Interphylal Product Splicing: The First Total Syntheses of Cephalostatin 1, the North Hemisphere of Ritterazine G, and the Highly Active Hybrid Analogue, Ritterostatin G_N1_N[1]

Thomas G. LaCour,*,† Chuangxing Guo,† Sudhakar Bhandaru,† Michael R. Boyd,‡ and P. L. Fuchs*,†

Contribution from the Department of Chemistry, Purdue University, West Lafayette, Indiana 47907, and Laboratory of Drug Discovery, Research and Development, National Cancer Institute, Frederick, Maryland 21702

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Abstract: Convergent total syntheses of the extremely potent cell growth inhibitor cephalostatin 1 and two hybrid analogues, ritterostatins G_N1_N and G_N1_S, have been achieved. *Ritterostatin G_N1_N displays sub-nanomolar activity in the 60 cell line human tumor panel of the National Cancer Institute.* The North hemisphere of ritterazine G was efficiently constructed from hecogenin acetate in 15% yield over 13 steps. Extension of a key photolysis/Prins sequence to intermediates **19** and **32** proceeded in excellent yield, leading to installation of the Δ^{14} moiety in the North G and South 1 steroidal subunits. Application of a method for directed unsymmetrical coupling furnished the natural and analogue pyrazines in good yield from the cephalostatin and ritterazine components.

Introduction

Cephalostatin 1 (**1**)[2] is among the most powerful anticancer agents ever tested by the National Cancer Institute. Cephalostatin 7 (**2**)[3a] and ritterazine G (**3**)[4] are other very potent (sub-nanomolar antineoplastic activity) members of an expanding group of trisdecacyclic pyrazines isolated from *different phyla*: from the marine tube worm *Cephalodiscus gilchristi* in the Indian Ocean,[2,3] by Pettit at Arizona State University, and from the tunicate *Ritterella tokioka* off the coast of Japan,[4] by Fusetani at the University of Tokyo (Figure 1). Surprisingly, they are structurally related, featuring the union of two C_{27} steroids (referred to as North and South) taken from an array of six basic skeletal units which may be seen as substituted isomers of the abundant plant-derived steroid hecogenin.[5] Clinical trials of cephalostatins 1 (**1**) and 7 (**2**) have stalled because of severe difficulties in harvesting these rare materials (0.1 g of **1**, <60 mg of **2**, from 450 kg of worm) by SCUBA operations at 60−80 m in the white shark infested waters off East Africa.[6] This scarcity will be nontrivial to alleviate via synthesis due to the complexity of their steroid substructures, as evidenced by the heroic preparations of cephalostatin 7 (**2**)[7] and the 14′α,15′-dihydro analogue of **1**,[1] wherein each of the 3-ketosteroid precursors required 28−33 steps (1−3% overall yield) from hecogenin acetate. Interestingly, several of the ritterazines exhibit cytotoxicities approaching the same nanomolar range as the most active of the cephalostatins, although the ritterazines are far less oxygenated. Their relative simplicity promises

* To whom correspondence should be addressed. E-mail: pfuchs@chem.purdue.edu or lacourtg@chem.purdue.edu. Fax: (765) 494-7679.

† Purdue University.

‡ National Cancer Institute.

(1) Cephalostatin synthesis. 11. Portions of this work have been communicated in ref 12a and in paper 10 of this series: Guo, C.; Bhandaru, S.; Fuchs, P. L.; Boyd, M. R. *J. Am. Chem. Soc.* **1996**, *118*, 10672; see also references therein for syntheses of the cephalostatin subunit precursors **29** and **37** from hecogenin acetate.

(2) Pettit, G. R.; Inoue, M.; Kamano, Y.; Herald, D. L.; Arm, C.; Dufresne, C.; Christie, N. D.; Schmidt, J. M.; Doubek, D. L.; Krupa, T. S. *J. Am. Chem. Soc.* **1988**, *110*, 2006, reported $ED_{50} < 10^{-4}$ ng/mL, corrected = 0.01−0.001 ng/mL (P388 leukemia). Mean GI_{50} 1.2 nM (ref b), 2.3 nM (ref 1), 3.5 nM (8/4/97 test, this work).

(3) (a) Pettit, G. R.; Kamano, Y.; Inoue, M.; Dufresne, C.; Boyd, M. R.; Herald, C. L.; Schmidt, J. M.; Doubek, D. L.; Christie, N. D. *J. Org. Chem.* **1992**, *57*, 429. Neither precise ED_{50} (P388 mouse leukemia) nor GI_{50} values were ever reported. The paper states that "Cephalostatins 7−9 displayed remarkable potency with TI_{50} (molar) values of 10^{-9} to $<10^{-10}$ against a number of (e.g. renal RXF-393 ... leukemia RPMI-8226) cell lines and values of 10^{-8}−10^{-9} for the breast MCF-7 cell line..." Our own testing results (8/4/97) with synthetic cephalostatin 7 (**2**) revealed a mean GI_{50} of −6.8 (150 nM) across the entire 60-cell line panel, with certain cell lines showing greater effect: GI_{50} −8.1 (7.9×10^{-9} molar = 7.9 nM) renal RXF-393, −8.0 (10 nM) leukemia RPMI-8226, but −6.6 (229 nM) for the breast MCF-7 line, also see ref 2. Finally, Professor Pettit has indicated that these latter results are in accord with his own and may be considered definitive. (Personal communication, G. R. Pettit, December 1997.) (b) Pettit, G. R.; Xu, J.; Schmidt, J. M.; Boyd, M. R. *Bioorg. Med. Chem. Lett.* **1995**, *5*, 2027 and references therein.

(4) (a) Fukuzawa, S.; Matsunaga, S.; Fusetani, N. *Tetrahedron* **1995**, *51*, 6707 and references therein; ritterazines B and G, IC_{50} 0.15 and 0.73 ng/mL (P388 leukemia). (b) Fukuzawa, S.; Matsunaga, S.; Fusetani, N. *J. Org. Chem.* **1997**, *62*, 4484. The southern hemisphere is acid sensitive and isomerizes even at 25 °C to several compounds, including some apparently related to North 5, the sixth and least active "basic"steroidal subunit: see refs 5, 7, and 11.

(5) "North" has also been designated "right side" by Pettit[2,3] and "East" by Fusetani.[4] Ranked by the bioactivities of the pyrazines containing them: North and South 1, South 7 and North G, North A, North 5. Cephalostatin basic units: North 1, South 1, South 7, and North 5. Ritterazine basic units: North A and North G, with 7βOH-South 7 ("South A", not a "basic" unit) appearing commonly. In fact, South 7 and North G may be viewed as deoxygenated spiroketal isomers of North 1, and 7β-OH-South 7 as a spiroketal isomer of North 1 with translation of one hydroxyl group. North G is considered a basic unit rather than North B (the 14β dihydro, 22*R* spiroketal isomer of North G since the Δ^{14} function permits access to all the D ring functions seen in the ritterazines; see ref 4.

(6) Pettit reports that ∼0.5 ton of the organisms yielded approximately 100 mg of cephalostatin 1 (**1**); ∼1 g of material is required for the initial phases of the trials. Personal communication, Professor G. R. Pettit, 12/94.

(7) (a) Jeong, J. U.; Sutton, S. C.; Kim, S.; Fuchs, P. L. *J. Am. Chem. Soc.* **1995**, *117*, 10157. (b) Kim, S.; Fuchs, P. L. *Tetrahedron Lett.* **1994**, *35*, 7163.

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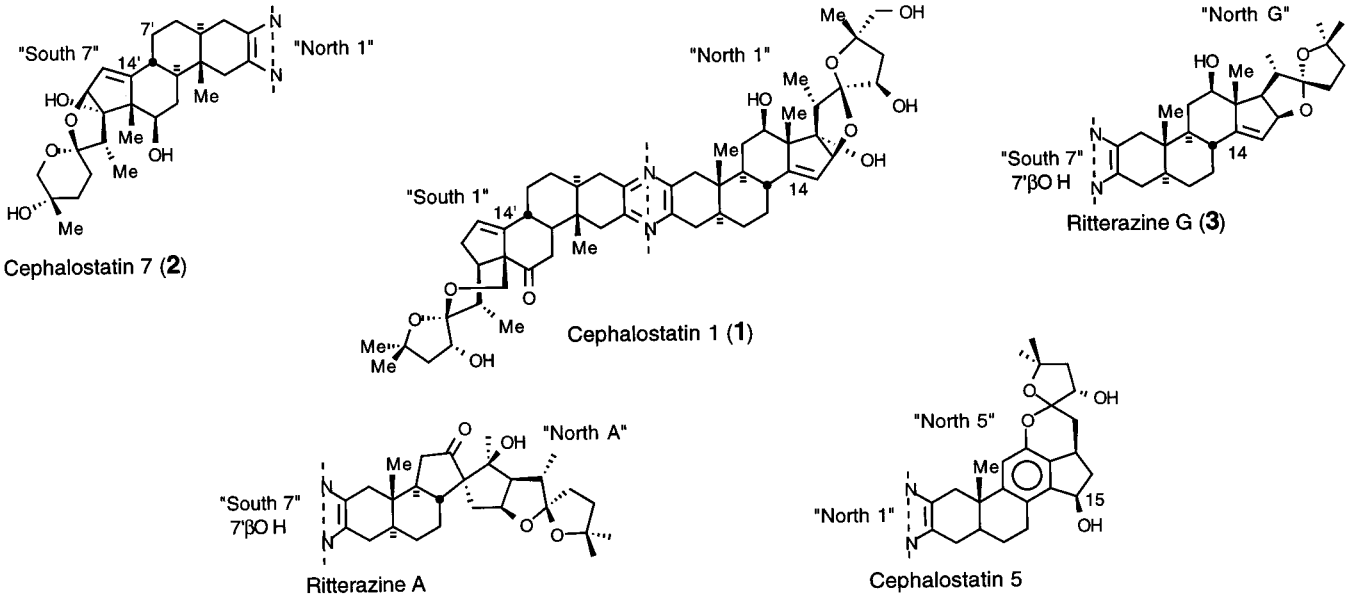


Figure 1.

greater synthetic accessibility with probable retention of significant bioactivity.

Nature has provided a wealth of SAR data in the form of the 43 variations on this theme so far isolated by Pettit[3] and Fusetani,[4] although a number of questions remain. Subtle variations of stereochemistry and substitution on the six basic steroid units display marked effects on bioactivity. We and others have addressed some of these questions by the syntheses of dihydrocephalostatin 1,[1] derivatives of ritterazine B,[4b] and pyrazines related more closely to hecogenin.[10] Provocatively, the 11 most potent (subnanomolar activity) pyrazines of the natural series utilize only the four basic units North 1, South 1, South 7, and North G (Figure 1),[5] in four of the six possible unsymmetric combinations, of which the South 1 / North 1 combination **1** is the most potent yet seen.

The unknown combinations are those of North G with North 1 and South 1, which would constitute the union of units from disparate organisms, hence "interphylal natural product splicing." Indeed, North 1 and 7βOH-South 7 (the southern hemisphere of many of the ritterazines, including ritterazine G **3**) have the same level of oxygenation, differing only in the location of one hydroxyl group (C_{23} vs C_7) and in their isomeric spiroketal arrangements. Since **1** is significantly more potent than **2** or **3**, which utilize South 7-type subunits, the question of such chemical "cross-breeding" takes on significance. Directed efforts to answer this and other remaining synthetic and SAR challenges may lead not only to new chemical insights but to practical and potent chemotherapeutic agents.

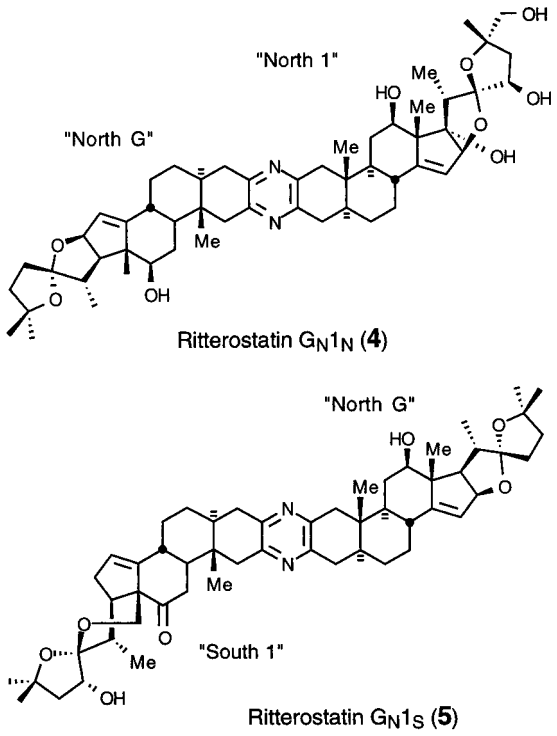


Figure 2.

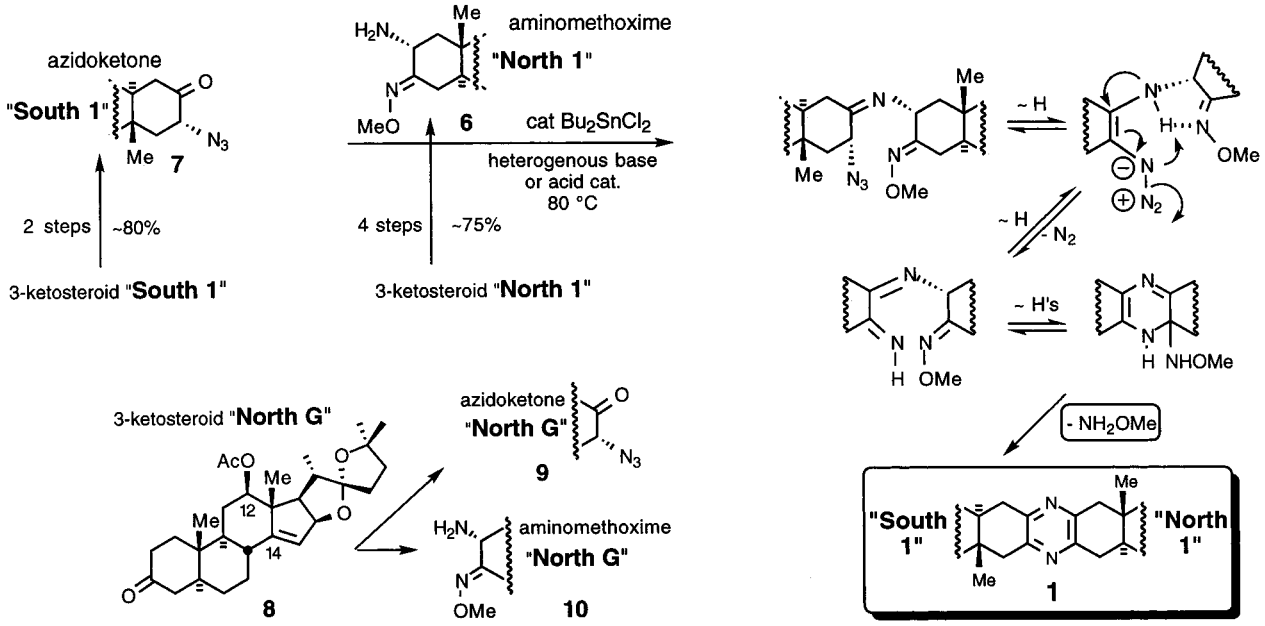
Thus, in addition to the important goal of completing the first total synthesis of the natural product **1**, and as part of general SAR explorations, we consider it useful to construct these hybrids composed of ritterazine and cephalostatin steroidal subunits (Figure 2). In the present study, we describe the first synthesis of the North hemisphere of ritterazine G via a formal isomerization of the spiroketal group of hecogenin acetate, extension of an optimized photolysis/Prins protocol to install the Δ^{14} functionality, use of the protocol to deliver the same moiety into the South hemisphere of cephalostatin 1 from an advanced saturated intermediate, and application of the unsymmetrical pyrazine formation method developed in these laboratories[1] to provide the first total syntheses of cephalostatin 1 (**1**) and the hybrid analogues ritterostatin G_N1_N (**4**)[8] and ritterostatin G_N1_S (**5**).[8]

(8) The naming scheme employed for the unnatural ritterazine−cephalostatin hybrids combines the lettering scheme of Fusetani[4] with the number designations of Pettit[2,3] and is necessarily further extended by adopting a "North−South" designator to indicate which hemisphere of each material is present in the new analogue. Thus, ritterostatin G_N1_N (**4**) unambigiously describes a material composed of the North steroidal unit of ritterazine G, combined with the North steroidal segment of cephalostatin 1. In concept, one could also name the same material cephalozine 1_NG_N. While this latter designation is extremely attractive in recognition of Pettit's seminal contributions to the discovery and structural elucidation of the cephalostatins, we ultimately opted for the ritterostatin appellation in deference to the "statin" suffix which provides more immediate information vis-à-vis the compound's biological activity.

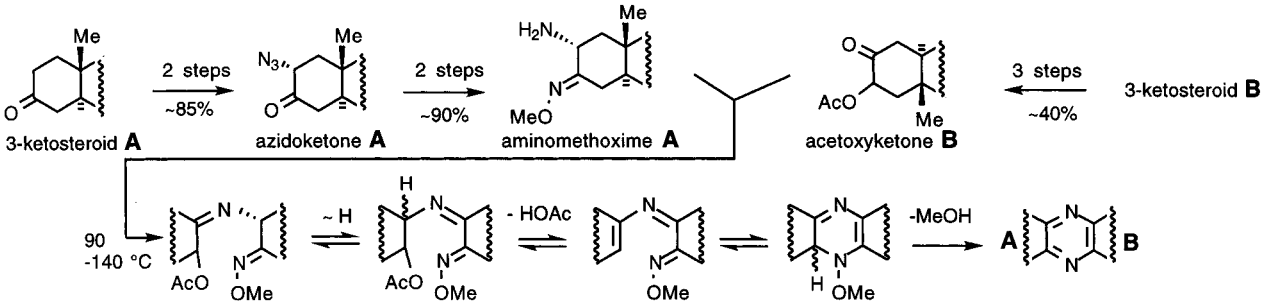
(9) (a) Smith, S. C.; Heathcock, C. H. *J. Org. Chem.* **1992**, *57*, 6379. (b) Heathcock, C. H.; Smith, S. C. *J. Org. Chem.* **1994**, *59*, 6828.

(10) Drogemuller, M.; Jautelat, R.; Winterfeldt, E. *Angew. Chem., Int. Ed. Engl.* **1996**, *35*, 1572.

Scheme 1. Guo, Bhandaru, and Fuchs (ref 1)



Scheme 2. Smith and Heathcock (ref 9)

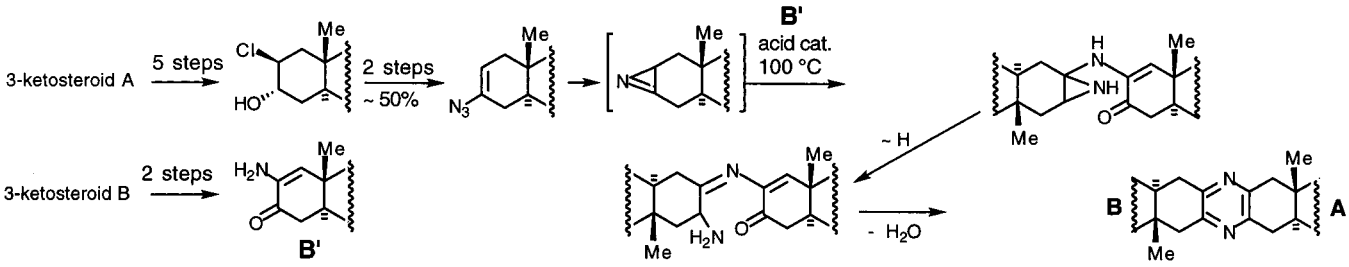


Since we wish to be in a position to construct an array of unsymmetrical pyrazines, the choice of method for coupling different subunits is of prime concern. We recently reported the first total synthesis of cephalostatin 7 (**2**) via a biomimetic approach in which the central pyrazine ring was constructed by a statistical coupling of the North and South α-amino 3-ketosteriods (produced in situ from the corresponding α-azido ketosteroids);[7] however, such a method is inappropriate for our present purpose. Rather, the central pyrazine ring of cephalostatin 1 (**1**) was envisioned as arising from the directed reaction of the α-aminomethoxime "North 1" (**6**) with α-azidoketone "South 1" (**7**) via the newer protocol that was successfully employed to synthesize dihydrocephalostatin 1, the 14′α,15′-dihydro analogue of **1** (Scheme 1).[1] Likewise, the appropriate derivatives of "North G" (**8**) would be coupled with **6** and **7** to give the analogues. This method for the synthesis of cross-coupled, "C_2-pseudosymmetric" (i.e., trans vs cis) pyrazines is much milder (80 °C, 3−6 h) and better yielding (60−90%) than the seminal procedure reported by Heathcock and Smith, which involved the reaction of an α-aminomethoxime with an α-acetoxy ketone at elevated temperatures (90−140 °C) for 2 days with yields of 29−43% for two cases (Scheme 2).[9] The seemingly trivial substitution of an azido ketone for the acetoxy ketone as the acceptor partner for imine formation has two important consequences. The first of these simply relates to a more efficient preparation of the acceptor (~80%, two steps vs ~40%, three steps). The more important difference pertains to a change of mechanism, for the reaction produces N_2 gas and becomes basic (instead of acidic, as in Heathcock's method) through the loss of methoxylamine.

More recently, Winterfeldt and co-workers reported construction of nonsymmetrical steroid pyrazines via the reaction of a steroidal azirine, formed in situ from a vinyl azide, with a steroidal amino enone (Scheme 3).[10] While the conditions and yields for the highly creative coupling step (100 °C, dioxane, PPTs, 51−67% for two cases) are more competitive with those of our methodology, the azirine strategy suffers some drawbacks: (i) a longer synthetic sequence to prepare the vinyl azide from a 3-ketosteroid, with attendant loss of precious late-stage material, and (ii) the use of homogeneous acid catalysis, which causes isomerization of labile spiroketals such as that present in the South 7 subunit.[4b,11] The coupling protocol described herein is milder, functions well with heterogeneous base catalysis, and permits rapid derivation (two to four steps) of the coupling partners from the parent 3-ketosteroids in high yield (vide infra). Schemes 1−3 illustrate plausible mechanisms for the three methods. For reasons of brevity, mildness and yield,

(11) (a) The Southern hemisphere of cephalostatin 7, "South 7", contains an acid-labile [5,6] spiroketal which undergoes extensive isomerization (<50% remaining South 7 after brief reflux at 80 °C with catalytic PPTs) when heated with acid and has exhibited a tendency to isomerize under even mild provocation to a mixture of spiroketals (Jeong, J. U.; Guo, C.; Fuchs, P. L. Synthesis of the South Hexacyclic Portion of Cephalostatin 7, manuscript in preparation, and see ref 23). The 20α,22α [5,5] spiroketal isomer, precisely "South C" (the southern hemisphere of ritterazine C, is calculated to lie only 0.04 kcal above that of the natural 20α,22α [5,6] spiroketal of South 7. (b) Calculations performed using CAChe 3.1; see the Supporting Information for the energies of all isomers.

Scheme 3. Drogemuller, Jautelat, and Winterfeldt (ref 10)



we therefore chose the same unsymmetrical coupling utilized in the synthesis of the dihydrocephalostatin 1 analogue.[1] In this way, the union of "North 1" aminomethoxime **6** with "South 1" azido ketone **7** leads to cephalostatin 1 (**1**), that of **6** with "North G" azido ketone **9** leads to ritterostatin G_N1_N (**4**), and that of **7** with "North G" aminomethoxime **10** leads to ritterostatin G_N1_S (**5**).

As part of our ongoing program to synthesize the most bioactive members of the ritterazines and cephalostatins, we desired a more direct route to functionalization of ring D than that employed in previous syntheses which also delivered C-17 oxygenation.[1,7] The Δ^{14} moiety is present in 55 of the 86 steroidal subunits of the known trisdecacyclic pyrazines, including the North half of ritterazine G (**3**) and the South half of cephalostatin 1 (**1**), neither of which require a 17-hydroxyl. To efficiently access this unsaturation from existing synthetic materials,[12] we inquired whether the known photolysis/Prins/elimination strategy of Welzel[13a] (recently employed on hecogenin acetate to generate pyrazine steroidal subunits by Winterfeldt)[10,14a] could be utilized for our substrates. The importance of at least one such unsaturation to bioactivity is indicated by the low activities of Winterfeldt's saturated analogues,[10] our own 14′β,15′-dihydrocephalostatin 1 analogue (still highly active with one Δ^{14} in the North and a trans-fused C/D junction in the South, but less active than **1** in which both hemispheres are unsaturated),[1] and the inferior activity of the natural fully saturated ritterazines.[4b] This moiety is also essential for synthetic access to the type of cis-fusion of the C/D rings found in the most potent ritterazines such as B and F, and other 14-hydroxylated or epoxidized subunits.[3,4]

Welzel's protocol drew from the pioneering work of Bladon.[15a] Specifically, Bladon found that irradiation of hecogenin acetate **11** affords lumihecogenin acetate **13** as the main product (~80% on 250 g scale), which on further irradiation undergoes Paterno−Büchi cyclization to photohecogenin acetate **14** (Scheme 4). Chinn[15b] demonstrated that oxetane **14** participated in the same acid-catalyzed ene and Prins reactions by prior conversion back to **13**. Welzel more fully explored these reactions, identifying both of the Prins products and correcting the assignment of the 14-hydroxy product stereochemistry. Additional pertinent transformations of the photoproducts reported by Bladon,[15a] Chinn,[15b] Welzel,[13a] and Winterfeldt[14b] are summarized in the scheme. Under Welzel's protocol, a mixture[13b] of **13** and **14** underwent the intramolecular Prins reaction[16] in modest yield to give a diastereomeric mixture of diols **15** (6.5:1 α/β, 52%), which on oxidation[17] afforded the keto alcohol **17** (67%; also accessible in one pot by oxidation of **13** in AcOH). Elimination of the 14β-hydroxyl gave the Δ^{14} ketone **18** (69%) contaminated by an alkyl chloride.[18] The calculated conversion from ketone **11** to **17** of 27%, 19% overall to impure **18**, was somewhat daunting. After our investigations of the **11** to **18** sequence, Winterfeldt has published an improved $BF_3{\cdot}OEt_2$-mediated ene reaction of **13**, achieving an 80% yield of Δ^{14} alcohol **16**α from ketone **11** in only two steps,[14b] now making **16**α extremely attractive as an alternate precursor to **18**.[37]

In light of studies on fundamental carbonyl photochemistry, insights of practical significance may be gleaned by examination of the factors that account for the formation of Norrish type I (α-cleavage) product **13** in such preparatively useful yield. It appears that structural features of **11**, both gross and subtle, play a most significant role, but the solvent must also be considered. The acyl and tertiary alkyl radicals produced (reversibly) by α-cleavage are relatively stable, and intramolecular 14α-H abstraction to give the secoaldehyde **13** may proceed without undue strain, so that 11-ketene is not formed;[15b] however, the stability of such radicals alone is insufficient to ensure exclusive type I cleavage. Type II reactions of excited ketones often proceed at a higher rate, and the well-known

(12) (a) Bhandaru, S.; Fuchs, P. L. *Tetrahedron Lett.* **1995**, *36*, 8351. (b) Bhandaru, S.; Fuchs, P. L. *Tetrahedron Lett.* **1995**, *36*, 8347.

(13) (a) Welzel, P.; Janssen, B.; Duddeck, H. *Liebigs. Ann. Chem.* **1981**, 546 and references therein; (b) In response to a reviewer's question, Welzel reports reactions on what he terms "Gemisches von **4** und **5**" [here **13** and **14**] obtained directly from photolysis of **11**; Winterfeldt (ref 14a) makes no mention of the composition of his photolysate, but simply references Bladon and Welzel. However, the crude product from photolysis demonstrably contains multiple products and is not merely a mixture of **13** and **14**. The amorphous mass in the mother liquor after crystallization of **13** contained little or no **14** by NMR, and its quantitative conversion to diols **15**, as well as the quantitative conversion of the crude photolysate, is in our opinion a repetition of neither Winterfeldt's nor Welzel's efforts but a useful improvement; (c) Welzel has also extended this protocol to the synthesis of cardenolides and bufadienolides from less-strained, tetracyclic steroids, although the yields in this series suffer by comparison to hecogenin acetate **11**; see ref 30.

(14) (a) Kramer, A.; Ullmann, U.; Winterfeldt, E. *J. Chem. Soc., Perkin Trans. 1* **1993**, 2865. (b) Jautelat, R.; Winterfeldt, E.; Muller-Fahrnow, A. *J. Prakt. Chem.* **1996,** *338*, 695.

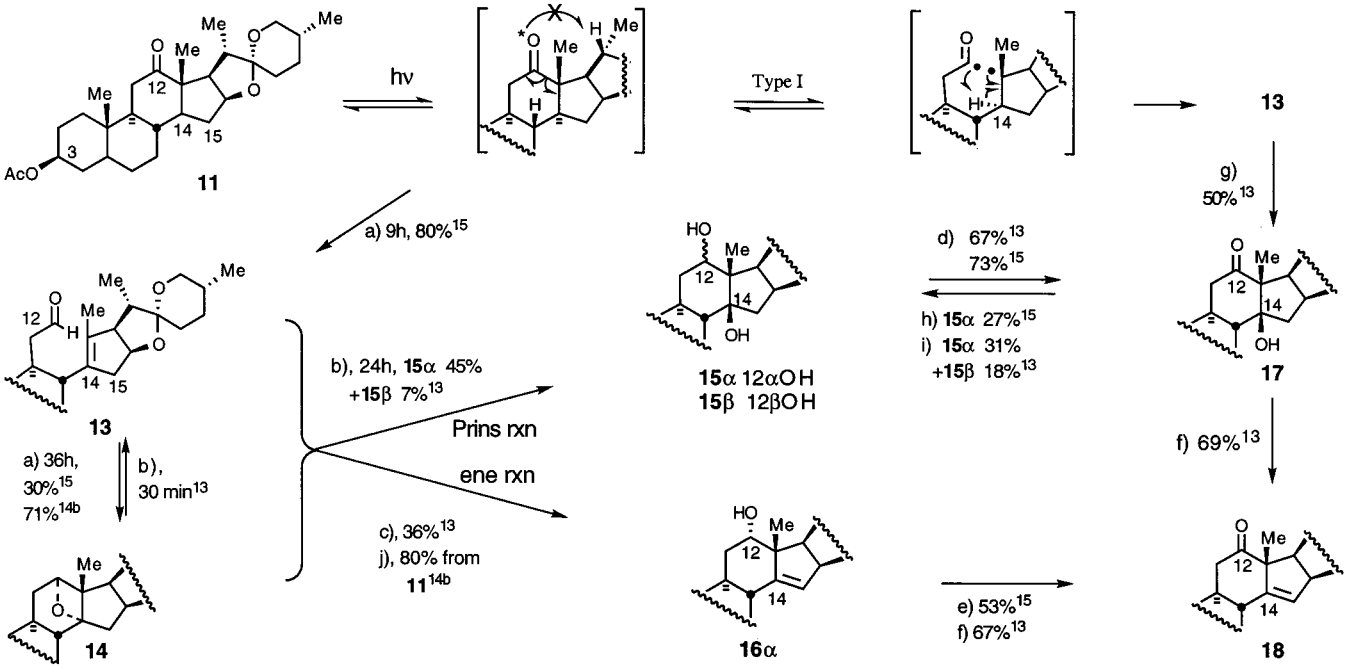
(15) (a) Bladon, P.; McMeekin, W.; Williams, I. A. *J. Chem. Soc.* **1963**, 5727 and references therein. (b) Chinn, L. J. *J. Org. Chem.* **1967**, *32*, 687, reported only **15**α. Chinn and Bladon (ref. 15a) both assigned the stereochemistry of the 14-OH group in **15** and **17** as "α" but were subsequently corrected by Welzel: the correct assignment is "β" as shown (ref 13).

(16) (a) Prins, H. J. *Chem. Weekbald* **1919**, *16*, 1510 (b) Arundale, F.; Mikeska, L. A. *Chem. Rev. (Washington, D.C.)* **1952**, *51*, 505 (c) Smissman, E. E.; Schnettler, R. A.; Portoghese, P. S. *J. Org. Chem.* **1965**, *30*, 797.

(17) "Jones" reagent, originally known as "Kiliani reagent", prepared as per Bladon et al. *J. Chem. Soc.* **1951**, 2402.

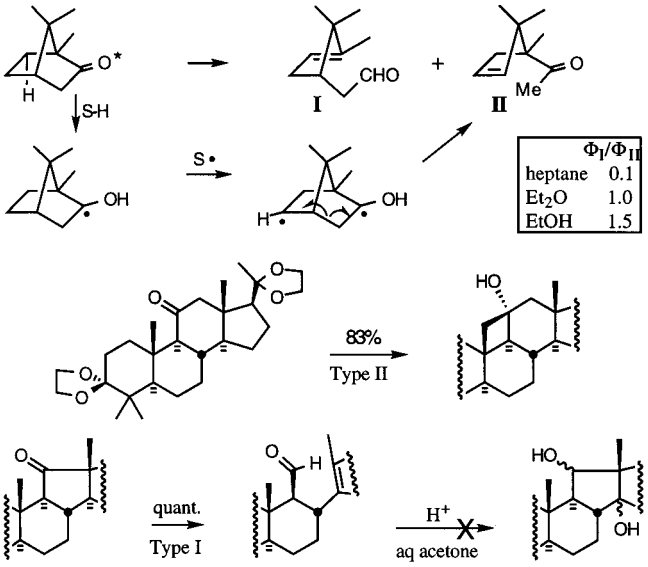
(18) Welzel reports that the product isolated after chromatography (69%) gave a peak in its mass spectrum which corresponds to formal HCl addition to **18**; the amount of this impurity was estimated to be ~10% of the isolated material. Under our modified conditions (−10 to 0 °C instead of room temperature, much less $SOCl_2$, early quench) the reaction was found to give a quantitative mass balance of material containing ~15−20% of such side product (varying by run, amount estimated by NMR), tentatively identified as the 14β chloride, which almost completely coeluted with desired **18** (TLC in several systems show a single spot; crystallization has not yet permitted separation). Careful chromatography furnished an 83% yield of material which was nearly pure (by sacrifice of later fractions, which contained more substantial amounts of the chloride mixed with the desired olefin) but which still contained ~6−9% of the chloride. While we have not yet been able to isolate this chloride wholly pure, MS and NMR comparisons to 14β chloride **25d** make its identification as the 14β chloride persuasive.

Scheme 4[a]



[a] (a) *hν*, dioxane; (b) 75% AcOH in H_2O, 25 °C; (c) BF_3·OEt_2, benzene, 25 °C; (d) CrO_3, aqueous H_2SO_4, acetone, 25 °C, 5 min; (e) PCC, CH_2Cl_2, 25 °C; (f) $SOCl_2$, Pyr, 0−25 °C, 1 h; (g) CrO_3 in aqueous AcOH, 25 °C; (h) $LiAlH_4$, THF, 25 °C; (i) $NaBH_4$, MeOH, 25 °C; (j) excess BF_3·OEt_2, toluene, 0 °C.

Scheme 5



propensity of α-trialkyl ketones to suffer α-scission was shown by Yang, Lewis, Wagner, and others to be subject to effective competition from type II pathways when γ-H (or even δ-H in suitable substrates)[19] abstraction is conformationally available.[20] In **11**, no such hydrogen atom is within reach of the excited carbonyl oxygen, and type I should predominate.[21] Yet even when *intramolecular* abstraction is structurally precluded, *intermolecular* participation by solvent can lead to reduction or *net* type II product as with camphor[22] (Scheme 5). The importance of ring-strain relief to successful dominance of α-cleavage is further demonstrated by the behavior of 17-oxo androstanes, which often display only epimerization (by recombination of type I acyl/tertiaryalkyl radicals) and reduction.[23] Excessive ring strain, while useful for maximizing type I cleavage even when the ketone is only α-disubstituted, as in 11-oxo-nor-C-steroids vs 11-oxosteroids of otherwise similar structure, may prevent subsequent recyclization (Scheme 5).[24] Ketone **11** apparently possesses the requisite balance of ring strain for both photolysis and cyclization to proceed. Its rigid structure might preclude any β-cleavage resulting from intermolecular, solvent assisted production of a type II 1,4-biradical (via net γ-hydrogen abstraction at C_{16}, C_{20}, or C_8) since considerable reorganization would be required for sufficient overlap of the developing p-orbitals.[21d] Thus, aside from reduction, we may surmise that *only products derived from the type I process might be expected from photolysis of* ***11***.

Results and Discussion

Synthesis of South 1 (7) and North G (8). Our initial approach to North G (**8**) from **11** relied upon this method to introduce the Δ^{14} moiety in **18**, with intended subsequent

(19) For a leading review, see: Wagner, P. J. *Acc. Chem. Res* **1989**, *22*, 83.

(20) (a) Yang, N. C.; Feit, E. D. *J. Am. Chem. Soc.* **1968**, *90*, 504. (b) Wagner, P. J.; McGrath, J. M. *J. Am. Chem. Soc.* **1972**, *94*, 3849. (c) Lewis, F. D.; Hilliard, T. A. *J. Am. Chem. Soc.* **1972**, *94*, 3852. For reviews, including discussion of the relative rates for Type II reaction, see: (d) Turro, N. J.; Dalton, J. C.; Dawes, K.; Farrington, G.; Hautala, R.; Morton, D.; Niemczyk, M.; Schore, N. *Acc. Chem. Res* **1972**, *5*, 92. (e) Wagner, P. J. *Acc. Chem. Res* **1983**, *16*, 461.

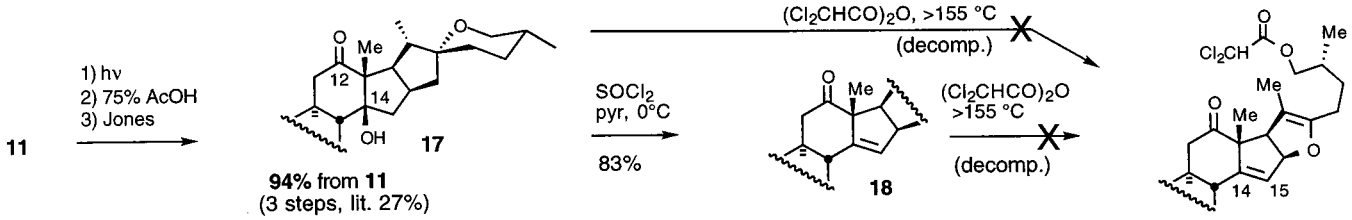
(21) (a) Lewis, F. D.; Johnson, R. W.; Johnson, D. E. *J. Am. Chem. Soc.* **1974**, *96*, 6090. For discussion of transition-state geometry for type II reaction and subsequent reactions of the 1,4 biradical, see: (b) Lewis, F. D.; Johnson, R. W.; Kory, D. R. *J. Am. Chem. Soc.* **1974**, *96*, 6100. (c) Wagner, P. J.; Kelso, P. A.; Kemppainen, A. E.; Zepp, R. G. *J. Am. Chem. Soc.* **1972**, *94*, 7500. (d) Wagner, P. J.; Kelso, P. A.; Kemppainen, A. E.; McGrath, J. M.; Schott, H. N.; Zepp, R. G. *J. Am. Chem. Soc.* **1972**, *94*, 7506. (e) Dawes, K.; Dalton, J. C.; Turro, N. J. *Mol. Photochem.* **1971**, *3*, 71. (f) Wagner, P. J. *Acc. Chem. Res.* **1971**, *4*, 168.

(22) (a) Yates, P. *Pure Appl. Chem.* **1968**, 93. (b) Srinivasan, R. *J. Am. Chem. Soc.* **1959**, *81*, 2604.

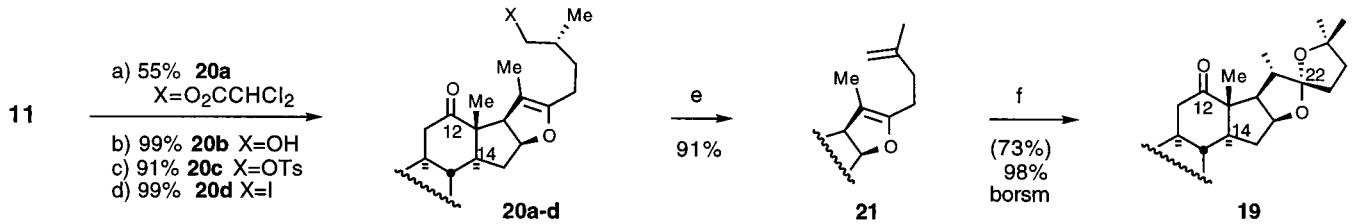
(23) Wehrli, H.; Schaffner, K. *Ber.* **1962**, *45*, 385 and references therein. (b) Butenandt, A.; Poschmann, L. *Ber. Deutsch. Chem. Ges.* **1944**, *77*, 392 and 394.

(24) Iriarte, J.; Schaffner, K.; Jeger, O. *Ber.* **1964**, *47*, 1255 and references therein.

Scheme 6



Scheme 7[a]



[a] (a) $(Cl_2CHCO)_2O$, 150−160 °C; (b) KOH/aqueous MeOH; (c) TsCl/pyr; (d) NaI; (e) DBU; (f) (i) 75% HOAc, (ii) Ac_2O/pyr.

opening of the [5,6] spiroketal and manipulation to the [5,5] system. This entry proved quite rewarding in its first stage, as several improvements in the protocol were developed (Scheme 6). We noted that the crude photolysate from **11** was composed of not only **13** and **14** as intimated by Welzel but rather many products (a fact alluded to by Bladon). Examination by NMR and MS revealed neither undesired vinyl (via net γ-hydrogen abstraction at C_{16}, C_{20}, or C_8) nor 12-OH (via reduction) compounds derived from solvent participation, indicating that dioxane remains effectively inert to this compound. Suspecting that the normal plethora of unidentified photo side products might, like oxetane **14**, be convertible to secoaldehyde **13** and thence to desired diols **15**, we first attempted such conversion on the complex mixture found in the mother liquor after crystallization of **13**. Several conditions (75% AcOH as per Welzel, dilute H_2SO_4, aqueous HBr/HOAc, aqueous TFA/CH_2Cl_2) gave inferior results, but extended exposure to 75% AcOH in a much more dilute reaction than indicated in the literature proved excellent, delivering pure diols **15** from the complex mixture. We further noted that **15**α (but not **15**β) is easily air-oxidized and that silica gel causes some degradation of both **15** and **17**, so care was taken to keep the oxidation cold and brief to achieve quantitative, clean conversion to **17** (the "milder" Brown−Jones method[25] gave inferior yields and purity). Subsequently applying these observations, and avoiding chromatography, we were pleased to find that *irradiation of a dioxane solution of* ***11*** *followed by slurry of the crude photolysate with 75% acetic acid (dilute, 25 °C, 24 h) smoothly and quantitatively effects the intramolecular Prins reaction to yield a ~5:1 mixture of diols* ***15***α*/****15***β.[26] *This crude mixture gave, upon Jones oxidation,*[17] *keto alcohol* ***17*** *in a remarkable 94% overall yield (99% when the 5% tigogenin (12-deoxyhecogenin) acetate present in commercial* ***11*** *is considered) for three steps from* ***11***. Dehydration of alcohol **17** to keto olefin **18** was accomplished in 83% yield[18] by modifying (cold, brief) Welzel's conditions. Other attempts to achieve improved conditions for elimination were totally unrewarding ($POCl_3$,[31a] Swern,[27] $BF_3{\cdot}Et_2O$,[28] sulfide or bromide installation during the Prins reaction, mesylation, xanthate formation, etc.). The chloride side product[18] always present in this alkene proved surprisingly resistant to elimination (DBU, hot pyridine, etc.). Further work on the transformation was suspended because, unfortunately, neither the 14β-OH (which resisted vigorous attempts at protection) nor the Δ^{14} moieties[37] were stable to the conditions necessary (>155 °C, acid anhydride) for spiroketal opening.[29]

Before commencing a potentially lengthy search for unprecedented spiroketal-opening conditions which would allow us to utilize D-ring functionalized materials **16**−**18**, we considered reversing the order of events. Our experience with spirostan-12-one **11** under the Welzel protocol encouraged attempted application to the related furostan-12-one **19**, which would arise from **11** by a prior spiroketal adjustment sequence (Scheme 7). The method had been extended by Welzel to 12-ketosteroids appropriate to the syntheses of cardioactive digitalins, bufadienolides, and cardenolides, but these tetracyclic precursors invariably gave poorer yields in the photolysis step,[30] possibly due to insufficient ring strain and type II competition. Indeed, a change of solvent from dioxane to CH_2Cl_2 was warranted. Further, there are many cases of substantial variance in the reactivities of distally differentiated steroids,[13,29,31] wherein what appears to be a minor perturbation in the steroid structure

(25) Brown, H. C.; Garg, C. P.; Liu, K.-T. *J. Org. Chem.* **1971**, *36*, 387.

(26) Interestingly, the Prins reaction produces a higher proportion of **15**β if allowed to stir for extended periods of time. The implied equilibrium between the diols was explored by resubjecting pure **15**α (the apparent kinetic product) to the reaction conditions (25 °C) for 15 h, whereupon a 6:1 ratio of **15**α/**15**β was obtained, presumably via a retro-Prins/Prins sequence. A 4:1 mixture of **15**α/**15**β was heated at 75 °C in 75% acetic acid for 15 h, at which time a 1:6 ratio of **15**α/**15**β was produced, the thermodynamic product **15**β greatly predominating; neither decomposition nor dehydration was observed. For similar evidence of such equilibrium in a related 1,3-diol, see ref 30a.

(27) Corey, E. J.; Gin, D. Y.; Kania, R. S. *J. Am. Chem. Soc.* **1996**, *118*, 9202.

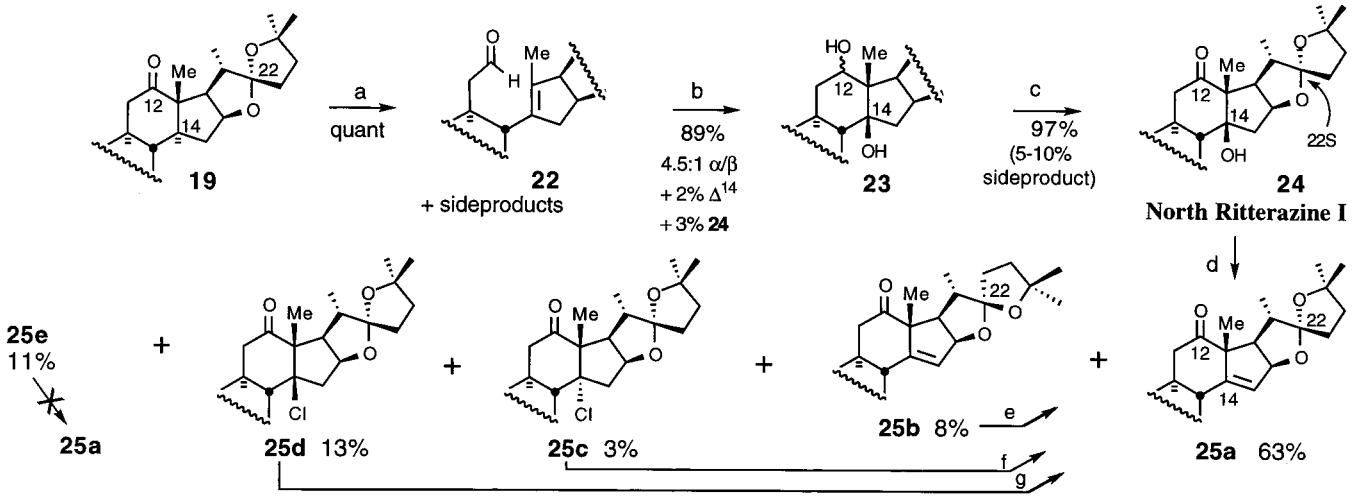
(28) Posner, G. H.; Shulman-Roskes, E. M.; Oh, C. H.; Carry, J.-C.; Green, J. V.; Clark, A. B.; Dai, H.; Anjeh, T. E. N. *Tetrahedron Lett.* **1991**, *32*, 6489.

(29) Jeong, J. U.; Fuchs, P. L. *J. Am. Chem. Soc.* **1994**, *116*, 773−774. See also: Cameron, A. F. B.; Evans, R. M.; Hamlet, J. C.; Hunt, J. S.; Jones, P. G.; Long, A. G. *J. Chem. Soc.* **1955**, 2807 for high-yield openings using fatty acid solvents (T > 150 °C) but which also fail on unsaturated steroids.

(30) (a) Digipurpurogenins I and II (~30% in the photostep): Welzel, P.; Moschner, R.; Ponty, A.; Pommerenk, U.; Sengewein, H. *Liebigs. Ann. Chem.* **1982**, 564. (b) Digitoxigenin (from hecogenin acetate): Milkova, T.; Stein, H.; Ponty, A.; Böttger, D.; Welzel, P. *Tetrahedron Lett.* **1982**, *23*, 413. (c) Milkova, T.; Stein, H.; Welzel, P. *Liebigs. Ann. Chem.* **1982**, 2119. (d) Bufalin (36% in the photostep): Hoppe, H.-W.; Welzel, P. *Tetrahedron Lett.* **1986**, *27*, 2459. (e) Digoxigenin (72% in the photostep): Stein, H.; Welzel, P. *Tetrahedron Lett.* **1981**, *22*, 3385 and ref 30c.

(31) For leading examples, see: (a) Fieser, L. F.; Fieser, M. *Steroids*; Rheinhold: New York, 1959, and references therein. (b) Djerassi, C. *Reactions of Steroids*; Holden-Day: San Francisco, 1963, and references therein.

Scheme 8[a]



[a] (a) *hν*, dioxane, 4 h; (b) 75% AcOH, 35 h; (c) CrO_3, H_2SO_4, acetone, 5 min; (d) 2 equiv of $SOCl_2$, 10 equiv of pyr, toluene, 5 min; (e) pTsOH, CH_3CN, 15 min, 85%; (f) DBU, CH_3CN, reflux, 30 min, 80%; (g) DBU, LiF, 100 °C, 1 h, 10%.

exercises profound changes in chemical behavior. The E/F ring strain difference expected between **11** and **19** which might be communicated to the C/D rings, and the possible increase in steric compression near the D ring in **19** were concerns, albeit ones which fortunately proved surmountable with respect to photolysis and Prins reaction (although all too well-founded with respect to ene or elimination reactions, vide infra).

The spiroketal of **11** was therefore opened and tosylate derivative **20c** obtained utilizing standard procedures[29] and then eliminated via the iodide **20d** with DBU to afford enol ether−olefin **21** in excellent yield. Several attempts to add water in Markownikoff fashion and form the [5,5] spiroketal were only marginally successful ($Hg(OAc)_2/NaBH_4$, aqueous CH_3CN with TFA or TsOH), but **21** hydratively cyclized through the action of hot aqueous acetic acid (with some loss of the 3-acetate, necessitating reacetylation) to furnish furostanone **19**[32] in 73% yield (98% based on recovered **21**).

Differences, sometimes dramatic, were noted in the reactivity of **19** compared to that of **11** throughout the remainder of the sequence. Photolysis of the furostanone to secoaldehyde **22** proceeded more quickly but less cleanly, and the Prins reaction yielded diols **23** more slowly (even as a solution rather than a slurry as for **13**, some unconverted photolysate remained after 36 h, but triol and Δ^{14} olefin were becoming evident).[33] These results bespeak the effect of increased C ring strain in **19** during the protocol compared to the corresponding steps from spirostanone **11**, although the yields remained high by faithfully carrying on the crude products to convergence (Scheme 8). By contrast, a tempting shortcut to diols **23** via photolysis of alkene **21** was unrewarding. It was hoped that the α-cleavage product would undergo one-pot successive Prins and hydrative spirocyclization reactions by temperature-controlled exposure to aqueous AcOH. Unfortunately, the desired cleavage was excruciatingly sluggish. After a 3 h irradiation of **21** in dioxane, only ∼7% aldehyde was produced, and after 20 h only a 1:2 ratio of aldehyde/starting ketone was obtained, with sizable buildup of low R_f byproducts already evident. The reaction did not approach completion even after 60 h. Dioxane participation as an H-atom donor was ruled out by conducting the reaction in acetonitrile,[34] but even less aldehyde and more byproducts were formed. Whether reduced C ring strain (from removal of the 22-spiro connection), alkene quenching of the excited ketone (by through-bond energy transfer[35] to the $C_{20,22}$ bond), type II β-abstraction[20] of the activated allylic 17α hydrogen (2.61 Å away), or a combination of these factors could account for the observations, it is clear that the method is no panacea applicable to all 12-ketosteroids.

Oxidation of diols **23** also proceeded less smoothly than had the analogous conversion of **15** to **17** which evidenced no byproducts. Jones oxidation of **23** gave 14β-hydroxyfurostanone **24**, the North segment of ritterazine I, containing 5−10% of an inseparable side product, possibly the 22*R* epimer.[36] However, dehydration to keto olefin **25a** was significantly more involved. Using even our modified conditions (vide supra), the reaction of **24** (stereochemistry confirmed by X-ray)[38] was lower yielding (42%) and much more complex than reaction of the 14-hydroxyspirostanone **17**. Revised conditions (dilute in toluene, 10 equiv of pyridine, 2 equiv of $SOCl_2$ required) and repeated fractionation by chromatography gave pure desired keto olefin **25a** (63%) and four side products **25b**−**e**. Repeated attempts to access the Δ^{14} moiety by ene reaction of **22** under Winterfeldt's conditions[14b] afforded only complex mixtures from which the desired homoallylic alcohol was obtained in poor yield.[37]

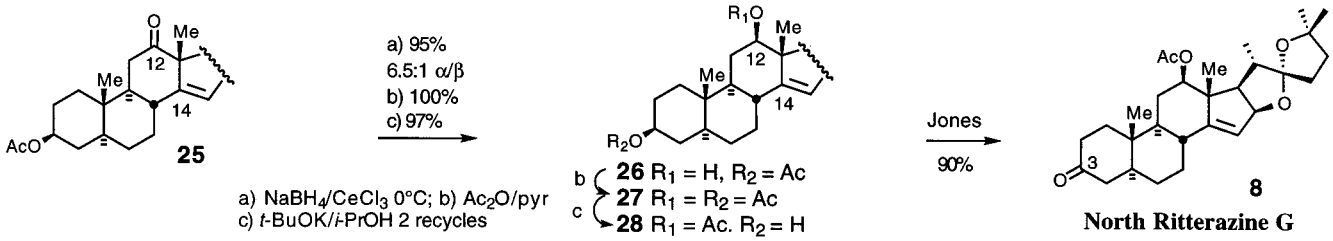
(32) The stereochemistry of **19** (20*S*,22*S*) was determined by Luche reduction at C-12 (13:1 β/α) followed by acetylation, and comparison of the resulting furostandiol diacetate to material previously synthesized by an independent route (S. Ma, unpublished results) for which single-crystal X-ray confirmation had been obtained.

(33) For production of a related homoallylic alcohol as a minor product during the Prins, see ref 30a. Attempted equilibration of **23**α by resubjection to the reaction conditions for 18 h gave unchanged **23**α (89%), **23**β (6%), and further 12α-hydroxy-14-alkene (5%). This confirmed both the acid-catalyzed equilibration of the diols (noted for the spirostanols **15**α/**15**β, vide supra) and the willingness of this 12α-furostanol to produce the formal ene adduct (also probably via a retro-Prins, but conceivably via direct dehydration). An equilibration attempt on **23**α at 95 °C resulted in extensive decomposition.

(34) Gong, J.; Fuchs, P. L. *Tetrahedron Lett.* **1997**, *38*, 787 and references therein.

(35) Agyin, J. K.; Timberlake, L. D.; Morrison, H. *J. Am. Chem. Soc.* **1997**, *119*, 7945. Since the excited endocyclic C20,22 π bond resulting from intraTTET cannot relax via rotation and has no H atom within abstractable range, we consider it possible that subsequent transfer, probably through space, to the terminal 26-ene (a "free rotor", see: Zimmerman, H. E.; Epling, G. A. *J. Am. Chem. Soc.* **1972**, *94*, 8749) completes the dissipation of the excitation energy. We cannot, at this point, exclude attack of the excited E ring olefin/biradical onto the 26-ene moiety, but we have no evidence for cyclic products or polymers expected from such addition, nor those from 17H abstraction. See Scheme 8a in the Supporting Information.

Scheme 9



The 22*R* epimer **25b**, for which we obtained single-crystal X-ray confirmation,[38] may arise from the side product in **24**, or directly from **25a** by Lewis acid catalysis in toluene.[36] Indeed, resubjection of **25a** to the reaction conditions gave a 10:1 ratio of **25a**/**b**. The epimer is rapidly (and completely!) consumed to give mainly **25a** with catalytic TsOH in acetonitrile (15 min, 25 °C), a solvent which we have found is superbly suited to production of the thermodynamically most stable 20,22 arrangement of the spiroketal moiety in several compounds. The presence of 14β-chloride **25d** was anticipated, based on earlier results with **17**.[18] Formation of the 14α-chloride **25c** (also confirmed by X-ray)[38] was not expected, as no evidence for such a product was found in the dehydration of 14β-hydroxyspirostanone **17**,[18] and implies a stronger carbocation-like character (E_1 rather than E_2) in the dehydration intermediate from **24** than is observed with **17**. Indeed, analysis of the dehydration of **24** run in a solvent expected to better stabilize such a carbenium ion (pyridine, as for **17**) reveals formation of much more (10%) of this chloride along with **25d** (11%). Elimination of 14α-chloride **25c** proceeded relatively well (DBU, CH_3CN, reflux 3h, 80%), but the epimer **25d** proved as recalcitrant as the chloride side product accompanying **18**. Finally, LiF in DBU solvent[39] (100 °C, 1 h) consumed **25d** but gave the desired elimination product in only poor yield, delivering mainly baseline material. The final side product **25e** resisted crystallization and attempts to convert it to known compounds; besides an apparent isomeric relationship to **25a/b** as a keto olefin, it has so far remained unidentified.

Further differences in this series from experiences with related steroids also deserve brief mention. Luche reduction[40] (Scheme 9) of Δ^{14}-furostenone **25** (6.5:1 β/α) suffered by comparison to reaction of 12-ketosteroids which retain a trans (saturated) C/D ring fusion or a [5,6] spiroketal: similar reductions of **11** (17:1 β/α) and **19** (13:1 β/α) were substantially more selective, as was Winterfeldt's uncatalyzed borohydride reduction of the 12-keto function in the pyrazine dimer of **18** (Δ^{14}-spirostenone).[14a] Reported reductions of cis-fused steroid **17** (Scheme 4) with LAH or borohydride (1:1.6 β/α) showed the opposite selectivity.[13,15] A greater divergence from previous experience occurred in the selective saponification of the 3β-acetate group of intermediate **27**, normally facile with $KHCO_3$ in aqueous methanol at reflux.[23] However, the 12β acetate of **27** proved surprisingly labile to these and several other conditions (LiOH, KOH, or $KHCO_3$ in numerous solvents, concentrations, and temperatures). Finally, potassium *tert*-butoxide (*t*-BuOK/*i*-PrOH, 5 °C) provided a useful 68% yield of the 3-hydroxy-12-acetate **28**; two recycles (reacetylation of monool **26** and the diol [not pictured], and resubjection to base) raised the cumulative yield to 96%. In addition, Jones oxidation of **28** to give **8** introduced 5% of an inseparable compound which appears (again!) to be its 22β epimer (not shown), a recurrent issue with this Δ^{14} [5,5] spiroketal system.[36b] A similar epimer of **27** was also evidenced following one reacetylation step (Ac_2O/pyridine, allowed to stir at 25 °C overnight) of the recycles producing additional **28** (vide supra), whereas acetylations conducted at 0 °C and quenched within 5 h caused no formation of the side product. By contrast, acetylation, selective hydrolysis (3,12-diacetate to 12-monoacetate), and oxidation reactions of the analogous homoallylic alcohol **16**β retaining a [5,6] spiroketal evidenced no such side products.

Notwithstanding these sometimes surprising side paths, conversion of hecogenin acetate **11** to the Δ^{14}-furostene North G **8** was accomplished in 15% yield over 13 steps, an encouraging gain over syntheses of the highly oxygenated South 7 and North 1 subunits (30−33 steps, 1−3%, vide supra) as well as that of South 1 (35 steps, 1%, vide infra), and optimizations are likely to further improve the process. Significantly, the key three-step Welzel sequence furnishing the 14β-OH of keto alcohol **24** in 86% overall yield from furostanone **19** had proceeded comparably to that from spirostanone **11** to keto alcohol **18** (94%).

A more adventurous application of this sequence was envisioned to introduce the Δ^{14} unsaturation present in the south half of cephalostatin 1 (**1**). The saturated ketone **32** (prepared rapidly and in good yield from the known advanced intermediate **29**,[12] Scheme 10) differs dramatically from ketones **11** and **19** in close proximity to the reacting center. The effects of the altered ring strain and steric repulsions on its reactivity during the photolytic opening and acid-catalyzed recyclization steps were nonobvious. The β-alkoxy moiety should destabilize formation of the tertiary alkyl radical,[41] and the E/D ring fusion

(36) (a) Only after we obtained the X-ray of **25b**, which we expected to be the 20β epimer of **25a**, did we suspect this possible identity. The NMR changes seen in going from **25a** to **25b** are similar to those visible for the side product in **24**. However, while **25a** is calculated to lie only 0.83 kcal/mol lower than **25b**, **24** is calculated to lie 3.81 kcal/mol lower than (22*R*)-*epi*-**24**, the Prins products **23**α 3.96 kcal/mol and **23**β 3.90 kcal/mol lower than their respective 22*R* isomers, and **19** 4.40 kcal/mol lower than (22*R*)-*epi*-**19**. In the face of such large energy differences, it seems rather more likely that any 22*R* epimer **25b** is produced from **25a** directly via *Lewis* acid catalysis *after* elimination. It remains difficult at this point to assign the identity of the side product in **24** with any certainty, and the possibility that it is the source of the unidentified side product **25e** remains viable. (b) For compounds **26**, **27**, **28**, 3,12-diol **8** and deacetylated **8**, each 22*S* isomer is calculated to lie 1.99−2.17 kcal/mol lower than its respective 22*R* isomer. (c) All calculations performed using CAChe version 3.5. (d) Brown−Jones oxidation produced even more of the impurity in **24**, see ref 25. Swern conditions gave less impure product but in lower yield.

(37) One referee questioned why we explored the Prins and elimination reactions to give **18** rather than access it more directly via **16**: to reiterate, we had already explored the chemistry proceeding from **11** and had moved on to an alternate approach via **19** (because substrates **17** and **18** proved unstable to spiroketal opening) several months prior to the appearance of ref 14b. The ene reaction of **22** was, however, attempted. These reactions were performed on crude photolysate **22** by Mr. Zhiwei Tong. Some adjustment of conditions was attempted in order to obtain yields more like those available from crude **13** without success, but more fine-tuning of the conditions may yet provide a synthetically useful reaction for this compound.

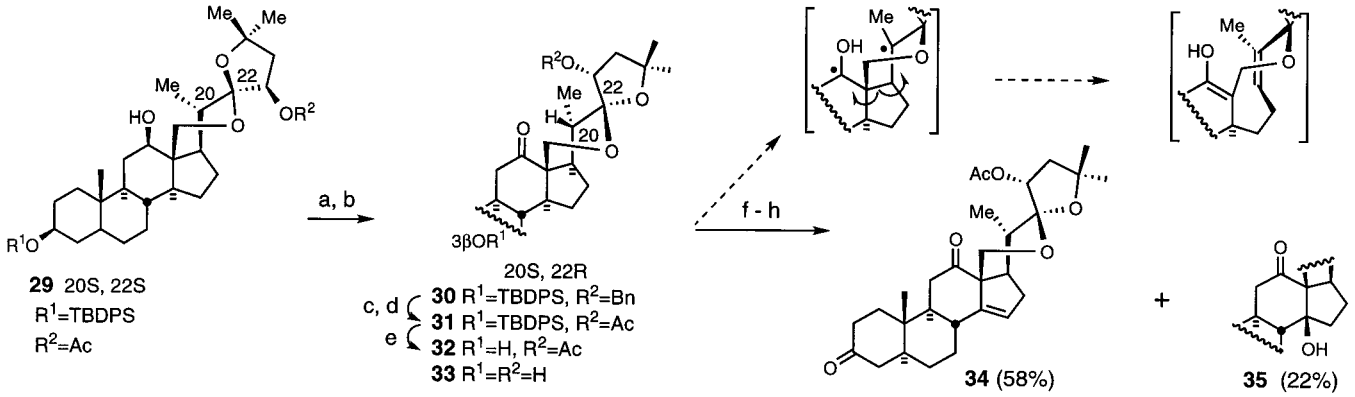
(38) X-ray data for compounds **24**, **25b**, and **25c** have been submitted to the Cambridge Crystallographic database.

(39) Kim, S. Ph.D. Thesis, Purdue University, 1994.

(40) Gemal, A. L.; Luche, J.-L. *J. Am. Chem. Soc.* **1981**, *103*, 5454.

(41) (a) Wagner, P. J.; Kemppainen, A. E. *J. Am. Chem. Soc.* **1972**, *94*, 7495. (b) Reference 21c.

Scheme 10[a]



[a] (a) H_2CrO_4, Et_2O, 25 °C, 15 min, 96%; (b) CSA, 1,2-DCE, 83 °C, 13 h, 87%; (c) 10% Pd/C (0.2 equiv), H_2 (1 atm), AcOH, 25% EtOH/EtOAc, 24 h, 88%; (d) Ac_2O, NEt_3, DMAP CH_2Cl_2, 25 °C, 1 h, quantitative; (e) TBAF, THF, 80 °C, 2.5 h, 87%; (f) 300 nm, *hν*, dioxane, 1.5 h; (g) 75% AcOH in H_2O, 25 °C, 2.5 d; (h) H_2CrO_4, Et_2O, 25 °C, 15 min.

of **32** could either hamper or aid collapse to desired secoaldehyde. Additionally, increased C ring strain would retard recyclization as seen for **19** and other steroids.[24] The greater concern, however, derived from the possibility of a type II abstraction of the 20β hydrogen, which lies, unavoidably, rigidly held well within reach of the excited carbonyl oxygen (only 2.60 Å away) via a nearly optimal six-membered transition state.[21,42] Of the three paths the resulting 1,4-biradical might take, reversion to ketone would be most facile due to the same rigid nature of the steroid and would afford renewed opportunity for the desired type I cleavage. Cyclobutanol formation should likewise be excluded structurally. However, reorganization to permit β-cleavage might not be so very difficult, as a 15° smaller change in dihedral angle to permit overlap of developing p-orbitals is needed in **32** than that required for **11** or **19**, with a calculated release of >9 kcal/mol by relief of the strain of D/E fusion and formation of the enol/nonene array (the 13α ketone lies a further 6 kcal/mol lower in energy).[11b,21]

In the event, the Welzel sequence was applied as before. A solution of **32** in dioxane was irradiated at 300 nm for a mere 1.5 h, at which time TLC revealed complete consumption of starting material. The crude photolysate was a complex mixture which evidenced a major spot by TLC, a minor, *less polar* spot, and a host of minor tailings. We note that oxetane **14** is *more* polar than **13**. NMR showed a smaller than expected aldehyde peak (in one run, none at all in another run) as well as minor (<10%) signals in the vinyl region, suggestive of the type II product. Unfortunately, isolation of the putative $\Delta^{17(20)}$ nonene proved elusive both at this stage and at the completion of the protocol, possibly because of enhanced spiroketal lability to silica gel (the 9-ring oxonium ion would be conjugated to the $C_{17(20)}$ double bond).[42] The mixture required much longer reaction times than crude **13** or **22** for the Prins reaction (at least 60h stirring with 75% acetic acid, perhaps much longer for consumption of all tailings) and afforded not two but a mixture of several products. Subsequent oxidation gave, much to our surprise and delight, an easily separable mixture of the diketo olefin **34** and diketo alcohol **35** in overall yields (for three steps from ketone **32**) of 58% and 22%, respectively. Chemical conversion therefore shows that type I cleavage had predominated in the unprecedented photolysis of **32**. That the majority of the "Prins" product contained the desired unsaturation (whether from spontaneous elimination of the kinetic Prins product or its intermediate carbocation, as was implied in the reaction of **23**, or from Bronsted acid-catalyzed ene reaction) is also unprecedented as well as serendipitous.[30a,33] It is instructive to note, as a consequence of substrate structure and ring strain, the striking divergence over the course of this three-step sequence of the proximally differentiated ketone **32** from spirostanone **11** and furostanone **19**, especially in terms of their rates of photolysis (much faster for **32**, some apparent type II reaction) and their product distributions (formal ene reaction versus Prins-reaction products) in the acid-catalyzed cyclization of their secoaldehyde photolysates.

Azido Ketone and Aminomethoxime Coupling Partners. Smooth elaboration of **34** to "South 1" azido ketone **7** occurred by application of the bromination (PTAB) / azide substitution (tetramethylguanidinium tribromide, TMGA) procedure established in these laboratories to give bromide **36** and azido ketone **7** successively in high yield (Scheme 11).[1] Azide substitution worked better with freshly distilled nitromethane: older solvent displayed an annoying tendency to generate β-nitro alcohol (Henry reaction at C_3)[43] as a side product. Equatorial azide was, as usual, the main product, presumably arising from enolization following substitution. The advanced North 1 intermediate azidoketone **37** ($78 \times 95 = 72\%$ from the ketone)[7] gave azidomethoxime **38** (99%), which was subjected to Staudinger reduction[44] to afford aminomethoxime **6** (80%).[1] Some improvements in conversion were achieved with North G ketone **8**, which was brominated using a modified procedure to furnish **39** (accompanied by ~5% axial bromide) in quantitative yield. Azide substitution operated very satisfactorily in acetonitrile,[45] giving azido ketone **9** (without Henry reaction complications) in 90% yield over two steps. Azidomethoxime **40** and aminomethoxime **10** were obtained from **9** via the protocol described above for the conversion of **37** to **6** (75% for two steps). Thus, all four coupling partners derived quickly and in good yield from their 3-ketosteroids although they contain substantial dissimilarities in rings D−F.

Unsymmetric Coupling and Deprotection. For the key coupling reaction leading to the first total synthesis of cepha-

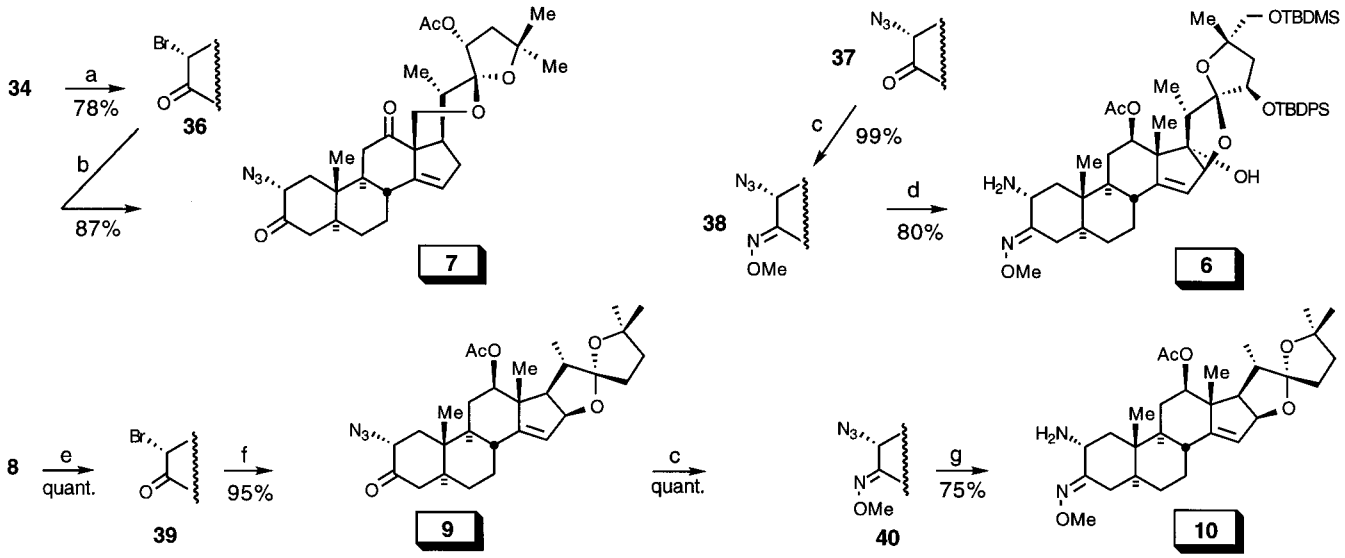
(42) See the expanded Scheme 10a and CAChe 3D drawings in the Supporting Information.

(43) For recent advances in the addition of nitroalkanes to carbonyl compounds, see: Simoni, D.; Invidiata, F. P.; Manfredini, S.; Ferroni, R.; Lampronti, I.; Roberti, M.; Pollini, G. P. *Tetrahedron Lett.* **1997**, *38*, 2749 and references therein.

(44) Procedure taken from the following: Nagarajan, S.; Ganem, B. *J. Org. Chem.* **1987**, *52*, 5044. For a review, see: Gololobov, Y. G.; Kasukhin, L. F. *Tetrahedron* **1992**, *48*, 1353.

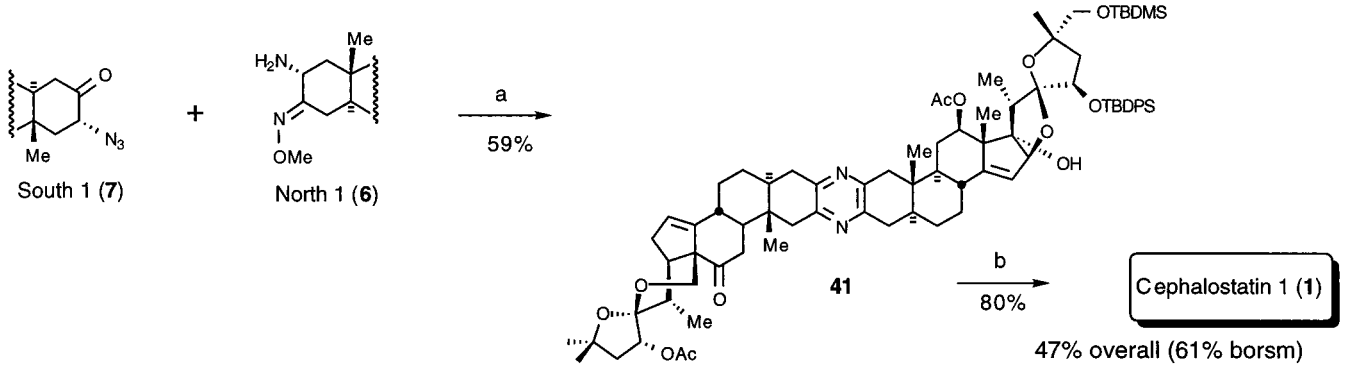
(45) The mixing order is critical: less than 3% of α-amino enone (not shown) and 5−10% of the axial azide were produced when a cold solution of bromide **39** was added to a cold solution of TMGA prior to warming, but the reverse order produced ~15% α-amino enone and >10% axial azide.

Scheme 11[a]



[a] (a) PTAB, THF, 25 °C, 6 min; (b) TMGA, CH_3NO_2, 25 °C, 2.5 h; (c) $NH_2OMe{\cdot}HCl$, 10% pyr in CH_2Cl_2, 0 °C to 25 °C, 4 h; (d) PPh_3, 3% aqueous THF, 25 °C, 24 h; (e) PTAB, THF, 0 °C, 10 min; (f) TMGA, CH_3CN, 0 °C to 25 °C, 6 h; (g) as (d), 48 h.

Scheme 12[a]



[a] (a) Bu_2SnCl_2 (10 mol %), PVP (100 wt %), benzene, 80−85 °C, 3 h (b) i. TBAF, THF, 83 °C, 2 h; ii. K_2CO_3, MeOH/H_2O (8:1), 0.5 h.

lostatin 1 (**1**), the North 1 α-aminomethoxime **6** (1 equiv) was heated with the south α-azido ketone **7** (1 equiv) in benzene in the presence of 10 mol % dibutyltin dichloride (a catalyst for imine formation)[46] and 100 wt % of polyvinylpyridine (PVP), with azeotropic removal of water for 3 h (Scheme 12). Chromatography gave protected cephalostatin 1 (**41**) in 59% yield (76% based on recovered **6**). Cleavage of the C_{23} and C_{26} silyl protecting groups (TBAF), followed by removal of the C_{12} and $C_{23'}$ acetate groups (K_2CO_3/aqueous MeOH), gave the first synthetic sample of (+)-cephalostatin 1 (**1**) in 80% yield from **41**. The TLC, ^1H and ^{13}C NMR, and HPLC profiles of synthetic and natural cephalostatin 1 (**1**) were found to be identical.[47]

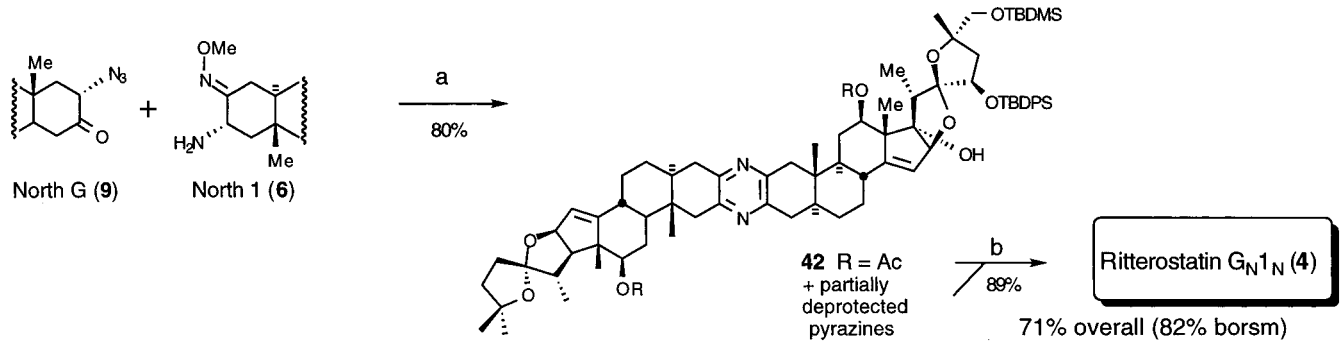
Similar conditions were employed in the coupling of North G azido ketone **9** with North 1 aminomethoxime **6** to give protected ritterostatin G_N1_N **42** as the main product in 49% yield after chromatography. In addition, attention was paid to a more polar pyrazine (31%) closely resembling **42**; 23% of aminomethoxime **6** was recovered unchanged (Scheme 13). The total yield of isolated coupled pyrazines was thus 80% (92% based on recovered **6**). This is more in line with the type of yields observed with model couplings[1] and in retrospect may explain the lower yields obtained in the cephalostatin 1 and dihydrocephalostatin 1 cases, for they also had evidenced similar TLC profiles. Regarding the origin of the more polar pyrazine product, suspicion fell on possible loss of the C_{12} acetate, which had shown some lability in the synthesis of North G ketone **8** (vide supra, Scheme 9), or possibly the $C_{12'}$ acetate; unprecedented formation of the cis-cross-coupled pyrazine seemed unlikely. Deprotection of **42** (TBAF then KOH in a single pot) gave ritterostatin G_N1_N (**4**) in 94% yield. Lacking unambiguous NMR confirmation of the more polar pyrazine's identity, the material was separately deprotected (TBAF, then KOH) and shown to give the desired ritterostatin. The combined yield of ritterostatin G_N1_N (**4**) was thus 71% overall (82% based on recovered **9**) for the complete coupling/deprotection sequence. Examination of the NMR spectra of the cephalostatins and ritterazines shows that the chemical shifts and coupling patterns of the steroidal subunits are essentially invariant, regardless of the partner to which they are coupled. The ^1H NMR and ^{13}C NMR spectra of ritterostatin G_N1_N (**4**) matched those of the North units of cephalostatin 1 (**1**) and ritterazine G (**3**), confirming the identity and trans orientation of the pyrazine-fused steroids.[48]

In the reaction of North G aminomethoxime **10** with South 1 azido ketone **7** to give protected ritterostatin G_N1_S **43**, Nafion H was employed in lieu of PVP in order to test the stability of

(46) Stein, C.; de Jeso, B.; Pommier, J. C. *Synth. Commun.* **1982**, *12*, 495.

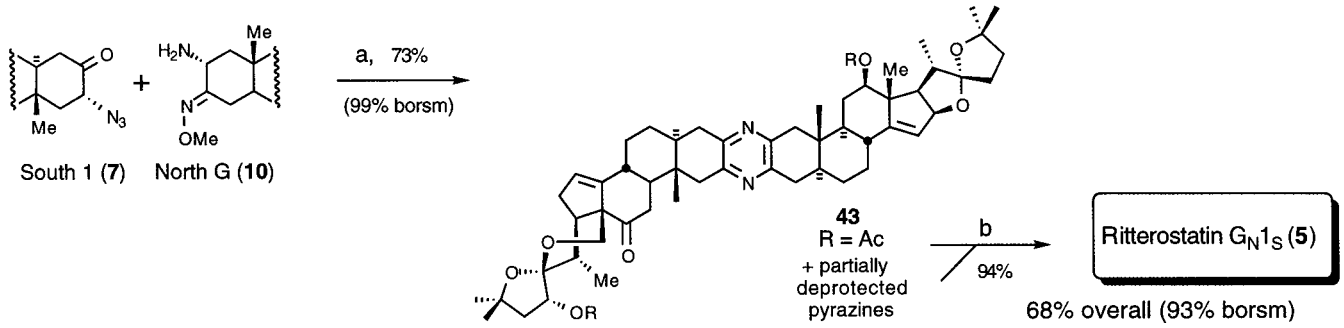
(47) We thank Professor G. R. Pettit of Arizona State University for kindly providing us with a sample of natural cephalostatin 1 (**1**) and Dr. Douglas Lantrip for performing the HPLC comparisons of synthetic and natural cephalostatin 1 (**1**).

Scheme 13[a]



[a] (a) 10% Bu_2SnCl_2, 100 wt % PVP, 100 wt % 4A sieves, benzene, reflux; (b) (i) TBAF, THF, reflux; (ii) KOH, MeOH added, reflux.

Scheme 14[a]



[a] (a) 10% Bu_2SnCl_2, 100 wt % Nafion H, benzene, reflux; (b) K_2CO_3, aqueous MeOH, reflux.

these partners' spiroketal moieties to *heterogeneous* Bronsted acid catalysis (Scheme 14). Couplings of model steroids have proceeded in somewhat higher yield for each partner set when Nafion H was utilized instead of PVP.[1] Such results might be understood in terms of the release during the course of the reaction of methoxylamine (see Scheme 1), which could diminish the effectiveness of the tin Lewis acid when left unneutralized, or capture azido ketone coupling partner as an azidomethoxime. The chemically resistant, hindered Δ^{14} functions were expected to survive unchanged. The natural 20α22α configuration of North G (as the 3-ketone) is calculated to lie 2.0 kcal/mol below its energetically nearest (20α22*β*) epimer, while the calculated energy of the natural 20α22α configuration of South 1 (as the 23-acetate) lies 3.6 kcal/mol below its closest (20α22*β*) epimer. For steroids which are thus expected to be stable to heterogeneous Bronsted acid (NOT South 7, for example, whose [5,6] spiroketal lies only 0.04 kcal below a [5,5] isomer: its 7*β*OH relative South B has been shown to isomerize extensively at 25 °C with HCl),[4b,11] such a protocol might prove advantageous in terms of yield in the coupling step.

In the event, North G aminomethoxime **10** (1.0 equiv) and South 1 azido ketone **7** (1.1 equiv) reacted as expected, and no evidence for further epimerization was found. Chromatography afforded a 52% yield of **43**; lower R_f pyrazines arising from loss of acetate at $C_{23'}$ (which had shown some lability, Scheme 10) and/or C_{12} amounted to another 21%. Unchanged aminomethoxime **10** (26%) was recovered, for a total yield of 73% (99% based on recovered **10**) in the coupling step. The actual yield of pyrazine was thus not substantially superior to that obtained in comparable PVP-mediated reactions, although a direct comparison for this case (keeping partners and equivalents of each constant) between PVP and Nafion H was not made. Saponification of the protected pyrazines afforded the hybrid analogue ritterostatin G_N1_S (**5**) in 94% combined yield for the deprotection, overall 69% (93% based on recovered **10**) for the two-step coupling/deprotection sequence. Again, the ^1H and ^{13}C NMR spectra of ritterostatin G_N1_S (**5**) closely matched those of its constituent subunits, the North hemisphere of ritterazine G and the South hemisphere of cephalostatin 1 (**1**).

Biological Activity. Testing of the analogues against natural cephalostatin 1 (**1**) in the National Cancer Institute's (NCI) in vitro, human cancer cell panel revealed that ritterostatin G_N1_N (**4**) displays exceptionally high potency. It is more potent than **1** for the leukemia K-562 line, equipotent for ovarian OVCAR-8, renal SN12C, and breast MCF7/ADR-RES cell lines, and nearly so for several others, including renal RXF-353 (**1**, GI_{50} 0.1 nM; **4**, 0.3 nM), with a mean $GI_{50} < -7.4$ (42 ± 7 nM, 60 of 60 cell lines affected) and an activity profile similar to that of **1** (mean $GI_{50} < -8.5$; i.e., 3.5 ± 0.7 nM, 60 of 60 cell lines affected).[49] Ritterostatin G_N1_N (**4**) possesses mean tumor inhibiting activity approaching that of taxol (mean GI_{50} −7.9; i.e., 12.6 nM), superior to that of cephalostatin 7 (**2**) in all categories of cancer cell lines tested, and superior to that of all standard chemotherapeutics, including adriamycin (mean GI_{50} −6.9), cisplatin (mean GI_{50} −5.7), 5-fluorouracil (mean GI_{50} −4.7), and cyclophosphamide (mean GI_{50} −3.7).[50]

That ritterostatin G_N1_N (**4**) retains most of the activity of cephalostatin 1 (**1**) represents a significant new advance. Synthesis of its southern subunit **8** (North G) was accomplished in only a third of the number of steps needed to construct the south hemisphere of **1** (South 1), with a 1500% increase in yield

(48) See the Supporting Information for a comparison of the NMR data of each ritterostatin with its constituent subunits as observed in the relevant ritterazine or cephalostatin.

(49) Originally, cephalostatin 1 (**1**) was reported to display a mean $GI_{50} < -8.9$ (i.e., 1.2 nM, *J. Nat. Prod.* **1994**, *57*, 52), but in head-to-head testing against dihydrocephalostatin a mean GI_{50} of −8.6 (i.e. 2.3 nM) was observed (ref 1). During head-to-head testing against the ritterostatin G_N1_N ($GI_{50} < -7.4$ [42 ± 7 nM] NSC D-699012) and G_N1_S ($GI_{50} > -6.1$ [900 nM] NSC D-699013) analogues, a mean GI_{50} of −8.45 (3.5 ± 0.7 nM) was observed.

from hecogenin acetate **11**. Ritterostatin G_N1_N (**4**) fulfills the desired increase in synthetic accessibility with retention of high potency which we hoped to find in such interphylal hybrids. The North G unit is thus shown to be an adequate analogue of South 1, and it is noteworthy that almost all of the most active cephalostatins include South 1 or a close relative thereof. The presence of a 12β-hydroxyl in North G probably contributes to its activity relative to South 1, since compounds with 12-keto or 12-acetoxy moieties are invariably less active than their direct 12β-hydroxyl counterparts.[51] The 10-fold decrease in activity in going from **1** to **4** may be more a reflection of the loss of the 23-hydroxyl (polarity loss)[4b] than either the change from an 18,-22-epoxy linkage to the 16,22-epoxide (a major spatial translation of the E/F rings) or the change from a [6,5] to [5,5] spiroketal arrangement. A short route to such a 23-hydroxy North G relative is currently being explored in order to test the theory. It will also be interesting to discover the effect of the [5,6] spiroketal arrangement present in hecogenin itself and its 14,15-dehydro relative, whose C rings and spiro connections have less strain energy to spend. Shorter routes to North 1 and analogues thereof to function as the North partner, especially those featuring a 17α-hydroxyl group, are also of prime interest and under current inquiry.

Ritterostatin G_N1_S (**5**), by contrast, was not expected to show high activity because it lacks a 17α-hydroxyl group, a feature present in at least one hemisphere of all of the most active ritterazines and cephalostatins. Ritterostatin G_N1_S (**5**) was significantly weaker than **4**, with a mean $GI_{50} > -6.1$, affecting only 10 of 60 cell lines at 900 nM concentration. Compounds which lack this moiety are dramatically less active than their direct 17-hydroxylated counterparts,[52] and the importance of this group to tumor inhibition has also been documented in angiostatic steroids such as 17α-hydroxyprogesterone and analogues.[53] Furthermore, the spiroketals of both South 1 and North G are calculated to lie at the bottom of their respective isomeric energy wells by at least 2 kcal/mol. *The most active pyrazines all contain at least one spiroketal which calculations (and in some cases, experimental evidence)[1,7,11,29] show is a less stable isomer*. Since there are indications that related steroids such as OSW-1 (which may be considered structurally analogous to a spiroketal, and the activity of which correlates well with that of the cephalostatins)[54] and solasodine act biologically in part by reaction at the spiro center,[55] the concept of a "cocked gun" may appropriately aid our understanding. North 1 (20α,22β), for example, is calculated to lie ~1 kcal/mol above the most stable (20β,22β) [5,5] spiroketal and *more than 6 kcal/mol above the most stable isomer*, the (20α,22α) [5,6] spiroketal. Interestingly, the latter happens to be the same isomeric arrangement (with an axial 23-hydroxyl instead of hydrogen) found in the South 7 steroid.[11]

On the other hand, if North G actually functioned as a very close mimic of South 1, not only would **4** be more active but the union **5** of these subunits would be expected to show low activity as do "symmetric" trisdecacyclic pyrazines (ritterazines K, N, and R, IC_{50}'s of 10, 460, and 2100 ng/mL, cephalostatin 12 and Winterfeldt's symmetric analogues, all with mean GI_{50}'s > -4.3). That is, it should display greatly diminished scope and level of activity, perhaps due to the lack of a polar/apolar pair of subunits as proposed by Fusetani.[4b] However, its activity level remains higher than expected (although its scope is diminished), likely due to the contribution of the 12βOH/Δ^{14} array in North G, and perhaps some polar/apolar pair character as a result of the difference between the steroid subunits imparted by the 23-hydroxyl present in South 1 and absent in North G.

Conclusion

We have successfully completed the first total synthesis of cephalostatin 1 (**1**), which remains among the most potent antineoplastic agents ever tested by the NCI. The lengthy sequence required to produce **1** has also been somewhat obviated by the synthesis of ritterostatin G_N1_N (**4**), which retains very high activity in the tumor panel but which is much more synthetically accessible. North G is shown to be a useful analogue of South 1, an important step toward the practical synthesis of extremely potent anticancer agents. These syntheses were made possible by logical modification and extension of a key photolysis/Prins sequence which was not known to be as general nor nearly as high yielding as has now been shown. New chemical insights and important new SAR data have been provided by these syntheses. The concept of interphylal product splicing is thus justified. Fruitful directions for future inquiry are indicated and are actively being pursued.

Experimental Section

General Methods. Unless otherwise stated, reactions were carried out under a positive argon atmosphere in flame-dried glassware using magnetic stirring. Diethyl ether (Et_2O) and tetrahydrofuran (THF) were distilled from sodium benzophenone ketyl. Dichloromethane (CH_2Cl_2), benzene, and toluene were distilled from calcium hydride. Deuterated NMR solvents ($CDCl_3$, C_6D_6, and 99.5% C_5D_5N) were stored over 4 Å molecular sieves for several days prior to use, with the exception of 99.99% C_5D_5N (used for <3 mg samples), which was used directly from ampules. All other chemicals were used as supplied from commercial sources unless otherwise specified. TLC plates were developed with anisaldehyde solution. Flash chromatography (hereafter sgc) was carried out as described by Still[56] (230−400 mesh silica gel). Melting points are uncorrected. ^{1}H and ^{13}C NMR spectra were obtained at 300 or 500 MHz and 75 or 125 MHz, respectively. ^{1}H NMR chemical shifts are reported in ppm relative to the residual protonated solvent resonance: $CHCl_3$, δ 7.26; C_6D_5H, δ 7.15; C_5HD_4N, δ 8.71. Splitting patterns are designated as s, singlet; d, doublet; t, triplet; q, quartet; p, pentet; m, multiplet; br, broadened; ap, apparent. Coupling constants (*J*) are reported in hertz. ^{13}C NMR chemical shifts are reported in ppm relative to solvent resonance: $CDCl_3$, δ 77.00; C_6D_6, δ 128.00; C_5D_5N, δ 135.50. Mass spectral data include the molecular ion designated as M.

Furostanone 19. Alkene **21** from combined runs (0.890 g, 1.86 mmol, contained 5% hecogenin acetate) was suspended in 75% acetic acid and heated at 90−95 °C during 40 h of stirring, when TLC analysis (3:1 hexane/EtOAc) of a worked up aliquot showed no remaining **21**. (In fact, some starting material did remain as the desacetate, but its high polarity obscured its identity, being mistaken for expected

(50) Detailed testing information for all standard therapeutic agents (~150) is available on the World Wide Web: http://epnws1.ncicrf.gov:2345/dis3d/itb/stdagnt/tab.html. Useful NSC numbers: cyclophosphamide (NSC 26271), 5-fluorouracil (NSC 19893), cisplatin (NSC 119875), adriamycin (NSC 123127), tamoxifen (NSC 180973), and paclitaxel (NSC 125973). Our compliments to Professor Winterfeldt for disseminating this site in his report (ref 10) as well.

(51) Compare, for example, ritterazines B (12βOH, ED_{50} 0.15 ng/mL against P388 leukemia), H (12-keto, ED_{50} 16 ng/mL, a 100-fold decrease in activity), and B-12-acetate (ED_{50} 3.5 ng/mL, a 23-fold decrease in activity). See ref 4b.

(52) Compare, for example, ritterazines A (17′-OH, IC_{50} 3.5 ng/mL) and T (17′-H, IC_{50} 460 ng/mL, a 330-fold decrease), or B (0.15 ng/mL) and Y (3.5 ng/mL).

(53) Schweiger, E. J.; Joullie, M. M.; Weisz, P. B. *Tetrahedron Lett.* **1997**, *38*, 6127 and references therein.

(54) Mimaki, Y.; Kuroda, M.; Kameyama, A.; Sashida, Y.; Hirano, T.; Oka, K.; Maekawa, R.; Wada, T.; Sugita, K.; Beutler, J. A. *Bioorg. Med. Chem. Lett.* **1997**, *7*, 633.

(55) Kim, Y. C.; Che, Q.-M.; Gunatilaka, A. A. L.; Kingston, D. G. I. *J. Nat. Prod.* **1996**, *59*, 283.

(56) Still, W. C.; Kahn, M.; Mitra, A. *J. Org. Chem.* **1978**, *43*, 923.

deacetylated spiroketal **19**.) The resulting solution was partitioned between EtOAc and water, and the organic layer was washed with saturated bicarbonate solution. The original aqueous layer (acidic) was back-extracted with EtOAc, the organic layer was washed with saturated bicarbonate, and the combined organic layers were dried over Na_2SO_4 and concentrated to give 0.90 g of pale yellow solids. The solids were acetylated in the usual way (CH_2Cl_2, TEA, Ac_2O, catalyst DMAP, 0 °C, 2 h) and worked up to give 0.91 g of pale orange solids. Sgc (40:1 CH_2Cl_2/THF) afforded 0.212 g of recovered alkene **21** (25%) and 0.646 g of furastanone **19** (73%, 98% borsm) as white solids: mp 230−232 °C (MeOH); ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.73−4.60 (1H, m, $H_{3\alpha}$), 4.43−4.35 (1H, m, $H_{16\alpha}$), 2.51 (1H, dd, J = 8.7, 6.9 Hz, $H_{17\alpha}$), 2.39 (1H, apt, J = 13.9 Hz, $H_{11\beta}$), 2.21 (1H, dd, J = 14.2, 5.1 Hz, $H_{11\alpha}$), 2.09 (1H, m), 2.00 (3H, s, H_{Ac}), 1.32 (3H, s, H_{27}), 1.16 (3H, s, H_{26}), 1.05 (1H, d, J = 6.4 Hz, H_{21}), 1.03 (3H, s, H_{18}), 0.91 (3H, s, H_{19}); ^{1}H NMR (300 MHz, C_6D_6) and ^{1}H NMR (300 MHz, pyr-d_5) see Supporting Information; ^{13}C NMR (75 MHz, $CDCl_3$) δ 214.0 (s), 171.0 (s), 120.1 (s), 82.6 (s), 79.1 (d), 73.4 (d), 55.6 (d), 55.4 (d), 55.3 (s), 53.3 (d), 44.5 (d), 39.0 (d), 37.8 (t), 37.1 (t), 36.3 (t), 36.2 (s), 34.4 (d), 33.8 (t), 31.5 (t), 31.2 (t), 30.2 (q), 28.5 (q), 28.2 (t), 27.3 (t), 21.5 (q), 16.0 (q), 13.6 (q), 11.9 (q); ^{13}C NMR (75 MHz, C_6D_6) see Supporting Information; MS *m*/*z* 472 (M^+, 3), 139 (100); HRMS calcd for $C_{29}H_{44}O_5$ 472.3189, found 472.3175.

Secoaldehyde 22: Ketone **19** (0.630 g, 1.33 mmol) in dioxane (70 mL) was deoxygenated with argon, and the solution was irradiated with 300 nm light (Rayonet apparatus) for 4 h, at which time TLC analysis (3:1 hexane/EtOAc) showed only a trace of **19** and the appearance of a major new spot of lower R_f accompanied by many faint tailing spots. The solvent was removed in vacuo to afford 0.75 g (119%, some solvent left) of photolysate as a white foam, 36 mg of which was purified by sgc to give an analytical sample: ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.47 (<1H, s, H_{12}), 4.73−4.60 (1H, m, $H_{3\alpha}$), 4.55−4.51 (1H, m, $H_{16\alpha}$), 2.70−2.64 (1H, apt, J = 7.3 Hz, $H_{17\alpha}$), 2.45−2.05 (4H, m), 2.02 (3H, s, H_{Ac}), 1.61 (3H, s, H_{18}), 1.35 (3H, s, H_{27}), 1.16 (3H, s, H_{26}), 1.06 (1H, d, J = 6.9 Hz, H_{21}), 0.84 (3H, s, H_{19}); ^{13}C NMR (75 MHz, $CDCl_3$): δ 201.4 (s), 170.7 (s), 135.5 (s), 135.3 (s), 117.0 (s), 81.9 (s), 77.4 (d), 73.2 (d), 61.3 (d), 46.3 (d), 45.3 (d), 43.8 (d), 43.4 (t), 37.9 (t), 37.8 (d), 37.4 (t), 37.3 (t), 36.5 (s), 34.0 (t), 33.6 (t), 30.9 (t), 30.2 (q), 28.6 (q), 28.3 (t), 21.5 (q), 14.6 (q), 12.8 (q), 12.2 (q); MS (CI, isobutane) *m*/*z* (%) 473 (100, M + H), 471 (61); HRMS calcd for $C_{29}H_{45}O_5$ (M + H) 473.3267, found 473.3257.

Diols 23: Crude photolysate containing mainly secoaldehyde **22** (0.714 g, 1.27 mmol maximum) was suspended in 75% AcOH (6 mL) and stirred for 35 h. The resulting solution was partitioned between EtOAc (30 mL) and water (90 mL), the aqueous layer was extracted with EtOAc (30 mL), and the combined organic layers were washed with water and then saturated bicarbonate and dried over sodium sulfate. Removal of solvent in vacuo afforded diols **23** (0.628 g, 101%) as white solids. ^{1}H NMR analysis indicated a 4.5:1 ratio of **23**α/**23**β (~90%) accompanied by traces of Δ^{14}-containing material, keto alcohol **24** (from dissolved O_2; degassed aqueous AcOH produced none of this material) and unphotolyzed furastanone **19**. Normally, this crude material was carried on without purification; in this instance, for characterization, sgc (gradient from 3:1 to 1:3 hexane/EtOAc) gave diols **23**α (0.400 g, 64%) and **23**β (0.077 g, 13%). In addition, there was obtained 24 mg (4%) of ketone **19**; 15 mg (2%) of keto alcohol **24**; 72 mg of material containing **23**α (59%, 7% of theory), **23**β (27%, 3% of theory), and what appeared to be the formal ene adduct (12αOH-Δ^{14}, 14%, 2% of theory); 19 mg (3%) of presumed unreacted photolysate side products, and 18 mg (3%) of mixed triols (3β,12α/β,14β-trihydroxy epimers).

Diol 23α: ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.71−4.61 (1H, m), 4.55 (1H, apt, J = 6.8 Hz, $H_{16\alpha}$), 3.62 (1H, brs, $H_{12\beta}$), 2.44−2.29 (3H, m), 2.00 (3H, s), 1.35 (3H, s, H_{27}), 1.16 (3H, s, H_{26}), 1.02 (3H, s, H_{18}), 0.95 (1H, d, J = 6.4 Hz, H_{21}), 0.80 (3H, s, H_{19}); ^{13}C NMR (75 MHz, $CDCl_3$) δ 171.1 (s), 116.8 (s), 86.2 (s), 82.3 (s), 81.2 (d), 76.1 (d), 73.7 (d), 57.9 (d), 50.9 (s), 44.4 (d), 43.0 (d), 42.8 (d), 40.8 (t), 40.1 (d), 37.3 (t), 36.8 (t), 35.4 (s), 34.0 (t), 33.6 (t), 30.2 (q), 28.7 (q), 28.6 (t), 28.5 (t), 27.4 (t), 27.3 (t), 21.6 (q), 15.4 (q), 15.1 (q), 12.2 (q); [α] = −12.8° (c = 1.0, $CHCl_3$); MS (EI) *m*/*z* 472 (M − H_2O, 56); (CI) 473 (M + H − H_2O, 84), 455 (100); HRMS (CI) calcd for $C_{29}H_{45}O_5$ (M + H − H_2O) 473.3267, found 473.3252.

Diol 23β: ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.70−4.59 (1H, m), 4.55 (1H, apt, J = 6.8 Hz), 3.09 (1H, dd, J = 11.8, 4.1 Hz, $H_{12\alpha}$), 2.48 (1H, apt, J = 7.7 Hz, $H_{17\alpha}$), 2.37−2.27 (2H, m), 2.00 (3H, s), 1.33 (3H, s), 1.16 (3H, s), 0.97 (1H, d, J = 6.4 Hz), 0.97 (3H, s, H_{18}), 0.80 (3H, s); ^{13}C NMR (75 MHz, $CDCl_3$): δ 170.8 (s), 116.8 (s), 86.7 (s), 82.5 (s), 79.5 (d), 74.6 (d), 73.5 (d), 57.0 (d), 53.2 (s), 46.3 (d), 44.6 (d), 42.8 (d), 39.3 (d), 38.8 (t), 37.2 (t), 36.9 (t), 35.8 (s), 33.9 (t), 33.6 (t), 30.2 (q), 29.4 (t), 28.5 (t), 28.5 (q), 27.7 (t), 27.4 (t), 21.5 (q), 14.8 (q), 12.2 (q), 7.6 (q); MS (EI) *m*/*z* 490 (M, 36); (CI) 491 (M + H, 84).

Keto Alcohol 24 (North I as 3-Acetate). Jones oxidation (see Supporting Information) of **23**α (0.245 g, 0.499 mmol) afforded keto alcohol **24** (0.237 g, 97%) as white solids, mp 231.5−232 °C (CH_2Cl_2), which by NMR contained ~8% of an impurity (tentatively identified as the 22*R* epimer). Oxidation of crude diols **23** proceeded in similar yield: ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.71−4.60 (1H, m), 4.46 (1H, apt, J = 6.6 Hz, $H_{16\alpha}$), 3.32 (1H, apt, J = 7.8 Hz, $H_{17\alpha}$), 2.78 (1H, brs, H_{OH}), 2.45 (1H, apt, J = 13.5 Hz, $H_{11\beta}$), 2.31−2.11 (3H, m), 2.00 (3H, s), 1.33 (3H, s), 1.23 (3H, s, H_{18}), 1.16 (3H, s), 0.96 (1H, d, J = 6.7 Hz), 0.88 (3H, s); ^{1}H NMR (300 MHz, pyr-d_5)[4] δ 5.46 (1H, brs, H_{OH}), 4.86−4.74 (1H, m, $H_{3\alpha}$), 3.74 (1H, apt, J = 6.6 Hz, $H_{16\alpha}$), 2.95−2.86 (1H, m, $H_{17\alpha}$), 2.55 (1H, apt, J = 13.4 Hz, $H_{11\beta}$), 2.38−2.30 (2H, m), 2.05 (3H, s, H_{Ac}), 1.48 (3H, s, H_{27}), 1.44 (3H, s, H_{18}), 1.20 (3H, s, H_{26}), 1.10 (1H, d, J = 6.7 Hz, H_{21}), 0.76 (3H, s, H_{19}); ^{13}C NMR (75 MHz, $CDCl_3$) δ 213.6 (s), 170.7 (s), 116.7 (s), 87.6 (s), 82.7 (s), 78.8 (d), 73.2 (d), 62.4 (s), 51.3 (d), 47.0 (d), 44.6 (d), 42.5 (d), 39.0 (d), 37.6 (t), 37.2 (t), 36.6 (t), 36.3 (s), 33.9 (t), 33.8 (t), 30.2 (q), 28.5 (q), 28.3 (t), 27.9 (t), 27.3 (t), 21.5 (q), 14.9 (q), 14.8 (q), 12.0 (q); [α] = −25.9° (c = 0.99, $CHCl_3$); MS (CI) *m*/*z* 489 (M + H, 91), 471 (100); HRMS (CI) calcd for $C_{29}H_{45}O_6$ (M + H) 489.3216, found 489.3206; single-crystal X-ray.

Keto olefin 25a. Keto alcohol **24** (0.2164 g, 0.443 mmol) was dissolved in toluene (3.4 mL). Pyridine (0.036 mL, 0.487 mmol, 10 equiv) was added, followed by dropwise addition of a solution of thionyl chloride (0.030 mL, 0.487 mmol, 1.1 equiv) in toluene (1.0 mL) over 1 min. After 5 min, and after 2 h, TLC showed ~40% **24** remaining. Additional $SOCl_2$ (0.025 mL, 0.399 mmol, 0.9 equiv) was added neat; after 5 min, TLC showed no **24** remaining. The reaction was quenched by the addition of ice (5 g) and partitioned between toluene (15 mL) and 0.003 M HCl (27 mL), and the aqueous layer was extracted with toluene. The organic layers were combined, washed with brine, and dried over sodium sulfate, and the solvent was removed in vacuo. The residue was fractionated repeatedly by sgc (gradient 50:1 to 10:1 CH_2Cl_2/THF) to afford (in order of elution) 14α-chloride **25c** (6.7 mg, 3%), (22*R*)-keto olefin **25b** (16.5 mg, 8%), desired keto olefin **25a** (130 mg, 63%), 14β-chloride **25d** (29 mg, 13%), and 23 mg (11%) of an unidentified product **25e**.

25a: mp 189−191 °C (CH_2Cl_2/hexane); ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.40 (1H, brs, H_{15}), 4.81 (1H, apd, J = 8.0 Hz, $H_{16\alpha}$), 4.70−4.61 (1H, m), 3.32 (1H, apt, J = 8.6 Hz), 2.54 (1H, apt, J = 14.2 Hz, $H_{11\beta}$), 2.49−2.38 (1H, m), 2.33 (1H, dd, J = 14.5, 4.4 Hz, $H_{11\alpha}$), 2.01 (3H, s, H_{Ac}), 1.99 (2H, aps), 1.35 (3H, s), 1.28 (3H, s, H_{18}), 1.18 (3H, s), 1.02 (1H, d, J = 6.6 Hz), 0.93 (3H, s); ^{13}C NMR (75 MHz, $CDCl_3$): δ 211.3 (s), 170.7 (s), 154.6 (s), 121.3 (d), 117.8 (s), 83.7 (d), 82.1 (s), 73.1 (d), 62.4 (s), 53.5 (d), 49.9 (d), 44.1 (d), 41.0 (d), 37.4 (t), 37.3 (t), 36.4 (s), 36.3 (t), 34.2 (d), 33.8 (t), 33.5 (t), 30.1 (q), 29.5 (t), 28.6 (q), 28.0 (t), 27.3 (t), 21.5 (q), 21.2 (q), 14.1 (q), 11.8 (q); COSY NMR (300 MHz, $CDCl_3$), ^{1}H NMR (300 MHz, pyr-d_5), COSY and CSCM NMR (300 MHz, pyr-d_5), ^{13}C NMR (75 MHz, pyr-d_5), see Supporting Information; [α] = +71.1° (c = 1.02, $CHCl_3$); MS (CI) *m*/*z* 471 (M + H, 100); HRMS (CI) calcd for $C_{29}H_{43}O_5$ (M + H) 471.3111, found 471.3117.

25b: ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.39 (1H, brs), 4.72−4.61 (1H, m), 4.69 (1H, ap brd, J = 7.0 Hz, $H_{16\alpha}$), 3.18 (1H, apt, J = 7.6 Hz, $H_{17\alpha}$), 2.56 (1H, apt, J = 13.9 Hz), 2.52−2.42 (1H, m), 2.31 (1H, dd, J = 14.5, 4.7 Hz), 2.11 (1H, dd, J = 13, 6.2 Hz, $H_{20\beta}$), 2.02 (3H, s), 1.38 (3H, s), 1.32 (3H, s, H_{18}), 1.15 (3H, s), 1.04 (1H, d, J = 7.0 Hz), 0.94 (3H, s); COSY NMR (300 MHz, $CDCl_3$) see Supporting Information; MS (CI) *m*/*z* 471 (M + H, 100); single-crystal X-ray.

25c: ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.75−4.65 (2H, m, $H_{3\alpha}$ &$H_{16\alpha}$), 3.15 (1H, dd, J = 8.0, 7.4 Hz, $H_{17\alpha}$), 2.54 (1H, dd, J = 13.7, 7.1 Hz), 2.38−2.30 (3H, m), 2.01 (3H, s), 1.35 (3H, s), 1.19 (3H, s, H_{18}), 1.17 (3H, s), 1.09 (1H, d, J = 6.9 Hz), 0.98 (3H, s); COSY NMR (300 MHz, $CDCl_3$) see Supporting Information; ^{13}C NMR (75 MHz, $CDCl_3$) δ 209.4, 170.5, 120.0, 96.3, 82.5, 79.4, 73.0, 61.1, 52.6, 49.1, 44.2, 41.6, 39.6, 39.2, 37.0, 36.5, 36.1, 33.9, 33.7, 30.2, 29.7, 28.4, 27.8, 27.5, 27.1, 20.6, 13.5, 12.0; MS (CI) *m*/*z* 507/509 (M + H, 3.4/2.2); HRMS (CI) calcd for $C_{29}H_{44}ClO_5$ (M + H) 507.2877, found 507.2856; single-crystal X-ray.

25d: ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.72−4.61 (1H, m), 4.47 (1H, apt, J = 7.5 Hz), 3.46 (1H, apt, J = 8.7 Hz, $H_{17\alpha}$), 2.66 (1H, apdq, J = 9.2, 6.8 Hz), 2.49 (1H, apt, J = 13.3 Hz, $H_{11\beta}$), 2.41 (1H, apdq, J = 13, 3.1 Hz), 2.32 (1H, dd, J = 13.1, 3.9 Hz, $H_{11\alpha}$), 2.23 (1H, d, J = 16.3 Hz), 2.20 (1H, dt, J = 11.9, 3.3 Hz), 2.01 (3H, s), 1.99 (2H, aps), 1.37 (3H, s, H_{18}), 1.35 (3H, s), 1.17 (3H, s), 0.96 (1H, d, J = 6.8 Hz), 0.89 (3H, s); ^{13}C NMR (75 MHz, $CDCl_3$) δ 211.1 (s), 170.6 (s), 117.4 (s), 88.5 (s), 82.0 (s), 77.8 (d), 73.0 (d), 63.6 (s), 52.9 (d), 48.4 (d), 44.2 (d), 42.1 (d), 37.3 (t), 37.2 (t), 36.5 (t), 36.5 (s), 33.8 (t), 33.5 (t), 30.3 (q), 28.6 (q), 28.6 (t), 28.2 (t), 27.2 (t), 21.5 (q), 18.0 (q), 14.9 (q), 11.9 (q); MS (CI) *m*/*z* 507/509 (M + H, 16/6); HRMS (CI) calcd for $C_{29}H_{44}ClO_5$ (M + H) 507.2877, found 507.2867.

Mono-ol 28. To a solution of diacetate **27** (101.6 mg, 0.197 mmol) in *i*-PrOH (4 mL) cooled to −15 °C was added *t*-BuOK (217 μL of a 1.0 M solution in *t*-BuOH, 0.217 mmol, 1.1 equiv). The solution was warmed to 0 °C, stirred for 8 h, and then placed in the freezer (−15 °C) for 14 h. TLC analysis (1:1 hexane/EtOAc) showed substantial **27** (R_f 0.60) remaining, with the appearance of a major new spot (desired mono-ol **28**, R_f 0.33), a minor spot (diol, R_f 0.16), and a trace spot (mono-ol **26**, R_f 0.43). The reaction was warmed to 0 °C and stirring continued for 10 h, at which time TLC showed only a trace of **27** remaining. The solution was partitioned between ether and 0.5% HCl, and the aqueous layer was extracted with ether. The combined organics were washed with bicarbonate, dried (Na_2SO_4), and concentrated, and sgc (1:1 hexane/EtOAc) afforded desired mono-ol **28** (63.0 mg, 68%) and diol (17.8 mg, 21%). The earlier fractions containing **27** and mono-ol **26** were combined with the diol and reacetylated to give 32.2 mg of diacetate **27**, which was resubjected to the above hydrolysis procedure (1.3 mL of *i*-PrOH, 69 μL of *t*-BuOK solution) to afford additional **28** (19.5 mg, 66% for this recycle). Reacetylation and hydrolysis of the remaining material afforded another 6.8 mg of desired mono-ol, bringing the total production of **28** to 89.3 mg (96%): ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.40 (1H, apt, J = 1.8 Hz), 4.92 (1H, dd, J = 8.2, 1.8 Hz), 4.35 (1H, dd, J = 11.3, 4.7 Hz, $H_{12\alpha}$), 3.64−3.53 (1H, m, $H_{3\alpha}$), 2.34 (1H, apt, J = 8.8 Hz), 2.04 (3H, s), 1.99 (2H, aps), 1.36 (3H, s), 1.18 (3H, s), 1.08 (3H, s, H_{18}), 0.98 (1H, d, J = 6.7 Hz), 0.86 (3H, s); COSY and CSCM NMR (300 MHz, $CDCl_3$) see Supporting Information; ^{13}C NMR (75 MHz, $CDCl_3$): δ 170.6 (s), 156.6 (s), 120.2 (d), 117.4 (s), 84.3 (d), 82.1 (s), 81.1 (d), 71.0 (d), 56.1 (d), 52.3 (d), 51.3 (s), 44.5 (d), 41.2 (d), 37.9 (t), 37.3 (t), 36.8 (t), 36.0 (s), 34.1 (d), 33.3 (t), 31.3 (t), 30.1 (q), 29.6 (t), 28.5 (q), 28.3 (t), 26.5 (t), 21.3 (q), 15.0 (q), 14.0 (q), 12.2 (q); MS (CI, isobutane) *m*/*z* 473 (M + H, 45), 455 (64), 413 (100); HRMS (CI, isobutane) calcd for $C_{29}H_{45}O_5$ (M + H) 473.3267, found 473.3281.

Ketone 8. Jones oxidation (see Supporting Information) of alcohol **28** (50.3 mg, 0.106 mmol) afforded ketone **8** (45.1 mg, 90%) as waxy white solids: ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.43 (1H, apt, J = 1.8 Hz), 4.92 (1H, dd, J = 8.3, 1.8 Hz), 4.37 (1H, dd, J = 11.3, 4.7 Hz), 2.39−2.22 (4H, m), 2.16−2.05 (3H, m), 2.05 (3H, s), 2.00 (2H, s), 1.37 (3H, s), 1.18 (3H, s), 1.11 (3H, s), 1.06 (3H, s, H_{19}), 0.99 (1H, d, J = 6.7 Hz); CSCM NMR (300 MHz, $CDCl_3$) see Supporting Information; ^{13}C NMR (75 MHz, $CDCl_3$) δ 211.2 (s), 170.6 (s), 155.9 (s), 120.8 (d), 117.5 (s), 84.2 (d), 82.2 (s), 80.8 (d), 56.1 (d), 51.8 (d), 51.4 (s), 46.2 (d), 44.5 (t), 41.2 (d), 38.2 (t), 38.0 (t), 37.3 (t), 36.1 (s), 34.0 (d), 33.3 (t), 30.1 (q), 29.3 (t), 28.6 (q), 28.5 (t), 26.7 (t), 21.3 (q), 15.0 (q), 14.0 (q), 11.4 (q); MS (CI) *m*/*z* 471 (M + H, 100); HRMS (CI) calcd for $C_{29}H_{43}O_5$ (M + H) 471.3111, found 471.3134.

Diketo Olefin 34 and Diketo Alcohol 35. A solution of **32** (130 mg, 0.27 mmol) in dioxane (12 mL) was irradiated as for **19** for 1.5 h at which time TLC (40% EtOAc/hexane, 2×) showed no **32**. The dioxane was evaporated and the crude photolysate dissolved in 75% aqueous acetic acid (3 mL) and stirred at 25 °C for 2.5 days. The reaction mixture was diluted with EtOAc, washed with water (4−5 times) and saturated $NaHCO_3$, and dried over sodium sulfate. The solvent was evaporated and the residue dissolved in ether. Brown−Jones oxidation (see Supporting Information) followed by sgc (25% EtOAc in hexane) gave 75 mg (58% for three steps from **32**) of diketo olefin **34** and 28 mg (22%) of the diketo alcohol **35** as white foams.

Diketo Olefin 34. ^{1}H NMR (300 MHz, $CDCl_3$): δ 5.43 (1H, br s, H_{15}), 5.14 (1H, dd, J = 6.6, 4.5 Hz, H_{23}), 3.83 (1H, d, J = 11.7 Hz, H_{18}), 3.76 (1H, d, J = 12 Hz, H_{18}), 2.07 (3H, s), 1.28 (3H, s), 1.26 (3H, s), 1.05 (3H, d), 1.04 (3H, s); ^{13}C NMR (APT, 75 MHz, $CDCl_3$) [EVEN (17)] δ 211.7, 210.9, 169.9, 148.1, 109.3, 82.3, 63.6, 61.4, 44.5, 44.0, 38.8, 37.9, 36.2, 31.9, 29.3, 28.2; [ODD (12)] δ 123.4, 82.8, 51.9, 45.7, 43.4, 35.7, 32.3, 29.6, 29.5, 21.4, 14.9, 10.9; [α] = +23.4° (c = 1.2, $CHCl_3$); MS (CI) 485 (M + H, 100); HRMS (CI) calcd for $C_{29}H_{40}O_6$ 485.2903, found 485.2912.

Diketo Alcohol 35: ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.15 (1H, apd, J = 6 Hz), 4.77 (1H, br s, OH), 4.04 (1H, d, J = 11.7 Hz, H_{18}), 3.38 (1H, d, J = 12 Hz, H_{18}), 2.06 (3H, s), 1.31 (3H, s), 1.27 (3H, s), 1.10 (3H, d, J = 6.6 Hz), 0.99 (3H, s); ^{13}C NMR (APT, 75 MHz, $CDCl_3$) [EVEN (18)] δ 211.9, 210.9, 170.1, 110.7, 87.5, 83.9, 64.0, 61.9, 44.5, 42.6, 41.0, 38.2, 37.8, 36.3, 34.6, 28.5, 27.7, 25.0; [ODD (11)] δ 81.0, 45.9, 44.8, 43.8, 43.0, 31.5, 30.9, 29.3, 21.4, 14.5, 11.2; MS (CI) 503 (M + H, 100); HRMS (CI) calcd for $C_{29}H_{42}O_7$ 503.3009, found 503.3024.

South 1 Azido Ketone 7. To a solution of **36** (29 mg, 0.05 mmol) in freshly distilled nitromethane (5.2 mL) was added tetramethylguanidinium azide (TMGA, 33 mg, 0.21 mmol). The mixture was stirred at 25 °C for 2.5 h, when a ^{1}H NMR of an aliquot of showed no **36**. After removal of solvent, sgc (15% EtOAc/hexane) gave 24 mg (87%) of **7** as a foam: ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.44 (1H, s), 5.16 (1H, dd, J = 6.3, 4.8 Hz), 3.98 (1H, dd, J = 12.9, 6.9 Hz, $H_{2\beta}$), 3.83 (1H, d, J = 12 Hz), 3.75 (1H, d, J = 12 Hz), 2.08 (3H, s), 1.29 (3H, s), 1.27 (3H, s), 1.12 (3H, s), 1.06 (3H, d, J = 6 Hz); ^{13}C NMR (APT, 75 MHz, $CDCl_3$) [EVEN (16)] δ 210.9, 204.4, 169.9, 147.4, 109.3, 82.3, 63.6, 61.4, 44.9, 44.0, 43.5, 38.7, 37.3, 32.0, 29.2, 27.7; [ODD (13)] δ 123.8, 82.8, 63.5, 51.5, 46.6, 44.4, 35.2, 32.2, 29.6, 29.5, 21.4, 14.8, 11.9; MS (FAB, DTT/DTE) 526 (M + H, weak), 500 (M − N2 + H).

Bromo Ketone 39. A solution of ketone **8** (34.8 mg, 73.9 mmol) in THF (0.75 mL) was cooled to 0 °C; PTAB (30.6 mg, 81.3 mmol, 1.1 equiv) in THF (1.5 mL) was cooled and then added to the solution of **8** rapidly via cannula. The resulting orange solution deposited copious precipitate and faded to a beige color within 2 min. After 10 min of stirring at 0 °C, the reaction was quenched with brine (10 mL) and extracted twice with ether. The combined organics were washed with brine, dried (Na_2SO_4), concentrated, and filtered through a plug of silica gel (2:1 hexane/EtOAc) to afford bromo ketone **39** (41 mg, quantitative) as off-white solids which by NMR contained ∼5% of the 3*S* (axial) epimer: ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.44 (1H, apt, J = 1.9 Hz), 4.92 (1H, dd, J = 8.3, 1.9 Hz), 4.71 (1H, dd, J = 13.4, 6.3 Hz, $H_{2\beta}$), 4.37 (1H, dd, J = 11.2, 4.7 Hz), 2.55 (1H, dd, J = 13.0, 6.3 Hz, $H_{1\beta}$), 2.48−2.42 (2H, m), 2.37 (1H, apt, J = 8.8 Hz), 2.06 (3H, s), 2.00 (2H, s), 1.36 (3H, s), 1.18 (3H, s), 1.14 (3H, s, H_{19}), 1.10 (3H, s), 0.99 (1H, d, J = 6.7 Hz); ^{13}C NMR (75 MHz, $CDCl_3$) δ 200.6 (s), 170.6 (s), 155.2 (s), 121.2 (d), 117.5 (s), 84.1 (d), 82.2 (s), 80.4 (d), 56.1 (d), 53.7 (d), 51.4 (d), 51.4 (s), 51.0 (t), 47.0 (d), 43.7 (t), 41.2 (d), 39.3 (s), 37.3 (t), 33.5 (d), 33.3 (t), 30.1 (q), 29.1 (t), 28.6 (q), 28.0 (t), 26.7 (t), 21.3 (q), 15.1 (q), 14.0 (q), 12.0 (q); [α] = +33.7° (c = 1.00, $CHCl_3$); MS (CI) *m*/*z* 551/549 (M + H, 10/12), 393/391 (100/35); HRMS (CI) calcd for $C_{29}H_{42}BrO_5$ (M + H) 549.2216, found 549.2226.

Azido Ketone 9: A solution of bromo ketone **39** (31.4 mg, 57 mmol) in CH_3CN (3.2 mL) was cooled to 0 °C and then rapidly added, via cannula, to a cold (0 °C) solution of TMGA (45.8 mg, 290 mmol, 5 equiv) in CH_3CN (2.6 mL). The reaction was allowed to warm slowly to 25 °C during 18 h of stirring and partitioned between EtOAc and brine, and the organic layer was dried (Na_2SO_4) and concentrated to give crude azido ketone **9** (30 mg, quantitative). ^{1}H NMR analysis indicated that the material contained 5% α-amino enone side product and ∼10% 2-epi-**9** (axial azide). Crude **9** coupled admirably in the next step, but in this run sgc (2:1 hexane/EtOAc) furnished pure **9** (24.7

mg, 85%) as white solids for analytical purposes: ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.43 (1H, apt, J = 1.9 Hz), 4.92 (1H, dd, J = 8.2, 1.9 Hz), 4.37 (1H, dd, J = 11.2, 4.7 Hz), 3.95 (1H, dd, J = 13.0, 6.7 Hz, $H_{2\beta}$), 2.42−2.32 (1H, m), 2.33 (1H, apt, J = 8.5 Hz), 2.29 (1H, dd, J = 14.4, 4.2 Hz, $H_{4\alpha}$), 2.22 (1H, dd, J = 12.6, 6.3 Hz, $H_{1\beta}$), 2.06 (3H, s), 1.99 (2H, aps), 1.36 (3H, s), 1.18 (3H, s), 1.13 (3H, s, H_{19}), 1.10 (3H, s), 0.99 (1H, d, J = 6.7 Hz); COSY NMR (300 MHz, $CDCl_3$) see Supporting Information; ^{13}C NMR (75 MHz, $CDCl_3$) δ 204.6 (s), 170.6 (s), 155.3 (s), 121.2 (d), 117.5 (s), 84.1 (d), 82.2 (s), 80.5 (d), 63.7 (d), 56.1 (d), 51.5 (d), 51.4 (s), 47.1 (d), 45.1 (t), 43.6 (t), 41.2 (d), 37.3 (s), 37.3 (t), 33.4 (d), 33.3 (t), 30.1 (q), 29.1 (t), 28.6 (q), 28.0 (t), 26.8 (t), 21.3 (q), 15.0 (q), 14.0 (q), 12.4 (q); MS (FAB, DTT/DTE) 512 (M + H); HRMS (FAB, DTT/DTE) calcd for $C_{29}H_{42}N_3O_5$ (M + H) 512.3124, found 512.3134.

Aminomethoxime 10: Azido ketone **9** (9.9 mg, 19 μmol) was converted to azidomethoxime **40** (10.4 mg, 19 μmol) followed by Staudinger reduction to give aminomethoxime **10** (7.4 mg, 75% from **9**) as a white foam, which resisted crystallization (see Supporting Information): ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.40 (1H, apt, J = 1.8 Hz), 4.91 (1H, dd, J = 8.3, 1.8 Hz), 4.36 (1H, dd, J = 11.2, 4.7 Hz), 3.84 (3H, s), 3.49 (1H, dd, J = 12.2, 5 Hz, $H_{2\beta}$), 3.01 (1H, dd, J = 14.3, 2.6 Hz, $H_{4\alpha}$), 2.34 (1H, apt, J = 8.8 Hz), 2.05 (3H, s), 1.99 (2H, aps), 1.36 (3H, s), 1.18 (3H, s), 1.08 (3H, s), 0.98 (1H, d, J = 6.7 Hz), 0.97 (3H, s, H_{19}); ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.5 (s), 156.1 (s), 120.6 (d), 117.5 (s), 84.2 (d), 82.1 (s), 80.9 (d), 61.6 (q), 56.1 (d), 51.9 (d), 51.4 (s), 49.7 (d), 48.8 (t), 45.5 (t), 41.2 (d), 37.3 (t), 37.0 (s), 33.6 (d), 33.3 (t), 30.1 (q), 29.3 (t), 28.6 (q), 27.9 (t), 27.3 (t), 26.5 (t), 21.3 (q), 15.1 (q), 14.0 (q), 12.4 (q); MS (FAB, DTT/DTE) 515 (M + H); HRMS (FAB, DTT/DTE) calcd for $C_{30}H_{47}NO_5$ (M + H) 515.3485, found 515.3468.

Protected Cephalostatin 1 (41). To a solution of α-azido ketone **7** (5 mg, 0.01 mmol) and α-aminomethoxime **6** (9.6 mg, 0.01 mmol) in benzene (3 mL) was added dichlorodibutylstannane (0.3 mg, 10 mol %) and polyvinylpyridine (15 mg). The reaction flask was equipped with a Dean−Stark trap, and the mixture was heated at reflux for 3 h (2−4 mL of fresh benzene was added twice to maintain the solvent level in the reaction vessel), at which time TLC (25% EtOAc/hexane) indicated no remaining **7**. The reaction mixture was cooled and filtered, and the solids were washed with CH_2Cl_2. Evaporation of the filtrate and sgc (15−20% EtOAc/hexane) of the residue gave 7.6 mg (59%) of pure **41** as a white solid and 2.8 mg of recovered α-aminomethoxime **6**: ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.86 (2H, d, J = 6.9 Hz), 7.75 (2H, d, J = 7.2 Hz), 7.48−7.38 (6H, m), 5.57 (1H, aps), 5.48 (1H, aps), 5.17 (1H, dd, J = 6.6, 4.2 Hz), 5.07 (1H, dd, J = 11.4, 5.4 Hz), 4.95 (1H, aps), 3.97 (1H, s), 3.90−3.79 (3H, m), 3.10 (1H, apd, J = 9.6 Hz), 2.98 (1H, apd, J = 10.2 Hz), 2.91−2.81 (4H, m), 2.09 (3H, s), 2.00 (3H, s), 1.31 (3H, s), 1.29 (3H, s), 1.26 (3H, s), 1.25 (3H, s), 1.12 (3H, d), 1.08 (3H, d, J = 6 Hz), 1.01 (9H, s), 0.86 (3H, s), 0.85 (3H, s), 0.76 (9H, s), −0.13 (3H, s), −0.14 (3H, s).

(+)-Cephalostatin 1 (1). To a solution of **41** (7 mg, 0.005 mmol) in THF (2 mL) was added a 1.0 M solution of tetrabutylammonium fluoride (TBAF) in THF (16 mL, 0.016 mmol), and the mixture was heated at reflux for 2 h and cooled and the solvent evaporated. The residue was dissolved in an 8:1 mixture of MeOH/H_2O (2 mL), and K_2CO_3 (7.5 mg, 0.054 mmol) was added. The resulting suspension was heated at reflux for 0.5 h, cooled, and concentrated. The residue was dissolved in EtOAc, washed with water (two to three times) and dried (Na_2SO_4). Evaporation of the solvent followed by sgc (3−5% MeOH in chloroform) of the residue gave 3.8 mg (80%) of pure cephalostatin 1 (**1**) as a white solid. The ^{1}H and ^{13}C NMR, TLC, and HPLC profiles of synthetic **1** and natural **1** were found to be identical.[2] For a comparative summary of ^{1}H and ^{13}C NMR of synthetic and natural cephalostatin 1 (**1**), see Supporting Information: ^{1}H NMR (500 MHz, C_5D_5N) δ 8.16 (1H, d, J = 7.5 Hz), 7.27 (1H, d, J = 4 Hz), 6.63 (1H, t, J = 5.5 Hz), 6.26 (1H, s), 5.64 (1H, s), 5.44 (1H, s), 5.25 (1H, s), 4.81 (2H, m), 4.71 (1H, d, J = 1 Hz), 4.08 (1H, d, J = 12 Hz), 4.06 (1H, m), 4.03 (1H, d, J = 12 Hz), 3.82 (1H, dd, J = 11, 5.5 Hz), 3.72 (1H, dd, J = 11.5, 5 Hz), 3.18 (1H, dq, J = 7, 6 Hz), 3.08 (1H, d, J = 16 Hz), 3.05 (1H, d, J = 17 Hz), 1.65 (3H, s), 1.47 (3H, s), 1.47 (3H, d, J = 6.5 Hz), 1.39 (3H, s), 1.35 (3H, d, J = 7 Hz), 1.33 (3H, s), 0.75 (3H, s), 0.72 (3H, s); ^{13}C NMR (150 MHz, C_5D_5N) δ 211.75, 152.72, 149.46, 148.99, 148.65, 148.43, 148.37, 123.17, 122.28, 117.15, 110.91, 93.14, 91.66, 82.79, 81.53, 81.13, 75.58, 71.51, 69.29, 64.19, 61.83, 55.40, 53.23, 52.22, 47.33, 45.98, 45.82, 44.50, 44.23, 41.81, 41.24, 39.52, 38.82, 36.33, 36.29, 35.79, 35.73, 35.61, 33.81, 32.87, 32.36, 29.75, 29.51, 29.46, 28.96, 28.69, 28.23, 27.95, 26.40, 15.47, 12.57, 11.73, 11.32, 8.99; [α] = +95° (c = 0.04, MeOH) [lit.[2] [α] = +102° (c = 0.04, MeOH)].

Protected Ritterostatin G_N1_N 42. North G azido ketone **9** (4.7 mg, 0.0092 mmol), North 1 aminomethoxime **6** (9.6 mg, 0.0105 mmol), polyvinylpyridine (14 mg), freshly crushed 4A molecular sieves (14 mg), dichlorodibutylstannane (catalyst), and 10 mL of benzene, procedure as for **41** except 7 mL of distillate was collected over 2 h without addition of fresh solvent. Sgc (gradient from 2:1 to 1.5:1 hexane/EtOAc, then 100:10:1 EtOAc/MeOH/TEA) afforded 5.9 mg (49%) of **42** as a white foam, 3.7 mg (31%) of material containing mainly what appeared by NMR to be partially deacetylated **42**, and 2.2 mg of aminomethoxime **6** (23%). The combined yield of pyrazines was thus 80% based on **9**, 92% based on recovered **6**: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.86 (2H, m), 7.74 (2H, dd, J = 7.9, 1.6 Hz), 7.46−7.28 (6H, m), 5.56 (1H, aps), 5.45 (1H, aps), 5.06 (1H, dd, J = 11.2, 5.1 Hz), 4.95 (1H, aps), 4.93 (1H, dd, J = 8.6, 1.9 Hz), 4.40 (1H, dd, J = 11.1, 4.5 Hz), 4.30 (1H, dd, J = 10.5, 7.9 Hz), 3.97 (1H, s), 3.10 (1H, d, J = 10.1 Hz), 2.97 (1H, d, J = 10.1 Hz), 2.87−2.82 (4H, m), 2.78−2.44 (5H, m), 2.38 (1H, dd, J = 8.8, 8.7 Hz), 2.06 (1H, s), 2.04 (1H, s), 1.37 (3H, s), 1.24 (3H, s), 1.19 (3H, s), 1.12 (3H, d, J = 6.0 Hz), 1.11 (6H, s), 1.00 (9H, s), 0.99 (1H, d, J = 7 Hz), 0.86 (3H, s), 0.85 (3H, s), 0.75 (9H, s), −0.14 (3H, s), −0.15 (3H, s); COSY NMR (300 MHz, $CDCl_3$) see Supporting Information; MS (FAB, DTT/DTE) 1134 (M + H).

Ritterostatin G_N1_N 4. To **42** (4.7 mg, 0.0035 mmol) in THF (1.5 mL) was added TBAF (0.0105 mL of 1.0 M THF solution, 3 equiv); the resulting yellow solution was heated at reflux for 2 h and then cooled. Methanol (1 mL) and 10% KOH (0.1 mL) were added, and the reaction mixture was heated at reflux for 45 min. The mixture was cooled and partitioned between EtOAc (10 mL) and brine (10 mL), and the organic layer was washed with 0.01% HCl. The combined aqueous layers were extracted with EtOAc (10 mL). The combined organic layers were washed with saturated $NaHCO_3$, dried over sodium sulfate, concentrated, and chromatographed (gradient 3−7% MeOH in CH_2Cl_2) to afford 2.9 mg of **4** (94%) as white solids.

Partially deacetylated **42** (3.7 mg, the second fraction from the coupling reaction) was treated as above to afford 2.0 mg of **4** as white solids (89% combined yield; 62% overall from **9**, 69% based on recovered **6**). See the Supporting Information for comparison of the ^{1}H and ^{13}C NMR spectra of **4** with signals of its constituent subunits in natural **1** and **3**: ^{1}H NMR (500 MHz, pyr-d_5) δ 8.12 (1H, apd, J = 7.2 Hz), 6.59 (1H, apt, J = 5.3 Hz), 6.29 (1H, d, J = 4.8 Hz), 6.24 (1H, s), 5.63 (1H, aps), 5.54 (1H, aps), 5.27 (1H, dd, J = 8.4, 1.7 Hz), 5.24 (1H, aps), 4.80 (1H, m), 4.70 (1H, d, J = 1.5 Hz), 4.04 (1H, dd, J = 10.6, 4.7 Hz), 3.80 (1H, dd, J = 11, 4.8 Hz), 3.71 (1H, dd, J = 11, 4.8 Hz), 3.48 (1H, m), 3.11−3.05 (3H, m), 2.92−2.83 (3H, m), 2.72 (1H, dd, J = 11.4, 8.0 Hz), 2.67−2.61 (4H, m), 2.32 (1H, apt, J = 11.1 Hz), 2.22 (1H, dq, J = 8, 7 Hz), 2.16−2.00 (7H, m), 1.92−1.72 (4H, m), 1.64 (3H, s), 1.46 (3H, s), 1.35 (3H, d, J = 7 Hz), 1.32 (3H, s), 1.31 (3H, s), 1.24 (3H, d, J = 6.7 Hz), 1.19 (3H, s), 0.94−0.83 (2H, m), 0.75 (3H, s), 0.73 (3H, s); ^{13}C NMR (125 MHz, pyr-d_5) δ 157.1, 152.7 ($C_{14'}$), 150.2, 148.9, 148.6, 148.5, 122.3, 120.4, 117.8, 117.2, 93.2, 91.6, 85.0, 82.8, 81.5, 78.5, 75.6, 71.5, 69.3, 56.3, 55.4, 53.2, 52.6, 46.1, 46.0, 44.5, 42.1, 41.8, 41.8, 39.5, 37.8, 36.3, 36.2, 35.8, 35.8, 34.0, 33.8, 33.6, 30.9, 30.3, 29.7, 28.8, 28.9, 28.7, 28.4, 27.9, 26.4, 14.5, 13.9, 12.6, 11.8, 11.7, 9.0; [α] = +166° (c = 0.01, $CHCl_3$); MS (FAB, DTT/DTE) 879 (M + H); HRMS (FAB, DTT/DTE) calcd for $C_{54}H_{75}N_2O_5Si$ (M + H) 879.5523, found 879.5449.

Protected Ritterostatin G_N1_S 43. To a 25 mL flask equipped with a Dean−Stark trap and condenser was charged North G aminomethoxime **10** (5.4 mg, 0.0105 mmol), South 1 azido ketone **7** (6.0 mg, 0.011 mmol), Nafion H (11.5 mg), dichlorodibutylstannane (catalyst), and 10 mL of benzene. The apparatus was heated to reflux, with removal of the contents of the Dean−Stark trap (3.5 mL) after 1 h and again after 3 h heating; a further 1.5 mL was removed after 4 h, leaving the final reaction volume at 1.5 mL, TLC analysis still showed

remaining **7** (3:1 hexane/EtOAc) and **10** (100:10:1 EtOAc/MeOH/TEA) with one main new spot and another more polar spot (1:1 hexane/EtOAc). The mixture was cooled, filtered, and chromatographed (gradient from 1:1 to 1:2 hexane/EtOAc, then 100:10:1 EtOAc/MeOH/TEA) to afford 1.3 mg (21%) recovered impure **7**, 5.2 mg (52%) protected ritterostatin G_N1_S **43** as white solids, 2.0 mg (21%) of material containing mainly what appeared by NMR to be partially deacetylated pyrazines **43**, and 1.5 mg (26%) of clean **10**. The combined yield of the pyrazines was thus 73% based on azido ketone **7**, 99% based on recovered aminomethoxime **10**: ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.48 (1H, aps), 5.44 (1H, aps), 5.16 (1H, dd, J = 6.7, 4.6 Hz,), 4.93 (1H, dd, J = 8.2, 1.8 Hz), 4.40 (1H, dd, J = 11.2, 4.7 Hz), 3.85 (1H, d, J = 8.0 Hz), 3.85 (1H, d, J = 8.0 Hz), 2.91−2.78 (4H, m), 2.71−2.28 (6H, m), 2.08 (3H, s), 2.05 (3H, s), 1.37 (3H, s), 1.30 (3H, s), 1.28 (3H, s), 1.18 (3H, s), 1.11 (3H, s), 1.07 (3H, dd, J = 6.2 Hz), 1.00 (3H, dd, J = 6.7 Hz), 0.85 (3H, s), 0.84 (3H, s); MS (FAB, DTT/DTE) 947 (M + H); HRMS (FAB, DTT/DTE) calcd for $C_{58}H_{79}N_2O_9$ (M + H) 947.5786, found 947.5737.

Ritterostatin G_N1_S 5. Diacetate **43** (5.1 mg, 0.0054 mmol) was dissolved in 88% aqueous MeOH (2 mL), and K_2CO_3 (4 mg) was added. The mixture was heated at reflux for 30 min and then cooled. The mixture was partitioned between CH_2Cl_2 (10 mL) and water (10 mL). The organic layer was dried over sodium sulfate and then concentrated to afford 5 mg of **5** (quantitative) as white solids.

Partially deacetylated **43** (2.0 mg, the second fraction from the coupling reaction) was treated as above to afford 2 mg of impure **5** as white solids. The two lots of **5** were combined and chromatographed (gradient 2−4% MeOH in CH_2Cl_2) to afford 6.0 mg of **5** as white solids (94% combined yield; 68% overall from **10**, 93% overall based on recovered **10**). See Supporting Information for comparison of the ^{1}H and ^{13}C NMR spectra of **5** with signals of its constituent subunits in natural **1** and **3**: ^{1}H NMR: (300 MHz, pyr-d_5) δ 7.19 (1H, brs), 5.55 (1H, aps), 5.54 (1H, aps), 5.28 (1H, brd, J = 8.0 Hz), 4.80 (1H, m), 4.07 (1H, d, J = 11.9 Hz), 4.04 (1H, d, J = 11.9 Hz), 3.49 (1H, m), 3.18 (1H, dq, J = 6.8, 6.8 Hz), 3.10 (1H, d, J = 17.5 Hz), 3.09 (1H, apq, J = 8.0 Hz), 3.08 (1H, d, J = 16.9 Hz), 2.93 (1H, dd, J = 18.1, 5.0 Hz), 2.91 (1H, d, J = 18.0, 5.0 Hz), 2.85 (1H, apdt, J = 12, 4 Hz), 2.78 (1H, apt, J = 13.7 Hz), 2.81−2.76 (1H, m), 2.71−2.61 (3H, m), 2.64 (1H, d, J = 16.1 Hz), 2.62 (1H, dd, J = 13.5, 3.2 Hz), 2.57 (1H, d, J = 17.5 Hz), 2.35 (1H, dd, J = 12.3, 7.1 Hz), 2.32 (1H, m), 2.23 (1H, dt, J = 13.4, 6.9 Hz), 2.17−1.80 (10H, m), 1.70−1.53 (6H, m), 1.47 (3H, s), 1.47 (3H, d, J = 6.8 Hz), 1.46 (3H, s), 1.38 (3H, s), 1.32 (3H, s), 1.25 (3H, d, J = 6.5 Hz), 1.19 (3H, s), 0.77 (3H, s), 0.73 (3H, s); ^{13}C NMR (125 MHz, pyr-d_5) δ 157.1, 149.4, 149.0, 148.7, 148.4, 148.3, 123.0, 120.4, 117.8, 110.9, 85.0, 81.5, 81.5, 81.1, 78.6, 64.2, 61.8, 56.4, 53.5, 52.6, 52.2, 47.3, 46.1, 45.8, 44.2, 42.1, 41.7, 41.2, 38.8, 37.8, 36.3, 36.2, 35.8, 35.8, 35.6, 34.0, 33.6, 32.9, 32.4, 30.9, 30.3, 30.0, 29.7, 29.7, 29.4, 28.7, 28.4, 28.0, 15.5, 14.5, 13.9, 11.8, 11.3; IR ($CHCl_3$) 1705; [α] = +66° (c = 0.05 MeOH); MS (FAB, DTT/DTE) 863 (M + H); HRMS (FAB, DTT/DTE) calcd for $C_{54}H_{75}N_2O_7$ (M + H) 863.5574, found 863.5548.

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Supporting Information Available: Experimentals for compounds **6**, **13**−**18**, **20a**−**d**, **21**, **27**−**32**, **36**−**38**, and **40**; NMR spectra and extensive signal assignments for all compounds; Schemes 8a and 10a; CAChe drawing of **32** (ground state); calculated energies of spiroketal isomers; and tables comparing ^{1}H and ^{13}C NMR data for natural versus synthetic pyrazines (155 pages). See any current masthead page for ordering and Internet access instructions.

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