

Synthesis of 3-(4-Amino-7H-pyrrolo[2,3-d]pyrimidin-7-yl)propanoic acid – A Functionalized Base Derivative of the Nucleoside Antibiotic Tubercidin

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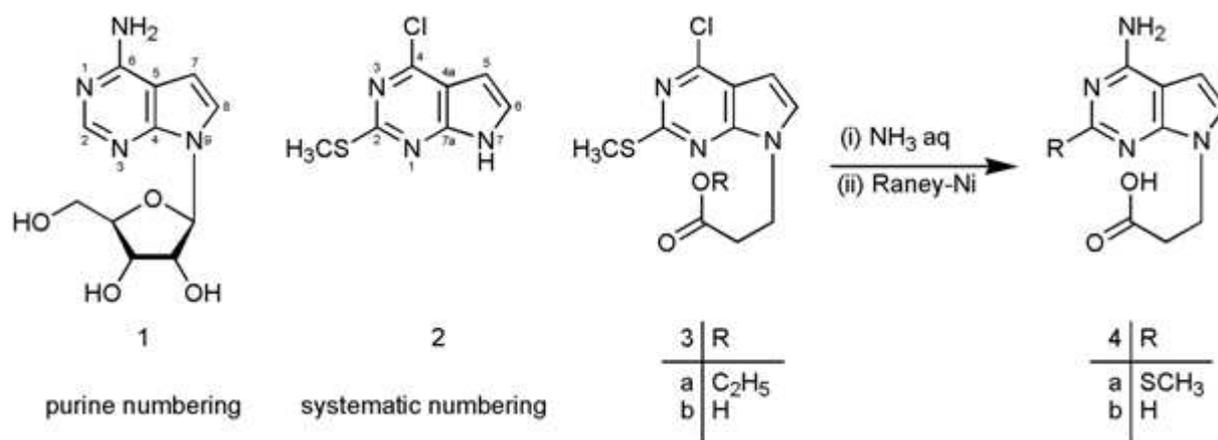
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Abstract: Regioselective N-alkylation of 4-chloro-2-(methylthio)-7H-pyrrolo[2,3-d]pyrimidine (**1**) with ethyl 3-bromopropionate under liquid-liquid phase-transfer reaction conditions and further saponification of the ester function under formation of compound **3b** is described. Subsequent S_NAr substitution of the 4-chloro substituent by an amino function and reductive removal of the 2-methylthio group of **3b** gives, via **4a**, the title compound **4b**.

Keywords: 7-Deazaadenine, Tubercidin, Functionalization

The adenine-isosteric 7-deazaadenine represents the heterocyclic nucleobase of the nucleoside antibiotic tubercidin (**1**) [1]. This base is able to form regular *Watson-Crick* but not *Hoogsteen* base pairs [2].



Functionalized derivatives of 7-deazaadenine such as compound **4b** are of considerable interest because they can be easily coupled to polymers or surfaces carrying amino functions lending them the functionality of a particular modified nucleobase [3]. Coupling of compounds **3b** or **4a,b** to amino-functionalized lipids [4] or phospholipids such as kephalines may lead to potential organo- or hydrogelators. Moreover, compound **4b** represents the 7-deaza analogue of 3-(adenine-9-yl)propanoic acid – a by-product of the antihypocholesteremic eritadenine isolated from the *Shiitake* mushroom *Lentinus edodes* Sing [5]. Furthermore, compounds **3b** and **4a,b** can be used for the synthesis of base-modified peptidyl nucleic acids (PNA) [6].



The preparation of **4b** started from 4-chloro-2-(methylthio)-7H-pyrrolo[2,3-d]pyrimidine (**2**) – synthesized in 5 steps according to the literature [7-10] - which was alkylated with ethyl 3-bromopropionate under liquid-liquid phase transfer catalysis conditions with tetrabutylammonium hydrogensulfate as catalyst to

yield compound **3a** [3]. Subsequent saponification of the ester group gave the acid **3b**. Nucleophilic displacement of the 4-chloro substituent by an amino group using aqueous ammonia (\rightarrow **4a**) followed by reductive removal of the 2-SCH₃ group with *Raney Nickel* gave the title compound **4b**; its 3D-optimized structure is shown in the figure. Studies on the coupling of **4b** to various carrier molecules is underway.

Experimental Procedures

General

Thin-layer chromatography (TLC): Silica Gel 60 F254 plates (*VWR*, Darmstadt, Germany). UV Spectroscopy: U-3200 spectrophotometer (*Hitachi*, Japan) λ_{\max} in nm, ϵ in dm³/mol. NMR Spectra were measured on AC-250 and AMX-500 spectrometers (*Bruker*, Rheinstetten, Germany). Operational frequencies: ¹H: 250.13, 500.14 MHz; ¹³C: 62.896, 125.700 MHz. Chemical shifts (δ values) are in parts per million relative to tetramethylsilane as internal standard. Microanalyses were performed by *Mikroanalytisches Labor Beller* (Göttingen, Germany). The 3D-optimized structure of compound **4b** shown in the figure was obtained using the program *ChemSketch/3D Viewer*, version 10.0, from *Advanced Chemistry Developments*, Toronto, Canada; <http://www.acdlabs.com>.

3-[4-Chloro-2-(methylthio)-7H-pyrrolo[2,3-d]pyrimidin-7-yl]propanoic acid (**3b**)

4-Chloro-2-(methylthio)-7H-pyrrolo[2,3-d]pyrimidine (**2**, 0.5 g, 2.5 mmol), suspended in a mixture of 20 ml of benzene and 20 ml of 50% aq sodium hydroxide, and tetrabutylammonium hydrogensulfate (85 mg, 0.25 mmol) were agitated for 5 min with a vibromixer. Thereupon, ethyl 3-bromopropionate (3.11 ml, 25 mmol) was added and mixing was continued for 60 min. Then, another portion of ethyl 3-bromopropionate (3.11 ml, 25 mmol) was added. After further mixing for 30 min the layers were separated and the aqueous phase extracted twice with benzene. The combined organic layers were washed with water, filtered and evaporated to dryness. The residue was dissolved in a small amount of MeOH, and ethyl 3-[4-chloro-2-(methylthio)-7H-pyrrolo[2,3-d]pyrimidin-7-yl]propanoate (**3a**) was crystallized by addition of water. Colorless crystals (523 mg, 69%); m.p. 62-63°C. TLC (silica gel, CHCl₃-MeOH, 9:1, v/v): R_f 0.9. UV (MeOH): λ_{\max} 252, 279, 309 nm (ϵ , 26.750, 5.600, 6.500). Anal. calcd. for C₁₂H₁₄N₃O₂SCl (299.785): C, 48.08; H, 4.71; N, 14.02. Found: C, 48.24, H, 4.81; N, 13.97. ¹H-NMR (d₆-DMSO): δ , 1.09 (3H, t, CH₃-ester, J = 7.0 Hz); 2.59 (3H, s, SCH₃); 2.93 (2H, t, CH₂N, J = 7.0 Hz); 4.01 (2H, q, CH₂-ester, J = 7 Hz); 4.47 (2H, t, CH₂C=O, J = 4.0 Hz); 6.53 (1H, d, H-C(5), J = 4.0 Hz); 7.59 (1H, d, H-C(6), J = 4.0 Hz). ¹³C-NMR (d₆-DMSO): δ , 13.8 (CH₃-ester); 13.9 (SCH₃); 34.0 (CH₂C=O); 39.1 (CH₂-ester); 60.2 (CH₂N); 98.7 (C-5); 113.5 (C-4a); 129.8 (C-6); 150.6 (C-7a); 151.4 (C-2); 163.0 (C-4); 170.5 (C=O). The ester **3a** (200 mg, 0.67 mmol) was dissolved in a mixture of 10 ml of EtOH and 10 ml of 1N NaOH and stirred for 30 min at room temperature. After dilution with 50 ml of water the reaction mixture was neutralized by addition of Amberlite IR-120 (H⁺-form, glass electrode). After filtration the ion exchange resin was thoroughly washed with EtOH/H₂O (1:1, v/v). The filtrate was evaporated to dryness, and the residue was taken up in a small amount of water. The title compound (**3b**) was crystallized by adding a few drops of glacial acetic acid. Colorless crystals (175 mg, 96%); m.p. 147-148°C. TLC (silica gel, CHCl₃-MeOH, 9:1, v/v): R_f 0.3. UV (MeOH): λ_{\max} 252, 279, 309 nm (ϵ , 26.300, 5.500, 6.000). Anal. calcd. for C₁₀H₁₀N₃O₂SCl (271.731): C, 44.20; H, 3.71; N, 15.47. Found: C, 44.36; H, 3.85; N, 15.29. ¹H-NMR (d₆-DMSO): δ , 2.59 (3H, s, SCH₃); 2.95 (2H, t, CH₂N, J = 7.0 Hz); 4.47 (2H, t, CH₂C=O, J = 7.0 Hz); 6.53 (1H, d, H-C(5), J = 4.0 Hz); 7.58 (1H, d, H-C(6), J = 3.8 Hz). ¹³C-NMR (d₆-DMDO): δ , 13.7 (SCH₃); 33.7 (CH₂C=O); 51.4 (CH₂N); 98.7 (C-5); 113.5 (C-4a); 129.8 (C-6); 150.6 (C-7a); 151.6 (C-2); 163.0 (C-4); 170.9 (C=O).

3-(4-Amino-7H-pyrrolo[2,3-d]pyrimidin-7-yl)propanoic acid (**4b**)

The acid **3b** (300 mg, 1.1 mmol) was dissolved in conc. aq. ammonia (25%) and heated to 120°C in an

autoclave for 12 h. After evaporation to dryness the residue was dissolved in dilute aq. ammonia, and the solution was acidified by addition of glacial acetic acid. Colorless needles of compound **4a** (183 mg, 66%); m.p. 267-270°C. TLC (silica gel, 0.25 M LiCl): R_f 0.5. UV (MeOH): λ_{max} 239, 284 nm (ϵ , 17.500, 12.200). Anal. calcd. for $C_{10}H_{12}N_4O_2S$ (252.30): C, 47.61; H, 4.79; N, 22.21. Found: C, 47.60; H, 4.95; N, 22.34. 1H -NMR (d_6 -DMSO): δ , 2.45 (3H, t, SCH₃); 2.74 (2H, t, CH₂N, J = 7.0 Hz); 4.26 (2H, t, CH₂C=O, J = 6.9 Hz); 6.43 (1H, d, H-C(5), J = 3.4 Hz); 6.99 (1H, d, H-C(6), J = 3.5 Hz); 7.01 (2H, s, NH₂). ^{13}C -NMR (d_6 -DMSO): δ , 13.0 (SCH₃); 34.4, 40.0 (2 x CH₂); 98.4 (C-5); 99.6 (C-4a); 122.7 (C-6); 150.1 (C-7a); 156.8 (C-4); 162.4 (C-2); 172.0 (C=O). The acid **4a** (300 mg, 1.19 mmol) was dissolved in 25% aq. ammonia (50 ml), and *Raney Nickel* suspension (2 ml) was added. After refluxing for 2 h, the catalyst was filtered off, thoroughly washed with hot aq. ammonia, and the filtrate was evaporated to dryness. The residue was dissolved in dilute aq. ammonia and chromatographed on Dowex 1x2 ion exchange resin (OAc⁻ form; column: 2.5 x 40 cm). By-products were eluted first by water; the title compound was eluted by dilute acetic acid (10 vol-%). Colorless crystals of compound **4b** (182 mg, 74%); m.p. 282-285°C. TLC (silica gel, 0.25 M LiCl): R_f 0.55. UV (MeOH, MeOH-H₂O, 1:1 v/v): λ_{max} 272 nm (ϵ , 7.300). Anal. calcd. for $C_9H_{10}N_4O_2$ (206.20): C, 52.42; H, 4.89; N, 27.17. Found: C, 52.47; H, 4.99; N, 27.12. 1H -NMR (d_6 -DMSO): δ , 8.05 (1H, s, H-C(2)); 7.12 (1H, d, H-C(6), J = 3.4 Hz); 6.95 (2H, s, NH₂); 6.49 (1H, d, H-C(5), J = 3.4 Hz); 4.31 and 2.75 (2 x 2H, 2 t, 2 x CH₂, J = 7.0 Hz, both). ^{13}C -NMR (d_6 -DMSO): δ , 171.9 (C=O); 157.2 (C-4); 151.2 (C-2); 149.3 (C-7a); 123.9 (C-6); 102.3 (C-4a); 98.1 (C-5); 40.0 and 34.3 (2 x CH₂).

References and Notes

1. Suhadolnik, R. J., *Nucleosides As Biological Probes*. John Wiley & Sons, New York **1979**, pp. 158-166.
2. Saenger, W. 'Principles of Nucleic Acids Structure', in 'Springer Advanced Texts in Chemistry', Ed. C. R. Cantor, Springer Verlag, New York – Berlin – Heidelberg – Tokyo, **1984**, p. 122-126, p. 243-248; and literature cited therein.
3. Rosemeyer, H.; Kaiser, K.; Seela, F. *Int. J. Biol. Macromol.* **1987**, *9*, 205-210.
4. Ahlers, M.; Ringsdorf, H.; Rosemeyer, H.; Seela, F. *Colloid Polym. Sci.* **1990**, *268*, 132-142.
5. Suhadolnik, R. J., *Nucleosides As Biological Probes*. John Wiley & Sons, New York **1979**, pp. 298-310.
6. Uhlmann, E.; Peymann, A.; Breipohl, G.; Will, D. W. *Angew. Chem.* **1998**, *110*, 2954-2983.
7. Lüpke, U., Thesis, Universität-GH Paderborn, Germany, **1979**, p. 36.
8. Noell, C. W.; Robins, R. K. *J. Heterocyclic Chem.* **1964**, *1*, 34-41.
9. Lüpke, U.; Seela, F. *Chem. Ber.* **1979**, *112*, 3432-3440.
10. Compound **2** is commercially available from: Activate Scientific GmbH, Regensburg, Germany, sales@activate-scientific.com