DOI 10.1007/s10600-019-02724-5
Chemistry of Natural Compounds, Vol. 55, No. 3, May, 2019

SYNTHESIS OF THE NATURAL PRODUCT IOTROCHAMIDE B

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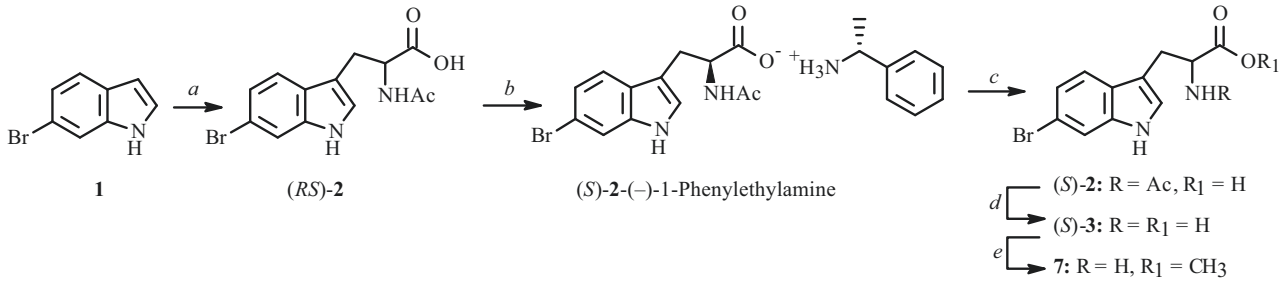
Iotrochamide B is the first cinnamoyl amino acid reported from the marine sponge Iotrochota *sp. The total synthesis of the marine indole alkaloid iotrochamide B was achieved by condensation of 6-bromo-L-tryptophan (**3**) and (*Z*)-2-methoxy-3-phenylacrylic acid (**6**). The key step was the synthesis of 6-bromo-L-tryptophan ((*S*)-**3**) from racemic* N*-acetyltryptophan by optical resolution using (*S*)-(–)-1-phenylethylamine. This work provides an efficient method for future synthesis of iotrochamide B derivatives.*

Keywords: marine natural product, bromoindole alkaloid, iotrochamide B, total synthesis.

A growing number of bromoindole alkaloids are being discovered from a variety of marine invertebrates, including bryozoans, coelenterates, sponges, and tunicates. Adding to their interest is the fact that they display a wide range of biological activity [1, 2]. The indole alkaloid iotrochamide B, found in the Australian sponge *Iotrochota* sp., was shown to inhibit *Trypanosoma brucei brucei* with IC_{50} values of 4.7 μM, and displayed medium cytotoxic activity to HEK293 (100 ± 0% inhibition at 70 μM). [3]. Iotrochamide B is the first cinnamoyl amino acid reported from the marine sponge *Iotrochota* sp. Since only small quantities of iotrochamide B (**8**) are available in nature, efficient methods for the total synthesis of these compounds have been required to provide these alkaloids and the related compounds for launching a thorough biological investigation. In this paper, we developed an efficient approach to synthesize iotrochamide B. 6-Bromoindole (**1**) is the starting material for 6-bromo-L-tryptophan [4]. Substitution reaction of **1** with L-serine in acetic acid and acetic anhydride gave racemic *N*-acetyl-6-bromotryptophan ((*RS*)-**2**) in 90% yield [5], followed by optical resolution using (*S*)-(–)-1-phenylethylamine which afforded *N*-acetyl-6-bromo-L-tryptophan (*S*)-**2** in 60% yield. The deacetylation of an *N*-acetyltryptophan (*S*)-**2** using aqueous NaOH afforded 6-bromo-L-tryptophan in 85% yield (Scheme 1) [6]. Condensation of benzaldehyde with *N*-acetylglycine in refluxing acetic anhydride and sodium acetate gave the oxazole intermediate **4** in 72% yield. Hydrolysis of this oxazole intermediate with 3 M HCl provided (*Z*)-2-hydroxy-3-phenylacrylic acid (**5**) in 68% yield. Methylation of **5** with dimethyl sulfate in 10% NaOH followed by acidification with HCl furnished the methylated product (*Z*)-2-methoxy-3-phenylacrylic acid (**6**) in 80% yield (Scheme 2) [7]. Condensation of 6-bromo-L-tryptophan methyl ester with (*Z*)-2-methoxy-3-phenylacrylic acid (**6**) with EDCI and HOBt in DMF gave iotrochamide B methyl ester in 50% yield [8]. The hydrolysis of iotrochamide B methyl ester using LiOH in THF–MeOH–H_2O afforded iotrochamide B in 70% yield (Scheme 2). As far as we know, the total synthesis of iotrochamide B has not been reported.



a. L-Serine, AcOH, Ac_2O, 73°C, 2h, 90%; *b*. (*S*)-(–)-1-phenylethylamine, MeOH, 60%; *c*. 1M NaOH, toluene, 1N HCl, pH 3; *d*. 25% NaOH, reflux, 6h, 85%; *e*. MeOH, $SOCl_2$, ice bath, 80%

Scheme 1

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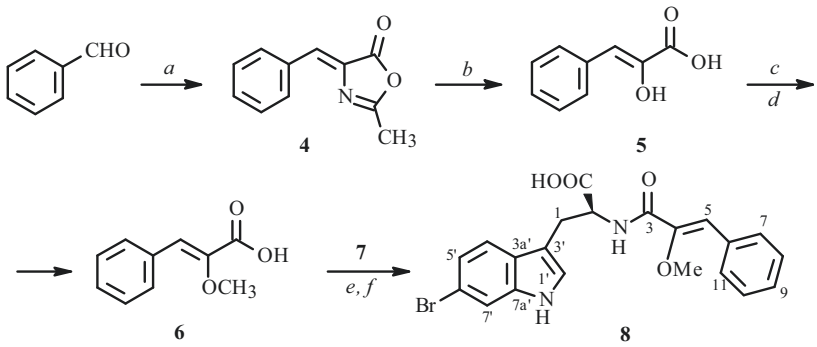
Published in *Khimiya Prirodnykh Soedinenii*, No. 3, May–June, 2019, pp. 428–430. Original article submitted December 6, 2017.



a. *N*-acetylglycine, Ac_2O, CH_3COONa, reflux, 1 h, 72% yield; *b*. 3M HCl, reflux, 3 h, 68%; *c*: 10% NaOH, $(CH_3)_2SO_4$, r.t., 4 h; *d*. 3M HCl, r.t., 0.5 h, 80%; *e*. EDC·HCl, HOBt, DMF, triethylamine, r.t., overnight, 50%; *f*. LiOH, H_2O, MeOH, THF, r.t., overnight, 70%

Scheme 2

EXPERIMENTAL

All reagents and solvents were of reagent grade or purified according to standard methods. Column chromatography (CC): silica gel (200–300 mesh; Qingdao Marine Chemical Factory, China). TLC: silica gel GF_{254} plates (10–40 μm; Qingdao Marine Chemical Factory, China). ^{1}H NMR was recorded on a Bruker Avance 400 spectrometer using TMS as internal standard. HR-ESI-MS was obtained on an Agilent Quadrupole-Time of Flight instrument.

Preparation of *N*-Acetyl-6-bromo-DL-tryptophan ((*RS*)-2). *L*-Serine (1.05 g, 10 mmol) was dissolved in a solution of 6-bromoindole (980 mg, 5 mmol) in AcOH (12 mL) and Ac_2O (4 mL), and the mixture was stirred for 2 h at 73°C under argon. After cooling, the reaction mixture was diluted with diethyl ether (30 mL) and adjusted to pH 11 with 30% NaOH. Ether (45 mL) was further added, and the whole partitioned. The ether layer was further extracted with 1 mol/L NaOH (30 mL × 2), and a small amount of $Na_2S_2O_4$ was added to the combined alkali solution, which was then neutralized with conc. HCl, concentrated to 1/2 volume, acidified with 5% HCl to adjust to pH 3 using congo red as an indicator, and extracted with EtOAc (100 mL × 3). The EtOAc layer was washed with H_2O (30 mL × 2), dried over Na_2SO_4, and concentrated *in vacuo* with addition of benzene several times to remove the vapor of HCl gas to give (*RS*)-**2** (1.45 g, 90.0%) as light brown crystals, mp 84–86°C (acetone). ^{1}H NMR (400 MHz, acetone-d_6, δ, ppm, J/Hz): 1.89 (3H, s, $COCH_3$), 3.18, 3.31 (each 1H, dd, J = 8, 16, H-3), 4.76 (1H, dt, J = 5.5, 7.5, H-2), 7.14 (1H, dd, J = 4, 8, H-5′), 7.16 (1H, d, J = 3.0, H-2′), 7.56 (1H, d, J = 8, H-4′), 7.58 (1H, d, J = 4, H-7′), 10.27 (1H, br, H-1′). HR-ESI-MS *m/z* 325.0188 $[M + H]^+$ (calcd for $C_{13}H_{14}O_3N_2Br^{79}$, 325.0188).

Preparation of *N*-Acetyl-6-bromo-L-tryptophan (*S*)-2. A solution of (*RS*)-**2** (1.4 g, 4.3 mmol) in methanol (20 mL) and (*S*)-(–)-1-phenylethylamine (907.5 mg, 1.5 equiv.) was refluxed for 4 h in a 50-mL round bottom flask. The mixture was ice-cooled to allow crystallization, and then the precipitates were filtered and dried. The crystals afforded the salt of (*S*)-*N*-acetyl-6-bromo-*L*-tryptophan-(*S*)-(–)-1-phenylethylamine. The salt was dissolved in H_2O and adjusted to pH 10 with 1 M NaOH. The alkali solution was further extracted with toluene (45 mL); then the water layer was acidified with 1 N HCl to pH 3 and concentrated to 1/2 volume to allow crystallization. The solid formed was *N*-acetyl-6-bromo-L-tryptophan (*S*)-**2** (972 mg, 60%). $[\alpha]_D^{25}$ +8.20° (*c* 0.73, acetone). The ^{1}H NMR data coincided with that of (*RS*)-**2**.

Preparation of 6-Bromo-L-tryptophan (*S*)-3. *N*-Acetyl-6-bromo-L-tryptophan ((*S*)-**2**) (900 mg, 2.77 mmol) was dissolved in 25% NaOH (5 mL) and refluxed for 6 h. The solution was acidified with 1 mol/L HCl to pH 3. Filtration afforded 6-bromo-L-tryptophan (*S*)-**3** (688.1 mg, 85%). $[\alpha]_D^{25}$ –22.44° (*c* 0.73, acetone). ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm, J/Hz): 2.96 (1H, dd, J = 8.0, 12.0, Ha-3), 3.22 (1H, dd, J = 4.0, 12.0, Hb-3), 3.83 (1H, dd, J = 4.0, 9.0, H-2), 7.10 (1H, dd, J = 2.0, 8.0, H-5′), 7.20 (1H, br.s, H-2′), 7.53 (1H, d, J = 2.0, H-7′), 7.62 (1H, d, J = 8.0, H-4′). ^{13}C NMR (100 MHz, DMSO-d_6, δ, ppm): 27.35 (t, C-3), 55.15 (d, C-2), 110.49 (s, C-3′), 114.14 (s, C-7′), 114.26 (s, C-6′), 120.83 (d, C-4′), 121.57 (d, C-5′), 125.65 (d, C-2′), 126.88 (s, C-3a′), 137.65 (s, C-7a′), 170.14 (s, COOH). HR-ESI-MS *m/z* 283.0092 $[M]^+$ (calcd for $C_{11}H_{12}O_2N_2Br^{79}$, 283.0082).

Preparation of (*Z*)-2-Hydroxy-3-phenylacrylic Acid (5). A mixture of benzaldehyde (2.12 g, 20 mmol), *N*-acetylglycine (3.56 g, 30 mmol), and sodium acetate (2.46 g, 30 mmol) in acetic anhydride (15 mL) was stirred at reflux for 1 h. The reaction was quenched with ice water and vigorously stirred for 1 h in an ice bath to allow precipitation. Filtration afforded **4** (yellow powder, 4.03 g, 72%). A suspension of oxazole derivative **4** (3.74 g, 20 mmol) in aq. HCl (3 M, 20 mL) was

stirred at reflux for 3 h. The mixture was cooled to room temperature to allow crystallization. Filtration afforded (*Z*)-2-hydroxy-3-phenylacrylic acid (**5**). Pale yellow crystal, yield 68%. ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm, J/Hz): 6.39 (1H, s, CH), 7.23–7.37 (3H, m, ArH), 7.75 (2H, d, J = 7.2, ArH), 9.24 (1H, s, OH), 13.21 (1H, s, COOH). ESI-MS *m/z* 165 $[M + H]^+$.

Preparation of (*Z*)-2-Methoxy-3-phenylacrylic Acid (6). Dimethyl sulfate (5 mL) was added dropwise into a suspension of compound **5** (10 mmol) in 10% NaOH (15 mL) in an ice-water bath, and the mixture was stirred at room temperature for 4 h. After that, 3 M HCl (15 mL) was added and the whole stirred for another 0.5 h to precipitate. Filtration afforded compound **6**, yield 80%, pale yellow powder. ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm, J/Hz): 3.74 (3H, s, OCH_3), 6.93 (1H, s, CH), 7.34–7.45 (3H, m, ArH), 7.78 (2H, d, J = 7.2, ArH), 13.00 (1H, s, COOH). ESI-MS *m/z* 179 $[M + H]^+$.

Preparation of Iotrochamide B (8). 6-Bromo-L-tryptophan (566 mg, 2 mmol) was suspended in methanol, and the suspension was stirred in an ambient ice bath under thionyl chloride atmosphere until the suspension became clear. The product solution was rotary evaporated under reduced pressure. Methanol was then added and the whole rotary evaporated to remove the hydrogen chloride. A mass of white solid was obtained **7** (475 mg, 80%). A solution of (*Z*)-2-methoxy-3-phenylacrylic acid (**6**) (178 mg, 1 mmol) in DMF was added to triethylamine (2 mmol), EDC·HCl (286.5 mg, 1.5 mmol), and HOBt (202 mg, 1.5 mmol). After addition of the appropriate amine **7** (356.4 mg, 1.2 mmol), the mixture was stirred overnight, then dissolved in H_2O and extracted with EtOAc. The EtOAc layer was washed with H_2O, dried over Na_2SO_4, and evaporated under vacuum to afford a crude substance, which was purified by column chromatography using CH_2Cl_2–EtOAc (1:1) to provide the methyl ester of iotrochamide B (228.5 mg, 50%). A preparation of iotrochamide B methyl ester (220 mg, 0.48 mmol) and LiOH (72 mg, 3 mmol) in THF–MeOH–H_2O (4:2:1 mL) was stirred overnight. All the volatiles were removed, and the white solid residue was acidified with 7 mL of HCl (1 N), filtered, and washed with H_2O to afford the titled compound, which was purified by column chromatography using CH_2Cl_2–EtOAc (1:1) to provide brown crystals (309.5 mg, 70%). $[\alpha]_D^{25}$ –21.5° (*c* 0.1, MeOH). ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm, J/Hz): 3.20 (1H, dd, J = 9.0, 15.0), 3.26 (1H, dd, J = 4.8, 15.0), 3.46 (3H, s), 4.59 (1H, ddd, J = 4.8, 7.8, 9.0), 6.58 (1H, s), 7.10 (1H, dd, J = 1.8, 8.4), 7.20 (1H, d, J = 2.4), 7.29 (1H, dd, J = 7.8, 7.8), 7.37 (2H, dd, J = 7.8, 7.8), 7.50 (1H, d, J = 8.4), 7.52 (1H, d, J = 8.4), 7.62 (2H, d, J = 7.8), 8.19 (1H, br.d, J = 7.8), 10.97 (br.s). HR-ESI-MS m/z 465.0416 $[M + Na]^+$ (calcd for $C_{21}H_{19}O_4N_2NaBr^{79}$, 465.0420).

ACKNOWLEDGMENT

This work was supported by Scientific Research Fund of Xinxiang Medical University grants [2013QN121].

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