HHS Public Access

Author manuscript

J Am Chem Soc. Author manuscript; available in PMC 2017 March 17.

Published in final edited form as:

J Am Chem Soc. 2011 May 18; 133(19): 7260–7263. doi:10.1021/ja200034b.

11-Step Enantioselective Synthesis of (−)-Lomaiviticin Aglycon

Seth B. Herzon[*], **Liang Lu**[†], **Christina M. Woo**[†], and **Shivajirao L. Gholap**

Department of Chemistry, Yale University, New Haven, Connecticut 06520, United States

Abstract

Lomaiviticins A and B are complex antitumor antibiotics that were isolated from a strain of *Micromonospora*. A confluence of several unusual structural features renders the lomaiviticins exceedingly challenging targets for chemical synthesis. We report an 11-step, enantioselective synthetic route to lomaiviticin aglycon. Our route proceeds by late-stage, stereoselective dimerization of two equivalent monomeric intermediates, a transformation that may share parallels with the natural products' biosyntheses. The route we describe is scalable and convergent, and it lays the foundation for determination of the mode of action of these natural products.

Lomaiviticins A and B (**1** and **2**, Figure 1) are complex dimeric bacterial metabolites that were obtained as minor constituents from the fermentation broth of *Micromonospora lomaivitiensis*.[1] The lomaiviticins, and the related monomeric isolates known as the kinamycins (**4**–**6**),[2] constitute a small family of natural products which contain a diazo functional group. In **1**–**6**, this function is embedded in a highly oxidized tetracyclic carbon skeleton, forming a diazotetrahydrobenzo[*b*]fluorene (diazofluorene, see structure **7**). Simple diazo-containing molecules, such as diazomethane, are prone to detonation.[3] Thus, the presence of this functional group within a natural product, itself formed under conditions of microbial metabolism, is remarkable. Several additional structural features of the lomaiviticins—deoxyglycoside residues, highly oxidized napthoquinones, and a sterically congested carbon–carbon bond bridging the two moieties of each metabolite—elevate their molecular complexity and render their structures singular among all known natural products.

Both **1** and **2** exhibit powerful antimicrobial activities (MICs ≈ 6–25 ng/spot, plate assay), and lomaiviticin A (**1**) is reported to inhibit the growth of 25 cancer cell lines at low nanomolar to picomolar concentrations. The activity profile of **1** differs from those of other cancer chemotherapeutics, such as doxorubicin and mitomycin, suggesting that a distinct mechanism of action is operative.[1] Model studies have provided evidence that the lomaiviticins form reactive intermediates under reducing conditions.[4] However, investigations of **1** and **2** directly have been impossible to initiate because the lomaiviticins are obtained in minute quantities from their natural source,[1] and a total synthesis has not yet been realized.[5,6]

Corresponding Author: seth.herzon@yale.edu.

[†]Author Contributions

These authors contributed equally to this work.

Supporting Information. Experimental procedures and detailed characterization data for all new compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

Herein, we report an 11-step, enantioselective synthesis of lomaiviticin aglycon (**3**). This research addresses the decade-long problem of synthesis of the dimeric diazofluorene structure of the lomaiviticins. Many steps in our route may be executed efficiently on gram scales, as shown. The brevity and convergence of our synthesis renders it amenable to incorporation of affinity probes for target identification experiments and to the synthesis of analogues for structure–function studies.[7]

Our strategy to synthesize **3** was guided by the hypothesis that nature prepares the lomaiviticins by direct oxidative dimerization of monomeric diazofluorenes, as depicted in Scheme 1a. The merit of this approach in a synthetic setting is that it prognosticates the union of two identical monomers late-stage in the synthesis, thereby vastly simplifying the route to **3**. However, several challenges to this bond construction are readily identified. First, under strongly acidic or basic conditions, monomers such as **8** are susceptible to a β-elimination-aromatization pathway (Scheme 1b), and this reaction manifold severely limits the chemical reagents that are compatible with such substrates. Second, as a consequence of the steric hindrance about the incipient bond, it was anticipated that the rate of dimerization may be too slow to produce workable yields of coupled products (**9**). This substitution pattern also made it difficult to predict the stereochemical outcome of the coupling event. Finally, although the oxidative couplings of ketones,[8] their enolates,[9] and their enoxysilanes[10] have been used with great success in synthetic chemistry, the oxidative dimerization of diazofluorenes, such as **8**, was unknown at the outset of these studies.

Our pathway to lomaiviticin aglycon (**3**) began with synthesis of the monomeric diazofluorene **18** by the sequence outlined in Scheme 1c. Our route to **18** follows a pathway originally developed for the synthesis of kinamycin F (**6**),[6c] with several critical modifications to increase flexibility and material throughput. The synthesis began with silylation of the inexpensive commercial reagent 3-ethylphenol (96%, >1 mol scale).[11] Birch reduction of the resulting silyl ether **10** followed by regio- and stereoselective dihydroxylation[12] afforded the enoxysilane **11** in 91% ee and 61% yield over two steps (>60 g scale). In a departure from previous studies, the diol **11** was oxidized directly to the α,β-unsaturated ketone **12** by treatment with palladium acetate (10 mol %) under an atmosphere of dioxygen (92%, 9.6 g scale).[13] This modification delayed installation of the protecting group, which accelerated the development of this sequence. The α,β-unsaturated ketone **12** was then protected as its mesitylaldehyde acetal (85%, 1:1 mixture of diastereomers, 4.4 g scale). The mixture of diastereomeric acetals **13** was homologated to the β-(trimethylsilylmethyl)-α,β-unsaturated ketones **14** by copper-mediated 1,4-addition of trimethylsilylmethylmagnesium chloride, trapping with chlorotrimethylsilane, and reoxidation (82%, 1.5 g scale).

The β-(trimethylsilylmethyl)-α,β-unsaturated ketones **14** and 2,3-dibromo-5,8-bis(methoxymethyloxy)naphthoquinone (**15**, prepared in one step from 2,3-dibromo-5,8-dihydroxynaphthoquinone)[11,14] were efficiently coupled by treatment with tris-(diethylamino)sulfonium trimethyldifluorosilicate [TASF(Et)], to afford the γ-quinonylation products **16** in 81% yield (1 g scale). The products **16** were then cyclized by heating in the presence of palladium acetate and polymer-supported triphenylphosphine. Lowering the ratio of phosphine to palladium from 2.5:1 to 1.5:1 increased the yield of the cyclized

products **17** to 95% (compared to 66% yield in our kinamycin synthesis;[6c] 1.4 g scale). Finally, diazo transfer to the cyclized products **17** (TfN_3, DMAP) afforded the diazofluorenes **18** and **19** in 51% combined yield, and these were readily separated by flash column chromatography (550 mg scale).[15]

Transformation of **18** (or **19**) to lomaiviticin aglycon (**3**) proved to be a formidable challenge. An exhaustive evaluation of conditions (>1500 experiments) using **18**, **19**, and various derivatives of each was unsuccessful. Ultimately, however, a short sequence was developed (Scheme 2a). First, the *exo*-mesityl diazofluorene **18** was converted to its trimethylsilyl enol ether derivative **20** by exposure to trimethylsilyl trifluoromethanesulfonate and triethylamine at 0 °C. The unpurified enoxysilane **20** was immediately re-dissolved in benzene and treated with a solution of manganese tris(hexafluoroacetylacetonate) (**21**)[16] in benzene at 21 °C. Under these conditions, the oxidative coupling products **22** and **23** were obtained in 38% combined yield on a 100 mg scale (26% isolated yield of **22**, 12% isolated yield of **23**, 30% of **18** recovered).[17] A third dimeric product, tentatively identified as the C_s-symmetric dimer, was detected by LC/MS analysis of the unpurified product mixture, but this compound was not formed in quantities sufficient for detailed characterization.

The relative stereochemistry of the dimeric products **22** and **23** was deduced by conversion of **22** to lomaiviticin aglycon (**3**, vide infra). The stereoselectivity in their formation (**22:23** = 2:1) is believed to originate from a nonbonded interaction between the *ortho*-methylarene substituents of **20** and the C-3 ethyl substituent (Scheme 2b). This interaction is proposed to orient the ethyl residue over the *Si* face of the enoxysilane, guiding the approach of the enoxysilanes **20** from their respective *Re* faces. Dimerization of the enoxysilane of **19**, which contains the mesitylaldehyde acetal in the *endo* orientation, forms the (1*S*,1′*S*)-dimer exclusively (36% yield).[11,18] This result is consistent with our stereochemical model and the inherent preference for addition *exo* to the *cis*-fused 5–6 system.

The deprotection of the (1*R*,1′*R*)-dimer **22** was successfully executed under carefully optimized conditions. Thus, slow addition of trifluoroacetic acid to a mixture of **22** and excess anhydrous *tert*-butylhydroperoxide[19] in dichloromethane–decane solution at −35 °C, followed by extractive workup and purification, afforded lomaiviticin aglycon (**3**) in 39% yield (10 mg scale, 2.5 mg of **3** isolated; >15 mg of **3** has been prepared to date). Careful examination of this reaction established that the open-chain ketone isomer of lomaiviticin aglycon (**25**) is the product that is first formed in the deprotection of **22** (Scheme 2c). The ratio of the ketone isomer **25** to **3** was >8:1 throughout the course of the reaction (LC/MS analysis, retention times of **25** and **3** = 1.55 and 1.26 min, respectively), but these mixtures quantitatively converted to **3** upon purification by preparative thin-layer chromatography. Mixtures of **25** and **3** could be obtained by rapid isolation using flash-column chromatography,[11] and HMBC experiments unequivocally established the presence of **25** [a correlation was observed between H-1/H-1′ (δ 3.34) and C-2/C-2′ (δ 190.8)]. The open-chain isomer (**25**) slowly converted to lomaiviticin aglycon (**3**) on standing in chloroform (ca. 50% conversion of **25** after 12 h at 24 °C) or, more rapidly, in methanol (full conversion within 10 min). The C-2/C-2′ carbon resonance of **3** (δ 99.4, $CDCl_3$), which was coupled to

the tertiary hydroxyl proton and to H-1/H-1′ in the HMBC spectrum, agrees well with that of lomaiviticin B (**2**, δ 96.5, CD_3OD).[1,11] These data support the assignment of the relative stereochemistry of **3** and, by inference, that of **22** and **23**, as shown.

By comparison, intermediates in the (1*S*,1′*S*)-series were appreciably more robust. The (1*S*, 1′*S*)-dimer **23** was deprotected by heating with trifluoroacetic acid in methanol at 50 °C, to afford the diastereomeric lomaiviticin aglycon (**24**, 41%). The increased stability of **24** may be attributed to the *cis* arrangement of the α-hydrogen and β-oxygen substituents, which is expected to decrease the rate of β-elimination. As expected, the (1*S*, 1′*S*)-diastereomer **24** showed no evidence of cyclization after standing for 2 days at 24 °C in methanol-d_4.

Several additional observations that arose during the course of our studies are noteworthy. First, we found that enoxysilanes derived from diazofluorenes bearing acyclic protecting groups on the vicinal diol function were unstable above 0 °C. Attempts to induce their direct coupling (at lower temperatures) invariably resulted in elimination and aromatization, without formation of detectable levels of dimeric products. Additionally, conventional oxidants, such as copper chloride[9a] or ceric ammonium nitrate (CAN),[10] led to rapid aromatization of the enoxysilane **20**, even at −35 °C. An extensive evaluation of alternative oxidants ultimately led to the identification of manganese tris(hexafluoroacetylacetonate) (**21**) as uniquely effective in this bond construction. The synthesis of **21** proceeds in one step from commercial reagents,[16] and the volatility of the complex allows purification of multigram quantities by sublimation (60–70 °C, 200 mTorr). The application of **21** in the oxidative coupling of enoxysilanes has not been described, to our knowledge, although Mayer has conducted detailed studies of its reactivity toward activated C–H bonds.[16b] The success of **21** in our studies may be attributed to two factors. First, because the manganese atom in **21** is coordinatively saturated, it cannot behave as a Lewis acid toward the β-oxygen of **20**. Second, in contrast to copper chloride or CAN, the manganese complex **21** is fully soluble (and stable) in benzene. Use of this solvent in the oxidative coupling reaction may attenuate competing elimination pathways.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

Financial support from Yale University, the National Science Foundation (Graduate Research Fellowship to C.M.W.), Eli Lilly, and the Searle Scholars Program is gratefully acknowledged. We thank Dr. Haiyin He (Pfizer Pharmaceuticals) and Prof. Andrew J. Phillips (Yale University) for helpful discussions. We thank Patrick Lynch for design of the cover art.

References

1. He H, Ding WD, Bernan VS, Richardson AD, Ireland CM, Greenstein M, Ellestad GA, Carter GT. J Am Chem Soc. 2001; 123:5362. [PubMed: 11457405]
2. (a) Ito S, Matsuya T, Omura S, Otani M, Nakagawa A. J Antibiot. 1970; 23:315. [PubMed: 5458310] (b) Hata T, Omura S, Iwai Y, Nakagawa A, Otani M. J Antibiot. 1971; 24:353. [PubMed: 5091211] (c) Omura S, Nakagawa A, Yamada H, Hata T, Furusaki A, Watanabe T. Chem Pharm Bull. 1971; 19:2428.(d) Omura S, Nakagawa A, Yamada H, Hata T, Furusaki A. Chem Pharm Bull.

1973; 21:931. [PubMed: 4727361] (e) Seaton PJ, Gould SJ. J Antibiot. 1989; 42:189. [PubMed: 2925510] For a review of kinamycin biosynthesis, see:(f) Gould SJ. Chem Rev. 1997; 97:2499. [PubMed: 11851467]

3. DeBoer, TJ., Backer, HJ. Organic Syntheses. Vol. 36. Wiley; New York: 1956. p. 16
4. (a) Laufer RS, Dmitrienko GI. J Am Chem Soc. 2002; 124:1854. [PubMed: 11866589] (b) Zeng W, Ballard TE, Tkachenko AG, Burns VA, Feldheim DL, Melander C. Bioorg Med Chem Lett. 2006; 16:5148. [PubMed: 16870443] (c) Feldman KS, Eastman KJ. J Am Chem Soc. 2006; 128:12562. [PubMed: 16984207] (d) Khdour O, Skibo EB. Org Biomol Chem. 2009; 7:2140. [PubMed: 19421453]
5. For studies toward the syntheses of 1 and 2, see:(a) Nicolaou KC, Denton RM, Lenzen A, Edmonds DJ, Li A, Milburn RR, Harrison ST. Angew Chem Int Ed. 2006; 45:2076.(b) Krygowski ES, Murphy-Benenato K, Shair MD. Angew Chem Int Ed. 2008; 47:1680.(c) Zhang W, Baranczak A, Sulikowski GA. Org Lett. 2008; 10:1939. [PubMed: 18410121] (d) Gholap SL, Woo CM, Ravikumar PC, Herzon SB. Org Lett. 2009; 11:4322. [PubMed: 19719089] (e) Nicolaou KC, Nold AL, Li H. Angew Chem Int Ed. 2009; 48:5860.(f) Morris WJ, Shair MD. Tetrahedron Lett. 2010; 51:4310. [PubMed: 20802782] (g) Lee HG, Ahn JY, Lee AS, Shair MD. Chem–Eur J. 2010; 16:13058. [PubMed: 20976820]
6. For syntheses of various kinamycins, see:(a) Lei X, Porco JA. J Am Chem Soc. 2006; 128:14790. [PubMed: 17105273] (b) Nicolaou KC, Li H, Nold AL, Pappo D, Lenzen A. J Am Chem Soc. 2007; 129:10356. [PubMed: 17676854] (c) Woo CM, Lu L, Gholap SL, Smith DR, Herzon SB. J Am Chem Soc. 2010; 132:2540. [PubMed: 20141138] For synthesis of (±)-O-methyl-kinamycin C, see: (d) Kumamoto T, Kitani Y, Tsuchiya H, Yamaguchi K, Seki H, Ishikawa T. Tetrahedron. 2007; 63:5189.For studies toward the synthesis of kinamycin F (6), see:(e) Chen N, Carriere MB, Laufer RS, Taylor NJ, Dmitrienko GI. Org Lett. 2008; 10:381. [PubMed: 18183994]
7. Wender PA, Verma VA, Paxton TJ, Pillow TH. Acc Chem Res. 2008; 41:40. [PubMed: 18159936]
8. Hawkins EGE, Large R. J Chem Soc, Perkin Trans. 1974; 1:280.
9. For selected examples, see:(a) Ito Y, Konoike T, Harada T, Saegusa T. J Am Chem Soc. 1977; 99:1487.(b) Baran PS, DeMartino MP. Angew Chem, Int Ed. 2006; 45:7083.(c) DeMartino MP, Chen K, Baran PS. J Am Chem Soc. 2008; 130:11546. [PubMed: 18680297]
10. For selected examples, see:(a) Baciocchi E, Casu A, Ruzziconi R. Tetrahedron Lett. 1989; 30:3707. (b) Avetta CT, Konkol LC, Taylor CN, Dugan KC, Stern CL, Thomson RJ. Org Lett. 2008; 10:5621. [PubMed: 19053737]
11. See Supporting Information.
12. Kolb HC, VanNieuwenhze MS, Sharpless KB. Chem Rev. 1994; 94:2483.
13. (a) Ito Y, Hirao T, Saegusa T. J Org Chem. 1978; 43:1011.(b) Larock RC, Hightower TR, Kraus GA, Hahn P, Zheng D. Tetrahedron Lett. 1995; 36:2423.
14. Huot R, Brassard P. Can J Chem. 1974; 52:838.
15. This reaction proceeds in 75% yield, as determined by ^{1}H NMR analysis of the unpurified reaction mixture against an internal standard. However, under all purification conditions that we have examined, the products 18 and 19 partially decompose. [Under optimized conditions, elution of analytically pure samples of 18 (or 19) resulted in 70% recovery.]
16. (a) Evans S, Hamnett A, Orchard AF, Lloyd DR. Faraday Discuss Chem Soc. 1972; 54:227.(b) Bryant JR, Taves JE, Mayer JM. Inorg Chem. 2002; 41:2769. [PubMed: 12005502]
17. The ratio of 22 and 23 varied somewhat between experiments but was consistently within the range 1.5–3:1. For example, in a separate 100 mg scale experiment, 22, 23, and 18 were obtained in 33%, 23%, and 15% yield, respectively.
18. Deprotection of the (1*S*,1′*S*)-dimer derived from 19 (TFA, CH_3OH) provided 24 in 38% yield; see Supporting Information.
19. Myers AG, Fundy MAM, Lindstrom PA. Tetrahedron Lett. 1988; 29:5609.

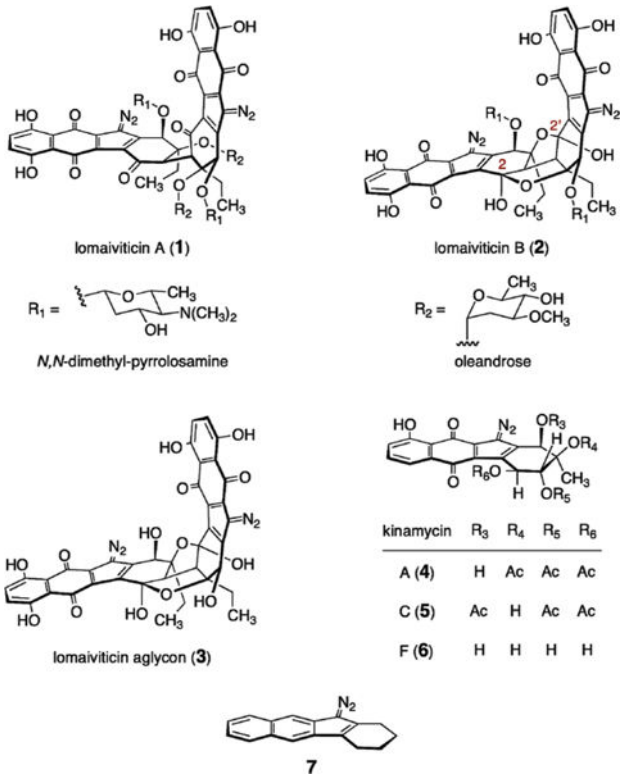
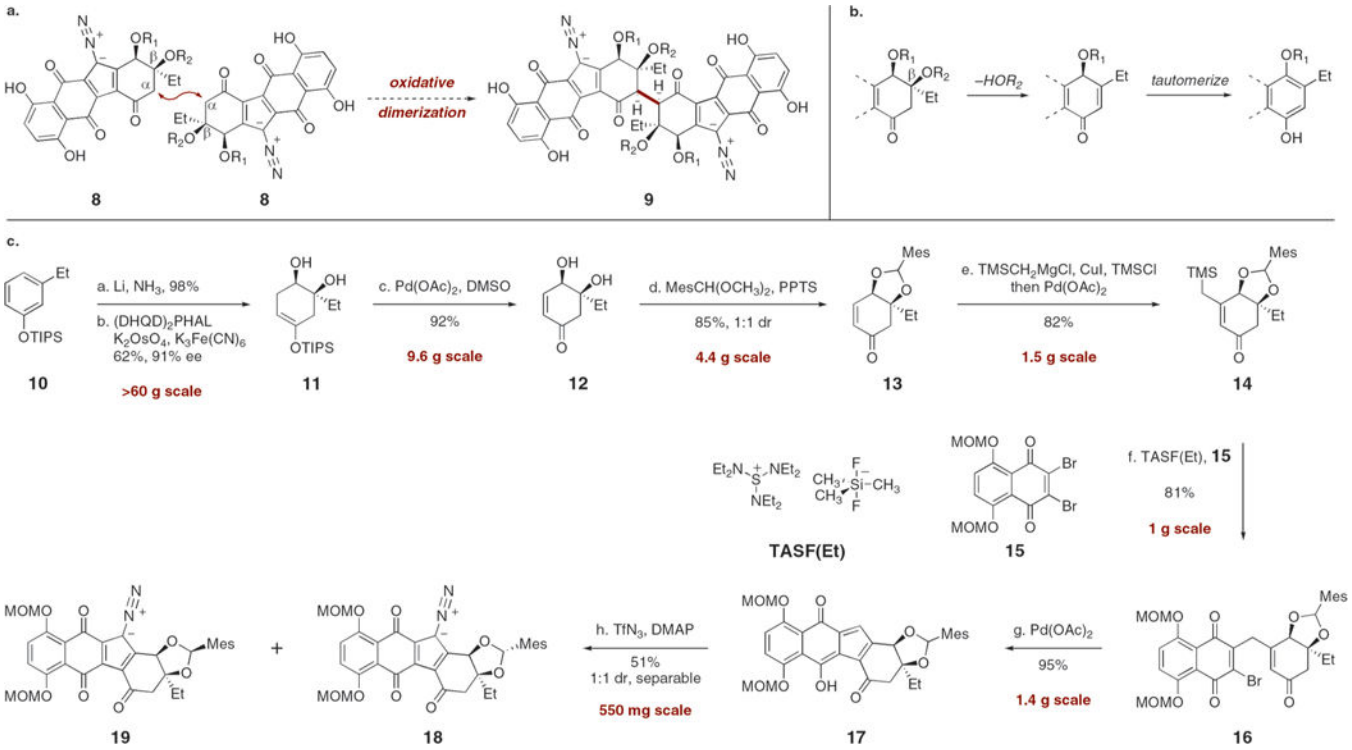


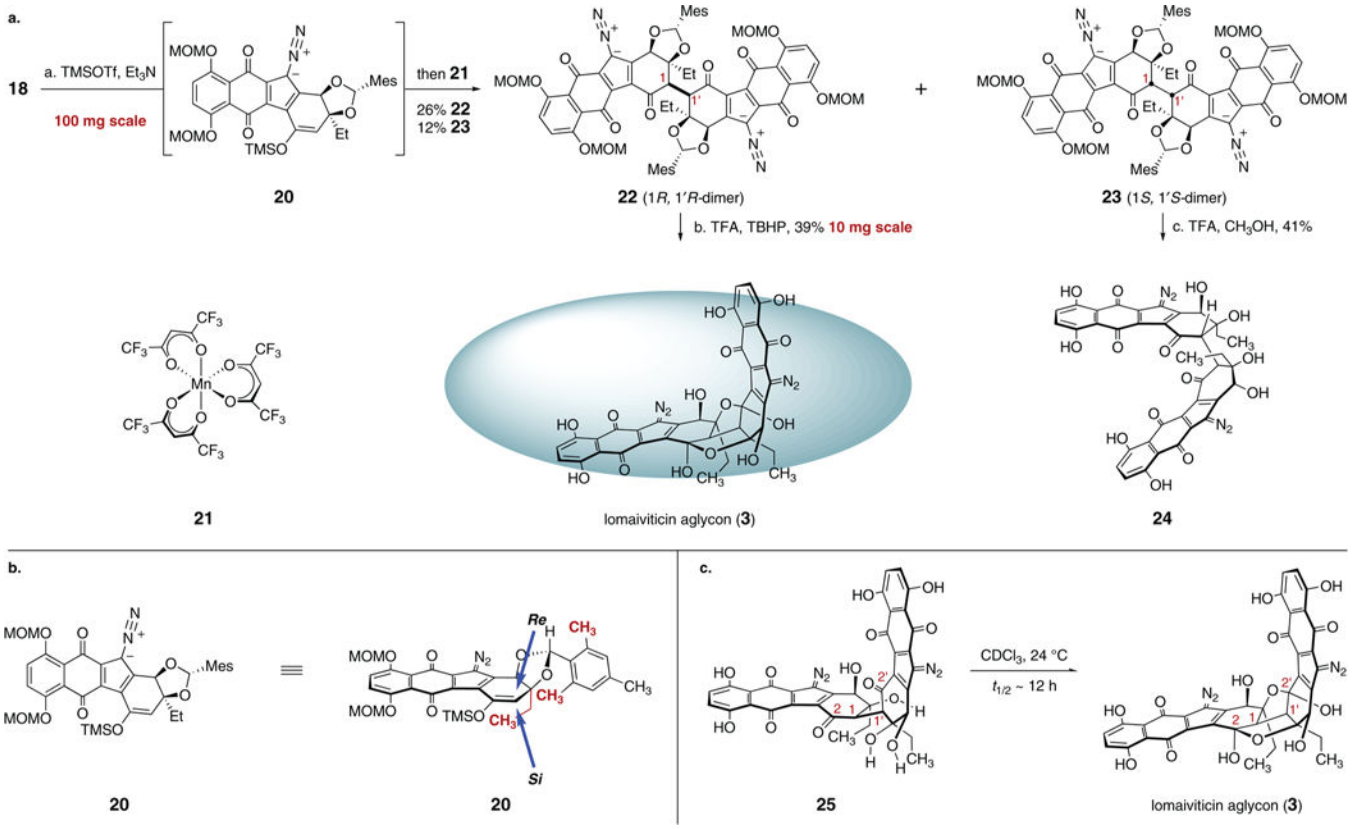
Figure 1.

Structures of lomaiviticins A and B (**1** and **2**), lomaiviticin aglycon (**3**), kinamycins A, C, and F (**4**–**6**), and diazotetrahydrobenzo-[*b*]fluorene (diazofluorene, **7**).



Scheme 1. (a) Proposed Oxidative Dimerization To Form Lomaiviticins A and B (1 and 2), (b) Elimination–Aromatization Pathway, and (c) Syntheses of the Diazofluorenes 18 and 19[a]

[a]Conditions: (a) Li, NH_3, *t*-BuOH, THF, −78 → −33 → −78 °C, 98%. (b) $(DHQD)_2PHAL$, K_2OsO_4, $CH_3SO_2NH_2$, *t*-$BuOCH_3$–*t*-BuOH-H_2O, −5 °C, 62%, 91% ee. (c) $Pd(OAc)_2$, O_2, DMSO, 24 °C, 92%. (d) $MesCH(OCH_3)_2$, PPTS, CH_3CN, 24 → 50 °C, 85%. (e) $TMSCH_2MgCl$, CuI, HMPA, Et_3N, TMSCl, THF, −30 → −60 → −78 °C; $Pd(OAc)_2$, CH_3CN, 24 °C, 82%. (f) TASF(Et), CH_2Cl_2, −78 °C, 81%. (g) $Pd(OAc)_2$, polymer-supported PPh_3, Ag_2CO_3, toluene, 80 °C, 95%. (h) TfN_3, DMAP, CH_3CN, −20 °C, 51%.



Scheme 2. (a) Dimerization of the *exo*-Mesityl Diazofluorene 18,[a] (b) Stereochemical Model for the Dimerization Event, and (c) Cyclization of the Ketone Isomer (25) of Lomaiviticin Aglycon (3)

[a]Conditions: (a) TMSOTf, Et_3N, CH_2Cl_2, 0 °C, then **21**, PhH, 21 °C, 26% **22**, 12% **23**, 30% **18**. (b) TFA, TBHP, CH_2Cl_2, −35 °C, 12 h, 39%. (c) TFA, CH_3OH, 50 °C, 41%.