Tetrahedron 66 (2010) 4805–4810

Contents lists available at ScienceDirect

Tetrahedron

journal homepage: www.elsevier.com/locate/tet

Synthesis and bioactivity of (±)-tetrahydrohaliclonacyclamine A

Brian J. Smith [a], Tao Qu [b,d], Matthew Mulder [b], Meredith J. Noetzel [b], Craig W. Lindsley [a,b], Gary A. Sulikowski [a,c,*]

[a] Departments of Chemistry, Institute of Chemical Biology, Vanderbilt University, Nashville, TN 77842–3012, USA
[b] Departments of Pharmacology, Institute of Chemical Biology, Vanderbilt University, Nashville, TN 77842–3012, USA
[c] Departments of Biochemistry, Institute of Chemical Biology, Vanderbilt University, Nashville, TN 77842–3012, USA
[d] Departments of Chemistry, Texas A&M University, College Station, Texas 77482, USA

ARTICLE INFO

Article history:
Received 28 January 2010
Received in revised form 29 March 2010
Accepted 30 March 2010
Available online 3 April 2010

Keywords:
Marine alkaloid
Ring-closing metathesis
Stereoselective hydrogenation
Stille cross-coupling
Muscarinic M1 antagonist

ABSTRACT

The total synthesis of tetrahydrohaliclonacyclamine A (**5**) is described. A key step involves the hydrogenation of an unsaturated bis-piperidine incorporated into a 17-membered macrocycle to provide the *cis–syn–cis* stereochemistry common to haliclonacyclamines A–D. The hydrogenation product is advanced to the title compound following a five-step reaction sequence. Tetrahydrohaliclonacyclamine A is shown to bind to a variety of ion channels/GPCRs and act as a muscarinic M_1 antagonist.

1. Introduction

The alkylpiperidine alkaloids are a group of natural products isolated primarily from marine sponges and biosynthetically derived from nicotinic acid.[1,2] The simplest family members are monomeric and dimeric 3-alkylpyridines. Architecturally unique 3-alkylpiperidine alkaloids incorporate an array of ring systems including tricyclic, tetracyclic, and pentacyclic structures. Pentacyclic members have attracted attention from synthetic chemists culminating in the total syntheses of the manzamines and (−)-sarain A.[3,4] Little progress has been reported toward the total synthesis of tetracyclic class of alkylpiperidines such as haliclonacyclamines A–D (Fig. 1) despite their unique molecular structure and reported biological activity.[5] Tetracyclic alkaloids possess the general structure of a 3,4-linked bis-piperidine core appended to two aliphatic macrocycles as represented by the halicyclamines,[6] haliclonacyclamines,[7] arenosclerins,[8] and halichondramine.[9] Over sixteen tetracyclic class members have been isolated from a variety of marine sponges with the haliclonacyclamines constituting the largest sub-group.

Haliclonacyclamines A–D (**1**–**4**) share a common molecular constitution incorporating a core 3,4-bis-piperidine fused to ten (N1 to C7, **1**) and twelve (N2 to C2, **1**) carbon bridges. Haliclonacyclamines A–D differ in the number and position of double bonds located within bridging carbons. The bis-piperidine core of haliclonacyclamines A–D possess identical relative stereochemistry that we describe as *cis–syn–cis* configuration; cis denoting relative stereochemistry

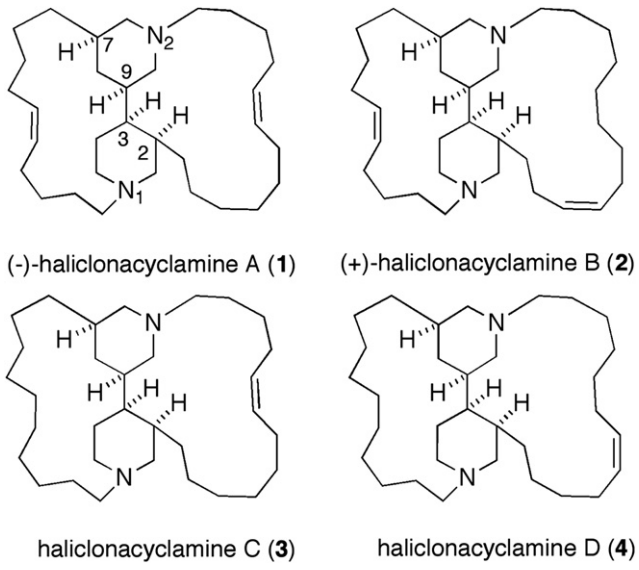


Figure 1.

* Corresponding author. E-mail address: gary.a.sulikowski@vanderbilt.edu (G.A. Sulikowski).

0040-4020/$ – see front matter © 2010 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tet.2010.03.117

within the piperidine rings and *syn* across C9 and C3 stereocenters interconnecting the piperidine rings (haliclonacyclamine numbering). Recently the absolute stereochemistry of haliclonacyclamines A and B has been assigned by Garson and co-workers as shown in Figure 1 based on single-crystal X-ray analysis.[7c] The Garson group has demonstrated hydrogenation of haliclonacyclamines A–C lead a common reduction product, tetrahydrohaliclonacyclamine A (**5**, Fig. 2), thereby confirming a common bis-piperidine core.[7a,b] Herein we provide a full account of our synthesis of the tetrahydrohaliclonacyclamine A (**5**) and preliminary studies into its activity against select ion channels and GPCR's.[10]

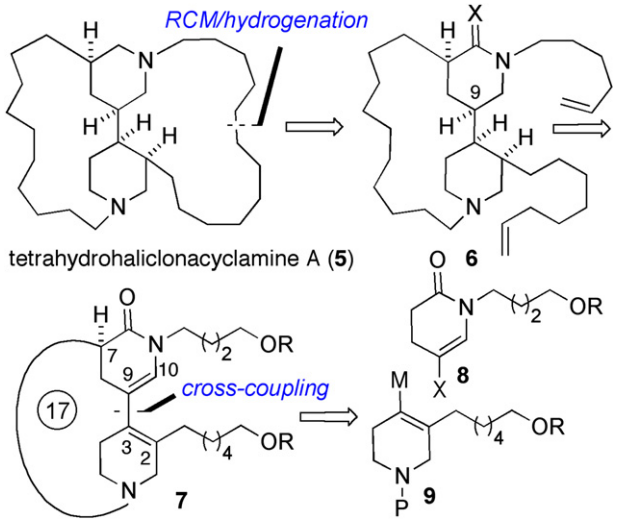
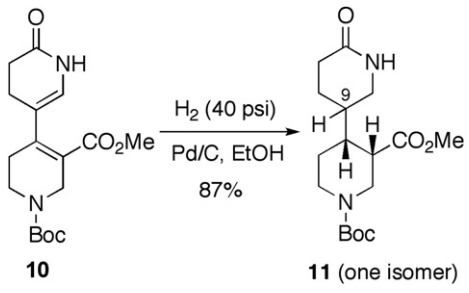


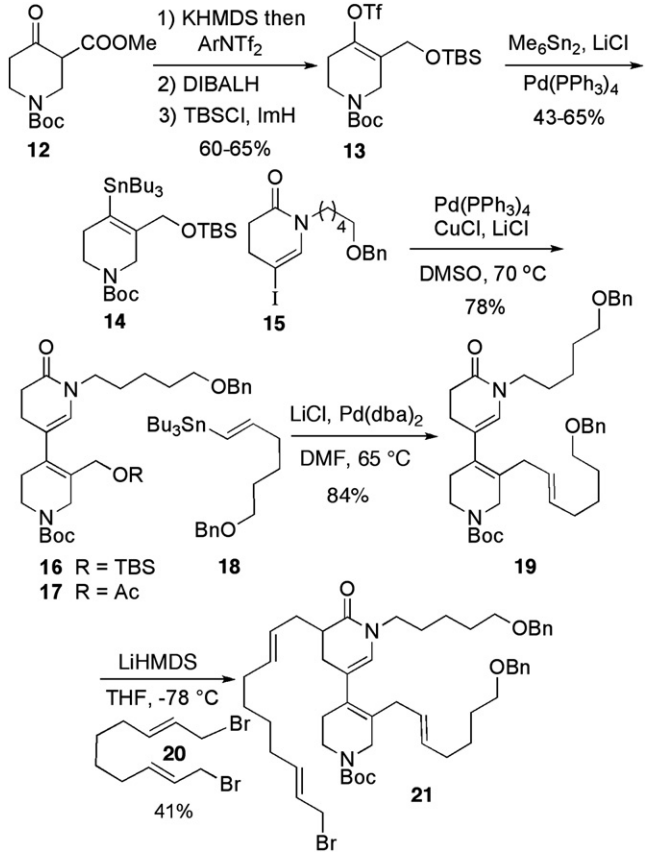
Figure 2.

2. Results and discussion

Our synthetic strategy to tetrahydrohaliclonacyclamine A (**5**) is shown retrosynthetically in Figure 2. We anticipated tetrahydrohaliclonacyclamine A (**5**) to be derived from bis-alkene **6** by employing a ring-closing metathesis reaction followed by alkene reduction.[11] In order to set the *cis–syn–cis* relative stereochemistry of the bis-piperidine core we elected to examine the hydrogenation of a 17-membered macrocycle (**7**). While the stereoselective hydrogenation of a macrocyclic diene represented by **7** was unprecedented, this approach was selected based in part upon the observed stereoselective hydrogenation of diene **10** that led to **11** as a single isomer (C9 stereochemistry not assigned).[12] Furthermore, we anticipated incorporation of an equivalent 1,3-diene into a 17-membered macrocycle (**7**) would enforce peripheral hydrogenation leading to the desired *cis–syn–cis* relationship C2/C3 and C7/C9.[13] Assembly of the macrocyclic **7** was envisioned to be initiated by Stille cross-coupling of a vinyl halide (**8**) and stannane (**9**) (Fig. 2).



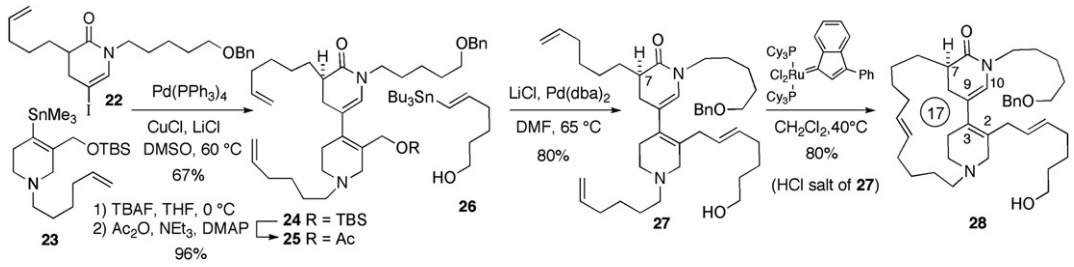
Our first approach to macrocycle **7** started from known β-ketoester **12**,[14] which was converted to stannane **14** by way of intermediate enol triflate **13**. To this end, the potassium enolate derived from **12** was reacted with Comins' triflating agent[15] and the resulting unsaturated ester reduced with DIBAL-H to afford the corresponding allyl alcohol that was protected as a TBS ether (**13**). Stille coupling of enol triflate **13** with hexamethyldistannane provided **14** in 43–65% yield.[16] The latter was coupled with iodoenamide **15**[10] to provide bis-piperidine **16** in 67% yield (Scheme 1).[17] The TBS ether was then exchanged for an acetate (TBAF, THF, 0 °C then Ac_2O, TEA, 86%) to set the stage for a third Stille cross-coupling this time between **17** and vinyl stannane **18**[18] to provide **19** in 84% yield.



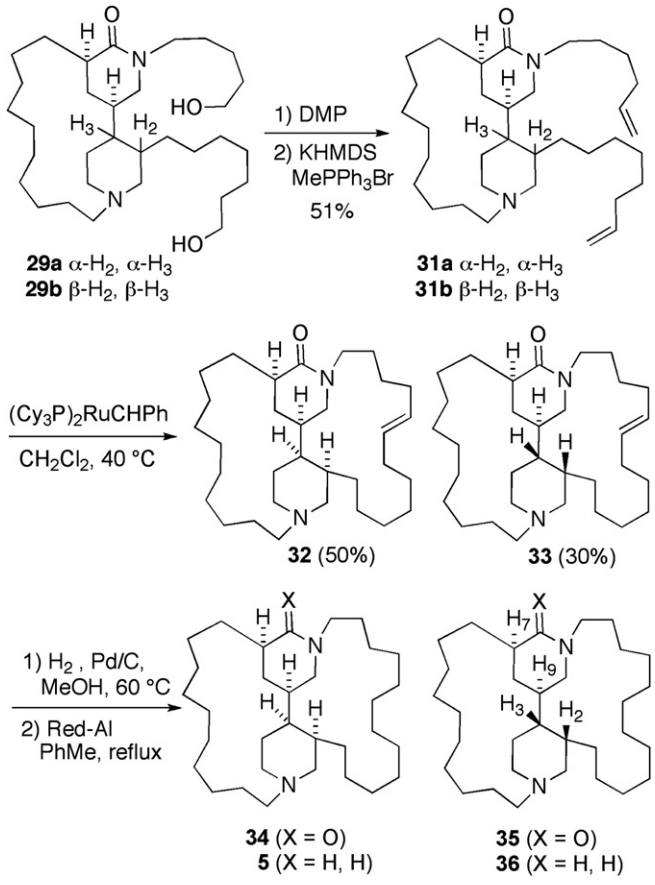
Scheme 1. First approach to 17-membered macrocycle.

Our initial approach to fashioning the 17-memberd macrocycle was to alkylate the enolate derived from **19** with (*E*,*E*)-dibromide **20**[19] followed by Boc removal and intramolecular N-alkylation. Unfortunately, while enolate alkylation proceeded in 41% yield we were unable to remove the Boc protecting group under a variety of acidic and thermal conditions observing instead decomposition. Rather then examining other nitrogen protecting groups we turned our attention to a ring-closing metathesis (RCM) strategy to form the key 17-membered macrocycle (Scheme 2).

To this end, bis-piperidine **27**, the substrate required for the RCM reaction leading to 17-membered ring formation, was prepared starting from iodoenamide **22** and vinyl stannane **23**.[10] The four-step reaction sequence leading to **27** utilized two Stille cross-coupling reactions in a manner identical to the coupling series described earlier in Scheme 1. With all carbons now in place we turned our attention to formation of the 17-membered macrocycle spanning C7 and the southern piperidine nitrogen. Initially, we found the RCM reaction failed to proceed using the free amine, leading primarily to unreacted starting material. Fortunately, when the derived hydrochloride salt of **27** was treated with Fürstner's



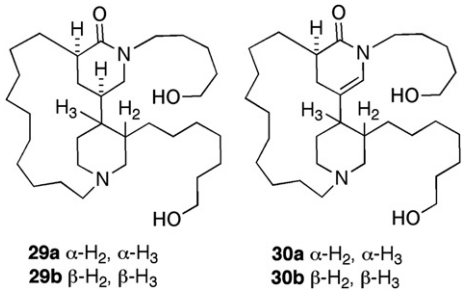
Scheme 2.



Scheme 3.

ruthenium indenylidene catalyst in refluxing dichloromethane macrocyle **28** was obtained in 80% yield (>90% trans).[20] The RCM reaction proceeded with equal efficiency when employing Grubbs first-generation catalyst.[21]

With the key 17-membered macrocycle in hand, we next examined a range of reductive conditions to effect benzyl ether hydrogenolysis and alkene saturation of macrocycle **28**. Homogeneous hydrogenation of **28** employing Wilkinson catalysis primarily produced starting material accompanied by hydrogenation of one alkene as determined by LC/MS analysis. Hydrogenation of the TFA salt of **28** under one atmosphere hydrogen in methanol over Pearlman's catalyst led to variable results. However, when the same reaction was repeated in ethanol at 500 psi at a temperature of 70 °C for 8 days a reproducible 1.3:1 mixture of two isomers tentatively assigned the structures of **29a** and **29b** was produced in 79% yield. It was further determined if the reduction was prematurely stopped enamide **30** was isolated as an approximate 1:1 mixture of **30a** and **30b**. These observations led us to conclude hydrogenation of the C2–C3 carbon–carbon double bond occurred first in a non-stereoselective fashion followed by a stereoselective reduction of the C9–C10 enamide olefin, leading to a tentative assignment of the reduction products as *cis–syn–cis* (**29a**) and *cis–anti–cis* (**29b**). The non-stereoselective reduction of the C2–C3 olefin of the southern piperidine is attributed to the incorporation of a stereochemically dynamic tertiary amine that due to rapid inversion equally exposes the diastereotopic faces of the C2–C3 to the periphery of the macrocyclic structure and hydrogen addition (Scheme 2).



The mixture of diols (**29**) was oxidized with Dess–Martin periodinane to give a moderately stable bis-aldehyde and without purification was treated with 10 equiv of methylenetriphenylphosphorane to provide bis-alkene **31** (Scheme 3). The tetracyclic ring system common to the haliclonacyclamines was completed upon treatment of the TFA salt derived from **31** with Grubbs' first-generation catalyst to provide **32** (6:1 *trans*/*cis*) and **33** (>95:5 *trans*/*cis*), separated by flash chromatography. Each isomer was subjected to alkene hydrogenation (100 psi, $Pd(OH)_2$, EtOH) followed by lactam reduction (Red-Al, PhMe, reflux). Hydrogenation of **32** provided lactam **34**, which was subjected to a single-crystal X-ray analysis revealed the *cis–syn–cis* stereochemistry of the bis-piperidine core common to haliclonacyclamines A–D (Fig. 1).[10] Indeed Red-Al reduction of **34** followed by passing the reaction product thru and SCX column provided tetrahydrohaliclonacyclamine A (**5**), identical to an authentic sample by ^1H and ^{13}C NMR comparison.[22] The *cis–anti–cis* isomer (**33**) was converted to amine **36** by way of the same two-step reduction process (Scheme 3). The stereochemistry of amine **36** was assigned based on NMR analysis. Examination of the NOSY spectrum of **36** revealed significant NOE between H7–H9 and H2–H3 (i.e., cis stereochemistry). This observation supported the stereochemical assignment of bis-piperidine core of **36** as *cis–anti–cis*; partly by the process of elimination as isomer **5** possessed the *cis–syn–cis* relationship.

Haliclonacylamines A and B have been reported to display antibiotic, antifungal, and cytotoxic properties.[7a] Berlinck and co-workers have also reported on the antibiotic and cytotoxic properties of haliclonacyclamine E and structurally related arenosclerins A–C.[23] We submitted the bis-TFA salt of tetrahydrohaliclonacyclamine A (**5**) to a panel of ion channel and GPCR receptors for evaluation in radioligand biochemical assays.[24] At a concentration of 10 μM tetrahydrohaliclonacyclamine A (**5**) inhibited radioligand binding of a variety of receptors including muscarinic M_1 (82%), opiate k (83%), and

potassium channel hERG (98%). Since ligand binding assays provide no information on functional activity (i.e., agonist or antagonist effects), we then performed a functional assay on the hM_1 mAChR with tetrahydrohydrohaliclonacyclamine A and the unnatural *cis–anti–cis* isomer (**36**). Interestingly, tetrahydrohaliclonacyclamine A (**5**) was found to be a full antagonist (ACh EC_{80} reduced to baseline) of hM_1 (albeit with modest potency, EC_{50}=5 μM). The isomeric bis-piperidine (**36**) was a partial antagonist (ca. 60% of an ACh EC_{80}) with similar potency.

3. Conclusion

The synthesis of the tetrahydrohydrohaliclonacyclamine A (**5**) and the unnatural *cis–anti–cis* isomer (**36**) have been completed in 16 steps (longest linear sequence). The former was determined to bind to a range of ion channels and receptors, likely related to its reported cytotoxic properties. In a functional assay tetrahydrohaliclonacyclamine A was shown to act as an antagonist against the muscarinic M_1 receptor.

4. Experimental

4.1. General

All non-aqueous reactions were performed in flame-dried or oven dried round-bottomed flasks under an atmosphere of argon. Reaction temperatures were controlled using a thermocouple thermometer and analog hotplate stirrer. Reactions were conducted at room temperature (rt, approximately 23 °C) unless otherwise noted. Flash column chromatography was conducted using silica gel 230–400 mesh. Where necessary, silica gel was neutralized by treatment of the silica gel prior to chromatography with the eluent containing 1% triethylamine. Where indicated ammonium salts were converted to free-amines using Strong Cation Exchange (SCX) cartridges purchased from Varian. Analytical thin-layer chromatography (TLC) was performed on E. Merck silica gel 60 F_{254} plates and visualized using UV, ceric ammonium molybdate, potassium permanganate, and anisaldehyde stains. Yields were reported as isolated, spectroscopically pure compounds. ^{1}H NMR spectra were recorded on Bruker 300, 400, 500, or 600 MHz spectrometers and are reported relative to deuterated solvent signals. ^{13}C NMR spectra were recorded on Bruker 75, 100, 125, or 150 MHz spectrometers and are reported relative to deuterated solvent signals. LC/MS was conducted and recorded on an Agilent Technologies 6130 Quadrupole instrument. High-resolution mass spectra were obtained from the Department of Chemistry and Biochemistry, University of Notre Dame using either a JEOL AX505HA or JEOL LMS-GCmate mass spectrometer or from Vanderbilt Institute of Chemical Biology Drug Discovery Program laboratory using a Waters Acquity UPLC and Micromass Q-Tof Ultima API.

4.2. Stille cross-coupling of 22 and 23

A solution of vinyl stannane **22** (2.47 g, 5.23 mmol) and vinyl iodide **23** (2.05 g, 4.26 mmol) in dimethyl sulfoxide (38 mL) was treated with copper chloride (2.26 g, 22.8 mmol), lithium chloride (1.15 g, 27.3 mmol, flame dried under argon), and tetrakis(triphenylphosphine)palladium (526 mg, 0.46 mmol) at room temperature. The mixture was immediately degassed (3×) under high vacuum with an argon purge. The mixture was then warmed to room temperature and stirred for 2 h, followed by heating to 60 °C for 14 h. The black mixture was quenched with brine, and the resulting solution was filtered over Celite. The filtrate was washed with brine (100 mL) and 5% ammounium hydroxide (5 mL), extracted with ethyl acetate (4×50 mL), and the combined organic extracts were washed again with brine (25 mL). The combined extracts were dried over $MgSO_4$, filtered, and concentrated. The residue was purified by flash column chromatography on silica gel (gradient elution: 2:1 to 1:1 to 1:1:0.01 hexanes/ethyl acetate/triethylamine) to yield bis-piperidine **24** (1.90 g, 67%) as a yellow oil: IR (neat) 2930, 2855, 1670, 1403 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.32–7.26 (m, 5H), 5.91 (s, 1H), 5.79 (m, 2H), 4.94 (m, 4H), 4.47 (s, 2H), 4.13 (s, 2H), 3.43 (m, 4H), 3.04 (m, 2H), 2.54 (m, 2H), 2.40–2.36, (m, 2H), 2.20 (m, 2H), 2.10–2.02 (m, 5H), 1.82, (m, 2H), 1.62–1.50 (m, 6H), 1.43–1.31 (m, 8H), 0.88 (s, 9H), 0.02 (s, 6H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 171.1, 138.8, 138.6, 138.5, 131.0, 130.3, 128.3, 127.5, 127.4, 126.8, 116.7, 114.4, 114.3, 72.8, 70.1, 62.5, 58.4, 54.8, 50.2, 46.2, 40.4, 33.6, 33.5, 29.8, 29.3, 29.1, 28.9, 28.8, 28.5, 26.8, 26.6, 26.5, 25.8, 23.3, 18.2, −5.8. HRMS calculated for $C_{41}H_{67}N_2O_3Si$ $(M+H)^+$ *m/z*: 663.4954, measured: 663.4921.

4.3. Stille cross-coupling of 25 and 26

To a solution of allylic acetate **25** (2.50 g, 4.23 mmol) in dimethylformamide (35 mL) at room temperature was added (*E*)-6-(tributylstannyl)hex-5-en-1-ol (**26**) (2.47 g, 6.34 mmol), lithium chloride (888 mg, 21.2 mmol), and bis(dibenzylidieneacetone)palladium (245 mg, 0.42 mmol). The mixture was heated to 65 °C and stirred for 22 h total with 0.01 equiv of bis(dibenzylidieneacetone)palladium (24.5 mg, 0.04 mmol) added after 14 h. The reaction was filtered through Celite and the filtrate was washed with brine (20 mL). The aqueous layer was extracted with ethyl acetate (3×40 mL) and the combined organic extracts were washed with brine (2×20 mL), dried over $MgSO_4$, filtered, and concentrated. The residue was purified by flash column chromatography on silica gel (gradient elution with 2:1 to 1:1:0.01 hexanes/ethyl acetate/triethylamine) to yield tetraene **27** (2.13 g, 80%) as a yellow oil. IR (neat) 3625, 2928, 2856, 1666, 1404 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.30–7.24 (m, 5H), 5.85 (s, 1H), 5.78 (m, 2H), 5.00–4.92 (m, 4H), 4.46 (s, 2H), 3.57 (m, 2H), 3.43–3.32 (m, 4H), 2.88 (m, 2H), 2.73 (m, 2H), 2.52, (m, 2H), 2.39–2.35 (m, 3H), 2.18 (m, 2H), 2.18, (m, 2H), 2.07–2.02 (m, 6H), 1.62–1.50 (m, 10H), 1.41–1.33 (m, 12)H; ^{13}C NMR ($CDCl_3$, 100 MHz) δ 171.1, 138.8, 138.6, 138.5, 134.4, 129.3, 129.0, 128.5, 128.3, 127.6, 127.5, 125.8, 117.6, 114.4, 114.3, 72.8, 70.1, 62.5, 58.3, 56.0, 50.4, 46.2, 40.5, 35.5, 33.5, 33.5, 32.2, 29.8, 29.6, 29.4, 29.2, 28.8, 28.5, 27.8, 26.9, 26.8, 26.5, 25.6, 23.3, 17.5, 13.5. HRMS calculated for $C_{41}H_{63}N_2O_3$ $(M+H)^+$ *m/z*: 631.4839, measured: 631.4850.

4.4. Ring-closing metathesis of 27

A hydrochloric acid solution (5 mL of 2 M solution in ether) was added to a solution of tetraene **27** (220 mg, 0.35 mmol) in dichloromethane (5 mL), and the resulting solution was stirred for 30 min at ambient temperature. The solution was then concentrated and dried in vacuo. The viscous, bright yellow hydrochloride salt was dissolved in dichloromethane (1 L) and bis(tricyclohexylphosphine)-3-phenyl-1*H*-inden-1-ylidene ruthenium(II) dichloride (32 mg, 0.035 mmol) was quickly added in one portion. The solution was brought to reflux for 2 h, at which point an additional (32 mg, 10 mol%) of the catalyst was added. The solution was maintained at reflux for an additional 20 h, cooled to room temperature and concentrated. The resulting residue was dissolved in methanol and passed through a Varian SCX ion exchange column to remove ruthenium byproducts, followed by eluting diene **28** with 2 N ammonia in methanol to release the free amine. Additionally, the residue was purified by Biotage (KP-C18-HS) reverse phase chromatography eluting with H_2O (0.1% TFA)/Acetonitrile (20–60% acetonitrile over 10 column volumes). The aqueous fractions were concentrated by Genevac and the resulting material analyzed by LC/MS to yield 134 mg, 64% of diene **28**. The material was typically kept as the trifluoroacetic acid salt for use in the next step. The salt was converted to the free amine passage through a Varian SCX ion

exchange column for calculation of yield and analytical data. IR (neat) 3530, 2932, 2846, 1666 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 7.30–7.24 (m, 5H), 5.87 (s, 1H), 5.44–5.30 (m, 4H), 4.46 (s, 2H), 3.62–3.57 (m, 2H), 3.44 (app. t, J=6.4 Hz, 2H), 3.32 (m, 2H), 3.06 (m, 2H), 2.73 (m, 2H), 2.60 (m, 2H), 2.50, (m, 1H), 2.37 (m, 2H), 2.07–2.02 (m, 6H), 1.61–1.52 (m, 10H), 1.42–1.35 (m, 7H), 1.23 (m, 6H), 0.88–0.83 (m, 2H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 172.2, 133.1, 132.7, 132.0, 131.9, 130.8, 130.7, 130.6 (2C), 128.7, 128.2, 128.0, 127.9, 73.3, 70.5, 62.8, 51.8, 46.4, 41.1, 36.2, 32.8, 32.7, 31.9, 31.8, 31.0, 30.1 (2C), 29.8, 28.9 (2C), 27.6, 27.5, 26.2, 26.1 (2C), 25.1, 23.8. HRMS calculated for $C_{39}H_{59}N_2O_3$ $(M+H)^+$ m/z: 603.4526, measured: 603.4528.

4.5. Hydrogenation of 28

The trifluoroacetic acid salt of tetraene **28** (340.0 mg, 0.56 mmol, free amine weight) was dissolved in ethanol (30 mL), treated with palladium hydroxide (79.0 mg, 0.11 mmol), and transferred to a Parr hydrogenator. Once the vessel was tightly secured, the solution was purged with hydrogen, evacuated, and back-filled a total of five times. The pressure was set to 500 psi and the mixture was heated to 70 °C with vigorous stirring. The progress of the reaction mixture was monitored by LC/MS. After 8 days the reaction was filtered through Celite and concentrated. The resulting residue was purified by reverse phase HPLC chromatography eluting with H_2O (0.1% TFA)/Acetonitrile (12–40% acetonitrile) to afford a non-separable mixture of **29a** and **29b**. Fractions were analyzed using LC/MS and concentrated by Genevac. The products were converted to their free amines by passing through a Varian SCX ion exchange column by eluting first with methanol then 2 N ammonia in methanol to release 230 mg [79% yield of **29a** and **29b** (1.3:1 determined by ^{13}C NMR), characterized as a mixture]: IR (neat) 3400, 2920, 1622, 1494, 1461 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 3.61 (app. t, J=6.4 Hz, 4H), 3.37–3.27 (m, 2H), 3.23–3.17 (m, 1H), 3.03 (m, 1H), 2.85–2.77 (m, 3H), 2.65–2.53 (m, 5H), 2.26 (m, 1H), 2.11 (m, 2H), 1.93–1.86 (m, 3H), 1.67 (m, 1H), 1.63–1.55 (9H), 1.38–1.26 (28H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 172.1, 171.9 (two diastereomers); 62.6 (2C); 62.3, 56.9, 56.8, 54.5, 53.9, 52.9, 52.2, 47.2, 47.0, 46.0, 42.0, 41.6, 41.0, 40.1, 39.9, 36.0, 34.7, 34.6, 33.7, 33.3, 32.9, 32.6, 32.5, 32.2, 31.4, 30.9, 30.6, 29.7, 29.3 (2C), 28.1, 27.9, 27.5, 27.4, 27.2, 27.1 (2C), 26.9 (2C), 26.6, 26.5, 26.3, 26.1, 25.9, 25.7 (2C), 25.3, 22.8, 21.8, 21.6. HRMS calculated for $C_{32}H_{61}N_2O_3$ $(M+H)^+$ m/z: 521.4682, measured: 521.4682.

4.6. Ring-closing metathesis of 31

To a solution of dienes **31a**/**31b** (20.0 mg, 0.039 mmol, free amine weight) in dichloromethane (2.0 mL) was added trifluoroacetic acid (two drops). The solution was stirred for 30 min and concentrated. The residue was then dissolved in dichloromethane (250 mL) and bis(tricyclohexylphosphine)benzylidine ruthenium(IV) chloride (3.3 mg, 0.004 mmol) was added. The solution was refluxed for 2 h, cooled to room temperature, and treated with additional catalyst (3.3 mg, 0.004 mmol). This solution was heated at reflux for 16 h and concentrated. The resulting residue was purified by a SCX ion exchange column; eluting with methanol, then 2 N ammonia in methanol. This residue was further purified by reverse phase HPLC chromatography eluting with H_2O (0.1% TFA)/Acetonitrile (30–60% acetonitrile). The resulting fractions were concentrated by Genevac. The purified TFA salt was converted to the free amine by running the residue through an SCX ion exchange column. The fractions were concentrated and the two pure diastereomers were separated by flash column chromatography on silica gel (eluent 3:6.5:0.25 hexanes/ethyl acetate/triethylamine) to yield 9.5 mg of **26** and 5.6 mg of **27** (80% combined yield).

Compound **32**: light yellow oil: IR (neat) 2925, 2853, 1639 cm^{-1}. ^1H NMR ($CDCl_3$, 600 MHz) δ 5.31–5.23 (m, 2H), 4.34 (m, 1H), 3.53 (app t, J=12.0 Hz, 1H), 2.99 (m, 1H), 2.87 (app. t, J=10.8 Hz, 1H), 2.73 (m, 1H), 2.71–2.58 (m, 4H), 2.37 (m, 1H), 2.25 (m, 1H), 2.07–1.95 (m, 7H), 1.69 (m, 1H), 1.71 (m, 2H), 1.54 (m, 4H), 1.42–1.24 (m, 29H); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 171.8, 131.0, 130.8, 57.0, 54.7, 52.3, 47.7, 46.3, 42.7, 42.0, 41.4, 36.0, 35.2, 34.2, 33.1, 32.4, 31.6, 31.3, 29.6, 28.4, 28.2, 27.4, 27.3, 27.2, 27.1, 27.1, 26.8, 26.5, 26.3, 26.2, 25.6, 21.6. HRMS calculated for $C_{32}H_{57}N_2O$ $(M+H)^+$ m/z: 485.4471, measured: 485.4471.

Compound **33**: light yellow oil: IR (neat) 2923, 2851, 1644 cm^{-1}; ^1H NMR ($CDCl_3$, 600 MHz) δ 5.31–5.25 (m, 2H), 4.14 (m, 1H), 3.18, (m, 1H), 2.99 (app. t, J=11.4 Hz, 1H) 2.77 (m, 1H), 2.68 (m, 3H), 2.55 (m, 1H), 2.37 (app t, J=11.4 Hz, 1H), 2.29 (m, 2H), 2.17 (m, 1H), 2.05–1.97 (m, 5H), 1.84 (m, 3H), 1.51 (m, 3H), 1.33–1.29 (m, 32H); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 171.7, 131.1, 130.1, 56.7, 55.3, 52.4, 47.4, 47.1, 41.0, 40.8, 32.3, 32.3, 31.7, 31.6, 31.4, 30.5, 29.7, 29.3, 28.3, 28.0, 27.6, 27.4, 27.3, 27.2, 27.2, 27.0, 26.9, 26.8, 26.5, 26.4, 26.0. HRMS calculated for $C_{32}H_{57}N_2O$ $(M+H)^+$ m/z: 485.4471, measured: 485.4469.

4.7. Tetrahydrohaliclonacyclamine A

A solution of lactam **34** (20.0 mg, 0.041 mmol) in toluene (4 mL) was cooled to 0 °C and sodium bis(2-methoxyethoxy)aluminium hydride (130 μL, 0.410 mmol of a 65 wt.% solution in toluene) was added dropwise. The solution was then placed into a pre-heated oil bath at 130 °C and stirred for 6 h. The resulting solution was cooled to 0 °C and slowly quenched with a saturated aqueous solution of potassium sodium tartarate (4 mL) and stirred for 5 min. The mixture was then diluted with ethyl acetate, warmed to room temperature, and stirred for 30 min. The aqueous layer was extracted with ethyl acetate (4×10 mL) and the combined extracts were dried over $MgSO_4$, filtered, and concentrated. The residue was purified by passing through a Varian SCX ion exchange column by eluting first with methanol then 2 N ammonia in methanol. After concentration, the residue was subjected to flash column chromatography on silica gel eluting with 3:6.5:0.5 (hexanes/ethyl acetate/triethylamine) to yield tetrahydrohaliclonacyclamine A (**4**) (15.8 mg, 90%). ^1H NMR ($CDCl_3$, 600 MHz) δ 2.96 (app. t, J=11.1 Hz, 1H), 2.80–2.79 (m, 2H), 2.75–2.64 (m, 3H), 2.59 (m, 1H), 2.55–2.51 (m, 1H), 2.46–2.39 (m, 3H), 2.15 (app. t, J=11.2 Hz, 1H), 1.95 (m, 1H), 1.85–1.76 (m, 4H), 1.72–1.69 (m, 2H), 1.53–1.50 (m, 5H), 1.39–1.20 (m, 34H), 0.92–0.88 (m, 2H); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 60.7, 60.3, 58.4, 57.1, 53.2, 47.0, 45.5, 41.4, 38.3, 37.8, 36.4, 35.6, 34.1, 33.5, 29.3, 27.9, 27.8 (2C), 27.7, 27.6, 27.2, 27.1, 26.8 (2C), 26.5, 26.3, 26.1, 25.7 (2C), 25.6, 22.0, 21.5. HRMS calculated for $C_{32}H_{61}N_2$ $(M+H)^+$ m/z: 473.4835, measured: 473.4833.

Acknowledgements

We thank the National Institutes of Health (GM067726) and the Vanderbilt Institute of Chemical Biology for their support of this research. We also thank Joseph Reibenspies (Center for Chemical Characterization and Analysis, Texas A&M University) for determining the X-ray crystal structure of 32. We gratefully acknowledge Professor Mary Garson (University of Queensland) for providing a sample of tetrahydrohaliclonacyclamine A and spectral data.

Supplementary data

Supplementary data associated with this article can be found in the online version, at doi:10.1016/j.tet.2010.03.117.

References and notes

1. (a) Berlinck, R. G. S. *Top. Heterocycl. Chem.* **2007**, *10*, 211–238; (b) Andersen, R. J.; Van Soest, R. W. M.; Kong, F. In *Alkaloids: Chemical and Biological Perspectives*; Pelliter, S. W., Ed.; Pergamon: London, 1996; Vol. 10, pp 301–355.

2. (a) Cutignano, A.; Cimino, G.; Giordano, A.; D'Ippolito, G.; Fontana, A. *Tetrahedron Lett.* **2004**, *45*, 2627–2629; (b) Cutignano, A.; Tramice, A.; De Caro, S.; Villani, G.; Cimino, G.; Fontana, A. *Angew. Chem., Int. Ed.* **2003**, *42*, 2633–2636.
3. (a) Humphrey, J. M.; Liao, Y. S.; Ali, A.; Rein, T.; Wong, Y. L.; Chen, H. J.; Courtney, A. K.; Martin, S. F. *J. Am. Chem. Soc.* **2002**, *124*, 8584–8592; (b) Winkler, J. D.; Axten, J. M. *J. Am. Chem. Soc.* **1998**, *120*, 6425–6426.
4. Garg, N. K.; Hiebert, S.; Overman, L. E. *Angew. Chem., Int. Ed.* **2006**, *45*, 2912–2915.
5. (a) Gil, L.; Gateauolesker, A.; Marazano, C.; Das, B. C. *Tetrahedron Lett.* **1995**, *36*, 707–710; (b) Sanchez-Salvatori, M. D.; Marazano, C. *J. Org. Chem.* **2003**, *68*, 8883–8889; (c) Wypych, J. C.; Nguyen, T. M.; Nuhant, P.; Benechie, M.; Marazano, C. *Angew. Chem., Int. Ed.* **2008**, *47*, 5418–5421.
6. (a) Harrison, B.; Talapatra, S.; Lobkovsky, E.; Clardy, J.; Crews, P. *Tetrahedron Lett.* **1996**, *37*, 9151–9154; (b) Jaspars, M.; Pasupathy, V.; Crews, P. *J. Org. Chem.* **1994**, *59*, 3253–3255.
7. (a) Charan, R. D.; Garson, M. J.; Brereton, I. M.; Willis, A. C.; Hooper, J. N. A. *Tetrahedron* **1996**, *52*, 9111–9120; (b) Clark, R. J.; Field, K. L.; Charan, R. D.; Garson, M. J.; Brereton, I. M.; Willis, A. C. *Tetrahedron* **1998**, *54*, 8811–8826; (c) Mudianta, I. W.; Garson, M. J.; Bernhardt, P. V. *Aust. J. Chem.* **2009**, *62*, 667–670.
8. (a) de Oliveira, J.; Nascimento, A. M.; Kossuga, M. H.; Cavalcanti, B. C.; Pessoa, C. O.; Moraes, M. O.; Macedo, M. L.; Ferreira, A. G.; Hajdu, E.; Pinheiro, U. S.; Berlinck, R. G. S. *J. Nat. Prod.* **2007**, *70*, 538–543; (b) Torres, Y. R.; Berlinck, R. G. S.; Magalhaes, A.; Schefer, A. B.; Ferreira, A. G.; Hajdu, E.; Muricy, G. *J. Nat. Prod.* **2000**, *63*, 1098–1105.
9. Chill, L.; Yosief, T.; Kashman, Y. *J. Nat. Prod.* **2002**, *65*, 1738–1741.
10. Preliminary communication Smith, B. J.; Sulikowski, G. A. *Angew. Chem., Int. Ed.* **2010**, *49*, 1599–1602.
11. For reviews,see: (a) Grubbs, R. H.; Miller, S. J.; Fu, G. C. *Acc. Chem. Res.* **1995**, *28*, 446–452; (b) Grubbs, R. H.; Chang, S. *Tetrahedron* **1998**, *54*, 4413–4450; (c) Martin, S. F. *Pure Appl. Chem.* **2005**, *77*, 4490–4527; (d) Nicolaou, K. C.; Bulger, P. G.; Sarlah, D. *Angew. Chem., Int. Ed.* **2005**, *44*, 4490–4527.
12. Qu, T.M.S. Thesis, Texas A&M University, August 2004.
13. For examples of stereocontrol employing peripheral functionalization of medium and large rings, see: (a) Still, W. C. *J. Am. Chem. Soc.* **1979**, *101*, 2493–2495; (b) Vedejs, E.; Gapinski, V. *J. Am. Chem. Soc.* **1983**, *105*, 5058–5061; (c) Schreiber, S. L.; Sammakia, T.; Hulin, B.; Schulte, G. *J. Am. Chem. Soc.* **1986**, *108*, 2106–2108; (d) Vedejs, E.; Dent, W. H.; Gapinski, D. M.; McClure, C. K. *J. Am. Chem. Soc.* **1987**, *109*, 5437–5438; (e) Mulzer, J.; Kirstein, H. M.; Buschmann, J.; Lehmann, C.; Luger, P. *J. Am. Chem. Soc.* **1991**, *113*, 910–923; (f) Neeland, E. G.; Ounsworth, J. P.; Sims, R.; Weiler, L. *J. Am. Chem. Soc.* **1994**, *59*, 7383–7394.
14. Christoffers, J.; Scharl, H. *Eur. J. Org. Chem.* **2002**, 1505–1508.
15. Comins, D. L.; Dehghani, A. *Tetrahedron Lett.* **1992**, *33*, 6299–6302.
16. (a) Scott, W. J.; Stille, J. K. *J. Am. Chem. Soc.* **1986**, *108*, 3033–3040; (b) Wulff, W. D.; Peterson, G. A.; Bauta, W. E.; Chan, K.-S.; Faron, K. L.; Gilbertson, S. R.; Kaesler, R. W.; Yang, D. C.; Murray, C. K. *J. Org. Chem.* **1986**, *51*, 277–279.
17. Han, X.; Stoltz, B. M.; Corey, E. J. *J. Am. Chem. Soc.* **1999**, *121*, 7600–7605.
18. (a) Kalivretenos, A.; Stille, J. K.; Hegedus, L. S. *J. Org. Chem.* **1991**, *56*, 2883–2894; (b) Del Valle, L.; Stille, J. K.; Hegedus, L. S. *J. Org. Chem.* **1990**, *55*, 3019–3023.
19. Tokuda, M.; Satoh, K.; Suginome, H. *Bull. Chem. Soc. Jpn.* **1987**, *60*, 2429–2433.
20. Fürstner, A.; Grabowski, J.; Lehmann, C. W. *J. Org. Chem.* **1999**, *64*, 8275–8280.
21. Fu, G. C.; Nguyen, S. T.; Grubbs, R. H. *J. Am. Chem. Soc.* **1993**, *115*, 9856–9857.
22. We are very grateful to Professor Mary Garson (University of Queensland) for providing an authentic sample of tetrahydrohaliclonacyclamine C.
23. Torres, Y. R.; Berlinck, R. G. S.; Nascimento, G. G. F.; Fortier, S. C.; Pessoa, C.; de Moraes, M. O. *Toxicon* **2002**, *40*, 885–891.
24. Screening was conducted at MDS Pharma Services.