Total Synthesis of Gypsetin

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The regulation of cholesterol levels in humans remains a major focus of drug development. Connectivity between excess cholesterol and susceptibility to coronary heart disease is now generally accepted, though the rigor of the linkage is still open to debate.[1] Interference either with the dietary absorption of cholesterol-containing foodstuffs or with the *de novo* biosynthesis of cholesterol has been used to lower plasma cholesterol content. Indeed, early strategies for containment of hypercholestemia involved the use of the bile acid sequestrant cholestyramine resin.[2] However, the difficulties associated with this form of medication prompted a search for other approaches. A milestone in the contemporary management of cholesterol levels arose from the discovery of lovastatin, a powerful inhibitor of HMG-CoA reductase.[3] This enzyme mediates the rate-limiting enzymatic step in cholesterol biosynthesis. Agents based on the concept of HMG-CoA inhibition have proven to be effective in lowering both LDL and total cholesterol levels in primary hypercholestemic patients. Inhibitors of squalene synthase such as the zaragozic acids have also been identified as possible therapeutic agents but have not yet found clinical application.[4]

It is believed that the progression from abnormally high cholesterol levels to myocardial infarction begins with the accumulation of intracellular esterified cholesterol in macrophages. This is followed by subsequent foam cell formation and, ultimately, by the appearance of atherosclerotic plaques in arteries.[5] The enzyme acylCoA:cholesterol acyltransferase (ACAT) has been identified as the rate-limiting enzyme in the absorption of cholesterol. Thus, the inhibition of ACAT has received much attention due to its potential in moderating the effect of elevated cholesterol levels.[5,6] Gypsetin (**1**), recently isolated from *Nannizzia gypsea* var. *incurvata* IFO 9228, was found to be a competitive inhibitor of ACAT with respect to oleoyl-CoA with a K_i value of 5.5 μM. Furthermore, inhibition of cholesterol ester formation in cultured macrophages with an IC_{50} of 0.65 μM was observed.[7]

Our selection of gypsetin as a goal structure for total synthesis arose from the confluence of several considerations, starting with its obvious potential as a drug discovery lead. We were drawn to the diketopiperazine moiety which is formally composed of two hypothetical amino acids **2** and **3**, differing only in the relationship of the cis-fused [2,3-*b*]hexahydropyrroloindole chirality at C_{3a} and C_{8a} to the *S*-configured amino acid center (C_2) (Figure 1). For the sake of convenience, we refer to **2** as the "pre-anti" system and **3** as the "pre-syn" moiety, anticipating the backbone relationship of the full heptacyclic ensemble of gypsetin itself. Constructs **2** and **3** share an obvious "L-tryptophan connection" provided that methodology could be developed for the introduction of a 1,1-dimethylallyl (reverse prenyl) function at C_2. In addition, cyclization of N_b of the amino acid side chain to C_2 of the indole and hydroxylation at C_3 of the indole through the agency of formal $^+$OH would be necessary.

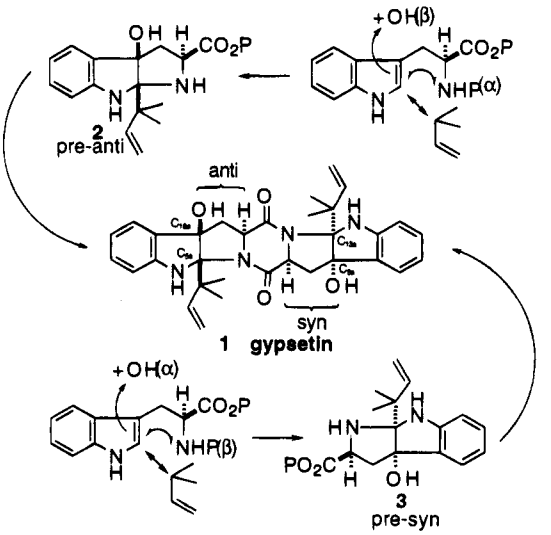

Figure 1. Formulation of the gypsetin problem.

In our recently described total synthesis of *N*-acetylardeemin, we had documented a method for the introduction of such a reverse prenyl group into the C_{3a} of a prebuilt [2,3-*b*]hexahydropyrroloindole moiety.[8] This transformation was accomplished via a C_{3a} phenylseleno precursor. While powerful for its intended purpose, the ardeemin protocol appeared ill suited to effect the introduction of the reverse prenyl groups at C_{5a} and C_{13a} and the hydroxyl functions at C_{8a} and C_{16a} of gypsetin (**1**). *In fact, straightforward solutions to these chemical issues have been discovered, thus enabling a highly concise total synthesis of gypsetin.*

We first worked out a simple method to introduce the "reverse prenyl" function at C_2 of the indole in the presence of a suitably protected amino acid side chain. Recalling the oxidative conversion of 2,3-disubstituted indoles to chloroindolenines and the use of such chloroindolenines for functionalization of the carbon benzylic to C_2,[9] we asked whether an intermediate such as **4** would have transient viability even if C_2 were unsubstituted.[10] We hoped that **4** might suffer nucleophilic attack at C_2 leading to **5** and thence, after tautomerization, to **6** (Figure 2). Application of this protocol might allow for a rapid preparation of 2,3-disubstituted indoles. This proposition has been broadly realized and will be reported elsewhere.[11]

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(7) (a) Shinohara, C.; Hasumi, K.; Takei, Y.; Endo, A. *J. Antibiot.* **1994**, *47*, 163. (b) Nuber, B.; Hansske, F.; Shinohara, C.; Miura, S.; Hasumi, K.; Endo, A. *J. Antibiot.* **1994**, *47*, 168.
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(10) For the only published example of a carbon-based nucleophilic addition to a C_2-unsubstituted chloroindolenine, see: Parsons, R. L.; Berk, J. D.; Kuehne, M. E. *J. Org. Chem.* **1993**, *58*, 7482. In proposing the conversion **4** → **5** for purposes of illustration, we do not rule out a two-step sequence (i.e., addition to the imine and loss of HCl).
(11) Schkeryantz, J. M.; Woo, J. C. G.; Danishefsky, S. J. Manuscript in preparation.

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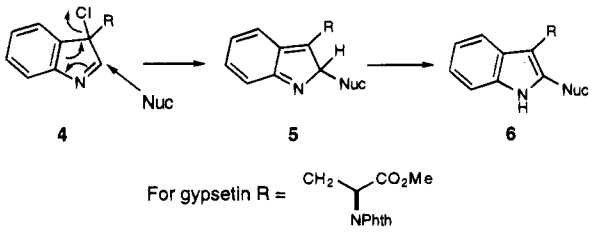
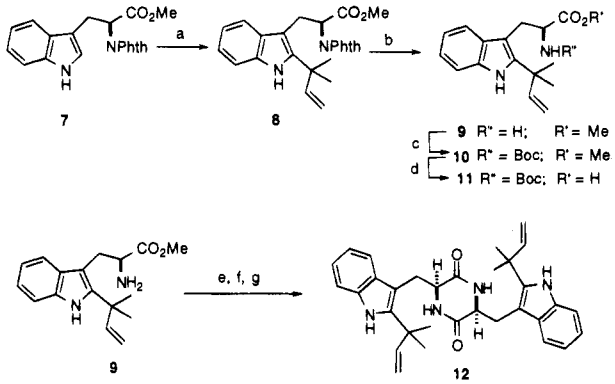


Figure 2. Preparation of 2,3-disubstituted indoles by addition of nucleophiles to chloroindolenines.

Scheme 1[a]



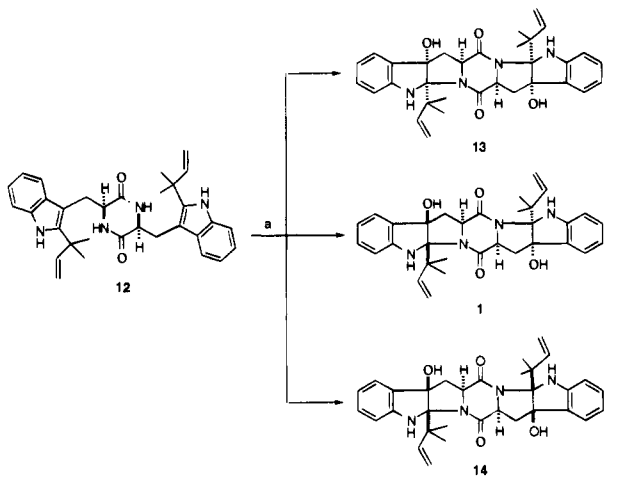
[a] Reagents and conditions: (a) *tert*-butyl hypochlorite, Et_3N, THF, −78 °C, 30 min, then prenyl-9-BBN (3 equiv) −78 °C, 6 h, 95%. (b) N_2H_2, EtOH, room temperature, 3 days, 65%. (c) $(Boc)_2O$, Et_3N, THF, 1 h, quantitative. (d) LiOH/THF/MeOH/H_2O, room temperature, 3 h, 94%. (e) **11** (0.91 (equiv), BopCl (3 equiv), CH_2Cl_2, −78 to 0 °C, 1 h. (f) TFA, CH_2Cl_2, room temperature, 1 h. (g) NH_3, MeOH, reflux, 12 h, 87% from **9**.

For the synthesis of gypsetin, we started with *N*-phthaloyltryptophan methyl ester[12] (**7**) (Scheme 1), which was converted in 95% yield to **8**, utilizing the logic described above, with prenyl-9-BBN as the nucleophile.[13] Hydrazinolysis led to the C_2-reverse prenylated tryptophan derivative **9**. We are currently exploring a range of methods by which monoacylated versions of **9** might be converted with high stereoselectivity to systems of type **2** or **3**. Such attempts will be disclosed in due course.

In the interim, an interesting alternative presented itself. It was recognized that, in the absence of diastereofacial governance in the conversion of a system such as **10** to **2** or **3**, oxidative cyclization of a prebuilt stereochemically uncommitted (with respect to the syn−anti issue) diketopiperazine (**12**) would be of significant advantage (vide infra). Therefore, compound **9** was converted to **11** by first protecting the amine function with a BOC group and saponifying with LiOH/THF/MeOH/H_2O. Coupling of **9** and **11** led, as shown, to diketopiperazine **12**.

We hoped to effect a one-step conversion of diketopiperazine **12** to gypsetin (**1**). A stereochemically random oxidative cyclization process would benefit from the truism that the

Scheme 2[a]



[a] Reagents and conditions: (a) dimethyldioxirane, CH_2Cl_2/acetone, −78 to 0 °C.

hypothetical ratio of gypsetin to "syn"−"syn" product **13** and "anti"−"anti" product **14** would be 2:1:1. Many oxidants have been surveyed, but at this writing, only one has proven to be successful. Thus, reaction of **12** with 4 equiv of dimethyldioxirane[14] afforded a 40% isolated yield of fully synthetic gypsetin (**1**) along with double syn product **13** (ca. 18%) and double anti product **14** (ca. 20%) (Scheme 2). The spectral properties (^1H NMR, ^{13}C NMR, MS, and IR) of synthetic gypsetin (**1**) were consistent with those of natural material. In addition, the chromatographic properties were identical and the melting point (159 °C) was in accord with that reported for naturally derived material. The optical rotation of synthetic gypsetin was $[\alpha]^{24}_D = -113.4°$ (*c* 0.20, $CHCl_3$), which was in good agreement with that of natural gypsetin, $[\alpha]^{24}_D = -116.9°$ (*c* 0.14, $CHCl_3$).

In summary, the total synthesis of a potentially important drug lead has been achieved. This synthesis, which can be conducted in as few as four steps from *N*-phthaloyltryptophan methyl ester (**7**),[15] is gratifyingly concise for a target of this apparent complexity. The chemistry developed here also serves to enhance methods for the synthesis of other complex indoles.[16]

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(12) Prepared from L-tryptophan methyl ester by modification of Bodansky and Bodansky: Bodansky, M.; Bodansky, A. *The Practice of Peptide Synthesis*; Springer-Verlag: Berlin, 1984; p 10.

(13) Kramer, G. W.; Brown, H. C. *J. Organomet. Chem.* **1977**, *132*, 9. The reaction of prenyl-9-BBN on the chloroindolenine (**4**) does not require the use of a catalyst. With other nucleophiles catalysis is needed.[11]

(14) For the reaction of N_a-acylated indoles with dimethyldioxirane, see: (a) Zhang, X.; Foote, C. S. *J. Am. Chem. Soc.* **1993**, *115*, 8867. (b) Adam, W.; Ahrweiler, M.; Peters, K.; Schmiedeskamp, B. *J. Org. Chem.* **1994**, *59*, 2733 and references therein.

(15) Diketopiperazine **12** can be prepared directly, albeit only in 35% yield, by heating tryptophan methyl ester (**9**) at 140 °C for 3 h, thus saving four steps in the synthetic sequence described above.

(16) There are relatively few direct methods for the preparation of 2,3-disubstituted indoles. For some current methods, see: (a) Saulnier, M. G.; Gribble, G. W. *J. Org. Chem.* **1982**, *47*, 2810. (b) Fukuyama, T. F.; Chen, X.; Peng, G. *J. Am. Chem. Soc.* **1994**, *116*, 3127 and references therein.