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Synthesis of 4-Aminosalicylglycine

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Abstract: To search for a better prodrug of 4-aminosalicylic acid that is expected to deliver stably parent drug to colon against the inflammatory bowel disease, a novel 4-aminosalicylic acid derivative was designed and synthesized from 4-aminosalicylic acid. 4-Aminosalicylglycine was prepared from 4-aminosalicylic acid by protecting amino and hydroxyl groups with benzyloxycarbonyl and acetyl, respectively, then the carboxylic acid was converted to acyl chloride which was treated with glycine. After removing the protection groups, 4-aminosalicylglycine was obtained. All the compounds were characterized by FT-IR, ^{1}H-NMR, ^{13}C-NMR spectra. *In vivo* experiment on rats suggested that the curative effect of 4-aminosalicylglycine was more effective than that of 4-aminosalicylic acid.

Keywords: 4-Aminosalicylic acid, 4-aminosalicylglycine, inflammatory bowel disease.

The cases of inflammatory bowel disease (IBD) are chronic bowel diseases. The exact etiology of the IBD is not yet clearly understood. Salicylates, corticosteroids, antibiotics and immunosuppressants are most common therapeutic agents to relieve the symptoms of such ailment[1]. A number of clinical studies have shown that 4-aminosalicylic acid (4-ASA) is highly effective and safe in topical treatment of active ulcerative proctitis or left sided ulcerative colitis, yet it is not adequate to be used in the treatment of IBD because it is absorbed rapidly and extensively through the upper intestine and excreted in the urine before it reaches the colonic site[2-4]. Therefore, 4-ASA was modified and 4-aminosalicylglycine (4-ASA-Gly) was synthesized as a prodrug of 4-ASA. 4-ASA-Gly was expected to be chemically and biochmically stable in the environment of upper intestine. It was reported that N-acyl amide bond derived from an aromatic carboxylic acid and an amino acid was chemically and biochemically stable in the upper intestine and dissociated in the large intestine by the microbial action[5]. Thus orally administered 4-ASA-Gly might be safely delivered to the colon, and act as prodrug of 4-ASA. For this purpose, 4-ASA-Gly was designed and synthesized.

One route for preparing 4-ASA-Gly was started from 4-ASA, protecting amino and hydroxyl groups with benzyloxycarbonyl and acetyl respectively, then the carboxylic acid was converted to acyl chloride, which was treated with glycine. After removing the protection groups, 4-aminosalicylglycine[6-7]was obtained (**Scheme 1**).

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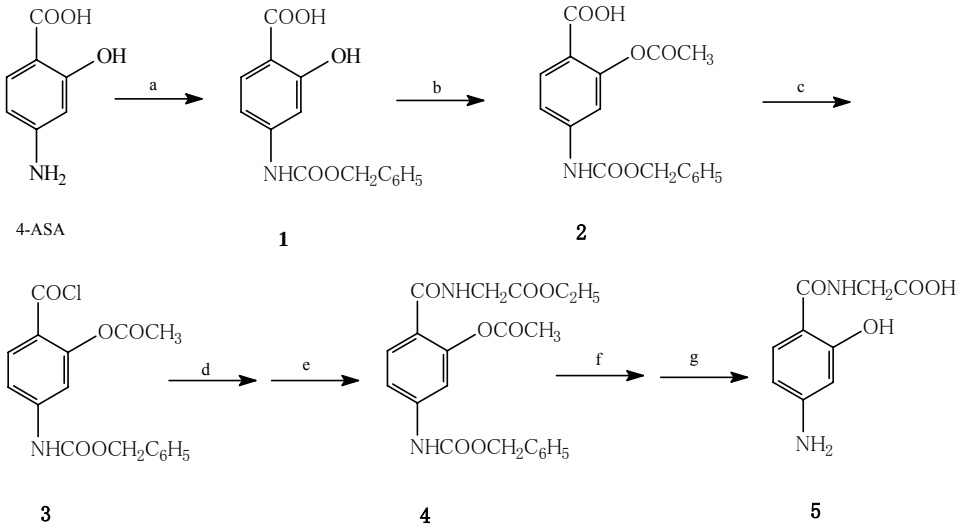
Scheme 1 The synthetic route of 4-aminosalicylglycine from 4-aminosalicylic acid



Reagents and conditions: (a) BzOCl, 0℃; (b) $(CH_3CO)_2O$, CH_3COOH; (c) $SOCl_2$, reflux; (d) $H_2NCH_2COOC_2H_5$, OH^-; (e) H^+; (f) Pd/C, H_2; (g) NaOH, H^+.

Experimental

Benzylchloroformate and 10%Pd/C were purchased from Sigma Chemical Co. (USA). All other chemicals and solvents were analytical reagent grade.

IR spectra was recorded with PE1730 spectrophotometer. ^{1}H-NMR and ^{13}C-NMR spectra were taken on a Varian (300MHz) spectrometer. The melting point was measured with X-4 digital melting point apparatus. Catalytic hydrogenation was conducted in pressure reactor (Parr WDF).

*Preparation of 4-[N-(benzyloxycarbonyl)amino]salicylic acid **1***

Benzylchloroformate (13.3 g, 78 mmol) was added dropwise to the suspension of 4-ASA (10 g, 65 mmol) in saturated solution of $NaHCO_3$ (250 mL). The mixture was reacted at 0℃ for 10 hours, then was filtered. The filterate was washed with ether and acidified with 3 mol/L HCl to pH=4, then extracted with ethyl acetate. After drying with anhydrous Na_2SO_4, the solvent was removed. The residue was recrystyllized with ethyl acetate and chloroform (1:3) to give compound **1** (14 g, 75% yield). mp: 191℃-194℃; IR (KBr, υ, cm^{-1}): 3385 (N-H) , 1263（C-N）, 1744 (C=O); ^{1}H-NMR (DMSO-d_6, δ ppm): 5.16 (s, 2H, -CH_2-), 7.00-7.70 (m, 3H, H-Ph), 7.37 (m, 5H,-C_6H_5), 10.13 (br.s, 1H, -NH-CO-); ^{13}C-NMR δ ppm: 66.3 (-CH_2-Ph), 104.8-128.6 (8C, Ph), 109.5 (Ph-COOH), 145.9 (Ph-NH-), 153.2 (Ph-OH), 136.4 (Ph-CH_2-), 162.3 (-NH-CO-), 171.7 (Ph-COOH).

*Preparation of 4-[N-(benzyloxycarbony)amino]2-acetoxysalicylic acid **2***

To the suspension of compound **1** (10 g, 35 mmol) in acetic acid (60 mL), acetic anhydride (6 mL, 63.5 mmol) and pyridine (0.3 mL) were added and the mixture was stirred for 24 hours. The precipitate was filtered, which was recrystyllized from methanol to give compound **2** (8.3 g, 72.5% yield). mp: 180℃-182℃; IR (KBr, υ, cm^{-1}): 1430, 1375 (-CH_3), 1696 (-COOH), 3295 (-NH-); ^{1}H-NMR (DMSO-d_6, δ ppm): 2.23 (s, 3H, -CH_3), 5.17 (s, 2H, -CH_2-Ph), 7.34-7.90 (m, 8H, H-Ph), 10.30 (br.s, 1H, -NH-CO-), 11.30 (s, 1H, -COOH); ^{13}C-NMR δ ppm: 21.0 (-CH_3), 66.4 (-CH_2-Ph), 112.3-132.6 (8C,Ph), 117.2 (Ph-COOH), 144.3 (Ph-NH-), 151.5 (Ph-OH), 136.3 (Ph-CH_2-), 153.2 (-CO-CH_3), 165.1 (-NH-CO-), 169.3 (Ph-COOH).

*Preparation of 4-[N-(benzyloxycarbony)amino]2-acetoxysalicylicylchloride **3***

With isolating water vapor with calcium chloride tube and absorbing gases with sodium hydroxide water solution, dried compound **2** (3 g, 9 mmol) and newly distilled $SOCl_2$ (4 mL, 6.46 g, 55 mmol) were refluxed for 3 hours. After reaction, the excessive $SOCl_2$ was removed *in vacuo* to give compound **3** (2.98 g, 94% yield), which was dried in vacuum desiccator over night.

*Preparation of 4-[N-(benzyloxycarbony)amino]salicylglycine ethyl ester **4***

Glycine ethyl ester hydrochloride (0.85 g, 6 mmol) solved in water (2 mL) was neutralized by adding Na_2CO_3 solution (10%, g/mL) dropwise at 0 ℃, adjusting pH 8.5-9.0, and stirred for 2 hours. Compound **3** (1 g, 3 mmol) solved in anhydrous acetone (15 mL) was added to the glycine ethyl ester solution, keeping pH 8.5-9.0. The reaction mixture was stirred mechanically at 0 ℃ for 8 hours. The precipitate was filtered and recrystallized from methanol and water (2:1) to give compound **4** (0.85 g, 79% yield). mp: 155℃-156℃; IR (KBr, υ, cm^{-1}): 1379 (-CH_3), 1644 (-CO-O-), 3416, 3386 (-NH-CO-), 3344 (-OH); ^{1}H-NMR (DMSO-d_6, δ ppm): 1.19 (t, 3H, -CH_3), 4.01 (d, 2H, J=6, NH-CH_2-COO-), 4.09 (q, 2H, -CH_2-CH_3), 5.15 (s, 2H, -O-CH_2-Ph), 6.95-7.75 (m, 8H, H-Ph), 9.06 (s, 1H, Ph-CO-NH-), 10.02 (s, 1H, Ph-NH-CO-), 12.36 (s, 1H, Ph-OH); ^{13}C-NMR δ ppm: 14.1 (-CH_3), 105.3-129.0 (8C, Ph), 41.1 (-NH-CH_2-COO-), 60.6 (-CO-O-CH_2-CH_3), 66.1 (-O-CH_2-Ph), 109.6 (Ph-CO-NH-), 144.1 (Ph-NH-), 153.1 (Ph-OH), 136.4 (Ph-CH_2-), 160.8 (-NH-CH_2-COO-), 168.6 (-NH-COO-), 169.8 (Ph-CO-NH-).

*Preparation of 4-aminosalicylglycine (**5**, 4-ASA-Gly)*

Compound **4** (1 g, 2.7 mmol) was dissolved in methanol (150 mL), adjusting pH=4, then hydrogenated at 0.3 MPa with 10% Pd/C to yield 4-aminosalicyglycine ethyl ester. The mixture was hydrolyzed with 1 mol/L NaOH (10 molar excess) at 25℃ for 3 hours, then neutralized to pH=7 with HCl (10%). The precipitate was extracted with ethyl acetate and the extract was condensed to 10 mL. The residue was separated with thin layer

chromatography to give compound **5** (0.29 g, 51% yield). mp: 168℃-170℃; IR (KBr, υ, cm^{-1}): 3495, 3388 (-NH_2), 3020 (-OH), 1654 (-CO-); ^{1}H-NMR (CD_3OD, δ ppm): 4.04 (s, 2H, -NH-CH_2-COO-), 6.14-7.50 (m, 3H, H-Ph); ^{13}C-NMR δ ppm: 41.7 (-NH-CH_2-COO-), 102.3-129.5 (3C, Ph), 107.3 (Ph-CO-NH-), 154.2 (Ph-NH_2), 163.4 (Ph-OH), 171.7 (Ph-CO-NH-), 173.4 (-NH-CH_2-COOH).

Conclusion

The 4-ASA-Gly was synthesized by a simple method. All the compounds were characterized by FT-IR, ^{1}H-NMR, ^{13}C-NMR spectra. Properties of 4-ASA-Gly acting as a colon-specific prodrug of 4-ASA was evaluated on rats with acetic acid-induced colitis. The expressions of cell factors, such as NF-κB, iNOS and TNF-α in colon tissue were determined by immunohistochemistry, and the injuries of colonic membrane were examined by pathomorphology. The experiment indicated that the differences were noticeable for the expressions of NF-κB, iNOS and TNF-α, and for the colon recovery between the rats administered 4-ASA-Gly and 4-ASA. The results showed that 4-ASA-Gly was more effective than 4-ASA for the treatment of ulcerative colitis. The results of our studies *in vivo* suggested that the prodrug, 4-ASA-Gly might have improved delivery properties over the parent drug, 4-ASA. Compared to 4-ASA, only a small fraction of 4-ASA-Gly has been absorbed in the upper intestine. The most fraction of 4-ASA-Gly was delivered to the large intestine and activated to liberate 4-ASA. Therefore, 4-ASA-Gly is a promising colon specific prodrug of 4-ASA and worthy of further study.

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