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Total Synthesis of 16-Membered Tetraene Macrolide Hygrolidin

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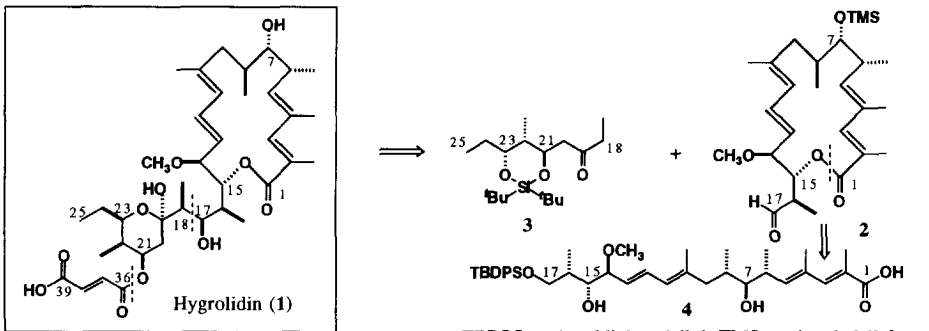
Abstract: *The first total synthesis of 16-membered tetraene macrolide antibiotic hygrolidin, a potent and relatively specific inhibitor of the vacuolar ATPases, has been achieved, incorporating macrolactonization by the modified Yamaguchi method and fragment assembly aldol reaction to establish the C17-C18 bond as key steps.* Copyright © 1996 Elsevier Science Ltd

Hygrolidin (**1**), a 16-membered tetraene macrolide isolated from the fermentation broth of *Streptomyces hygroscopicus* by Seto and co-workers in 1982,[2] is a prototypical member of a family of structurally related polyketide antibiotics, which includes other hygrolidins,[3] the bafilomycins,[4] and the concanamycins.[5] Among a variety of biological activities observed, it is worthwhile to mention that this family of macrolides exhibits potent and relatively specific inhibitory activity on the vacuolar ATPases.[6] Owing to these facts coupled with the 16- or 18-membered tetraene macrolide structure and the existence of a hydrogen-bonding network,[5b, 7] numerous studies have been directed toward this unique class of macrolides. Following the highly successful assembly of bafilomycin A_1 (**2**) by Evans and Calter,[8] Toshima and co-workers[9] have recently accomplished the total synthesis of **2**. In the preceding paper,[10] we outlined a convergent strategy for the total synthesis of hygrolidin (**1**) and described a stereocontrolled construction of the C1-C17 seco-acid fragment **4** and the C18-C25 masked hemiacetal subunit **3** of the molecule (Scheme 1). Herein, we wish to report the first total synthesis of **1** *via* macrolactonization of **4** and subsequent aldol fragment coupling between the macrocyclic fragment **2** and **3** to join the C17-C18 bond as pivotal steps.

In macrolide synthesis, it has become increasingly important to predict the viability of a macrolactonization by comparing the three-dimensional structure of a seco-acid bearing planarly remote carboxyl and hydroxyl groups with that of the corresponding macrolactone; a close similarity between their most stable conformations is one of the most critical factors responsible for an efficient macrolactonization.[11] Prior to the actual macro-



TBDPS = *t*-butyldiphenylsilyl; TMS = trimethylsilyl.

Scheme 1

lactonization of **4**, we thus carried out the conformational analysis of the model macrolactone **5** and the corresponding model seco-acid methyl ester **6** based on the MM2-CONFLEX3 method.[12] As shown in Figure 1, the most stable conformation of **5** has the following three-dimensional structural features: (1) the macrolactone ring has four corners at C6, C9, C14, and C15 positions; (2) the C2-C3-C4-C5 dihedral angle is +44°, with *s-cis* conformation of the conjugated diene being favored; (3) the other diene portion adopts *s-trans* conformation (the C10-C11-C12-C13 dihedral angle of θ= -178°). Likewise, the most stable conformation of **6** adopts two corners at C6 and C9, wherein the former is formed by minimization of 1,3-allylic strain between methyl groups at C4 and C6 as well as that of 1,3-*syn*-pentane interaction between C6 and C8 substituents, and the latter is formed mainly by a hydrogen bonding between C1 carbonyl and C15 hydroxyl groups. Furthermore, it adopts *s-cis* and *s-trans* conformations in two diene portions where the C2-C3-C4-C5 dihedral angle is +60° and the C10-C11-C12-C13 dihedral angle is +174°. Thus, these results suggested that this seco-acid **4** might cyclize smoothly to give the lactone without forced conformational changes.

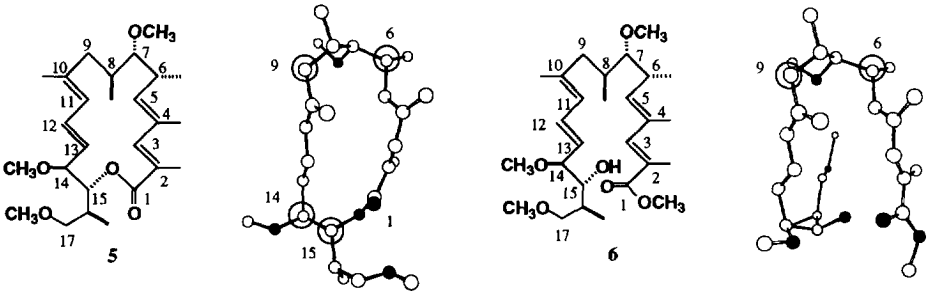
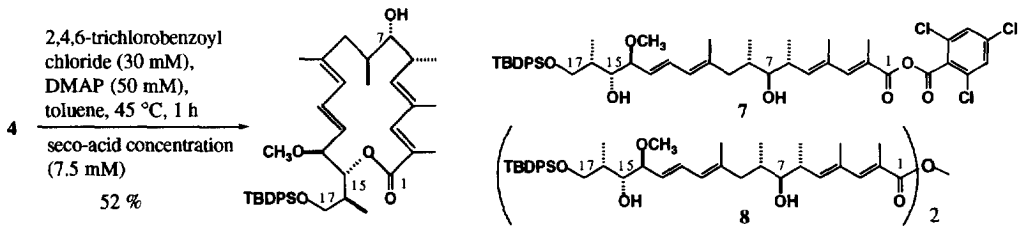


Figure 1. The most stable conformations of **5** and **6** (○: carbon atom, ● : oxygen atom)

With considerable anticipation from the above calculations, the seco-acid **4**[14] was submitted to the Yamaguchi conditions (Scheme 2).[13] To our surprise, however, we found that treatment of **4** with 2,4,6-trichlorobenzoyl chloride in the presence of triethylamine (THF, 23 °C, 15 h) afforded an approximately 6 : 4



Scheme 2

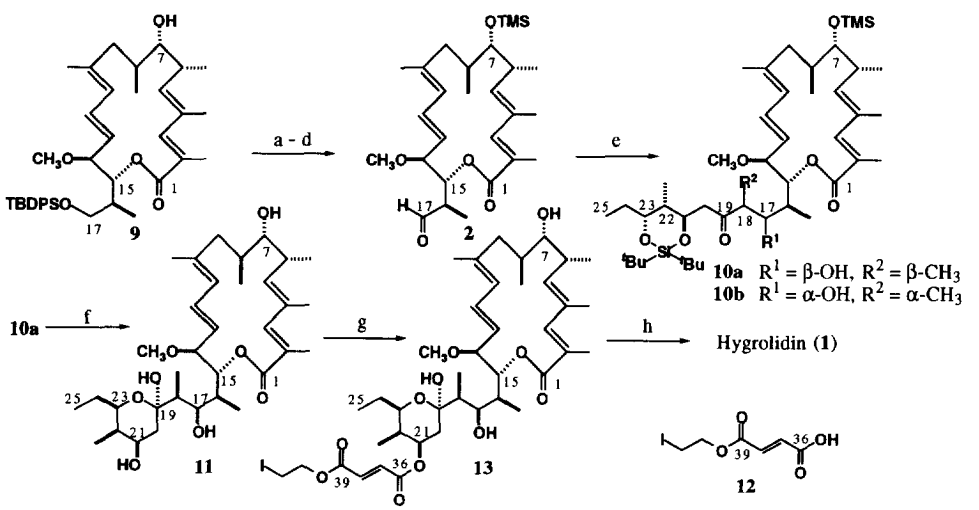
mixture[15] of the mixed 2,4,6-trichlorobenzoic anhydride **7** and the symmetrical anhydride **8**.[16] It should be noted that the formation of symmetrical anhydrides in the first step of Yamaguchi's protocol has never been documented. While we could neither find a valid reason for the unusual result nor improve the proportion of **7** to **8** under a variety of conditions, we were gratified to observe that the lactonization of **4** (7.5 mM) in toluene with the aid of 2,4,6-trichlorobenzoyl chloride (30 mM) and DMAP (50 mM) at 45 °C proceeded to completion within 1 h to give the desired

Table 1. Vicinal proton coupling constants (J : Hz) of the seco-acid methyl ester and macrolactone

$J_{H\text{-}H}$	Methyl ester of seco-acid (**4**)	Macrolactone (**9**)
5 - 6	9.7	9.0
6 - 7	1.0	1.5
7 - 8	5.7	5.7
8 - 9	3.5	1.0
8 - 9'	10.0	11.2
9 - 9'	13.3	14.4
13 - 14	8.2	7.7
14 - 15	6.3	5.7
15 - 16	5.0	5.7

lactone **9** in 52% yield,[11, 17] with no evidence of the formation of the corresponding diolide or triolide. Although the isolated yield does not necessarily come up to our expectation, the practical one-pot procedure which does not resort to the usual high-dilution technique demonstrates experimentally the power of the computation method in terms of conformational analysis. The positive proof of this was provided by ^{1}H NMR comparison of the methyl ester of the seco-acid **4** and the lactone **9** (Table 1); there is little, if any, difference in vicinal coupling constants between them. Needless to say, the difficulties in assessment of reactivities of the carboxyl and hydroxyl groups associated with macrolactonization leave room for considerable innovation in computation methods.

With cyclization of **4** realized, the stage was now set for the aldol fragment coupling to construct the C17-C18 bond and the completion of the synthesis as shown in Scheme 3. Toward this end, the macrolactone **9** was converted into the aldehyde **2** by sequential protecting group interchange and subsequent oxidation of the C17-hydroxyl group with the Dess-Martin periodinane in 69% yield. With regard to the crucial aldol coupling of the hygrolidin aldehyde and ketone fragments **2** and **3**, Evans and Calter recently achieved virtually complete stereocontrol for a related transformation in the synthesis of bafilomycin A_1.[8, 9b] Based on this precedent, the chlorophenylboryl enolate of **3** (2 equiv) generated by sequential treatment with $PhBCl_2$ (2 equiv) and diisopropylethylamine (2.2 equiv) in CH_2Cl_2 at -78 °C was reacted with **2** to give an easily separable 3:1 mixture of the desired *anti*-Felkin product **10a** and Felkin product **10b** in 60% yield, with complete *syn* aldol diastereoselectivity. According to the Evans model,[8] the modest selectivity of addition to the *anti*-Felkin aldehyde diastereoface might be attributed to the difference of the C22 stereocenter in chiral ketone fragments, which was confirmed with the model system.[18] Hemiacetal formation concurrent with removal of silyl ether and silylene acetal protection in **10a** was performed with TBAF-AcOH in THF to give the hemiacetal **11** quantitatively. Site-selective esterification of the C21 hydroxyl group in **11** with fumaric acid half ester **12** by the aid of *N*-ethyl-*N'*-3-dimethylaminopropyl carbodiimide (EDC) hydrochloride and DMAP followed by reductive removal of the 2-iodoethyl group completed the total synthesis of hygrolidin (**1**). The synthetic material was spectroscopically (IR, ^{1}H and ^{13}C NMR, HRMS) identical with natural hygrolidin,[2] and also had a specific rotation, $[\alpha]^{27}_D$ +42.6° (*c* 0.24, $CHCl_3$), in good agreement with the literature value [lit.,[2] $[\alpha]^{20}_D$ +43.3° (*c* 1.03, $CHCl_3$)]. Thus, the absolute stereochemistry of this natural product has been established.[22]



(a) TBAF, AcOH, THF, 15 h, quant.; (b) TMS-imidazole, CH_2Cl_2, 24 h,; (c) $NaHCO_3$, MeOH, 6.5 h, 81% (2 steps); (d) Dess-Martin periodinane, pyridine, CH_2Cl_2, 1.5 h, 85%; (e) $PhBCl_2$, **3**, $^iPr_2NEt$, CH_2Cl_2, -78 °C, 1 h, 45% of **10a** and 15% of **10b** (f) TBAF, AcOH, THF, 36 h, 99%; (g) **12**, EDC•HCl, DMAP, CH_2Cl_2, 6 h, 79%; (h) Zn, NH_4Cl, H_2O, EtOH, 3 h, -10 °C, 97%

Scheme 3

In conclusion, we have achieved the first total synthesis of macrolide antibiotic hygrolidin by a convergent route with an overall yield of 0.22% (based on 33 steps longest linear sequence), which unambiguously determined the absolute stereostructure of hygrolidin. Efforts to reveal mechanistic factors responsible for the formation of symmetrical anhydride in the first process of Yamaguchi's lactonization are currently underway.

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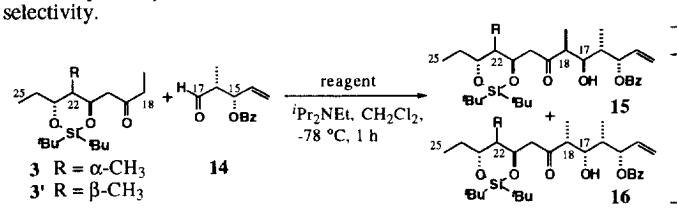
14. Since removal of even the triethylsilyl protecting group at C7 proved to be problematic after macrolactonization, the seco-acid without protection of the C7 hydroxyl group was used. No difference in lactonization was observed whether it was protected or not.
15. The ratio was determined by ^{1}H-NMR analysis of the crude mixture.
16. Symmetrical anhydride **8**: IR (neat) 3470, 2925, 1745, 1615, 1460, 1385, 1260, 1030 cm^{-1}; ^{1}H NMR : δ (500 MHz, C_6D_6) 7.80-7.86 (m, 4H, ArH), 7.50 (s, 1H, C_3-H), 7.27 (m, 6H, ArH), 6.58 (dd, *J* = 15.0, 11.0 Hz, 1H, C_{12}-H), 6.06 (d, *J* = 11.0 Hz, 1H, C_{11}-H) ,5.72 (dd, *J* = 15.0, 8.5 Hz, 1H, C_{13}-H), 5.71 (d, *J* = 9.0 Hz, 1H, C_5-H), 4.08 (m, 1H, C_{15}-H), 3.80 (dd, *J* = 10.0, 6.0 Hz, 1H, C_{17}-H), 3.77 (dd, *J* = 10.0, 5.0 Hz, 1H, C_{17}-H), 3.73 (brt, *J* = 7.0 Hz, 1H, C_{14}-H), 3.18 (s, 3H, C_{14}-OCH_3), 2.96 (m, 1H, C_7-H), 2.57 (m, 1H, C_6-H), 2.46 (dd, *J* = 13.0, 2.0 Hz, 1H, C_9-H), 2.26 (m, 1H, C_{16}-H), 2.07 (s, 3H, C_2-CH_3), 1.73 (m, 1H, C_8-H), 1.66 (s, 3H, C_4-CH_3), 1.62 (s, 3H, C_{10}-CH_3), 1.21 (brs, 12H, Si-C_4H_9, C_{16}-CH_3), 0.88 (d, *J* = 7.0 Hz, 3H, C_6-CH_3), 0.83 (d, *J* = 6.5 Hz, 3H, C_8-CH_3); HRMS (FAB): calcd for $C_{80}H_{114}O_{11}Si_2Na$ $(M + Na)^+$ 1329.7798, found 1329.7797.
17. The reaction is presumed to proceed through the common intermediacy of the acyl pyridinium salt formed from **7** or **8** with DMAP, wherein the regenerated seco-acid **4** in the latter reaction could be used for macrolactonization in the presence of large quantities of 2,4,6-trichlorobenzoyl chloride.
18. Double stereodifferentiating aldol reactions of boron[19, 20] or chlorotitanium[21] enolates of **3** and its C22 epimer **3'** (hygrolide numbering) with the chiral model aldehyde **14** were explored. As seen from the table, it is evident that both (*Z*)-boron and chlorotitanium enolates of **3'** exhibit an exceptionally high order of selectivity for *syn* aldol *anti*-Felkin diastereomer **15**, whereas those of **3** show a modest level of π-facial selectivity.



3 R = α-CH_3
3' R = β-CH_3
14
15
16

substrate	reagent	% yield	**15** : **16**
3	$PhBCl_2$	65	3.0 : 1
3	BCl_3	62	3.3 : 1
3	$TiCl_4$	68	1.4 : 1
3'	$PhBCl_2$	51	>15 : 1
3'	BCl_3	50	>15 : 1
3'	$TiCl_4$	58	>15 : 1

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