Total Synthesis of Spiroketal Alkaloids Lycibarbarines A–C

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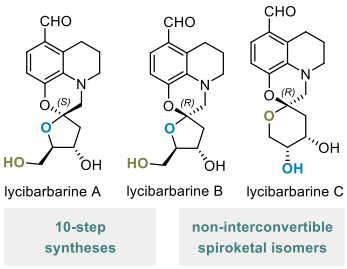
Supporting Information Placeholder

ABSTRACT: Lycibarbarines A–C are spirocyclic alkaloids with a unique tetracyclic framework, consisting of tetrahydroquinoline and spiro-fused oxazine-sugar spiroketal subunits. The first total syntheses of lycibarbarines A–C are reported, achieved over 10 steps (longest linear sequence) each. Through this work, it was discovered that the spiroketal unit of lycibarbarines A–C exhibits unusually high resistance to acid-mediated isomerisation and epimerisation, likely due to the basic nitrogen atom. As such, the lycibarbarines present an interesting case study of preventing the interconversion of spiroketal isomers, which may prove instructive in efforts to obtain non-thermodynamic spiroketal frameworks.

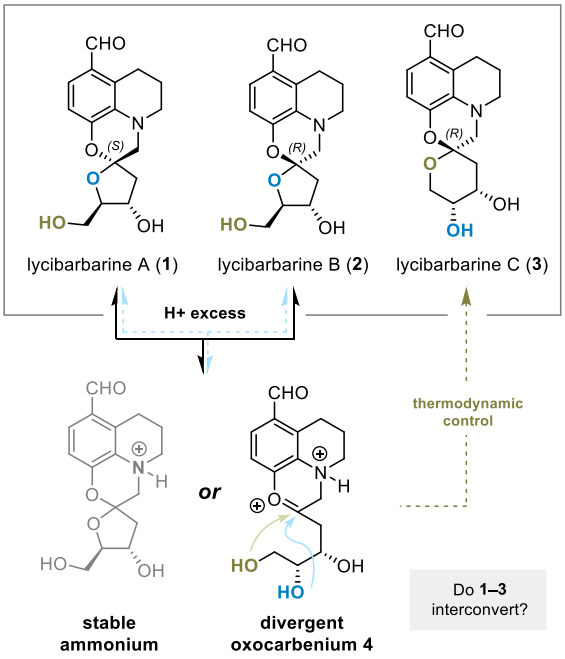


Lycibarbarines A–C (**1**–**3**) are spiroketal alkaloids isolated in 2021 by Chen *et al* from the fruits of *Lycium barbarum*, also known as goji berries (Scheme 1).[1] Goji berries have been used in traditional Chinese medicine for over 2000 years for the treatment of age-related conditions, including improved eyesight, improved liver and kidney functions, as well as anti-oxidant, immunomodulating, and antitumor activity.[2] Lycibarbarines A (**1**) and C (**3**) demonstrated neuroprotective activity by reduction of apoptosis in corticosterone-injured PC12 cells, and were found to reduce the expression levels of cleaved caspase-3 and caspase-9.[1] Lycibarbarines A–C (**1**–**3**) possess a unique tetracyclic structure, consisting of tetrahydroquinoline and spiro-fused oxazine-sugar spiroketal subunits. The oxazine-sugar spiroketal moiety is similar to the pyrrolomorpholine spiroketal compound class,[3,4] all of which demonstrate inhibition of high-glucose induced reactive oxygen species production in mesangial cells.[3,5]

Our group has had a longstanding interest in spiroketal-containing natural products, due to their attractive bioactivities,[3,6,7] and the privileged nature[8–10] of the spiroketal scaffold. We have accomplished the total synthesis of several spiroketal-containing natural products,[11–15] including acortatarin A[16] and pollenopyrroside A,[17] which share structural similarities with **1**–**3**.

We were intrigued by the lycibarbarines because they appeared to represent kinetic [5,6]- and thermodynamic [6,6]-spiroketal products derived from the same oxocarbenium precursor **4** (Scheme 1). This suggested **1**–**3** would likely interconvert under acidic conditions, or at least that the kinetic products (**1** and **2**) could potentially be isomerised to the thermodynamically-favored lycibarbarine C (**3**). This would be in line with the known tendency of spiroketals to epimerise or isomerise under mildly acidic conditions,[18–21] to the thermodynamically favored isomer, stabilised by the anomeric effect, minimisation of steric interactions, and intramolecular hydrogen bonding effects.[7,22] Whilst various synthetic strategies have been developed to access non-thermodynamic spiroketal frameworks,[22,23] such scaffolds remain labile to isomerisation under acidic conditions.

Scheme 1. Lycibarbarines A–C (**1**–**3**) and access from the same oxocarbenium precursor **4**.

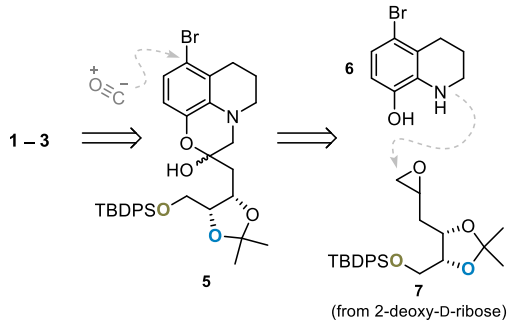


We noted, however, that the basic aniline moiety of **1**–**3** would be protonated under acidic conditions, therefore requiring a dicationic species (**4**) along the epimerisation/isomerisation pathway (Scheme 1). Formation of such a dication generally requires highly forcing conditions, due to the destabilising

effect of the two positive charges in close proximity.[24,25] It therefore seemed plausible that interconversion of the spiroketal core may effectively be prevented by the presence of the basic nitrogen atom, protecting the overall integrity of the spirocentre. Since the goji berry possesses an acidic environment (pH ~ 5.3),[26] an unusual spiroketal acid-stability of **1**–**3** may be important to enabling distinct roles of each isomer in the biochemistry of *L. barbarum*.[7,18]

Motivated by these questions, we undertook the total synthesis of the lycibarbarines (**1**–**3**) to investigate the properties of their spiroketal core and gain synthetic access to their unique structures for the first time.

Scheme 2. Retrosynthetic analysis of lycibarbarines A–C (**1**–**3**).



Our retrosynthetic strategy (Scheme 2) was targeted towards hemiketal **5**, an oxocarbenium precursor which could be used to access each lycibarbarine (**1**–**3**) by controlled acid-mediated spiroketalisation followed by late-stage formylation. We envisaged **5** could be accessed via an *N*-alkylation of **Scheme 3**. Total synthesis of lycibarbarine A (**1**) and B (**2**).

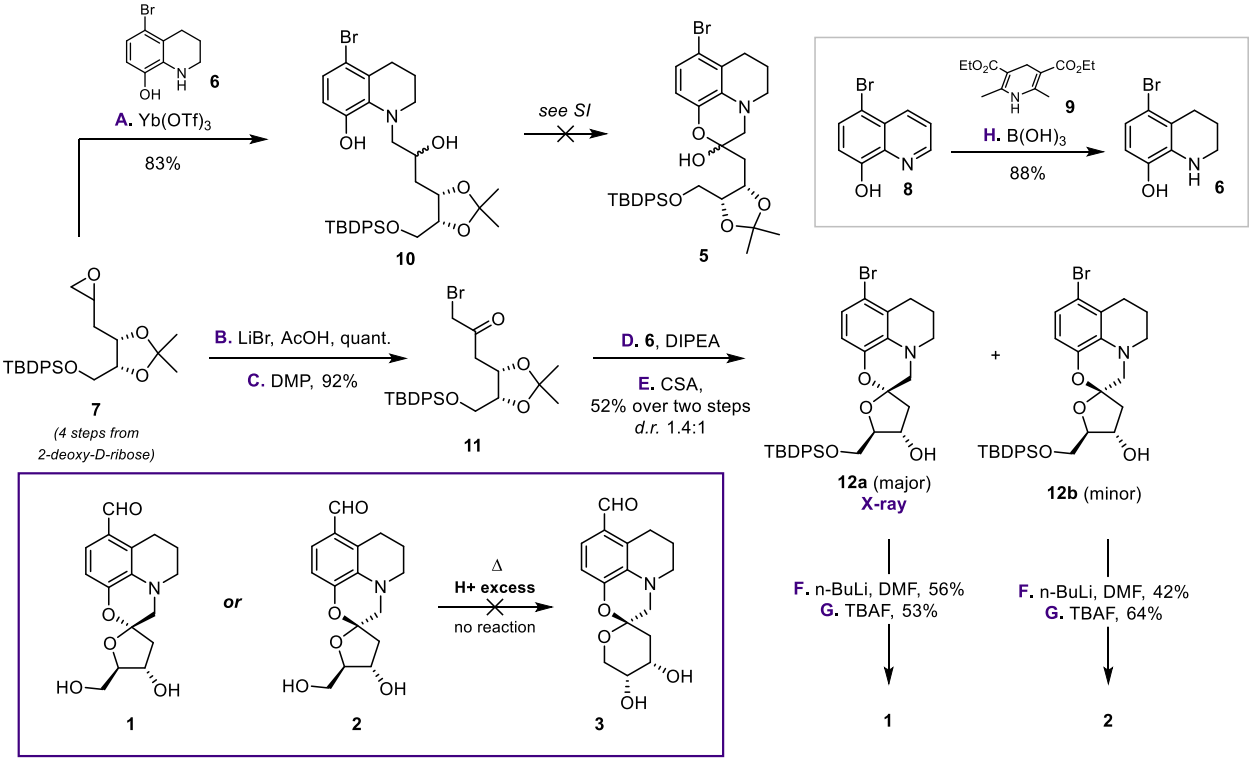
tetrahydroquinoline **6** with epoxide **7**, followed by oxidation. Tetrahydroquinoline **6** could be prepared by the reduction of the commercially available 5-bromo-8-hydroxyquinoline (**8**). Epoxide **7** would be accessed from 2-deoxy-D-ribose,[16,27] as this chiral pool material possesses the correct stereochemistry required for **1**–**3**.

Synthetic studies began with the construction of epoxide **7** — as developed for our synthesis of acortatarin A[16]— from 2-deoxy-D-ribose via sequential Wittig methylenation,[27] protection, and epoxidation. This protocol proceeded in good overall yield on a multi-gram scale (see Supporting Information for full details).

Tetrahydroquinoline **6** was accessed in one step from 5-bromo-8-hydroxyquinoline (**8**) *via* boric acid-catalysed reduction using the mild organic hydride donor Hantzsch ester (**9**) (Scheme 3).[28] Alternative hydride sources such as $NaBH_4$, $NaBH_3CN$, HBpin, and B_2pin_2 proved unreactive towards substrate **8**.

Aminolysis of epoxide **7** with tetrahydroquinoline **6** afforded alcohol **10** in good yield. However, the proposed oxidation and cyclisation of **10** to hemiketal **5** proved challenging (see Supporting Information for full details). This was likely due to sensitivity of the tetrahydroquinoline moiety to oxidative conditions, in addition to significant degradation of hemiketal **5** taking place during purification.

To circumvent the need for oxidation after *N*-alkylation, epoxide **7** was converted to α-bromoketone **11**, which would serve as an alternative electrophile for the key C–N coupling. Conversion of epoxide **7** to α-bromoketone **11** was



Reagents and conditions: A) **6**, $Yb(OTf)_3$ (25 mol%), DCE, 55 °C, 16 h, 83%; B) LiBr, AcOH, THF, 0 °C to rt, 1h, quant.; C) Dess-Martin periodinane, CH_2Cl_2, rt, 10 min, 92%; D) **6**, DIPEA, CH_2Cl_2, rt, 16 h; E) CSA, toluene, rt, 16 h, 52% over two steps (*d.r.* 1.4:1); F) *n*-BuLi, THF, -78 °C, 30 min, then DMF, 2.5 h, 56% (from **12a**) *or* 42% (from **12b**); G) TBAF, THF, 0 °C, 30 min, 53% **1** *or* 64% **2**; H) **9**, $B(OH)_3$ (25 mol%), DCE, 75 °C, 16 h, 88%. CCDC accession code of **12a**: CCDC 2244111.

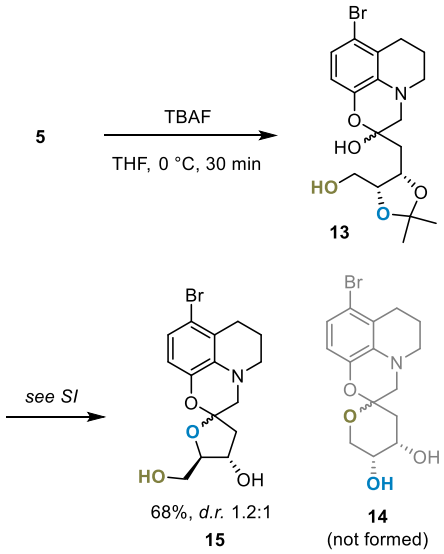
effected in excellent yield by bromide addition followed by DMP oxidation. *N*-Alkylation of **6** with bromoketone **11** afforded hemiketal **6**, initially in 46% isolated yield post-purification (see SI). Gratifyingly, degradation of **5** during purification could be avoided by using crude material in the subsequent acid-mediated spiroketalisation step, to give a mixture of the desired spiroketals **12a** and **12b** in an improved 52% yield over two steps. Epimers **12a** and **12b** were separable, and an X-ray structure of the major product **12a** was obtained. This structure unambiguously confirmed that spiroketal **12a** possessed the desired *S* stereochemistry at the spirocentre as in lycibarbarine A (**1**).

Bromides **12a** and **12b** were then advanced to lycibarbarine A (**1**) and B (**2**) separately, completing their first total syntheses and enabling confirmation of their structural assignments. Individual late-stage formylation of bromides **12a** and **12b** proceeded smoothly *via* the respective aryl lithium species. Subsequent silyl deprotection then afforded **1** and **2**. Pleasingly, the ^{1}H and ^{13}C NMR spectra, and optical rotation data were in agreement with those reported for the natural products lycibarbarine A (**1**) and B (**2**) respectively (Supporting Information).[1] Importantly, no epimerisation of the spiroketal centre was observed by NMR analysis during the final two steps, and it was concluded that the assigned *S* stereochemistry of the spirocentre in bromospiroketal **12a** was retained in lycibarbarine A (**1**) and similarly the *R* stereochemistry for lycibarbarine B (**2**), thus confirming the assigned absolute stereochemistry of these natural products.

With lycibarbarines A (**1**) and B (**2**) in hand, we next investigated their isomerisation to lycibarbarine C (**3**) and their interconversion by epimerisation. Treatment of **1** or **2** with an excess of either PPTS or PTSA (see Supporting Information) led to no detectable epimerisation or isomerisation to give **3**, until eventual degradation of **1** and **2** took place using more forcing conditions (see SI for details). This is in direct contrast to analogous carbohydrate 6,5-spiroketal structures, which undergo facile rearrangement to the 6,6-spiroketal isomer in acidic conditions.[21] These results provided the initial evidence that the lycibarbarines were not interconvertible, and that either the dication **4** does not form under the examined conditions, or it devolves to a decomposition pathway due to its unstable nature.

Attention was initially directed to silyl deprotection of **5**, followed by acid mediated spiroketalisation of **13**, to enable access to the 6,6-spiroketal scaffold of **3** (Scheme 4). While the deprotection of **5** using TBAF proceeded smoothly, subsequent treatment of **13** with a variety of Brønsted and Lewis acidic conditions failed to provide access to the desired 6,6-spiroketal **14** (Scheme 4 and Supporting Information). In many cases the substrate **13** was returned with partial degradation, but use of camphorsulfonic acid effected tandem acetonide deprotection/spiroketalisation, to afford **15** (*d.r.* 1.2:1) in up to 68% yield over two steps. Given that the acetonide moiety of **13** is labile under acidic conditions, and a clear kinetic preference for 6,5-spiroketal **15** was observed, we decided to change the protecting group strategy to prevent formation of the 5,6-spiroketal and selectively obtain the 6,6-spiroketal product.

Scheme 4. Selective formation of the 6,5-spiroketal **15** from hemiketal **5**.

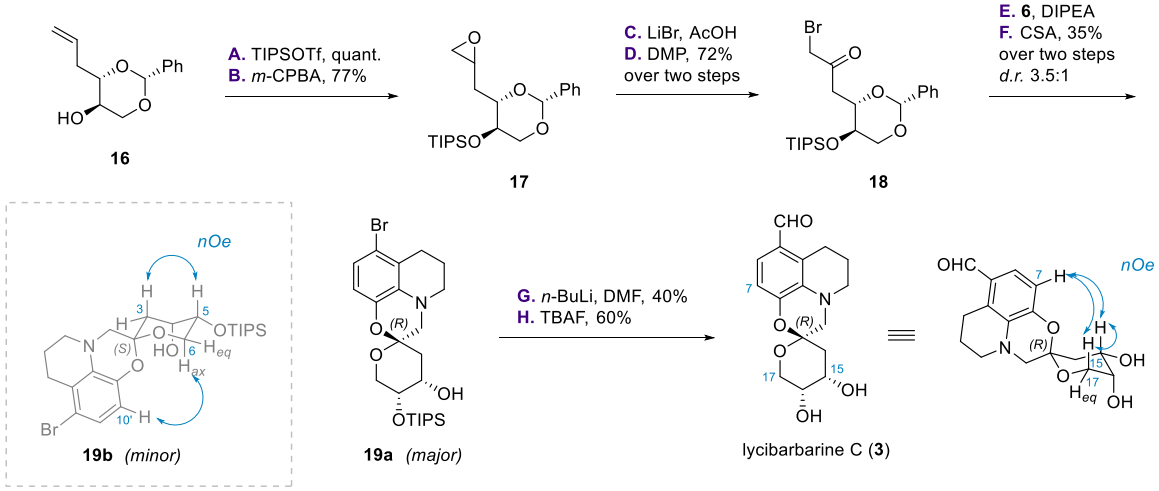


We proceeded to focus on the synthesis of the desired 6,6-spiroketal lycibarbarine C (**3**) *via* a modified protecting group strategy. To this end, alkene **16** was prepared in 2 steps from 2-deoxy-D-ribose (see Supporting Information for details).[27] We then installed the acid-stable *O*-TIPS protecting group at the key C-2 hydroxyl group, and subsequent epoxidation gave **17** in good yield (Scheme 5). Epoxide **17** was then advanced to bromoketone **18** in two steps. Subsequent *N*-alkylation of bromoketone **18** with tetrahydroquinoline **6** proceeded smoothly, and subsequent acid-mediated spiroketalisation afforded the desired 6,6-spiroketal **19**. This result marked the first synthetic access to the 6,6-spiroketal framework of lycibarbarine C (**3**).

The two epimers of the lycibarbarine C (**3**) scaffold, major product **19a** and minor product **19b**, were separable. The stereochemistry of the spiroketal core was determined using key nOe correlations observed for the minor isomer **19b**. The conformation of the tetrahydropyran ring was determined by nOe correlations between axial H-3 and H-5; the nOe correlation between H-10' and H_{ax}-6 established an *S* configuration of the spirocentre. This result confirmed that the major product **19a** possessed the same *R* stereochemistry of the spirocentre as the natural product **3**. Spiroketal **19a** was hence taken forward to complete the synthesis.

Elaboration of **19a** to the natural product **3** was achieved by late-stage formylation of the aryl bromide, followed by deprotection of the silyl ether (Scheme 5). Importantly, no isomerisation of **3** to **1** or **2** was observed upon deprotection of the silyl ether. The spectroscopic and optical rotation data were in agreement with those previously reported.[1] The stereochemistry of the spirocentre was confirmed from key nOe correlations for lycibarbarine C (**3**), which were consistent with those observed by Chen and co-workers in the isolation report.[1] The strong nOe correlation between axial H-15 and H_{ax}-17 confirmed the configuration of the tetrahydropyran ring, whilst weaker correlations between H-7 and both H-15 and H_{ax}-17 confirmed retention of the *R* stereochemistry of the spirocentre of **3**.

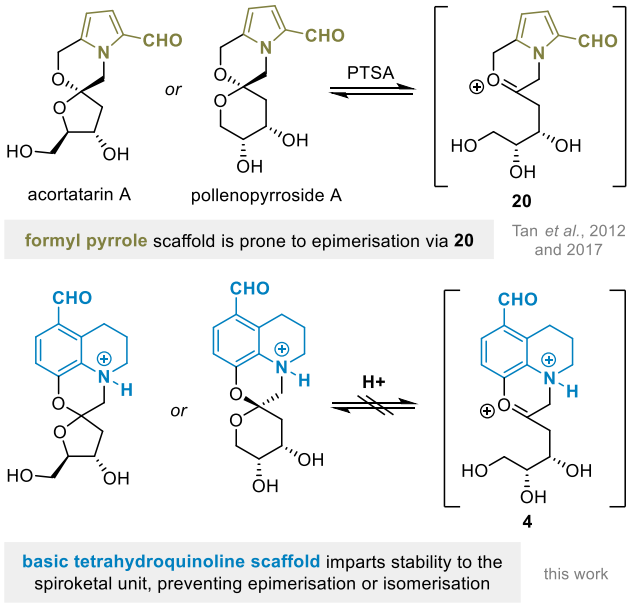
Scheme 5. Synthesis of lycibarbarine C (**3**).



Reagents and conditions: A) TIPSOTf, 2,6-lutidine, CH_2Cl_2, 0 °C to rt, 30 min, quant.; B) *m*-CPBA, CH_2Cl_2, 0 °C to rt, 16 h, 77%; C) LiBr, AcOH, THF, 0 °C to rt, 16 h; D) Dess-Martin periodinane, CH_2Cl_2, rt, 10 min, 72% over two steps; E) **6**, DIPEA, CH_2Cl_2, rt, 16 h; F) CSA, toluene/MeOH (1:1), rt, 48 h, 35% over two steps (*d.r.* 3.5:1); G) *n*-BuLi, THF, -78 °C, 30 min, then DMF, 2.5 h, 40%; H) TBAF, THF, 0 °C, 30 min, 60%.

With access to lycibarbarine C (**3**) established, we were able to probe the stability of the spiroketal motif towards epimerisation and isomerisation. It was found that treatment of **3** with an excess of PTSA did not lead to any discernible epimerisation of the spirocentre, nor the formation of lycibarbarine A (**1**) or B (**2**), although partial degradation was observed under reflux conditions in methanol (see SI for details). This result confirmed our hypothesis that indeed, the spiroketal units of **1**–**3** are unusually stable, likely due to a protective effect of the basic aniline nitrogen. When compared with the tendency of the related, but significantly less basic,[29] pyrrolomorpholine spiroketal alkaloids to undergo acid-catalysed epimerisation at the spirocentre *via* oxocarbenium ion **20** (see SI for details), the influence of scaffold alteration is highlighted.[17,19,20]

Scheme 6. Comparison of spiroketal stability between formyl pyrrole scaffold and tetrahydroquinoline scaffold.



In the broader context of spiroketals as privileged bioactive scaffolds, the lycibarbarines provide an interesting case study in preventing isomerisation of the spirocentre. In doing so, the lycibarbarines resist convergence to the thermodynamic isomer of the scaffold, allowing three distinct isomers to exist and exhibit unique bioactivity profiles. This lesson may hold value in the future design of synthetic bioactive spiroketals, to enable greater exploration of isomeric pockets of spiroketal chemical space. These insights were enabled by the development of a 10 step (longest linear sequence) synthesis of lycibarbarines A–C (**1**–**3**), which provided practical access to these unusual scaffolds for the first time.

ASSOCIATED CONTENT

Data Availability Statement

The data underlying this study are available in the published article and its Supporting Information.

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website.

Experimental procedures and analytical data (PDF)

Accession Codes

CCDC 2244111 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge via www.ccdc.cam.ac.uk/data_request/cif , or by emailing data_request@ccdc.cam.ac.uk , or by contacting The Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB2 1EZ, UK; fax: +44 1223 336033.

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Notes

The authors declare no competing financial interest.

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