis of a differently protected amino acid i.e. (*3R,4S*)-4,5-diamino-3-hydroxypentanoic acid to be introduced into edeine analogs instead of the A_2ha moiety.

In this paper we describe two procedures which allow to synthesize N-protected ethyl (*3R,4S*)- and (*3S,4S*)-4,5-diamino-3-hydroxypentanoates, **4a** and **4b** respectively, suitable for the syntheses of edeine D analogs. In the method A, the reduction of β-carbonyl compound **2**, obtained in the rection of acid **1** derivative with lithium enolate of ethyl acetate, afforded β-hydroxy-γ-amino product **4ab** as the mixture of two diastereoisomers: (*3R,4S*) and (*3S,4S*). In the procedure B, aldehyde **3** was condensed with the same enolate to give **4ab**. The mixtures of diastereoisomers **4ab** generated by both methods were easily separated by column chromatography.

The absolute configuration of new asymmetric carbon atoms C-3 in β-hydroxy-γ-amino products **4a** and **4b** was assigned, after their conversion into corresponding piperidin-2-ones **5a** and **5b**, on the basis of the ^{1}H NMR spectra following the literature procedure (Rodriguez et al., 1996).

Materials and methods

Thin layer chromatography was performed on DC – Alufolien Kieselgel 60 Merck. Column chromatography was carried out on Silica gel 60 (0.063–0.200 mm) Merck. All melting points are uncorrected. The optical rotations were measured on a POLAMAT A Carl Zeiss Jena polarimeter. Microanalyses were performed on a Carlo Erba CHNS-O-EA1108 instrument for C, H, N. ^{1}H NMR spectra were recorded on Gemini Varian 200 MHz or Gemini Varian 500 MHz spectrometers using TMS as an internal standard. Chemical shifts (δ) are given in ppm and coupling constants (J) in Hz. (*S*)-2-[(Benzyloxycarbonyl)amino]-3-[(*tert*-butyloxycarbonyl) amino]propanoic acid **1** was synthesized from (*S*)-asparagine according to the literature procedure (Zhang et al., 1997). (*S*)-2-[(Benzyloxycarbonyl)amino]-3-[(*tert*-butyloxycarbonyl)amino] propanal **3** was synthesized from **1** following the literature procedure (Schirlin and Altenburger, 1995).

Ethyl (*3R,4S*)-4-[(benzyloxycarbonyl)amino]-5-[(*tert*-butyloxycarbonyl)amino]-3-hydroxypentanoate **4a** and ethyl (*3S,4S*)-4-[(benzyloxycarbonyl)amino]-5-[(*tert*-butyloxycarbonyl) amino]-3-hydroxypentanoate **4b** – *Procedure A.*

To a solution of **1** (2.03 g, 6.0 mmol) in dry THF (36 mL) N,N′-carbonyldiimidazole (0.97 g, 6.0 mmol) was added at 0°C and the reaction mixture was stirred for 30 min under argon. Then the solution was cooled to −78°C and lithium enolate of ethyl acetate (prepared from lithium bis(trimethylsilylamide) (27.0 mL of a 1 M solution in THF, 27.0 mmol) and anhydrous ethyl acetate (2.64 mL, 27.0 mmol) at −78°C under argon, 20 min) was syringed in. After 60 min the reaction mixture was allowed to reach 0°C, quenched with 1N HCl (70 mL) and extracted with ethyl acetate. The organic phase was washed with brine and dried over $MgSO_4$. After evaporation of solvent 1.84 g (75%) of **2** was obtained as an oil. The crude oil of **2** (1.84 g, 4.5 mmol) was dissolved in dry THF (35 mL). Then sodium borohydride (0.17 g, 4.5 mmol) was added at 0°C and the mixture was stirred for 2 h. The reaction mixture was concentrated under reduced pressure, acidified with 0.5 M $KHSO_4$ to pH 4 and extracted with ethyl acetate. The combined extracts were washed with brine and dried over $MgSO_4$. Evaporation of solvent afforded the mixture of diastereoisomers **4ab** which was separated by column chromatography on silica gel with benzene : acetone (20 : 1) solvent system. In this manner 0.544 g (29%) of **4a** and 0.274 g (15%) of **4b** were obtained. **4a**: mp 101–104°C; TLC: R_f = 0.33 benzene : acetone (5 : 1); $[\alpha]_D^{20}$ = 0.0° (c = 1, MeOH).

^{1}H NMR (500 MHz, C_6D_6): δ = 0.88 (t, 3H, $OCH_2C\underline{H}_3$), 1.34 (s, 9H, $C(CH_3)_3$), 2.35 (dd, 1H, $J_{2a\text{-}2b}$ = 15.7, $J_{2a\text{-}3}$ = 4.4, H-2a), 2.45 (dd, 1H, $J_{2b\text{-}2a}$ = 15.7, $J_{2b\text{-}3}$ = 8.7, H-2b), 2.94 (m, 1H, H-5), 3.24 (m, 1H, H-5), 3.66 (m, 1H, H-4), 3.79 (m, 1H, H-3), 3.85 (q, 2H, $OC\underline{H}_2CH_3$), 4.07 (d, 1H, $J_{OH\text{-}3}$ = 5.2, OH), 4.22 (brs, 1H, N$\underline{H}$Boc), 4.99–5.07 (m, 2H, $C\underline{H}_2C_6H_5$), 5.21 (d, 1H, $J_{NH\text{-}4}$ = 9.6, N$\underline{H}$Z), 7.00–7.12 (m, 5H, C_6H_5).

Anal. Calc. for $C_{20}H_{30}O_7N_2$: C 58.52; H 7.37; N 6.82. Found: C 58.56; H 7.32; N 6.91.

4b: mp 91–93°C; TLC: R_f = 0.37 benzene : acetone (5 : 1); $[\alpha]_D^{20}$ = −21.7° (c = 1, MeOH).

^{1}H NMR (500 MHz, C_6D_6): δ = 0.89 (t, 3H, $OCH_2C\underline{H}_3$), 1.36 (s, 9H, $C(CH_3)_3$), 2.25 (dd, 1H, $J_{2a\text{-}2b}$ = 16.9, $J_{2a\text{-}3}$ = 5.3, H-2a), 2.52 (dd, 1H, $J_{2b\text{-}2a}$ = 16.9, $J_{2b\text{-}3}$ = 9.6, H-2b), 2.76 (m, 1H, H-5), 3.11 (m, 1H, H-5), 3.59 (m, 1H, H-4), 3.87 (q, 2H, $OC\underline{H}_2CH_3$), 4.04 (brs, 1H, OH), 4.07 (m, 1H, H-3), 4.16 (brt, 1H, N$\underline{H}$Boc), 4.98 (d, 1H, $J_{NH\text{-}4}$ = 9.6, N$\underline{H}$Z), 4.98–5.07 (m, 2H, $C\underline{H}_2C_6H_5$), 7.02–7.22 (m, 5H, C_6H_5).

Anal. Calc. for $C_{20}H_{30}O_7N_2$: C 58.52; H 7.37; N 6.82. Found: C 58.08; H 7.59; N 6.42.

Ethyl (*3R*,*4S*)-4-[(benzyloxycarbonyl)amino]-5-[(*tert*-butyloxycarbonyl)amino]-3-hydroxypentanoate **4a** and ethyl (*3S*,*4S*)-4-[(benzyloxycarbonyl)amino]-5-[(*tert*-butyloxycarbonyl)amino]-3-hydroxypentanoate **4b** – *Procedure B.*

Lithium bis(trimethylsilylamide) (16.52 mL of a 1 M solution in THF, 16.52 mmol) was placed in a dried, argon filled flask equipped with magnetic stirrer and cooled to −78°C, then anhydrous ethyl acetate (1.62 mL, 16.52 mmol) was syringed in and the mixture was stirred for 20 min. Next a solution of **3** (1.52 g, 4.72 mmol) in dry THF (8 mL) was syringed in. After 60 min the reaction mixture was allowed to reach 0°C, quenched with 1N HCl (35 mL) and extracted with ethyl acetate. The organic phase was washed with brine and dried over $MgSO_4$. Evaporation of solvent afforded the mixture of diastereoisomers **4ab** which was separated by column chromatography on silica gel with benzene : acetone (20 : 1) solvent system. In this manner 0.295 g (15%) of **4a** and 0.631 g (33%) of **4b** were obtained. **4a**: mp 99–101°C; TLC: R_f = 0.33 benzene : acetone (5 : 1); $[\alpha]_D^{20}$ = −0.5° (c = 1, MeOH).

^{1}H NMR (200 MHz, $CDCl_3$): δ = 1.27 (t, 3H, $OCH_2C\underline{H}_3$), 1.44 (s, 9H, $C(CH_3)_3$), 2.53–2.63 (m, 2H, H-2), 3.35 (m, 1H, H-5), 3.49 (m, 1H, H-5), 3.67 (m, 1H, H-4), 3.95 (m, 1H, H-3), 4.17 (q, 2H, $OC\underline{H}_2CH_3$), 4.92 (brt, 1H, N$\underline{H}$Boc), 5.10 (s, 2H, $C\underline{H}_2C_6H_5$), 5.39 (d, 1H, $J_{NH\text{-}4}$ = 9.6, N$\underline{H}$Z), 7.33–7.39 (m, 5H, C_6H_5).

4b: mp 88–91°C; TLC: R_f = 0.37 benzene : acetone (5 : 1); $[\alpha]_D^{20}$ = −21.7° (c = 1, MeOH).

^{1}H NMR (200 MHz, $CDCl_3$): δ = 1.26 (t, 3H, $OCH_2C\underline{H}_3$), 1.45 (s, 9H, $C(CH_3)_3$), 2.44 (dd, 1H, $J_{2a\text{-}2b}$ = 16.4, $J_{2a\text{-}3}$ = 4.8, H-2a), 2.60 (dd, 1H, $J_{2b\text{-}2a}$ = 16.4, $J_{2b\text{-}3}$ = 9.0, H-2b), 3.20 (m, 1H, H-5), 3.39 (m, 1H, H-5), 3.66 (m, 1H, H-4), 4.16 (q, 2H, $OC\underline{H}_2CH_3$), 4.21 (m, 1H, H-3), 4.92 (brt, 1H, N$\underline{H}$Boc), 5.10 (s, 2H, $C\underline{H}_2C_6H_5$), 5.23 (d, 1H, $J_{NH\text{-}4}$ = 9.7, N$\underline{H}$Z), 7.33–7.39 (m, 5H, C_6H_5).

(*4R*,*5S*)-5-[(Benzyloxycarbonyl)amino]-4-hydroxypiperidin-2-one **5a** and (*4S*,*5S*)-5-[(benzyloxycarbonyl)amino]-4-hydroxypiperidin-2-one **5b**.

A sample of **4a** or **4b** (25 mg, 0.061 mmol) was dissolved in TFA (0.5 mL). After 1 h TFA was evaporated to dryness under reduced pressure. The residue was washed with diethyl ether, dissolved in methanol (1 mL) followed by the addition of triethylamine (8.5 μL, 0.061 mmol) and allowed to react for 24 h at rt. Then methanol was evaporated and the corresponding product **5a** or **5b** was purified by column chromatography on silica gel with ethyl acetate : methanol (15 : 1) solvent system. **5a** – TLC: R_f = 0.21 acetone : benzene (10 : 1).

^{1}H NMR (500 MHz, DMSO): δ = 2.17 (dd, 1H, $J_{3e\text{-}3a}$ = 17.5, $J_{3e\text{-}4}$ = 4.0, H_e-3), 2.45 (dd, 1H, $J_{3a\text{-}3e}$ = 17.5, $J_{3a\text{-}4}$ = 4.2, H_a-3), 3.05 (m, 1H, H_e-6), 3.17 (m, 1H, H_a-6), 3.76 (m, 1H, H-5), 3.94 (m, 1H, H-4), 5.03 (s, 2H, $C\underline{H}_2C_6H_5$), 7.20 (d, 1H, $J_{NH\text{-}5}$ = 7.8, N$\underline{H}$Z), 7.32 (m, 1H, lactam NH), 7.35–7.39 (m, 5H, C_6H_5); modifications of some signals after simultaneous decoupling of lactam NH and N$\underline{H}$Z: δ = 3.05 (dd, 1H, $J_{6e\text{-}6a}$ = 11.5, $J_{6e\text{-}5}$ = 5.5, H_e-6), 3.16 (dd, 1H, $J_{6a\text{-}6e}$ = 11.5, $J_{6a\text{-}5}$ = 9.7, H_a-6), 3.75 (ddd, 1H, $J_{5\text{-}6a}$ = 9.7, $J_{5\text{-}6e}$ = 5.5, $J_{5\text{-}4}$ = 1.5, H-5), 3.93 (ddd, 1H, $J_{4\text{-}3a}$ = 4.2, $J_{4\text{-}3e}$ = 4.0, $J_{4\text{-}5}$ = 1.5, H-4).

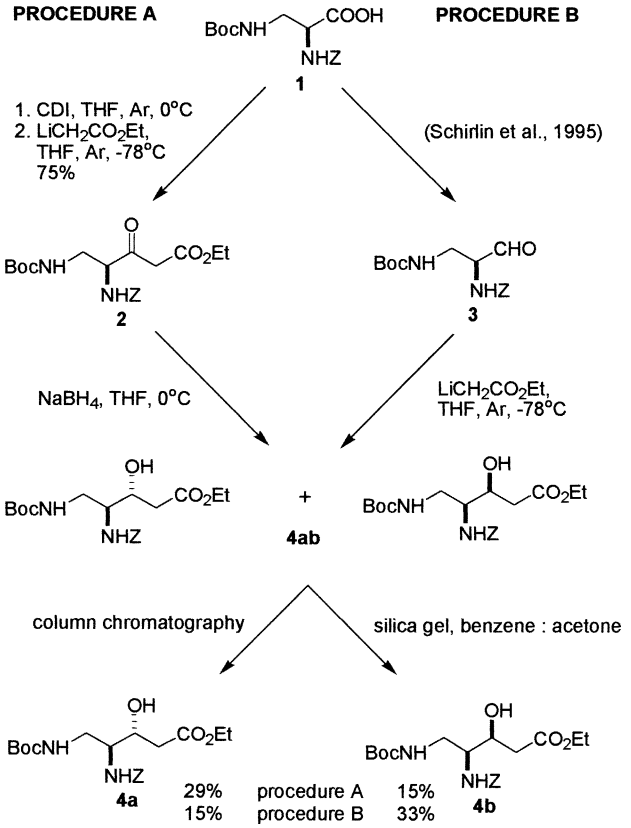
5b – TLC: R_f = 0.23 acetone : benzene (10 : 1).

^{1}H NMR (500 MHz, DMSO): δ = 2.07 (dd, 1H, $J_{3e\text{-}3a}$ = 17.5, $J_{3e\text{-}4}$ = 5.7, H_e-3), 2.51 (dd, 1H, $J_{3a\text{-}3e}$ = 17.5, $J_{3a\text{-}4}$ = 5.2, H_a-3), 2.91 (m, 1H, H_e-6), 3.34 (m, 1H, H_a-6), 3.57 (m, 1H, H-5), 3.78 (m, 1H, H-4), 5.02 (s, 2H, $C\underline{H}_2C_6H_5$), 5.18 (d, 1H, $J_{OH\text{-}4}$ = 4.4, OH), 7.32 (m, 1H, lactam NH), 7.35–7.39 (m, 5H, C_6H_5), 7.44 (d, 1H, $J_{NH\text{-}5}$ = 7.3, N$\underline{H}$Z); modifications of some signals after simultaneous decoupling of lactam NH, N$\underline{H}$Z and OH, t = 40°C: δ = 2.92 (dd, 1H, $J_{6e\text{-}6a}$ = 12.2, $J_{6e\text{-}5}$ = 6.1, H_e-6), 3.35 (dd, 1H, $J_{6a\text{-}6e}$ = 12.2, $J_{6a\text{-}5}$ = 4.8, H_a-6), 3.58 (ddd, 1H, $J_{5\text{-}4}$ = 6.7, $J_{5\text{-}6e}$ = 6.1, $J_{5\text{-}6a}$ = 4.8, H-5), 3.79 (ddd, 1H, $J_{4\text{-}5}$ = 6.7, $J_{4\text{-}3e}$ = 5.7, $J_{4\text{-}3a}$ = 5.2, H-4).

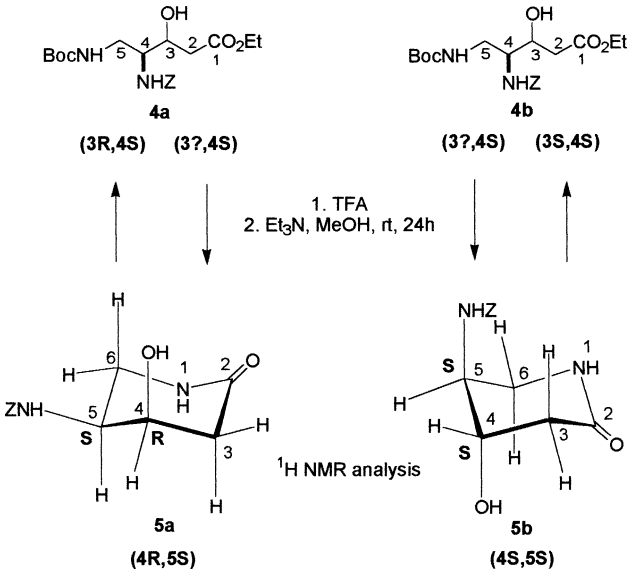
Results and discussion

The product of the reaction between protected acid **1** and N,N′-carbonyldiimidazole was not isolated but was reacted with the enol form of ethyl acetate to give compound **2**. Treatment of imidazolide with 50% excess of enolate did not afford **2** in good yield. Probably it was caused by the reaction of two amide protons and imidazole, as a byproduct of carboxyl group activation step, with enolate. The ratio of enolate to imidazolide 4.5 : 1 allowed to obtain **2** with 75% yield. In case of the procedure B, good results were observed when the ratio of enolate to aldehyde **3** was 3.5 : 1.

The analysis of ^{1}H NMR and ROESY spectra of piperidin-2-ones **5a** and **5b** allowed to assign the absolute configuration of C-3 in **4a** and **4b**. In case of **5a**, the diagnostic NOE correlation was observed between H_a-3 and H-5. It enabled us to deduce the chair conformation as shown on scheme 2. The large value of $J_{5\text{-}6a}$ =



Scheme 1. Synthesis of ethyl (*3R,4S*)- **4a** and (*3S,4S*)-4-[(benzyloxycarbonyl)amino]-5-[(*tert*-butyloxycarbonyl)amino]-3-hydroxypentanoates **4b** by two procedures



Scheme 2. Determination of the absolute configuration of *β*-hydroxy-*γ*-amino compounds **4a** and **4b**

9.7 Hz indicated the axial position of H-5. From the small coupling constant values $J_{4\text{-}5a}$ = 1.5 Hz and $J_{4\text{-}3a}$ = 4.2 Hz the equatorial position of H-4 was deduced and (*4R,5S*) configuration was assigned for **5a**. Thus, the corresponding compound **4a** was (*3R,4S*) and the second diastereoisomer **4b** was (*3S,4S*). It was confirmed by the ^{1}H NMR data of compound **5b**. The NOE correlation between NHZ and H_a-3 indicated its chair conformation and lack of NOE's between protons on C-3 and C-6 excepted the boat form of **5b**. From the small value of $J_{5\text{-}6a}$ = 4.8 Hz the equatorial position of H-5 was established. On the basis of the small value of $J_{4\text{-}3a}$ = 5.2 Hz and the NOE correlation between NHZ and H-4 the equatorial position of H-4 was determined. These data indicated (*4S,5S*) configuration of **5b** and (*3S,4S*) of corresponding **4b**.

In conclusion, the determination of the absolute configuration of substituted piperidin-2-ones making the analysis of coupling constants and NOE correlations in ^{1}H NMR spectra is a convenient method.

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References

Gumieniak J, Andruszkiewicz R, Czerwiński A, Grzybowska J, Borowski E (1983) Synthesis of the decarboxy analog of edeine D. J Antibiot 36: 1239–1241

Gumieniak J, Andruszkiewicz R, Borowski E (1984) Syntheses of edeine antibiotics and their analogs. Part VI. Synthesis of protected (*3R,4S*) and (*3S,4S*)-4,8-diamino-3-hydroxyoctanoic acid. Polish J Chem 58: 881–885

Hettinger TP, Kuryło-Borowska Z, Craig LC (1968) Edeine. III. The composition of the antibiotic peptide edeine B. Biochemistry 7: 4153–4159

Mazerski J, Wojciechowska H, Zgoda W, Woynarowska B, Borowski E (1981) Esters and amides of edeine A. J Antibiot 34: 28–33

Rodriguez R, Estiarte MA, Diez A, Rubiralta M, Colell A, Garcia-Ruiz C, Fernandez-Checa J (1996) Conformationally restricted analogues of methionine: synthesis of chiral 3-amino-5-methylthio-2-piperidones. Tetrahedron 52: 7727–7736

Schirlin D, Altenburger JM (1995) Synthesis of crystalline orthogonally N-protected (*S*)- or (*R*)-2,3-diaminopropionaldehyde from L- or D-aspartic acid. Synthesis: 1351–1352

Zhang L, Kauffman GS, Pesti JA, Yin J (1997) Rearrangement of Nα-protected L-asparagines with iodosobenzene diacetate. A practical route to *β*-amino-L-alanine derivatives. J Org Chem 62: 6918–6920

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