Scientia Pharmaceutica www.scipharm.at

Research article Open Access

Short Total Synthesis of the Marine Alkaloid Subarine

Matthias LOTTER, Franz BRACHER *

Department Pharmazie – Zentrum für Pharmaforschung, Ludwig-Maximilians-Universität München, Butenandtstr. 5–13, 81377 München, Germany

* Corresponding author. E-mail: Franz.Bracher@cup.uni-muenchen.de (F. Bracher)

Sci Pharm. 2009; 77: 1–7 doi:10.3797/scipharm.0901-14

Published: February 5th 2009 Received: January 9th 2009
Accepted: February 4th 2009

This article is available from: http://dx.doi.org/10.3797/scipharm.0901-14

Abstract

The marine benzo[*c*][2,7]naphthyridine alkaloid subarine is prepared in 4 steps, starting from commercially available 1,10-phenanthroline, *via* oxidative cleavage to the bipyridine-dicarboxylate, conversion to the mono(2-iodoanilide), and radical cyclization. The alkaloid does not show any significant antimicrobial or cytotoxic activity.

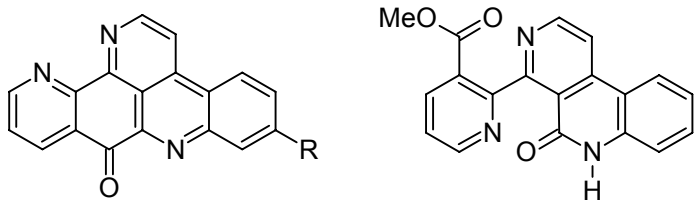
Keywords

Subarine • Alkaloid • Radical cyclization • Benzo[*c*][2,7]naphthyridine • Marine natural products

Introduction

Tunicates (ascidians) are sessile marine organisms which have intensely been investigated for the occurrence of low molecular, bioactive natural products [1]. Among the numerous types of compounds isolated from tunicates, the pyridoacridone type alkaloids [2, 3] have attended greatest interest, due to their significant cytotoxic activities [1, 4, 5].

In continuation of our research on the synthesis and biological evaluation of marine polycyclic aromatic alkaloids [6–10], e. g. ascididemin (**1a**) and 2-bromoleptoclinidinone (**1b**), we focussed our interest on the pyridyl-benzo[*c*][2,7]naphthyridine alkaloid subarine (**2**). This alkaloid was isolated from an unidentified Singaporean ascidian by Nilar et al. [11]. The authors supposed that there could be a biosynthetic link to the pyridoacridone alkaloids, like ascididemin (**1a**), since subarine (**2**) formally represents a seco-analogue of the pyridoacridone type alkaloids.



1a: R = H
1b: R = Br

2

Sch. 1.

A first total synthesis of subarine (**2**) has been published by the Delfourne group [5], but this synthesis requires multistep synthesis of the central intermediates, and no information about biological activities is given in this publication.

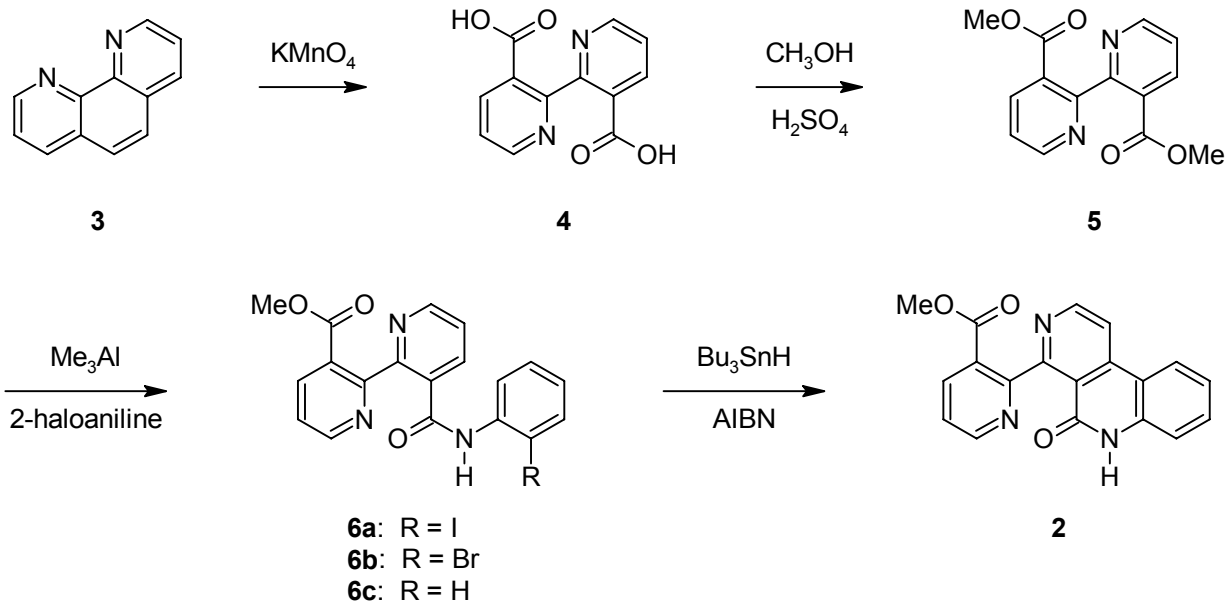
Our work was aimed at a more convenient approach to the alkaloid, in order to evaluate the biological activities, and to gain further evidence on structure-activity relationships in this class of natural products.

Results and Discussion

No matter that Delfourne [12] failed to get an access to the pyridyl-benzo[*c*][2,7]-naphthyridine ring system from bipyridine precursors, we searched for a short, new entry to this target structure starting from a bipyridine-dicarboxylate. Known dimethyl 2,2'-bipyridyl-3,3'-dicarboxylate (**5**) [13] was prepared in 2 steps from 1,10-phenanthroline (**3**). Oxidative cleavage with $KMnO_4$ gave dicarboxylate **4** [14], which was esterified with methanol/conc. sulphuric acid to give **5** in 73% overall yield. This symmetric diester was converted to the mono(2-haloanilides) **6a/b** under controlled Weinreb conditions [15]. Thus, 2-bromoaniline or 2-iodoaniline were pre-incubated with trimethylaluminum to give the corresponding dimethylaluminumanilides, which in turn were reacted with 1 equivalent of diester **5** to give 2-iodoanilide **6a** (40% yield) and 2-bromoanilide **6b** (35% yield). In both reactions the desired monoanilides were accompanied by considerable amounts of the corresponding dianilides, and unreacted starting material **5**; these mixtures were readily separated by column chromatography.

The final step of the total synthesis of subarine (**2**) required an intramolecular biaryl coupling starting from 2-haloanilides **6a/b**. It seemed most convenient to perform this reaction in a palladium-catalyzed coupling reaction. Numerous examples of intramolecular Pd-catalyzed biaryl syntheses starting from haloarenes have been published in literature, including examples starting from halogenated benzanilides [16, 17]. In our hands, 2-haloanilides **6a/b** completely failed to cyclize to subarine (**2**) under Pd(II) catalysis in the presence of diverse phosphine ligands (triphenylphosphine, tri(*o*-tolyl)phosphine, tri(*n*-butyl)phosphine, 1,3-bis(diphenylphosphino)propane), and bases like silver, potassium and sodium carbonates. Besides unreacted starting material only varying amounts of dehalogenated anilide **6c** could be isolated. The structure of **6c** was confirmed by synthesis from aniline and diester **5** under Weinreb conditions, as described above.

Finally, we investigated cyclization of **6a** under radical conditions. Following the protocol described by Ganguly et al. [18], reaction of iodoanilide **6a** with tributyltin hydride (Bu_3SnH) and a catalytic amount of azobisisobutyronitrile (AIBN) in refluxing benzene gave alkaloid subarine (**2**) in poor yield (7%). Once again, dehalogenation to give anilide **6c** was the dominating reaction. The ^1H and ^{13}C NMR data of synthetic subarine are in perfect accordance with those published for the natural product [11].



Sch. 2.

Despite the poor yield in the last step of the synthesis, this new approach wins over by the small number of reaction steps. Sufficient amounts of subarine (**2**) could be obtained for investigation of the antimicrobial and cytotoxic activities.

In an *in vitro* screening for antimicrobial activities against a number of gram-positive, gram-negative bacteria and yeasts, and for cytotoxic activity against HL 60 cells in an MTT assay [19], subarine did not show significant activity. Thus, the intact polycyclic ring system of the pyridoacridone alkaloids seems to be indispensable for antimicrobial and cytotoxic activity, seco-analogues like subarine are default of significant activity.

Experimental

NMR spectra were recorded on JEOL Eclipse plus NMR workstations (Jeol GSX 400 or JNMR GX 500 instrument) at 500 MHz or 400 MHz for ^1H MNR and 125 MHz or 100 MHz for ^{13}C NMR. Spectra were calibrated using residual undeuterated solvent ($HCCl_3$, ^1H 7.25 ppm, ^{13}C 77.00 ppm). Mass Spectra were recorded on a Hewlett Packard 5989 A MS-engine. Elemental analyses were performed on a CHN-Analyser Rapid (Heraeus). IR spectra were obtained on a Perkin Elmer Paragon 1000 spectrometer. Melting points were determined on a Büchi 540 apparatus. Flash column chromatography (FCC) was performed using Kieslegel 60, 230–400 mesh (Merck).

Methyl 3'-[(2-iodophenyl)carbamoyl]-2,2'-bipyridine-3-carboxylate (6a)

A solution of 1.10 g (5.00 mmol) 2-iodoaniline in 5 ml dichloromethane under N_2 atmosphere is treated dropwise with 2.50 ml (5.00 mmol) of a 2.0 molar solution of trimethyaluminum in heptane, and stirred at ambient temperature for 15 min. Then a solution of 1.36 g (5.00 mmol) dimethyl 2,2'-bipyridine-3,3'-dicarboxylate (**5**) in 10 ml dichloromethane is added dropwise, and the mixture is stirred at 35 °C for 15 h. Then 5% 2M HCl is added carefully, until gas evolution ceases, and then the mixture is extracted with dichloromethane (3 × 30 ml). The combined organic layers are dried over $MgSO_4$, and evaporated after filtration. The residue is purified by FCC (ethyl acetate-hexane, 9:1) to give 918 mg (40%) of **6a** as colourless needles, mp 152 °C. IR: 3001, 2925, 1715, 1666, 1580, 1414, 1300, 763 cm^{-1}; MS (CI): 460 (26, M^++1), 334 (6), 241 (100), 166 (30); ^1H NMR ($CDCl_3$): δ 8.74 (dd, 1H, J = 5.0, 1.7 Hz, 6'-H), 8.73 (dd, 1H, J = 5.1, 1.7 Hz, 6-H), 8.25 (dd, 1H, 8.8, 1.7 Hz, 4-H), 8.22 (dd, 1H, J = 7.9, 1.7 Hz, 4'-H), 8.20 (br s, 1H, NH), 8.11 (dd, 1H, J = 8.1, 1.5 Hz, 6''-H), 7.73 (dd, 1H, J = 7.9, 1.6 Hz, 3''-H), 7.49 (dd, 1H, J = 7.9, 5.0 Hz, 5'-H), 7.41 (dd, J = 8.0, 5.1 Hz, 5-H), 7.31 (ddd, 1H, J = 8.1, 7.8, 1.6 Hz, 5''-H), 6.83 (ddd, 1H, 7.9, 7.8, 1.5 Hz, 4''-H), 3.72 (s, 3H, CH_3); HRMS: calcd. for $C_{19}H_{14}IN_3O_3$: 459.0080, found: 459.0089.

Methyl 3'-[(2-bromophenyl)carbamoyl]-2,2'-bipyridine-3-carboxylate (6b)

This compound was prepared in an analogous manner as described for **6a** from 2-bromoaniline and dimethyl 2,2'-bipyridyl-3,3'-dicarboxylate (**5**). Yield: 35%, mp 153 °C. IR: 3005, 2954, 1718, 1660, 1581, 1299, 765 cm^{-1}; MS (CI): 414 (20, M^++1), 412 (21, M^++1), 241 (100); ^1H NMR ($CDCl_3$): δ 8.74 (dd, 1H, J = 4.8, 1.7 Hz, 6'-H), 8.72 (dd, 1H, J = 5.0, 1.7 Hz, 6-H), 8.25 (dd, 1H, 7.9, 1.7 Hz, 4-H), 8.22 (dd, 1H, J = 7.8, 1.7 Hz, 4'-H), 8.39 (br s, 1H, NH), 8.29 (dd, 1H, J = 8.2, 1.5 Hz, 6''-H), 7.46 (dd, 1H, J = 8.0, 1.5 Hz, 3''-H), 7.49 (dd, 1H, J = 7.8, 4.8 Hz, 5'-H), 7.41 (dd, J = 7.9, 5.0 Hz, 5-H), 7.28 (ddd, 1H, J = 8.2, 7.8, 1.5 Hz, 5''-H), 6.95 (ddd, 1H, 8.0, 7.8, 1.5 Hz, 4''-H), 3.73 (s, 3H, CH_3); Elemental analysis: Calcd.: C, 55.36; H, 3.42; N, 10.19%; found: C, 55.61; H, 4.44; N, 10.08%.

Methyl 3'-(phenylcarbamoyl)-2,2'-bipyridine-3-carboxylate (6c)

This compound was prepared in an analogous manner as described for **6a** from aniline and dimethyl 2,2'-bipyridine-3,3'-dicarboxylate (**5**). Yield: 37%, mp 155 °C. IR: 2950, 1731, 1664, 1538, 1417, 1282 cm^{-1}; MS (CI): 334 (100, M^++1), 241 (93); ^1H NMR ($CDCl_3$): δ 8.95 (br s, 1H, NH), 8.75 (dd, 1H, J = 4.9, 1.6 Hz, 6-H), 8.69 (dd, 1H, J = 4.8, 1.9 Hz, 6'-H), 8.27 (dd, 1H, 7.9, 1.6 Hz, 4-H), 8.24 (dd, 1H, J = 8.0, 1.5 Hz, 4'-H), 7.46 (dd, 1H, J = 8.0, 4.6 Hz, 5'-H), 7.44 (dd, J = 7.9, 4.9 Hz, 5-H), 7.39 (m, 2H, 2''-H and 6''-H), 7.26 (m, 2H, 3''-H and 5''-H), 7.06 (m, 1H, 4''-H), 3.72 (s, 3H, CH_3); Elemental analysis: Calcd.: C, 68.46; H, 4.54; N, 12.61%; found: C, 68.05; H, 4.64; N, 12.24%.

Methyl 2-(5-oxo-5,6-dihydrobenzo[c][2,7]naphthyridin-4-yl)pyridine-3-carboxylate (Subarine, 2)

A solution of 2-iodoanilide **6a** (2.04 g, 4.44 mmol) in 560 ml anhydrous benzene is treated with 1.75 g (6.00 mmol) tributyltin hydride and 82 mg (0.50 mmol) azobisisobutyronitrile (AIBN), and heated at reflux for 20 h. Then another 1.75 g (6.00 mmol) tributyltin hydride and 82 mg (0.50 mmol) azobisisobutyronitrile (AIBN) is added, and the mixture is refluxed for further 20 h. After cooling 170 ml of a saturated aqueous potassium fluoride solution and 170 ml water is added, and the mixture is shaken vigorously for 20 h. Then the

organic layer is separated, dried over $MgSO_4$, and evaporated. The residue is purified by FCC (chloroform-methanol, 19:1) to give 104 mg (7%) of subarine (**2**) as a pale yellow powder, mp >300 °C (decomp.). IR: 2924, 1729, 1679, 1577, 1271, 1086, 759 cm^{-1}; MS (CI): 332 (100, M^++1), 272 (12); ^1H NMR ($CDCl_3$): δ 8.93 (d, 1H, J = 5.6 Hz, 1'-H), 8.86 (dd, J = 4.8, 1.7 Hz, 6-H), 8.52 (dd, 1H, J = 8.0, 1.7 Hz, 4-H), 8.24 (dd, 1H, J = 8.1, 1.2 Hz, 10'-H), 8.18 (d, 1H, J = 5.6 Hz, 1'-H), 7.56 (dd, 1H, J = 8.0, 4.8 Hz, 5-H), 7.52 (ddd, 1H, 8.2, 7.4, 1.2 Hz, 8'-H), 7.34 (ddd, 1H, J = 8.1, 7.4, 1.2 Hz, 9'-H), 6.83 (dd, 1H, J = 8.2, 1.2 Hz, 7'-H), 3.62 (s, 3H, CH_3). ^{13}C NMR ($CDCl_3$): δ 165.4, 161.6, 161.5, 161.3, 151.8, 150.4, 142.6, 138.2, 137.6, 131.7, 124.6, 123.9, 123.1, 122.3, 119.0, 116.6, 116.5, 115.5, 52.3. HRMS: calcd. for $C_{19}H_{13}N_3O_3$: 331.0918, found: 331.0957.

Authors' Statement

Competing Interests

The authors declare no conflict of interest.

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Eine kurze Totalsynthese des marinen Alkaloids Subarin

Zusammenfassung

Das Benzo[*c*][2,7]naphthyridin-Alkaloid Subarin wird in 4 Syntheseschritten ausgehend von kommerziell erhältlichem 1,10-Phenanthrolin synthetisiert. Schlüsselschritte sind die oxidative Spaltung des Phenanthrolins zur Bipyridindicarbonsäure, die Überführung ihres Dimethylesters in das Mono-2-iodanilid, sowie eine radikalische Cyclisierung zum Alkaloid Subarin. Der Naturstoff zeigt keine nennenswerte antimikrobielle oder zytotoxische Aktivität.