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Mccreamycins A-D, Geldanamycin-Derived Cyclopentenone Macrolactams from an Eastern Kentucky Abandoned Coal Mine Microbe

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Mccreamycins A–D, Geldanamycin-Derived Cyclopentenone Macrolactams from an Eastern Kentucky Abandoned Coal Mine Microbe

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Conflict of interest

J.S.T. is a co-founder of Centrose (Madison, WI).

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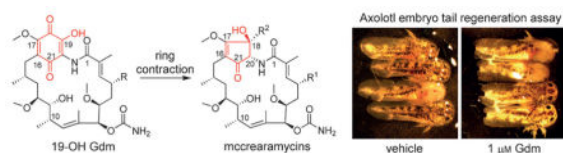
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Abstract

Four cyclopentenone-containing ansamycin polyketides (mccreamycins A–D), and six new geldanamycins (Gdms B–G, including new linear and mycothiol conjugates), were characterized as metabolites of *Streptomyces* sp. AD-23-14 isolated from the Rock Creek underground coal mine acid drainage site. Biomimetic chemical conversion studies using both simple synthetic models and Gdm D confirmed that the mccreamycin cyclopentenone derives from benzylic acid rearrangement of 19-hydroxy Gdm, and thereby provides a new synthetic derivatization strategy and implicates a potential unique biocatalyst in mccreamycin cyclopentenone formation. In addition to standard Hsp90 α binding and cell line cytotoxicity assays, this study also highlights the first assessment of Hsp90 α modulators in a new axolotl embryo tail regeneration (ETR) assay as a potential new whole animal assay for Hsp90 modulator discovery.

Mining for geldanamycins



Six new geldanamycins (Gdms) and four new ring-contracted cyclopentenone macrolactams are reported as metabolites of an abandoned Kentucky coal mine-associated microbe. A biosynthetic pathway via benzylic acid rearrangement is proposed and an axolotl embryo tail regeneration assay is utilized to assess the Hsp90 inhibitory activities of the metabolites.

Keywords

ansamycin; axolotl; biomimetic synthesis; Hsp90; regeneration

Geldanamycin (Gdm)-type polyketides are prototypical microbial benzoquinone ansamycin anticancer agents that target the N-terminal ATP-binding domain of heat shock protein 90 (Hsp90; Figure 1A).^[1] While a number of elegant and efficient Gdm synthetic and biosynthetic production and derivatization strategies have been developed,^[2] C-17 semi-synthetic Gdm modification was a key to both first (tanespimycin/17-AAG^[3] and orally bioavailable alvespimicin/17-DMAG^[4]) and second (retaspimycin hydrochloride/IPI-504)^[5] generation analogues advanced to the clinic (Figure 1A), the latter of which displayed improved solubility and reduced hepatotoxicity.^[6] More recent medicinal chemistry efforts have focused on C-19 substitution to prohibit non-specific alkylation (a putative contributor to non-selective toxicity), analogues of which were found to opportunistically favor the *cis*-amide conformer observed in the Gdm-Hsp90 ligand-bound complex.^[7] As part of a microbial natural products discovery effort from coal-mining-associated environments in Kentucky, USA,^[8] herein we describe the isolation and structure elucidation of six new Gdm analogues (**1–6**), and four unprecedented ring-contracted cyclopentenone macrolactams (mccrearamycins A–D, **7–10**) from the Rock Creek (McCreary County) underground coal mine acid drainage isolate *Streptomyces* sp. AD-23-14 (Figure 1B). Biomimetic studies using both simple synthetic models and isolated Gdm analogues revealed the *ortho*-quinone to undergo a facile benzilic acid rearrangement to provide the ring-contracted cyclopentenone scaffold, presenting both a new synthetic strategy and implicating the role of a potential novel biocatalyst for ansamycin ring contraction. In addition to expanding Hsp90 α inhibitor SAR, these studies also highlight the first assessment of Hsp90 α modulators in a new axolotl (*Ambystoma mexicanum*) embryo tail regeneration (ETR) assay.^[9]

Gdms B–G (**1–6**) were characterized as new Gdm analogues (including mycothiol conjugate **2** and linear Gdms **5–6**) based on NMR, MS, and comparison with literature precedent (see Figure 1 and the Supporting Information). While **7–10** also shared the signature spectral features of Gdm 19-membered macrolactams (Figures S2–S4), they notably lacked indicators of the corresponding Gdm 1,4-benzoquinone. Key HMBC correlations [for example, for **7**, from a nitrogen-bearing CH ($\delta_{\text{H}} = 5.12$ ppm, 20-H) to C-16 ($\delta_{\text{C}} = 116.2$ ppm), C-17 ($\delta_{\text{C}} = 177.0$ ppm) and C-18 ($\delta_{\text{C}} = 71.1$ ppm), and from 18-OH ($\delta_{\text{H}} = 6.16$ ppm) to C-17 and C-18] implicated an unprecedented alternative cyclopentenone ring (Figure 1B) in **7–10**. Determination of C-18 substitution (MSH in **7**; methyl formate in **8–10**) relied on HMBC correlations (Figures S2 and S3). The relative configurations of **7–10** were established through NOESY (Figures S3 and S4) where many observed modifications paralleled those of corresponding Gdm analogues. Namely, like **3** (Gdm D), hydration of the **8** C-4/C-5 double bond was observed, and similar to **6** (Gdm G), **9** and **10** were also identified as N-20-acyl (2-hydroxy-acetate) linear metabolites (Tables S1 and S4, Figures S2–S4). These cumulative analyses established **7–10** as new ring-contracted cyclopentenone macrolactams and thus were named mccrearamycins A–D in reference to the structural novelty and the producing strain's point of origin.

The similarities between Gdms and mccrearamycins from *Streptomyces* sp. AD-23-14 implicated Gdms as potential mccrearamycin progenitors (Scheme 1). In addition, while NOESY firmly established the cyclopentenone C-18/C-20 relative *trans*-configuration in **7**,

the key ^1H NMR resonance for 18-OH was lacking for **8–10**. For further validation, a model study was pursued to assess cyclopentenone formation via ring contraction of a 19-OH Gdm progenitor (Scheme 1) reminiscent of the classical cyclohexanone to cyclopentane-1-carboxylate benzilic acid rearrangement.^[10] While the corresponding Gdm rearrangement is unprecedented, the analogous Hooker oxidation rearrangement of hydroxynaphthoquinones to indane carboxylic acids served as related precedent.^[11] For this study, the synthesis of the Gdm model surrogate 2-hydroxyquinone **21** (Scheme 2) commenced with aryl lithiation–alkylation of benzyl methyl ether **12**. DDQ-mediated oxidation of **13** followed by hydroxy-directed iodination provided phenol **15**. The iodide was then treated with copper powder in basic medium to provide catechol **16**, which was selectively methylated by Me_2SO_4 . Methoxymethyl protection of the remaining phenolic hydroxyl followed by Baeyer–Villiger oxidation produced the key intermediate **18**. Consistent with challenges associated with hexasubstituted benzene syntheses,^[12] amination, amidation, and nitration of **18** directly, or of corresponding halogenated derivatives using transition-metal catalysts, failed to give desired aniline **19** or amide **20**. However, azo coupling with sulfanilic acid,^[13] followed by dithionite reduction, gave aniline **19** in 62% yield. Sequential acetylation, hydrolysis, oxidation, and deprotection furnished template **21** in 73% yield, and methylation of **21** further afforded the corresponding 2-methoxy quinone **22** as an additional comparator.

Consistent with the impact of CuCl_2 on benzilic acid rearrangement stereoselectivity and yield,^[14] evaluation of the putative **21** benzilic acid rearrangement in the presence of transition metal salts and various other known benzilic acid rearrangement promoters revealed CoCl_2 to afford the best overall yield and stereoselectivity (Table 1). Single-crystal X-ray diffraction of the isolated product **23** further established the relative C-2/C-3 *trans*-configuration (Table 1 and S7, CCDC 1496415), consistent with the signature **23** 2-OH to 3-CH NOE and corresponding 18-OH to 20-CH NOE of mcrearamycin A (**7**). A putative mechanism for Co^{2+} -assisted benzilic acid rearrangement is depicted in Scheme 1. Consistent with this mechanism, the substitution of CH_3OH with CD_3OD as solvent led to selective isotopic label incorporation in **25** (entry 18, Table 1). Importantly, the 2-methoxy model **22** and the prototypical Hooker reaction substrate lawsone failed to give the desired benzilic acid rearrangement under the optimized conditions (Scheme S1).

To probe the relevance to mcrearamycins, this biomimetic model study was subsequently extended to the corresponding 19-hydroxy-substituted Gdm D (**3**). Remarkably, reaction of **3** under the same optimized conditions led to 50% conversion to mcrearamycin B (**8**; entry 19, Table 1 and Figures S6–S8). The established stereoselectivity of the model reaction implicates an **8** cyclopentenone C-18/C-20 *trans*-configuration identical to that of **7** and **23**. Comparison of select ^{13}C NMR chemical shifts in mcrearamycins B–D (**8–10**) to that of the *trans*- and *cis*-configured models (**23** and **24**, respectively) provide further support of a common benzilic acid rearrangement-derived C-18/C-20 *trans*-configuration in all of the mcrearamycins (Table S5). Subsequent indirect mcrearamycin absolute configuration assignment was accomplished through electronic circular dichroism (ECD) analysis. Specifically, comparison of the ECD spectra of **8** in MeOH to the theoretical ECD spectra [generated using time-dependent density functional theory (TDDFT)]^[8a,15] for two possible

isomers of **8** (**8a**: 4*R*, 6*S*, 7*S*, 10*S*, 11*R*, 12*S*, 14*R*, 18*S*, 20*S* and **8b**: 4*R*, 6*S*, 7*S*, 10*S*, 11*R*, 12*S*, 14*R*, 18*R*, 20*R*), revealed that of **8a** as providing the best spectral match (Figure S9).

Based on the established mechanism of Gdm and related analogues, all of the isolated compounds were evaluated in standard Hsp90 α inhibition^[8b] and cancer cell line (human non-small cell lung A549) cytotoxicity assays (Table S7). This cumulative analysis revealed the parental prototypes (Gdm, reblastatin, and 17-*O*-demethyl-reblastatin) to afford greatest Hsp90 α inhibition (IC₅₀s of 5–30 nM) with notably divergent corresponding cytotoxicities (Gdm IC₅₀ 1 nM, reblastatin IC₅₀ 0.7 μ M, and 17-*O*-demethyl-reblastatin IC₅₀ > 50 μ M), suggesting the oxidation state and substitution pattern contribute to differences in cellular uptake and/or alternative cytotoxicity mechanisms consistent with prior Gdm SAR studies.^[7a,16] Similar to that of the parental prototypes, the corresponding cytotoxicity of the new *Streptomyces* sp. AD-23-14 metabolites did not correlate with Hsp90 α inhibitory potential in some cases.

Streptomyces sp. AD-23-14 metabolites were also evaluated using a highly regenerative salamander model, the Mexican axolotl (*Ambystoma mexicanum*).^[17] Previous transcriptional studies found *hsp90aa1* to be significantly upregulated 12 hours after axolotl limb and tail amputation, suggesting a role for Hsp90 in tissue regeneration.^[9] To investigate this further, we used the axolotl embryo tail regeneration (ETR) assay^[9b] to test Gdm for an inhibitory effect on tail regeneration. Tail-amputated axolotl embryos were incubated in microtiter plates in the absence (vehicle control, DMSO) or presence of 10 μ M agent (Gdm, reblastatin, 7-*O*-demethyl-reblastatin, and **1–10**) and imaged on day 1 (pre-treatment) and day 7. An initial single dose screen revealed Gdm to completely inhibit tail regeneration with no effect observed for all of the other test agents. Subsequent studies revealed a clear dose-response for Gdm, with developmental abnormalities and toxicity observed at the highest dose (10 μ M), inhibition of regeneration at intermediate doses, and no effect on regeneration at the lowest dose (0.1 μ M; Figure 2).

In summary, metabolic profiling led to the discovery of new Gdm analogues and a set of cyclopentenone macrolactams. The development and implementation of a cobalt-mediated benzilic acid rearrangement served as a key feature in mcrearamycin structure validation and highlights the potential synthetic utility in the context of 2-hydroxyquinone-containing complex natural products. That cyclopentenone formation requires distinct conditions may also implicate a unique biosynthetic pathway. These metabolites, together with the parental prototypes, also served as a test set to assess the impact of Hsp90 inhibitors in vivo using an axolotl ETR assay. While developmental abnormalities have been observed in many organisms (including zebrafish administered Gdm^[18]) when Hsp90 activity is reduced below critical levels,^[19] our results demonstrate that Gdm can be administered at a dose that blocks regeneration without overtly affecting development. This study implicates Gdm as a useful reagent to probe the role of Hsp90 in axolotl tail regeneration and suggests low dose Gdm could be used in a sensitized, ETR chemical genetic screen to identify new Hsp90 modulators.

Supplementary Material

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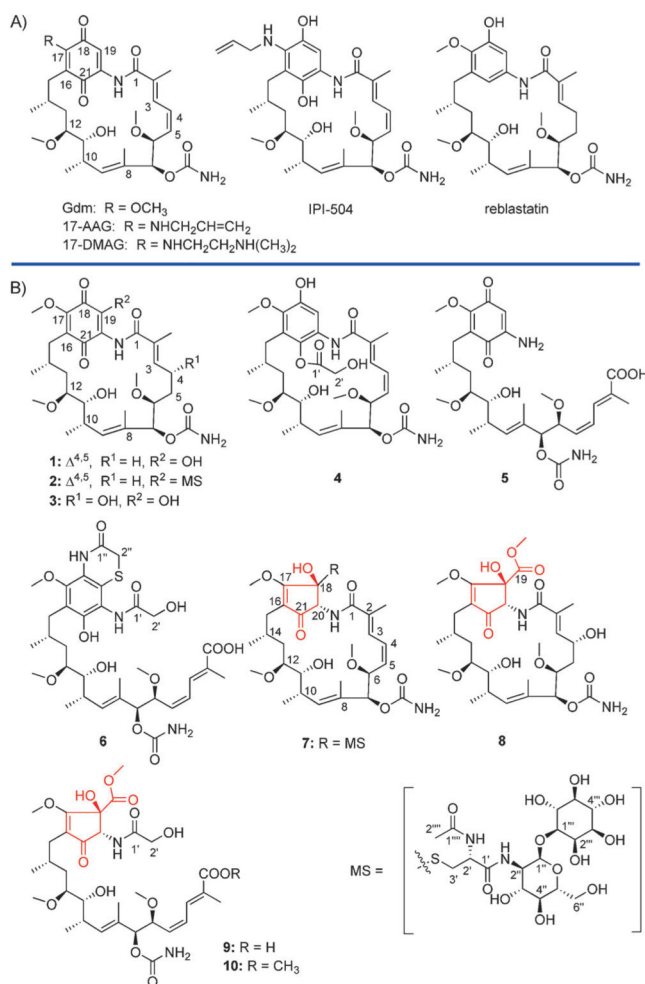
Acknowledgments

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**Figure 1.**

A) Chemical structures of representative Gdm-type ansamycins and B) new compounds isolated from *Streptomyces* sp. AD-23-14. The unique cyclopentenone ring structure of mcrearamycins A–D is highlighted in red.

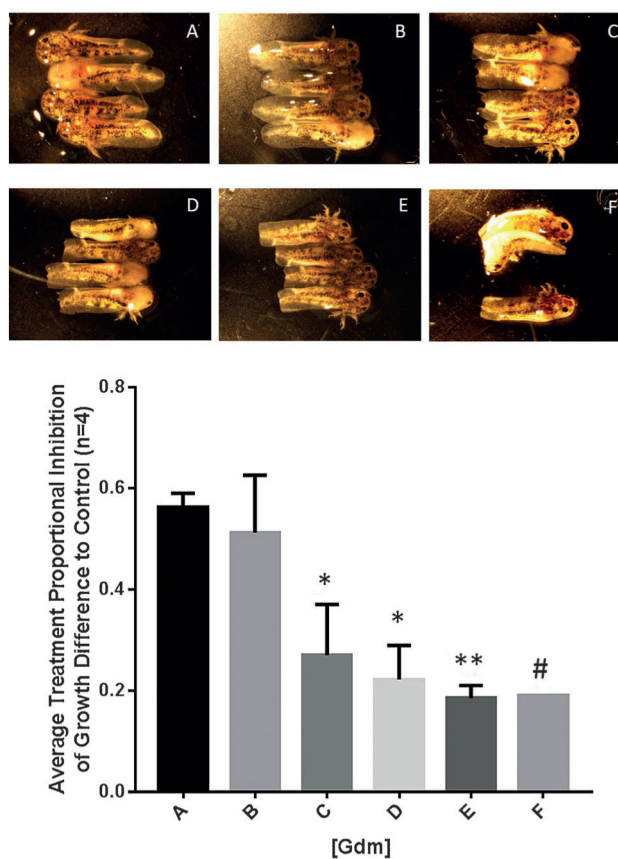
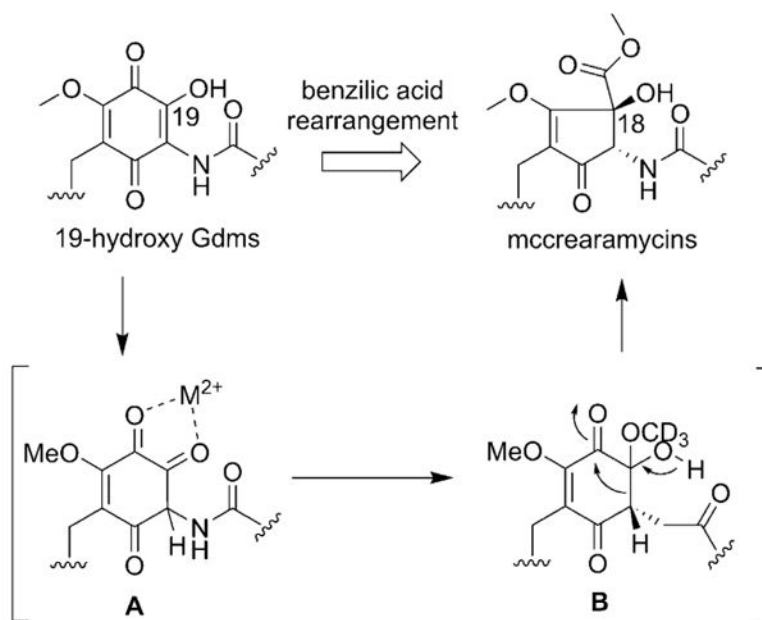
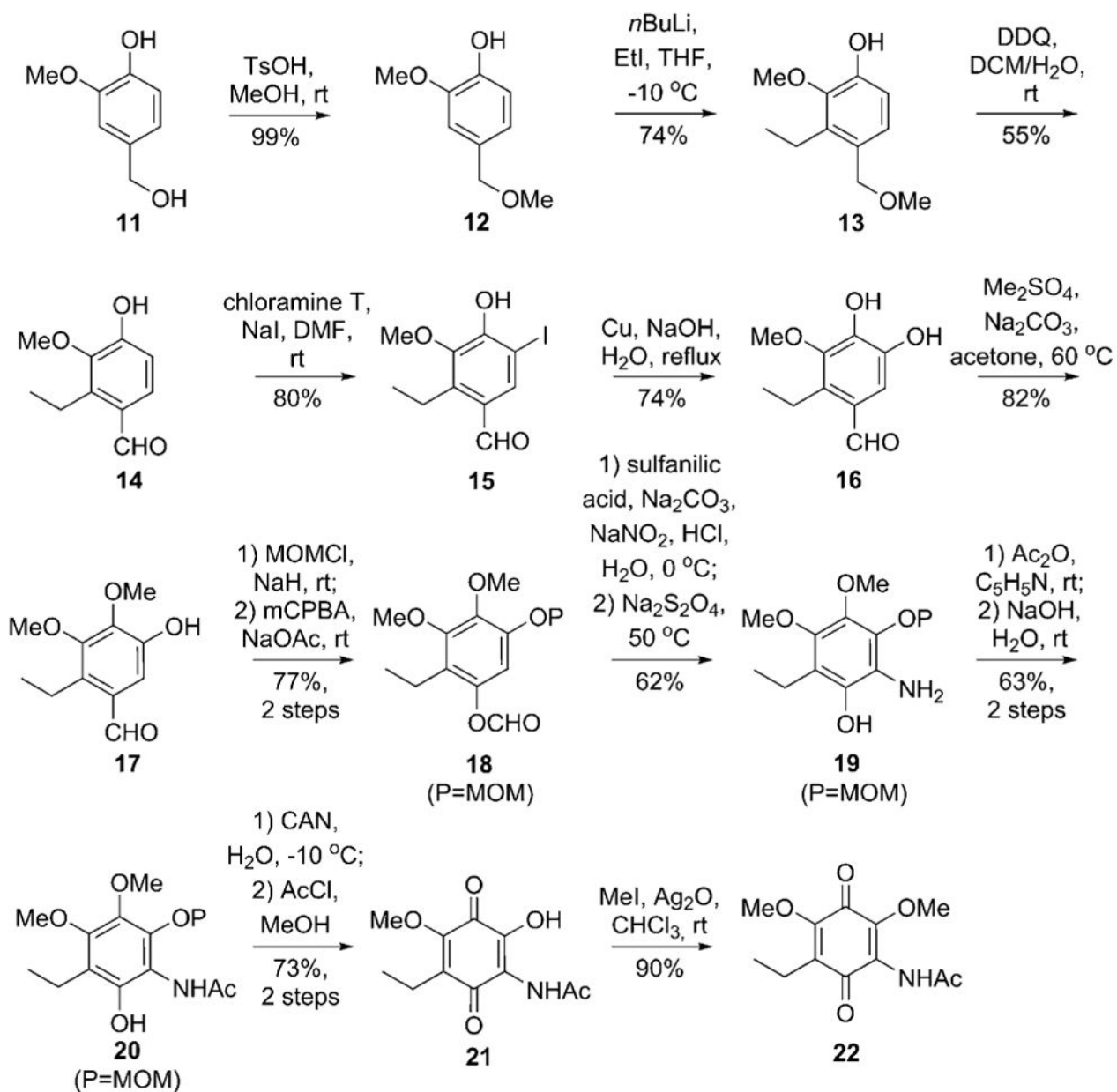


Figure 2. The impact of [Gdm] on axolotl embryo tail regeneration as determined by the ETR assay (* $p < 0.005$, ** $p < 0.0001$, $n = 4$; #: 3 axolotls were dead at day 7; A: DMSO control; B: 0.1 μM ; C: 1 μM ; D: 2.5 μM ; E: 5 μM ; F: 10 μM).

**Scheme 1.**

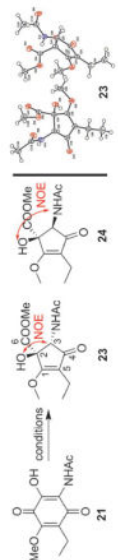
Proposed metal (M^{2+})-mediated benzilic acid rearrangement of the Gdm hydroxyquinone to afford the mcrearamycin cyclopentenone.



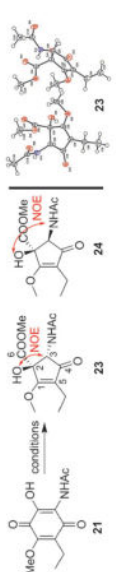
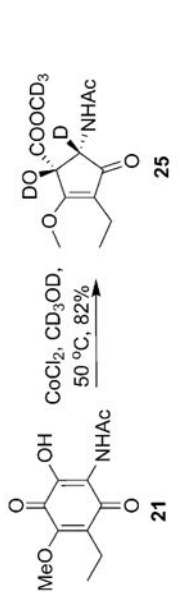
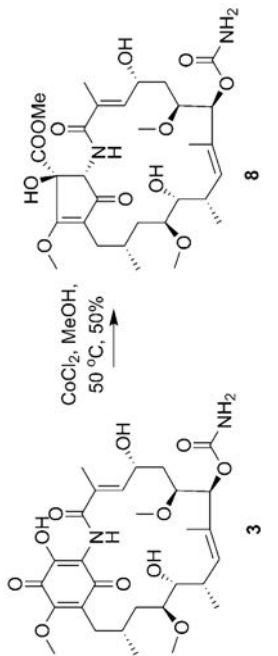
Scheme 2.
Synthesis of templates **21** and **22**.

Table 1

Optimization of benzylic acid rearrangement and biomimetic conversion of **3** to **8**.



Entry[a]	Additives	Temp. [°C]	Solvent	Time [h]	Ratio ^b (23/24)	Yield ^c of 23
1	none	50	MeOH/CH ₂ Cl ₂	16	—[d]	
2	KOBu	50	MeOH/CH ₂ Cl ₂	16	—[d]	
3	DBU	50	MeOH/CH ₂ Cl ₂	16	2:1	51%
4	TEA	50	MeOH/CH ₂ Cl ₂	16	5:1	60%
5	DABCO	50	MeOH/CH ₂ Cl ₂	16	1:1	
6	DIPEA	50	MeOH/CH ₂ Cl ₂	16	4:1	55%
7	CoCl ₂	50	MeOH/CH ₂ Cl ₂	16	>10:1	73%
8	NiCl ₂	50	MeOH/CH ₂ Cl ₂	16	—[e]	
9	CuCl ₂	50	MeOH/CH ₂ Cl ₂	16	—[e]	
10	AgOTf	50	MeOH/CH ₂ Cl ₂	16	1:5	39% ^[f]
11	Au(PPh ₃)Cl	50	MeOH/CH ₂ Cl ₂	16	—[d]	
12	Co(OAc) ₂	50	MeOH/CH ₂ Cl ₂	16	ND ^[g]	24%
13	Co(acac) ₂	50	MeOH/CH ₂ Cl ₂	16	ND ^[g]	15%
14	CoCl ₂	50	MeOH/CHCl ₃	16	ND ^[g]	29%
15	CoCl ₂	50	MeOH/DCE	16	>10:1	31%
16	CoCl ₂	50	MeOH	40	>10:1	82%
17	CoCl ₂	80	MeOH	16	>10:1	85%

Entry ^[a]	Additives	Temp. [°C]	Solvent	Time [h]	Ratio ^[b] (23/24)	Yield ^[c] of 23
18						
19		50 °C	CD ₃ OD		82%	
		50 °C	MeOH		50%	

^[a] Reactions contained substrate (0.05 mmol) and additives (0.1 mmol) in 0.5 mL solvent under specified conditions with product formation subsequently assessed by analytical HPLC and NMR (key NOE signatures are highlighted, see the Supporting Information for experimental details).

^[b] Based on analytical HPLC peak integration.

^[c] Yields of isolated products.

^[d] No reaction.

^[e] Undefined mixture.

^[f] Yield of isolated product **24**.

^[g] Not determined.