CHEMISTRY
A EUROPEAN JOURNAL

DOI: 10.1002/chem.200901732

Expeditious Synthesis of Hippuristanol and Congeners with Potent Antiproliferative Activities

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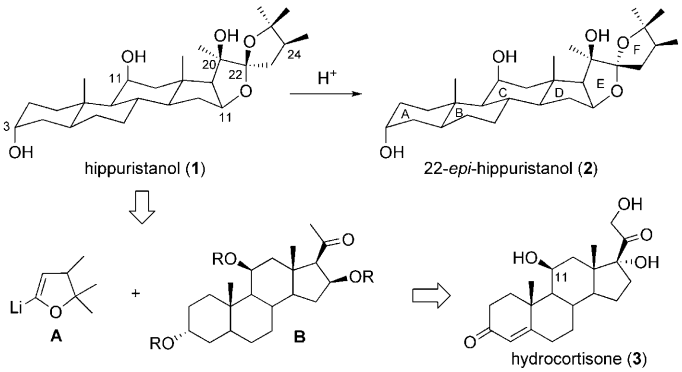
Hippuristanol (**1**) and its congeners comprise a family of structurally unique polyoxygenated steroids isolated from the Gorgonian *Isis hippuris* with potent antiproliferative activity against different cancer cell lines in vitro.[1] The molecular basis of their antiproliferative activity, however, remained a mystery until recently. Hippuristanol was reported to target the eukaryotic translation initiation factor (eIF)4A,[2a,b] an ATP-dependent RNA helicase that plays a pivotal role in translation in eukaryotic cells. The discovery of hippuristanol, along with another marine sponge-derived natural product pateamine A, as inhibitors of eukaryotic translation, not only offered new molecular probes to study eukaryotic translation initiation mediated by eIF4A, but also had important implications in targeting the translation process for the development of novel anticancer and antiviral drugs.[2] Fascinated by its unusual chemical architecture, especially the bicyclic spiroketal appendage (rings E and F) to the conventional steroid core, and its novel biological activity, we embarked on the synthesis of hippuristol. Herein, we report the first synthesis of hippuristanol and its congeners with structural and stereochemical variations on E and F rings, along with their characterization in both cell proliferation and in vitro translation assays.

In a retrosynthetic analysis, we envisioned an expeditious approach to hippuristanol (**1**) via addition of racemic 5-lithio-2,2,3-trimethyl-2,3-dihydrofuran (**A**) to a 5α-pregn-3α,11β,16β-trihydroxy-20-one derivative, such as **B** (Scheme 1);[3] the resulting adduct would have all skeletal carbons and functional groups in place for further elaboration. The nascent stereochemistry (at C20 and C22) would be determined largely by the pre-existing stereocenters on the steroidal substrate **B**. Thus, modification of the substrates to effect the natural stereochemistry might be required. The control, or lack of it, of the stereochemical outcome of the reaction leading to different stereoisomers could also be taken advantage of to explore the structure–activity relationship of the E and F rings of hippuristanol. In addition, by employing a variety of the 2,3-dihydrofuran derivatives (such as 2,3-dihydrofuran **18** and 2,2-dimethyl-2,3-dihydrofuran **19**), we could access the corresponding congeners with altered substitutions at the F ring. Hydrocortisone (**3**) was chosen as the starting precursor to the steroidal substrate **B** due to its commercial availability and low cost. It possesses the steroidal ABCD-ring skeleton and the required oxy-function (of **B**) at C3, C20, and C11. In particular, the β-OH at C11 of **3** would otherwise be difficult to introduce onto steroids core structure by conventional chemistry.



Scheme 1. Hippuristanol (**1**) and 22-*epi*-hippuristanol (**2**), and the retrosynthetic analysis.

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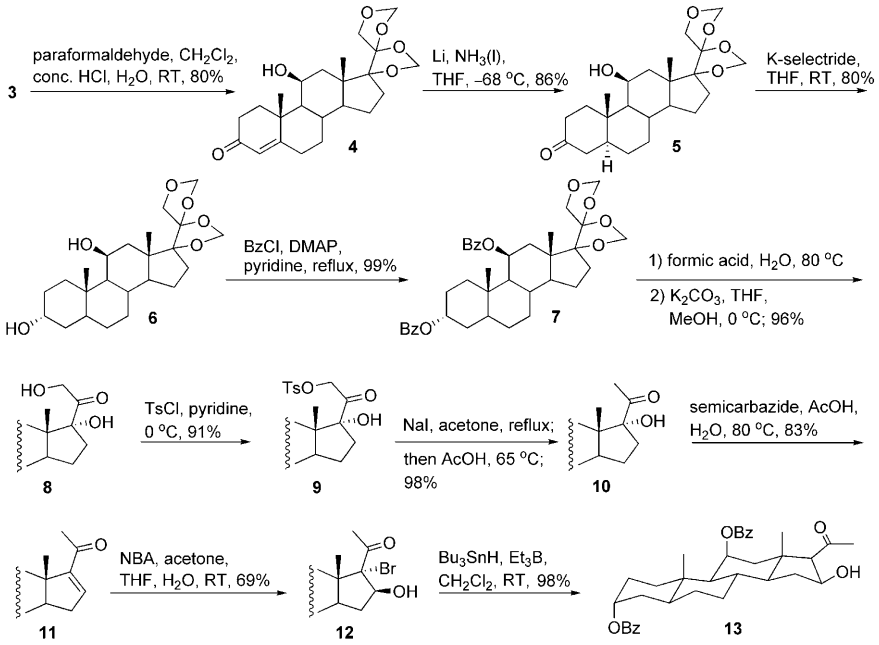
Supporting information for this article is available on the WWW under http://dx.doi.org/10.1002/chem.200901732.

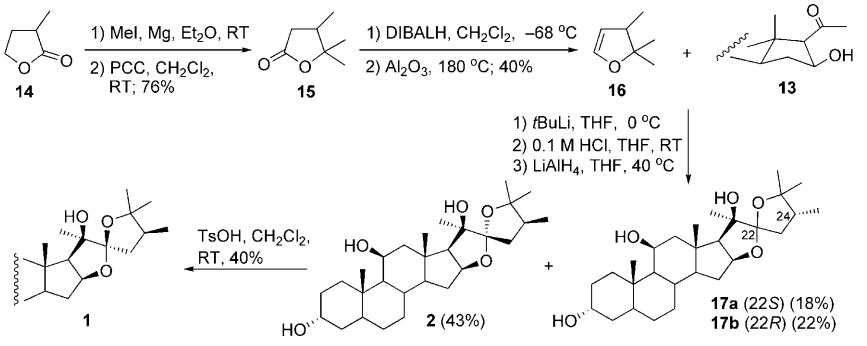
The synthesis commenced with conversion of hydrocortisone (**3**) into the 3α,11β,16β-trihydroxy-5α-pregn-20-one derivative **13** (Scheme 2). Treatment of hydrocortisone (**3**) with lithium in liquid NH_3 led to a complex mixture.[4] This could be attributed to undesirable reactions on the hydroxyacetyl-carbinol residue at C17, which was thus protected with a robust bismethylenedioxy function to give compound **4**.[5] Reduction of **4** with lithium in liquid NH_3 provided the desired 5α-H-3-one derivative **5** in good yield. Ketone **5** was then subjected to reduction with K-selectride to provide 3α-ol **6** stereoselectively (80%).[6,7] Removal of the bismethylenedioxy protecting group in **6** with formic acid resulted in the elimination of the 11β-OH to give the corresponding $\Delta^{9,11}$ derivatives.[5] Therefore, the 11β-OH (in **6**) was subjected to protection before further elaboration. Treatment of **6** with benzoyl chloride under harsh conditions (reflux in pyridine in the presence of DMAP) provided the 3,11-di-*O*-benzoyl derivative **7** (99%); under milder conditions, the 3-OH (in **6**) could be selectively benzoylated. Removal of the bismethylenedioxy protection from **7** with formic acid (80% in H_2O at 80°C) afforded compound **8** together with its 21-formate derivative, which was converted to **8** with K_2CO_3 in a mixed solvent of THF and MeOH at low temperature (0°C). Removal of the 21-OH in **8** was achieved via tosylate formation, giving **9** (91%), and replacement of the tosylate with iodide, followed by reduction (NaI, acetone, reflux; then AcOH, 65°C),[8] furnishing the desired **10** in 98% yield. Under the action of semicarbazide in aqueous acetic acid at 80°C,[8c,9] the α-hydroxy-ketone **10** was converted to enone **11** in 83% yield. A rearranged compound (**S1**)[10] was detected in trace amount, which had been the major product upon treatment of **10** with $SOCl_2$ in pyridine at room temperature.[11] It is worth noting that a substrate without the benzoyl protection at the 11-OH (namely, 3α-benzoyloxy-11β,17α-dihydroxy-5α-pregn-20-one) gave rise to the corresponding enone and rearranged product in nearly 1: 1 ratio upon treatment with semicarbazide (data not shown).



Scheme 2. Synthesis of the 3α,11β,16β-trihydroxy-5α-pregn-20-one (**13**).

Introduction of the 16-β-OH onto enone **11** was initially met with difficulties. Michael addition or hydroboration–oxidation of enone **11** under a variety of conditions provided the 16-α-hydroxy products.[12c,13] Fortunately, under the action of *N*-bromoacetamide (NBA),[12] enone **11** could be transformed into the 16β-hydroxyl-17α-bromide **12** in 69% yield. Removal of the 17-bromide under radical conditions (Bu_3SnH, Et_3B, CH_2Cl_2, RT) afforded the desired 16β-hydroxy-20-ketone **13** cleanly (98%).

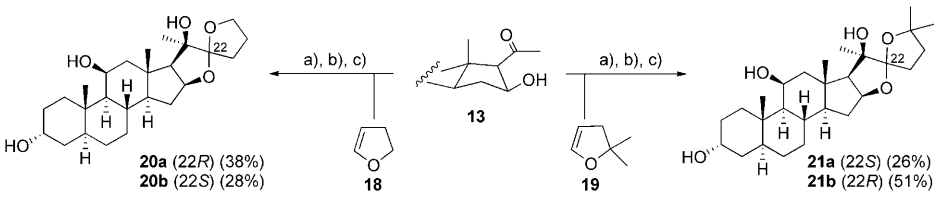
The volatile 2,2,3-trimethyl-2,3-dihydrofuran (**16**) was readily prepared from the commercially available α-methyl-γ-butyrolactone **14** in four steps and in 30% overall yield (Scheme 3).[3c,14] Treatment of ketone **13** with dihydrofuran **16** in the presence of *t*BuLi in THF at 0°C followed with 0.1 M HCl at RT provided a rather complex mixture, partly because of partial cleavage of the two *O*-benzoyl groups. Nevertheless, complete removal of the benzoyl groups with $LiAlH_4$ (THF, 40°C) afforded three stereoisomeric products, which were characterized as 22,24-di-*epi*-hippuristanol (**17a**) (18%), 24-*epi*-hippuristanol (**17b**) (22%), and 22-*epi*-hip-



Scheme 3. Elaboration of hippuristanol (**1**), 22-*epi*-hippuristanol (**2**), and stereoisomers **17a**/**17b**.

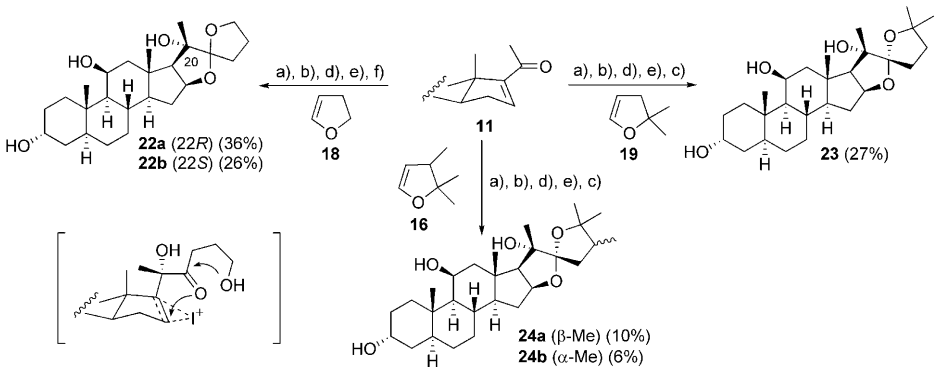
puristanol (**2**) (43%), in 83% overall yield. Hippuristanol (**1**) was obtained in ≈40% yield via epimerization of 22-*epi*-hippuristanol **2** under the action of a catalytic amount of TsOH in CH_2Cl_2 at RT. The corresponding $\Delta^{9,11}$ product from elimination of the 11-OH was not detected (cf. the acidic treatment of compound **6**).

The same synthetic route to hippuristanol was easily adapted to the synthesis of a variety of hippuristanol congeners (Scheme 4). Thus, addition of 22-ketone **13** with 2,3-dihydrofuran **18** (*t*BuLi, THF, −68°C) followed by further elaboration (0.1 M HCl, RT; then $LiAlH_4$, THF, 40°C) provided a pair of the C22 epimers **20a** and **20b** in 38 and 28% yield, respectively. Compounds **20a** and **20b** lack the three methyl groups of hippuristanols on the F ring. Similarly, treatment of **13** with 2,2-dimethyl-2,3-dihydrofuran (**19**) through three steps afforded **21a** (26%) and **21b** (51%), which lack the 24-methyl group of the natural product.



Scheme 4. Divergent assembly of hippuristanol analogues **20a/b** and **21a/b**. a) *t*BuLi, THF, −68°C (or 0°C for **13** + **19**). b) 0.1 M HCl, RT. c) $LiAlH_4$, THF, 40°C.

Contrary to the addition of 5-lithio-2,3-dihydrofurans to 16β-OH-22-one **13**, where the 20β-OH adducts predominated, their addition to enone **11** led to the corresponding 20α-OH products stereoselectively under similar conditions (α/β 8:1, 19:1, and 1:0, respectively, for addition with **18**, **19**, and **16**) (Scheme 5).[10] The resulting $\Delta^{16,17}$-22-one-25-ols underwent cyclization under the action of NIS,[15] providing the corresponding 16β-*O*, 17α-iodide, 22-*epi*-derivative as the sole stereoisomer, respectively. Subsequent removal of the 17-iodide (Bu_3SnH, AIBN, toluene, 70°C) followed by deprotection of the 3,11-*O*-benzoyl groups ($LiAlH_4$, THF, 40°C) furnished the final products (**22a/b**, **23**, and **24a/b**) in reasonable yields. The C22(*S*)-isomer **22b** resulted from the C22(*R*) **22a** upon acidic workup (5% HCl, RT).



Scheme 5. Divergent assembly of 20-*epi*-hippuristanol analogues **22a/b**, **23**, and **24a/b**. a) *t*BuLi, THF, −68°C. b) 0.1 M HCl, RT. c) $LiAlH_4$, THF, 40°C. d) NIS, CH_2Cl_2, RT. e) Bu_3SnH, AIBN, toluene, 70°C. f) $LiAlH_4$, THF, 40°C; then 5% HCl, RT.

Assignment of the structures of these synthetic congeners (**1**, **2**, **17a/b**, **20a/b**, **21a/b**, **22a/b**, **23**, and **24a/b**) proved to be extremely difficult by spectroscopic methods. Fortunately, the natural product hippuristanol (**1**) and 22-*epi*-hippuristanol (**2**) have been well characterized by extensive spectroscopic analysis as well as correlation with an X-ray structure of a natural congener (hippurin-1).[1] The ^1H and ^{13}C NMR spectra of the synthetic compounds **1** and **2** are identical to those of the authentic natural products. Besides, the structures of **22a/b** and **23** have been determined by X-ray diffraction of the relevant derivatives (compounds **S3** and **S5**).[7] Based on these unambiguous assignments, the structures of the synthetic congeners could be well correlated by NMR signals of the H16 and C22, as well as the $[\alpha]_D$ values (Table S1).[10]

With synthetic hippuristanol and congeners in hand, we determined their effects on the proliferation of transformed cancer cell line HeLa and on protein synthesis in vitro. In general, the two types of activities are in agreement with each other with synthetic hippuristanol exhibiting the highest activity in both assays. The synthetic hippuristanol inhibited HeLa cell proliferation with an IC_{50} of 72 nM, significantly lower than that (ca. 800 nM) reported previously for the natural product.[2a] A clear structure–activity relationship also emerged. First of all, an "R" configuration at C22 appears to be essential for the activity. Thus, inversion of the stereochemistry at C22 in hippuristanol (**2**) led to a significant decrease in activity (Table 1). A similar decrease in or loss of activity was also seen between congeners **21a** and **21b**, and between **17a** and **17b**. Given the opposite configuration at the D/E ring juncture and the relative rigidity of the E/F spiroketal functional group, it is tempting to speculate that the "R" stereochemistry at C22 would place the three oxygen atoms on the same side of a concave that are capable of multiple hydrogen-bonding interactions. Second, of the three methyl groups on the F ring, which occupy the convex surface opposite to the oxygen-rich concave, at least two are required for activity. Thus, congeners **20a**, **20b**, **22a** and **22b** are all inactive. In contrast, **21b** that contains the *gem*-dimethyl substitution on the F ring, is quite potent, sug-

Table 1. Effects of synthetic hippuristanol and its analogues on HeLa cell proliferation and in vitro translation.

Compound	IC_{50} [μM] HeLa	IC_{50} [μM] in vitro translation
1	0.072	0.20
2	3.59	14.45
17a	1.52	3.24
17b	0.13	0.96
20a/20b 1:1	>20	N/A
21a	>20	N/A
21b	0.36	0.60
22a	>20	N/A
22b	>20	N/A
23	>20	>50
24a	>20	>50
24b	>20	>50

gesting that those methyl groups are likely involved in hydrophobic interactions with the target eIF4A.

In summary, an expeditious route was established to synthesize the natural product hippuristanol and its congeners with structural alterations in its unique E and F rings. The determination of the biological activity of the synthetic analogues revealed the importance of both stereochemistry of the spiroketal group and the methyl substitutions in the F ring. These results allow future synthesis of new analogues to improve the potency of this family of inhibitors of eukaryotic translation initiation and molecule probes to further delineate the molecular recognition of hippuristanol by eIF4A.

Acknowledgements

We thank Prof. Carlos Jiménez of the Universidade de A Coruña (Spain) for providing NMR spectra of the authentic hippuristanol and 22-*epi*-hippuristanol. Financial support from the National Natural Science Foundation of China (20728202 and 20621062), the Chinese Academy of Sciences (KJCX2-YW-H08), and the Committee of Science and Technology of Shanghai (07DZ22001) are gratefully acknowledged.

Keywords: cancer · natural products · steroids · structure–activity relationships

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Received: June 23, 2009
Published online: September 11, 2009

