A Total Synthesis of Subarine, a Marine Alkaloid Related to the Pyridoacridine Family

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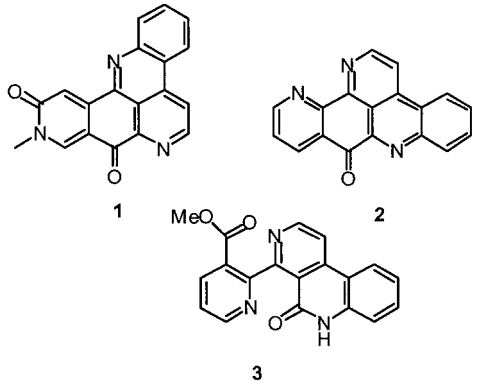
The synthesis of the marine alkaloid subarine has been accomplished in four steps in 70% overall yield starting from 4-bromo-1,10-phenanthroline. The natural compound and three intermediates in the synthesis were tested at six concentrations on six different human cancer cell lines, including various histopathological types. None of these compounds exhibits significant antitumor activity.

Introduction

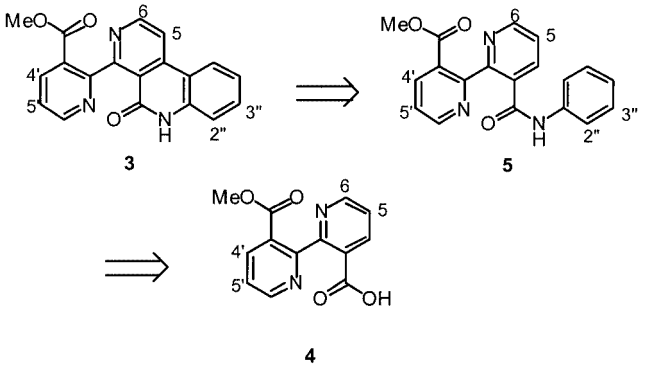
Since the isolation of the marine alkaloid amphimedine (**1**) by Schmitz in 1983,[1] there has been significant interest in compounds of the 11*H*-pyrido[4,3,2-*mn*]acridine family,[2] due to the range of potentially useful biological activities they exhibit, which include antifungal, antiviral and antitumor properties. Their role as pharmaceutical lead compounds, along with their limited availability from natural sources, makes them prime candidates for the development of total synthetic routes for alkaloids and their analogues. Among these compounds, ascididemin (**2**), isolated in 1988 by Kobayashi[3] from the ascidian *Didemnum sp.*, has been the subject of various investigations both to define new anticancer drugs[4] and to understand the mechanisms involved in tumor cytotoxicity.[5] Recently, subarine (**3**), a new alkaloid, has been isolated from a Singapore ascidian.[6] This compound has been suggested to be a significant biosynthetic link in the biosynthesis of ascididemin and other related pentacyclic pyridoacridines. We report herein the first total synthesis of subarine, which employs 4-bromo-1,10-phenanthroline **7** as the starting material.



Results and Discussion

Synthesis

In our first approach, we used the retrosynthetic analysis shown in Scheme 1, involving the monoester-monoacid **4**, the key step of the synthesis being the formation of an aryl−aryl bond, in order to construct the last ring.



Scheme 1

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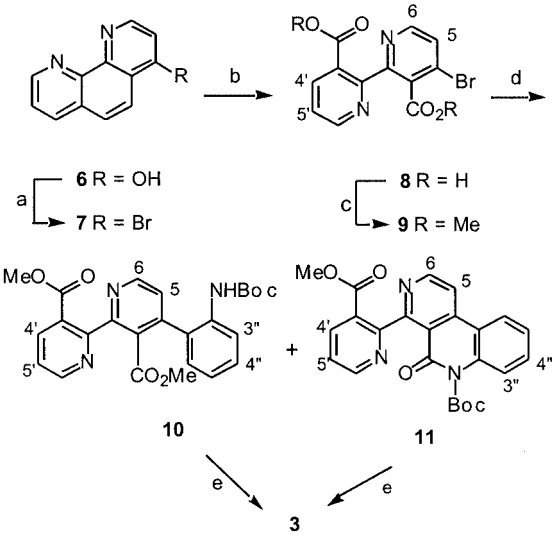
Compound **4** was prepared in two steps from 1,10-phenanthroline according to procedures previously described, namely, oxidation with a mixture of HNO_3 and H_2SO_4,[7] and reaction of the resultant 1,10-phenanthroline-5,6-dione with *m*-chloroperbenzoic acid.[8] Compound **4** was then transformed into the key amide intermediate **5** in 62% yield by the action of dicyclohexylcarbodiimide (DCC) and aniline. Unfortunately, attempts to cyclize this derivative **5**, by, amongst other methods, thermolysis, or the action of phenyliodine(III) bis(trifluoroacetate) (PIFA) in CH_2Cl_2 with $BF_3{\cdot}Et_2O$ as an activating agent,[9] failed.

An alternative route to **3** is presented in Scheme 2. This method was based on two key steps, including $KMnO_4$ oxidation of 4-bromo-1,10-phenanthroline **7** and a Stille aryl−aryl coupling.



Scheme 2. (a) $POBr_3$, PBr_3, 110 °C, 6 h. (b) $KMnO_4$, KOH, H_2O, reflux, 2.5 h. (c) DCC, MeOH, 0 °C, 2 h. (d) 2-(NHBoc)$C_6H_4SnMe_3$, $Pd(PPh_3)_4$, 1,4-dioxane, reflux, N_2, overnight. (e) TFA, CH_2Cl_2, room temp., overnight

Compound **7** was prepared as described previously, by action of a mixture of phosphorus oxybromide and phosphorus bromide on the corresponding hydroxy-derivative **6**,[10] itself synthesized in 79% yield from 8-aminoquinoline, according to the four-step procedure of Snyder and Freier.[11] The dicarboxylic acid **8** was obtained from **7** by alkaline permanganate oxidation according to the method reported by Wimmer and Wimmer[12] for the preparation of binicotinic acid from phenanthroline. It was then transformed into the corresponding diester **9** by action of DCC and methanol, in 88% yield over the two steps. The palladium(0)-catalyzed Stille cross-coupling reaction of **9** with *N*-(*tert*-butoxycarbonyl)-2-(trimethylstannyl)aniline[13] gave the expected adduct **10** (63%) together with the already-cyclized compound **11** (18%). Reaction of **10** with trifluoroacetic acid gave subarine (**3**) in 98% yield by hydrolysis of the Boc group and subsequent cyclization. This alkaloid was also obtained under similar conditions from compound **11** by cleavage of the protecting group. The spectroscopic data of **3** (^{1}H NMR and ^{13}C NMR) were identical to the values reported for the natural product.[6] The overall yield of this synthesis was 70%.

Biological results

The drug-induced inhibition of human cancer cell line growths was assayed for compounds **9**, **10**, **11** and subarine (**3**).[4] Six concentrations (ranging from 10^{-5} to 10^{-9} M) of each of these compounds were tested on six different cell lines of different histopathological types including glioblastomas (Hs683 and U-373MG), breast (MCF-7), colon (HCT-15 and LoVo) and lung (A549). None of these compounds presents cytotoxic activity ($IC_{50} > 10^{-5}$ M).

Experimental Section

General Procedures: All commercial chemicals were used without further purification. Flash chromatography was performed on flash silica gel 60 (Merck 0.015−0.040 mm). NMR spectra were recorded at 400 MHz for ^{1}H and at 100 MHz for ^{13}C.

Methyl 3′-(Anilinocarbonyl)-2,2′-bipyridine-3-carboxylate (5): DCC (309 mg, 1.5 mmol) was added to a solution of 3′-(methoxycarbonyl)-2,2′-bipyridine-3-carboxylic acid[7] (166 mg, 0.64 mmol) and aniline (60 mg, 0.64 mmol) in CH_2Cl_2 at 0 °C. The reaction was stirred for 2 h and filtered. The filtrate was concentrated under vacuum and the crude product purified by flash chromatography (CH_2Cl_2/MeOH, 95:5) to give **5** as a yellow solid (132 mg, 62%), m.p. 218 °C. ^{1}H NMR ($CDCl_3$): δ = 3.73 (s, 3 H, COOMe), 7.06 (t, *J* = 7.7 Hz, C4"-H), 7.25 (t, *J* = 7.7 Hz, 2 H, C3"-H and C5"-H), 7.39 (d, *J* = 7.7 Hz, 2 H, C2"-H and C6"-H), 7.44 (dd, *J* = 7.7, 4.8 Hz, 1 H, C5′-H), 7.48 (dd, *J* = 7.7, 4.8 Hz, 1 H, C5-H), 8.24 (dd, *J* = 7.7, 1.4 Hz, 1 H, C4′-H), 8.26 (dd, *J* = 7.7, 1.8 Hz, 1 H, C4-H), 8.69 (dd, *J* = 4.8, 1.4 Hz, 1 H, C6′-H), 8.76 (dd, *J* = 4.8, 1.8 Hz, 1 H, C6-H), 8.92 (br. s, 1 H, NH) ppm. ^{13}C NMR ($CDCl_3$): δ = 49.42, 119.66 (2C), 123.39, 123.41, 124.51, 124.55, 129.05 (2C), 132.12, 137.69, 137.91, 138.47, 150.20, 150.88, 156.72, 160.20, 165.16, 167.30 ppm.

4-Bromo-2,2′-bipyridyl-3,3′-dicarboxylic Acid (8): A solution of 4-bromo-1,10-phenanthroline (750 mg, 2.9 mmol), $KMnO_4$ (1.4 g, 8.7 mmol) and NaOH (232 mg, 5.8 mmol) in water (29 mL) was refluxed for 2.5 h. The reaction mixture was filtered and the filtrate was extracted with CH_2Cl_2 (30 mL). The aqueous layer was made acidic (pH 2) with 1 N HCl and concentrated to dryness. Methanol was added to the residue and the mixture was filtered to give **8** as a brown solid which was used in the next step without further purification. ^{1}H NMR ([D$_6$]DMSO): δ = 7.55 (dd, *J* = 4.8, 7.7 Hz, 1 H, C5′-H), 7.81 (d, *J* = 5.3 Hz, 1 H, C5-H), 8.10 (d, *J* = 7.7 Hz, 1 H, C4′-H), 8.41 (d, *J* = 5.3 Hz, 1 H, C6-H), 8.65 (d, *J* = 4.8 Hz, 1 H, C6′-H) ppm.

Dimethyl 4-Bromo-2,2′-bipyridinyl-3,3′-dicarboxylate (9): DCC (2.8 g, 13.56 mmol) was added to a solution of the diacid **8** in methanol (47 mL) at 0 °C. The reaction mixture was stirred for 2 h and filtered through Celite. The filtrate was concentrated and the crude product was purified by flash chromatography (CH_2Cl_2/MeOH, 99:1) to give the diester **9** as a white solid (748 mg, 88% from 4-bromo-1,10-phenanthroline), m.p. 222−224 °C. IR ($CHCl_3$): $\tilde{\nu}$ = 1656, 1734 cm^{-1}. ^{1}H NMR ($CDCl_3$): δ = 3.74 (s, 3 H, COOMe), 3.77 (s, 3 H, COOMe), 7.41 (dd, *J* = 4.8, 7.7 Hz, 1 H, C5′-H), 7.59 (d, *J* = 5.3 Hz, 1 H, C5-H), 8.12 (dd, *J* = 1.8, 8.1 Hz, 1 H, C4′-

H), 8.42 (d, J = 5.3 Hz, 1 H, C6-H), 8.68 (dd, J = 1.8, 4.8 Hz, 1 H, C6′-H) ppm. ^{13}C NMR ($CDCl_3$): δ = 52.56, 52.69, 123.45, 127.63, 128.26, 130.97, 131.82, 137.95, 149.22, 150.37, 155.09, 156.06, 166.47, 167.64 ppm. $C_{14}H_{11}N_2O_4Br$ (351.1543): calcd. C 47.86, H 3.13, N 7.98; found C 48.06, H 3.11, N 8.07.

Dimethyl 4-{[(2-*tert*-Butoxycarbonyl)amino]phenyl}-2,2′-bipyridinyl-3,3′-dicarboxylate (10) and *tert*-Butyl 4-[(3-Methoxycarbonyl)pyridin-2-yl]-5-oxo-5*H*−benzo[*c*][2,7]naphthyridine-6-carboxylate (11): A mixture of the bromo derivative **9** (220 mg, 0.63 mmol), *N*-(*tert*-butoxycarbonyl)-2-(trimethylstannyl)aniline[9] (404 mg, 1.13 mmol) and tetrakis(triphenylphosphane)palladium(0) (38 mg, 0.031 mmol) in 1,4-dioxane (14 mL) was refluxed under a nitrogen atmosphere overnight. After filtration through Celite, H_2O (50 mL) and EtOAc (100 mL) were added. The organic layer was separated and the aqueous layer was extracted with EtOAc (2 × 100 mL). The combined organic layers were dried over $MgSO_4$ and concentrated under vacuum. The crude product was purified by flash chromatography (CH_2Cl_2/MeOH, 98:2) to give **10** and **11**.

10: White-yellow solid (184 mg, 63%), m.p. 228 °C. IR ($CHCl_3$) $\tilde{\nu}$ = 1699, 1728 cm^{-1}. ^{1}H NMR ($CDCl_3$): δ = 1.44 (s, 9 H, CMe_3), 3.38 (s, 3 H, COOMe), 3.77 (s, 3 H, COOMe), 6.31 (s, 1 H, NH), 7.10 (m, 2 H, C6"-H and C5"-H), 7.31 (d, J = 5.0 Hz, 1 H, C5-H), 7.38 (dd, J = 8.4, 5.5 Hz, 1 H, C4"-H), 7.43 (dd, J = 4.8, 8.1 Hz, 1 H, C5′-H), 7.99 (d, J = 8.4 Hz, 1 H, C3"-H), 8.21 (dd, J = 1.6, 8.1 Hz, 1 H, C4′-H), 8.70 (dd, J = 1.6, 4.8 Hz, 1 H, C6′-H), 8.72 (d, J = 5.0 Hz, 1 H, C6-H) ppm. ^{13}C NMR ($CDCl_3$): δ = 28.25 (3C), 52.14, 52.43, 80.60, 121.81, 123.10, 123.45, 124.64, 127.71, 128.76, 128.92, 129.51, 135.31, 136.93, 138.00, 146.46, 149.51, 150.60, 153.04, 156.19, 156.59, 167.25, 167.41 ppm. $C_{25}H_{25}N_3O_6$ (463.5): calcd. C 64.79, H 5.40, N 9.07; found C 64.80, H 5.62, N 8.89.

11: White-orange solid (49 mg, 18%), m.p. > 164 °C. IR ($CHCl_3$) $\tilde{\nu}$ = 1675, 1727, 1771 cm^{-1}. ^{1}H NMR ($CDCl_3$): δ = 1.56 (s, 9 H, CMe_3), 3.64 (s, 3 H, COOMe), 7.10 (dd, J = 8.4, 1.2 Hz, 1 H, C6"-H), 7.37 (ddd, J = 7.8, 8.4, 1.2 Hz, 1 H, C4"-H), 7.45 (dd, J = 8.0, 4.8 Hz, 1 H, C5′-H), 7.58 (ddd, J = 7.8, 1.4, 8.4 Hz, 1 H, C5"-H), 8.13 (d, J = 5.5 Hz, 1 H, C5-H), 8.29 (dd, J = 1.4, 8.4 Hz, 1 H, C3"-H), 8.42 (dd, J = 1.8, 8.0 Hz, C4′-H), 8.80 (dd, J = 4.8, 1.8 Hz, 1 H, C6′-H), 8.91 (d, J = 5.5 Hz, 1 H, C6-H) ppm. ^{13}C NMR ($CDCl_3$): δ = 27.79 (3C), 52.49, 87.11, 115.25, 115.88, 117.08, 119.40, 123.19, 124.19, 125.07, 125.13, 132.72, 137.15, 139.12, 142.60, 151.39, 151.76, 152.92, 159.06, 161.75, 162.85, 166.47 ppm. $C_{24}H_{21}N_3O_5$ (431.4): calcd. C 66.82, H 4.87, N 9.74; found C 66.67, H 4.98, N 9.51.

Methyl 2-(5-Oxo-5,6-dihydrobenzo[*c*][2,7]naphthyridin-4-yl)nicotinate (Subarine) (3): TFA (1 mL) was added dropwise to a solution of compound **10** (156 mg, 0.34 mmol), or compound **11** (147 mg, 0.34 mmol) in CH_2Cl_2 (10 mL) The solution was stirred at room temperature overnight. A saturated solution of $NaHCO_3$ (10 mL) was added and the organic layer was separated. The aqueous layer was extracted with CH_2Cl_2 (2 × 10 mL), the combined organic layers were dried over $MgSO_4$ and concentrated under vacuum. The crude product was purified by flash chromatography (CH_2Cl_2/MeOH, 95:5) to give the alkaloid **3** as a pale-yellow solid (110 mg, 98% from **10**, 98% from **11**), m.p. > 260 °C. IR ($CHCl_3$): $\tilde{\nu}$ = 1646, 1683 cm^{-1}. ^{1}H NMR ($CDCl_3$): δ = 3.62 (s, 3 H, COOMe), 6.90 (d, J = 8.0 Hz, 1 H, C6"-H), 7.33 (dd, J = 8.9, 8.0 Hz, 1 H, C5"-H), 7.51 (dd, J = 7.9, 7.7 Hz, 1 H, C4"-H), 7.53 (dd, J = 7.1, 5.1 Hz, 1 H, 1 H, C5′-H), 8.15 (d, J = 5.5 Hz, 1 H, C5-H), 8.24 (dd, J = 7.7 Hz, C3"-H), 8.48 (dd, J = 1.8, 8.1 Hz, 1 H, C4′-H), 8.83 (dd, J = 5.1, 1.8 Hz, 1 H, C6′-H), 8.92 (d, J = 5.5 Hz, 1 H, C6-H), 10.10 (br. s, 1 H) ppm. ^{13}C NMR ($CDCl_3$): δ = 52.31, 115.45, 116.57, 116.80, 118.81, 122.19, 123.07, 123.79, 124.69, 131.47, 137.70, 138.01, 142.48, 150.58, 152.01, 161.66, 161.73, 162.13, 165.55 ppm. $C_{19}H_{13}N_3O_3$ (331.3): calcd. C 68.88, H 3.93, N 12.69; found C 68.38, H 3.88, N 12.61.

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