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Total Syntheses and Biological Reassessment of Lactimidomycin, Isomigrastatin and Congener Glutarimide Antibiotics

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Abstract: Lactimidomycin (**1**) was described in the literature as an exquisitely potent cell migration inhibitor. Encouraged by this claim, we developed a concise and scalable synthesis of this bipartite glutarimide-macrolide antibiotic, which relies on the power of ring-closing alkyne metathesis (RCAM) for the formation of the unusually strained 12-membered head group. Subsequent deliberate digression from the successful path to **1** also brought the sister compound isomigrastatin (**2**) as well as a series of non-natural analogues of these macrolides into reach. A careful biological re-evaluation of this compound collection showed **1** and progeny to be potently cytotoxic against a panel of cancer cell lines, even after one day of compound exposure; therefore any potentially specific effects on tumor cell migration were indistinguishable from the acute effect of cell death. No significant cell migration inhibition was observed at sub-toxic doses. Although these findings cannot be reconciled with some reports in the literature, they are in accord with the notion that lactimidomycin is primarily a ribosome-binder able to effectively halt protein biosynthesis at the translation stage.

Keywords: cell migration inhibition · cytotoxicity · metathesis · natural products · total synthesis

Introduction

Research into a small class of bipartite compounds of the glutarimide-polyketide estate gained considerable momentum once the cell-migration inhibition properties of migrastatin (**3**) had been recognized.[1–4] Since this mode of action might ultimately translate into medication to prevent cancer metastasis, clinically viable cell migration inhibitors could form a second line of defense against cancer, complementing conventional chemotherapy. The lead qualities of migrastatin also transpire from the fact that truncated analogues such as **6**–**10** with considerable structural remissions exhibit even better biological profiles (Scheme 1).[5,6] Most notably, compounds **9** and **10** show greatly improved metabolic stability and were found effective in suppressing the metastatic spreading of even highly invasive tumors in animal models.[7]

Independent biochemical and biophysical studies have identified fascin as the primary target of migrastatin and congeners.[8,9] This particular protein plays a pivotal role in regulating the assembly of the actin cytoskeleton and hence in cell motility and active cell movement.[10] An X-ray structure of fascin harboring compound **8** has been published[8] but was later withdrawn,[11] because of a serious discrepancy between the structure of the free and the bound ligand.[12] In any case, overexpression of fascin in malignant tumors is often correlated with poor prognosis.

Migrastatin (**3**) actually turned out to be a shunt metabolite of isomigrastatin (**2**),[2] which constitutes the genuine natural product formed by the producing *Streptomyces* strains.[13] Compound **2** transforms into **3** by a strain-relief-driven [3,3]-sigmatropic rearrangement that does not need any enzyme catalysis.[13,14] Hydrolysis (and partial isomerization) of **2** or **3** leads to the dorrigocins **5** and **4**, respectively,[13] which exhibit modest antifungal activity but do not interfere with cell migration inhibition to any appreciable extent.[2,15,16] Isomigrastatin, in turn, is very closely related to lactimidomycin (**1**) that had already been isolated from *S. amphibiosporus* ATCC-53964 prior to the discovery of the migrastatin family. **1** was originally described as a potently cytotoxic compound that exhibits promising selectivity for malignant cells over healthy tissue and shows even an appreciable antitumor activity in vivo.[17,18] In recognition of its obvious structural homology to **2** and **3**, however, lactimidomycin (**1**) was later also tested for cell migration inhibition and claimed to be "extremely potent".[19] Actually, it was de-

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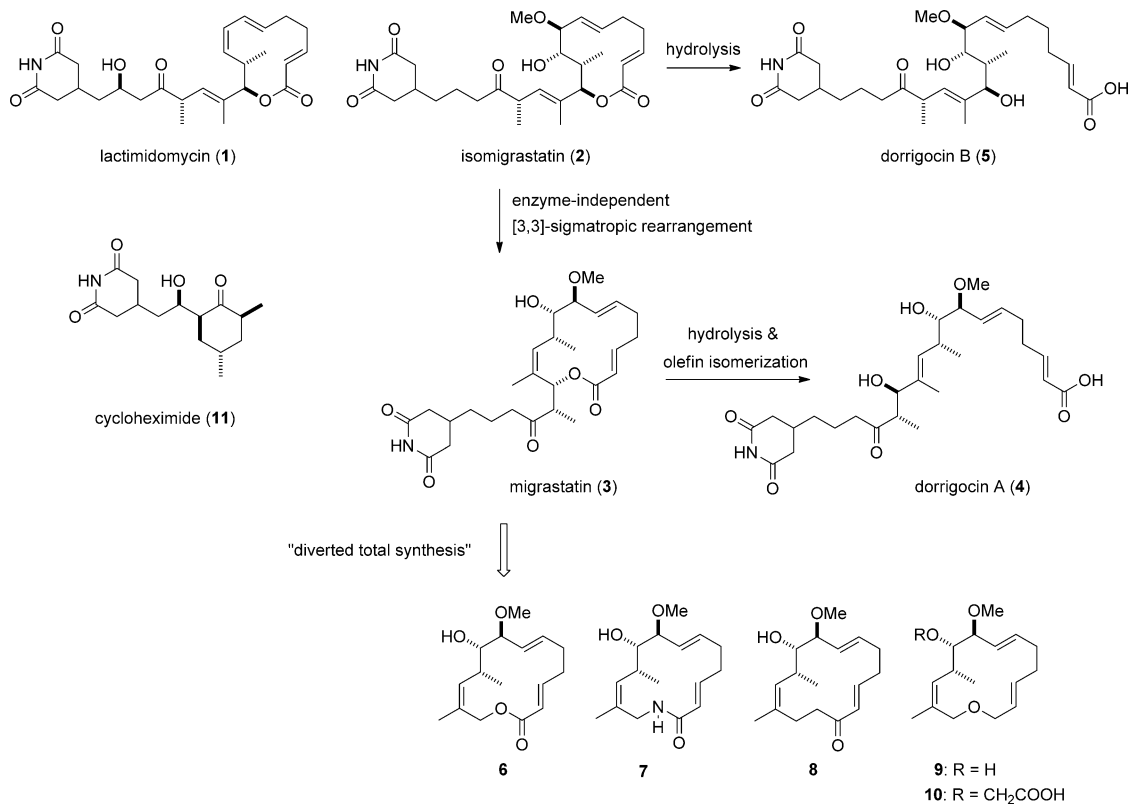


Scheme 1. Lactimidomycin, cycloheximide and the isomigrastatin/migrastatin pedigree.

scribed as the most effective agent of the entire series,[20] with an IC_{50} as low as 0.6 nM determined by a scratch-wound healing (SWH) assay using the highly invasive MDA-MB-231 human mammary adenocarcinoma cell line.[19] This remarkable level of activity clearly surpasses that of migrastatin (**3**) by more than one order of magnitude. Equally relevant is the claim that the cytotoxicity of **1**—though impressive per se—sets in only at concentrations well above those necessary for cell migration inhibition.[19]

A closer look at the available information, however, suggests that this claim requires scrutiny because it is difficult to accord with other biochemical data. Whereas the 14-membered lactone derivative migrastatin (**3**) binds to fascin as the main target,[8] convincing evidence has been published that its 12-membered relatives **1** and **2** primarily affect the ribosome.[21] Specifically, they were shown to inhibit protein biosynthesis by an interesting mechanism that effectively halts the translation of an RNA message at the first elongation step. This mode of action is similar to that of cycloheximide (**11**), a long known (but less potent) translation inhibitor with a long history as a laboratory research tool for the chemical interrogation of the eukaryotic ribosome.[22,23] While blockage of the ribosome allows the cytotoxicity of **1** and progeny to be rationalized with ease, effective inhibition of cell migration is much less obvious to explain by this primary mode of action and could hardly be an independent effect.[24,25]

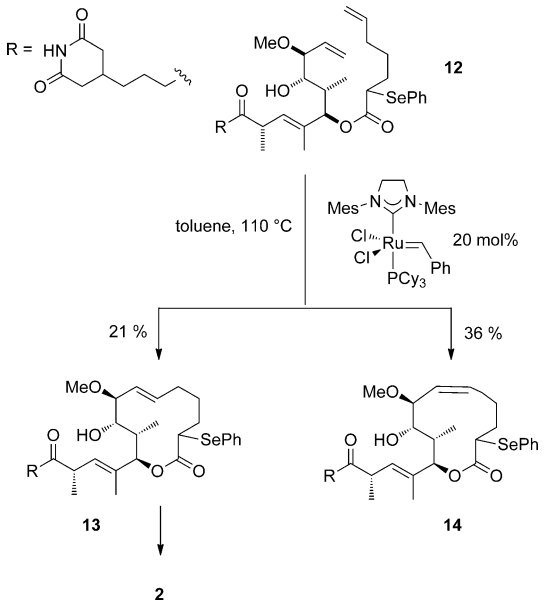
These somewhat ambiguous and, in part, even contradictory literature reports form the background against which our own investigations in this field have to be seen. The following major objectives were pursued by our group: first and foremost, an independent assessment of the bioactivities of lactimidomycin (**1**) as the presumably most potent agent of the entire series was intended, both with regard to its cytotoxicity as well as to the cell migration inhibition properties. To this end, a productive route to this quite challenging target had to be developed, which had never been conquered by total synthesis prior to our work. Ideally, the approach should be chemically innovative, programmed for a meaningful material supply,[26,27] and sufficiently flexible to give access to analogues with deep-seated structural mutations within their frame. Given the success of the simplified migrastatin offsprings **6–10**, this should include truncated variants devoid of the glutarimide tail, which cannot be easily obtained by chemical degradation of **1**. The results of our investigations along these lines are summarized below.

Results and Discussion

Prior art and strategic considerations: Migrastatin (**3**) had previously succumbed to total synthesis and subsequent molecular editing exercises;[3,5,6] yet, it seemed prudent to expect that lactimidomycin (**1**) poses larger problems. As

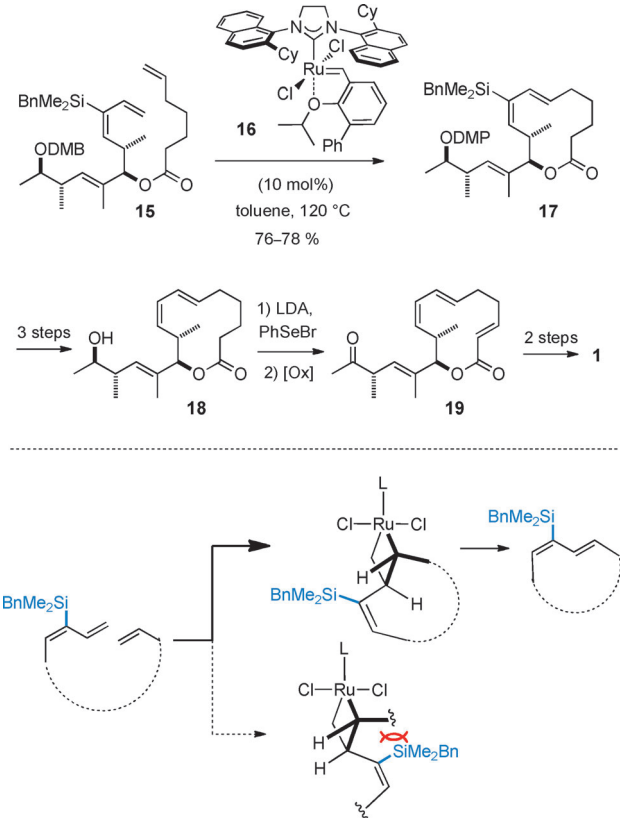
the 12-membered macrolide of **1** contains no less than seven sp^2-hybridized C-atoms, ring strain becomes a serious issue: it may make the closure of the macrocycle challenging and, at the same time, render the once-formed lactone ring labile towards solvolysis and/or strain-driven rearrangements.[28]

The only successful conquest of its sister compound isomigrastatin (**2**) known at the outset of our project unmistakably faced such problems (Scheme 2).[29,30] In a smart response, the Danishefsky group delayed the formation of both *E*-configured alkenes to the latest possible stages of the synthesis.[29] The first olefin was installed while forging the macrocyclic edifice by ring-closing alkene metathesis (RCM),[31] while the enoate moiety was procured in the subsequent step by an oxidative deselenation.[29,32] Still, the macrocyclization required very harsh conditions and a high catalyst loading but furnished the desired (*E*)-alkene **13** in only 21% yield (together with 36% of the undesired (*Z*)-isomer **14**). Diene **12** obviously constitutes a borderline case for contemporary RCM.[31,33]



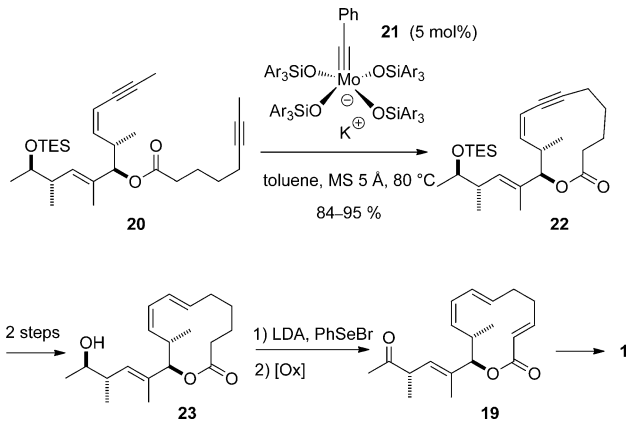
Scheme 2. The only known synthesis of isomigrastatin (**2**) reported by Danishefsky and co-workers (ref. [29]) provides valuable intelligence for the projected approach to the even more strained cousin lactimidomycin (**1**).

In view of these difficulties, it appeared prudent to us to consider more than one strategy in pursuit of lactimidomycin (**1**), which is arguably even more strained. One of our entries entailed the development of a conceptually novel, stereoselective RCM-based synthesis of conjugated dienes (Scheme 3):[34] specifically, exposure of compound **15** to complex **16** (10 mol%) furnished the macrocyclic product **17** in respectable yield as a single isomer. This favorable outcome is ascribed to the multitasking silyl residue, which protects the inner double bond in the substrate against attack by the catalyst, favors the cyclization-friendly s-*cis* configuration of the diene part, and serves as a stereodirecting substituent that ensures the selective formation of the required *E*-cycloalkene group.[34] After protodesilylation of **17**, the yet missing enoate was installed by selenation/oxidation in analogy to Danishefsky's lead from the isomigrastatin series.[29]

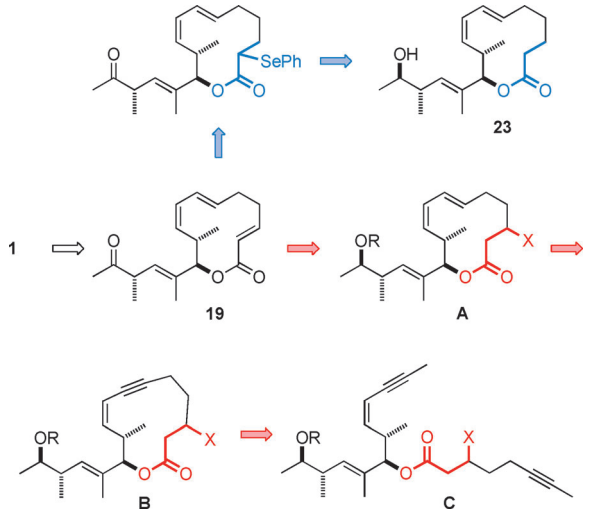


Scheme 3. Top: total synthesis of lactimidomycin (**1**) based on a novel RCM-based 1,3-diene synthesis and late-stage selenation/oxidative deselenation chemistry; bottom: rationale for the *E*-selective course of the RCM-based diene synthesis, cf. ref. [34]; Cy = cyclohexyl; DMB = 3,4-dimethoxybenzyl.

A second approach was equally successful, which relied on ring-closing alkyne metathesis (RCAM) as the key step (Scheme 4).[35] Although counterintuitive at first sight, we chose to temporarily increase (rather than decrease) the ring strain by formal replacement of the C6–C7 *E*-alkene by an alkyne unit. Trusting in the power of RCAM,[36–38] it was hoped that the enthalpic penalty would not be prohibitive as long as the enoate double bond is not in place. In fact, treatment of diyne **20** with catalytic amounts of the molybdenum alkylidyne complex **21**[39,40] afforded the desired product **22** in excellent yield. A ruthenium catalyzed *trans*-hydrosilylation/protodesilyation sequence[41,42] then gave **23** in readiness for installation of the enoate by the selenium chemistry alluded to above. At this stage, the RCM- and the RCAM-based routes converged and gave us first crops of the target compound lactimidomycin (**1**).[34,35]



Scheme 4. Original RCAM-based approach to lactimidomycin (**1**) (ref. [35]), which converges with the route shown in Scheme 3 at the stage of compound **19**.



Scheme 5. Comparison of the original (blue) and the refined retrosynthetic analysis (red), which is expected to allow for higher material throughput.

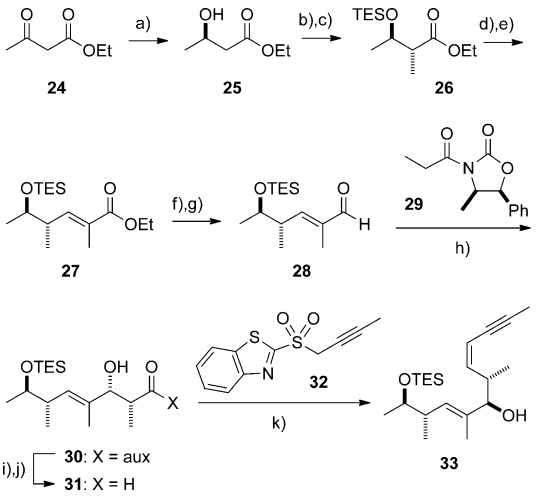
Scalable "second-generation" total synthesis of lactimidomycin: Although these syntheses were gratifying in methodological terms, they were not ideal for a larger material throughput, not least because of the late-stage selenium chemistry. This transformation mandates tedious purification in order to bring the selenium impurities to a tolerable level. Even more serious were sudden drops in yield when the selenation/oxidative deselenation sequence was performed on somewhat larger scales.

As a consequence, we planned to replace this chemistry altogether and to unveil the enoate in **19** in a more appropriate and benign manner. In so doing, however, one must make sure not to impose additional strain onto the projected cycloalkyne intermediate, otherwise RCAM is likely going to derail towards oligomerization. We conceived that this stringent condition could be met by elimination of an appropriate leaving group X located β to the ester linkage in a compound of type **A** (Scheme 5). This plan trusts in the chemoselectivity of the alkyne metathesis catalysts, which has to work efficiently with the polyfunctionalized cyclization precursor **C**, yet avoid any premature elimination of a sensitive aldol subunit in **B** and **C**. Although any effective alkyne metathesis catalyst must contain a high-valent and hence inherently Lewis acidic metal center as part of the operative metal-alkylidyne unit,[43] the acidity of complex **21** as a prototype member of the latest class of catalysts seems sufficiently tempered by the ancillary silanolate ligands;[39,40] in any case, this and related molybdenum alkylidynes display remarkable activity and an outstanding functional group tolerance at the same time,[44] and should therefore be able to meet the challenge. Provided that the projected RCAM (**C** → **B**) is successful, it appeared to us that the later elimination of the aldol subunit in **A** with formation of the signature enoate moiety could be favorably juxtaposed with the necessary protecting group manipulations to further shorten the longest linear sequence en route to lactimidomycin (**1**).

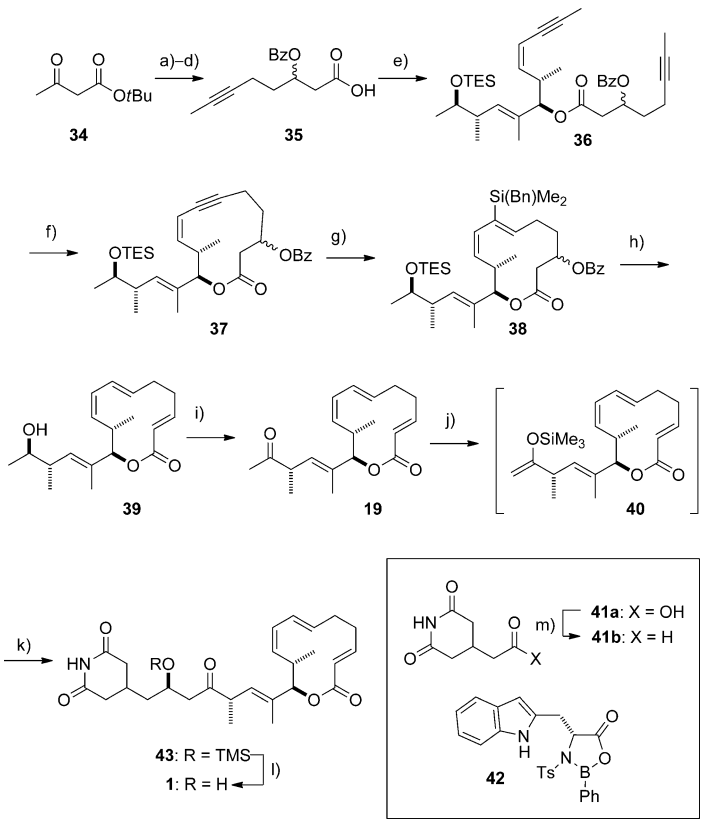
The preparation of the required alcohol segment **33** followed our previous route but was fully optimized for material throughput (Scheme 6). Specifically, an asymmetric hydrogenation of β-ketoester **24** furnished multigram quantities of the required aldol product **25** in excellent optical purity (98.8% *ee*).[45] The subsequent Fráter–Seebach alkylation could also be performed on a 10 gram scale.[46] In concord with a downstream Evans aldol reaction,[47] this robust route allowed the four chiral centers decorating this building block to be set with high selectivity.[48] Routine protecting group and oxidation state management led to aldehyde **31**, which was chain-extended by a Julia olefination with sulfone **32**[49] to give the required enyne motif. This transformation was also compliant to scale-up and furnished the *Z*-configured product **33** as the only detectable isomer, although the yield was somewhat variable and did not exceed 61% (over two steps).

Alcohol **33** was esterified under Yamaguchi conditions[50] with acid **35**, which derives from the commercial β-ketoester **34** as shown in Scheme 7. Note that **35** was used in racemic form since the chiral center is to be destroyed anyway in the projected elimination reaction. It was gratifying to note that the ring closure of the resulting substrate **36** by RCAM proceeded smoothly when catalyzed with the molybdenum alkylidyne ate-complex **21**,[39,40] furnishing the desired cycloalkyne **37** in 85% yield on a two gram scale (single largest batch). This product was subjected to a ruthenium-catalyzed *trans*-hydrosilylation[41,42] to give compound **38** that was treated with commercial TBAF in THF. This simple operation engendered a one-pot protodesilylation of the alkenylsilane,[51] deprotection of the TES ether and—most importantly—concomitant elimination of the benzoate group to give the fully functional macrolactone sector **39** of lactimidomycin. It is obvious that this sequence is considerably more practical, scalable and efficient than the previously chosen selenium chemistry.

The completion of the total synthesis followed our earlier work in that **39** was first oxidized to the corresponding ketone **19**, followed by formation of the respective silyl enol



Scheme 6. a) [((*R*)-Binap)$RuCl_2$] (0.2 mol%), H_2 (100 bar), EtOH, quant. (98.8% *ee*); b) LDA (2 equiv), THF/HMPA, MeI, −78 → 0°C; 86–94%; c) TESCl, pyridine, CH_2Cl_2, 91–98%; d) Dibal-H, CH_2Cl_2, −78°C; e) Ph_3P=C(Me)COOEt, THF, reflux, 86–92% (over two steps); f) Dibal-H, CH_2Cl_2, −78 → 0°C, 97%; g) PCC, CH_2Cl_2, MS 4 Å, 72–79%; h) **29**, Bu_2BOTf, Et_3N, CH_2Cl_2, −78°C → RT, 73–90%; i) MeNH(OMe)·HCl, Me_3Al, THF, −10°C, 81–83%; j) $LiAlH_4$, THF, −78 → 0°C; k) **32**, KHMDS, THF, −55°C, 47–61% (over two steps); Tf = trifluoromethylsulfonyl; PCC = pyridinium chlorochromate; MS = molecular sieves, TES = triethylsilyl; LDA = lithium diisopropylamide.



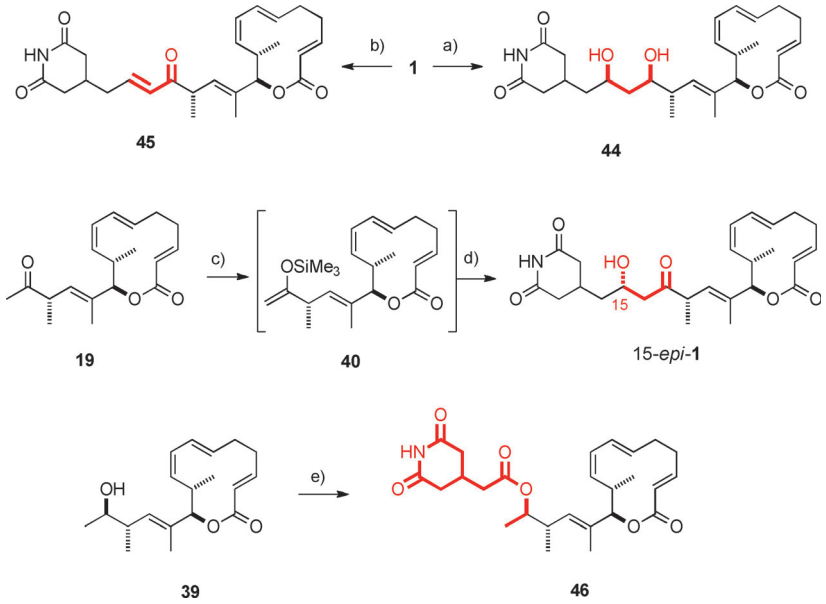
Scheme 7. "Second-generation" RCAM-based total synthesis of lactimidomycin: a) i) NaH, THF, 0°C; ii) *n*BuLi, 1-bromo-2-butyne, 0°C, 96%; b) $NaBH_4$, THF, 70%; c) benzoyl chloride, Et_3N, DMAP, CH_2Cl_2, 89%; d) TFA, CH_2Cl_2, quant.; e) 2,4,6-trichlorobenzoyl chloride, Et_3N, toluene, 0°C, then **33**, DMAP, 82%; f) **21** (5 mol%), MS 5 Å, toluene, 80°C, 85%; g) $[Cp^*Ru(MeCN)_3]PF_6$ (10 mol%), $BnMe_2SiH$, CH_2Cl_2, 0°C → RT; h) TBAF, THF, 0°C → RT, 73%; i) oxalyl chloride, DMSO, Et_3N, CH_2Cl_2, −78 → 0°C, 82%; j) TMSCl, Et_3N, THF, −78°C, then LiHMDS; k) **41b**, MS 4 Å, propionitrile, then **42**, −78°C; l) HF·pyridine, THF/pyridine, 0°C → RT, 50% (over three steps); m) i) HBTU, Et_3N, naphthalene-2-thiol, THF; ii) Pd/C cat., Et_3SiH, THF, 60% (over two steps); TFA = trifluoroacetic acid; DMAP = 4-dimethylaminopyridine; Cp* = pentamethylcyclopentadienyl; TMS = trimethylsilyl; LiHMDS = lithium hexamethyldisilazide; HBTU = *O*-(benzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate.

ether **40**. An asymmetric Mukaiyama aldol reaction[52] with the known aldehyde **41b**[53] under the aegis of the tryptophane-derived oxazaborolidinone **42**[54] gave product **43** as the only detectable isomer.[55] HF·pyridine in the presence of excess pyridine had to be used for the final deprotection, whereas more conventional fluoride sources led to substantial degradation. Like all other steps of the chosen route, this end game scaled well (50% overall yield from ketone **19**, 138 mg of **1** in the single largest batch). Suffice it to say that the spectral and analytical properties of synthetic lactimidomycin (**1**) thus formed were fully consistent with the data of our own earlier samples[34,35] and matched the data of the natural product reported in the literature.[17] Therefore we believe that the route presented above provides a sound basis for an in-depth evaluation of this precious natural product and/or for its use as a valuable research tool in chemical biology.

The first level of digression: Preparation of a collection of designer analogues: Whereas the derivatization of readily available natural products is standard practice in drug discovery, it is much less common to pursue the complementary approach, in which a de novo synthesis is turned into a "diverted total synthesis" campaign.[56–59] Deliberate digression from a given synthesis path, however, bears the chance of obtaining compounds with deep seated "structural point mutations" within their backbone that cannot be accessed by manipulation of the natural products themselves (at least not without undue efforts); the resulting analogues will also complement biosynthetic efforts of pathway engineering. This notion has already led to some remarkable success stories, including the development of a marketed drug compound.[60,61] The obvious requirement for any diverted total synthesis project is a robust and—at the same time—modular blueprint.

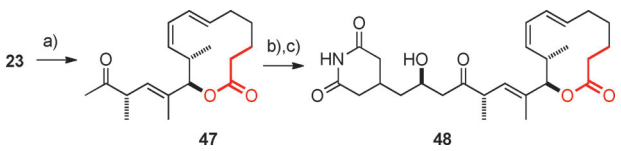
The access route to lactimidomycin outlined above seems to meet these basic criteria: it is reasonably short, has proven scalable, and contains several chemically feasible bifurcation points. Therefore we decided to prepare a small collection of analogues for screening purposes with an increasing level of chemical divergence from the natural lead, although not all steps were meticulously optimized during this campaign. In addition to the 1,3-*syn*-diol derivative **44** and the enone **45**, which are formed from **1** in a single step each, 15-*epi*-**1** was prepared by running the Mukaiyama aldol reaction of **19** and aldehyde **41b** with the enantiomeric oxazaborolidinone *ent*-**42** as the Lewis acid (Scheme 8). In-

terestingly, however, the new chiral center was set with a d.r. of only 85:15, showing that the chiral promoter is incapable of fully overriding the substrate bias. This outcome also implies that the excellent selectivity observed during the preparation of lactimidomycin (**1**) itself is the result of a matched case of double stereoinduction.[62] Yet another screening candidate is ester **46**, featuring a different connectivity between the macrolide portion and the glutarimide tail region.



Scheme 8. a) Catecholborane, THF, −5 °C, 58 %; b) methanesulfonyl chloride, Et_3N, CH_2Cl_2, 0 °C, 72 %; c) TMSCl, Et_3N, THF, then LiHMDS, −78 °C; d) i) **41b**, MS 4 Å, propionitrile, then *ent*-**42**, −78 °C; ii) HF·pyridine, THF/pyridine, 0 °C, 18 % (unoptimized, over three steps, d.r. 85:15); e) **41a**, EDCI, DMAP, THF, 55 % (brsm); EDCI = *N*′-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide.

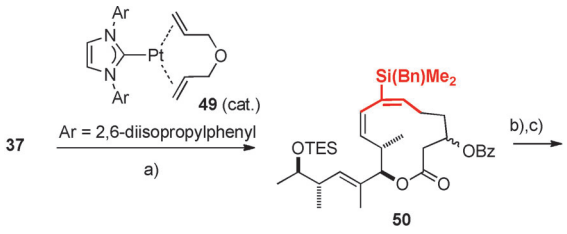
Whereas these analogues will allow the importance of the lateral aldol subunit for the biological activity to be probed,[19] it also seemed worthwhile to interrogate the role of the enoate function within the macrolactone ring, which might serve as a Michael acceptor for biological nucleophiles (Scheme 9).[16] To this end, it sufficed to subject ketone **47** to kinetic enolization/silylation, which in turn derives from the key intermediate **23** of our original approach to **1**.[35] The subsequent Mukaiyama aldol reaction of the resulting enol ether with aldehyde **41b** worked as expected, furnishing product **48** devoid of the enone motif.

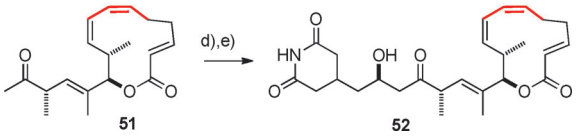


Scheme 9. a) Dess–Martin periodinane, CH_2Cl_2, 0 °C → RT, 89 %; b) TMSCl, Et_3N, THF, −78 °C, then LiHMDS; c) i) **41b**, MS 4 Å, propionitrile, then **42**, −78 °C; ii) HF·pyridine, THF/pyridine, 0 °C → RT, 31 % (unoptimized, over three steps).

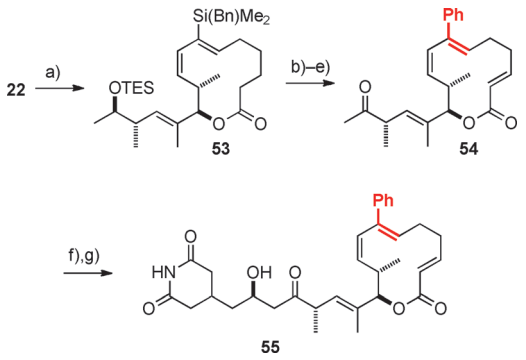
A more profound structural change is materialized in compound **52**, which is the 6*Z* isomer of the natural product (Scheme 10). All it took to reach this analogue was to change the catalyst in the hydrosilylation step from [Cp*Ru(MeCN)$_3$]PF_6 to the platinum complex **49**[63,64] and to pursue the established route from there on. In yet another variation, the silyl group in compound **53** was used to attach a substituent to the conjugated diene and hence drastically change the steric demand of this region. To this end, **53** was subjected to a Hiyama–Denmark coupling reaction[65] with iodobenzene; the resulting product **54** was elaborated into the substituted lactimidomycin analogue **55** in the customary manner (Scheme 11). The cytotoxicity and cell migration inhibition properties of these and related compounds are summarized in a later section of this paper.

The second stage of digression: Total synthesis of isomigrastatin and analogues: Having access to multigram amounts of cycloalkynes of type **A** (X = H, OBz), it was tempting to redirect the end game toward a total synthesis of the sister compound isomigrastatin (Scheme 12).[2] The preparation of different natural products from a single precursor is another incarnation of the concept of "diverted total synthesis". Moreover, the hybrid structure **56** was considered a lucrative target and a possible

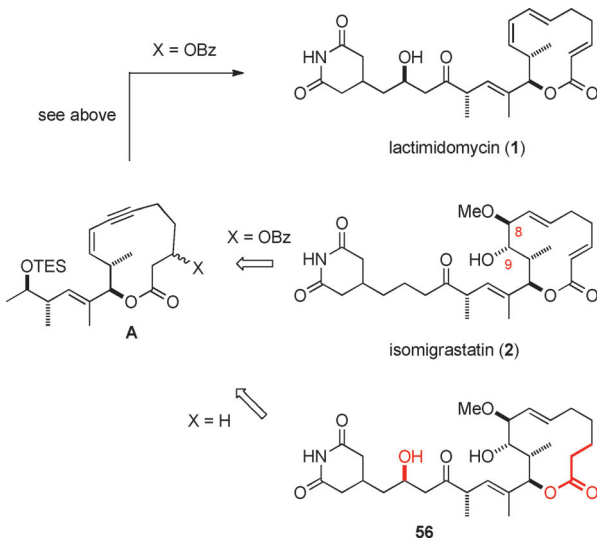




Scheme 10. a) $BnMe_2SiH$, **49** (5 mol %), THF, 60 °C; b) TBAF, THF, 82 % (over both steps); c) Dess–Martin periodinane, CH_2Cl_2, −78 → 0 °C, 77 %; d) TMSCl, Et_3N, THF, −78 °C, then LiHMDS; e) i) **41b**, MS 4 Å, propionitrile, then **42**, −78 °C; ii) HF·pyridine, THF/pyridine, 0 °C → RT, 69 % (over three steps).



Scheme 11. a) $[Cp^*Ru(MeCN)_3]PF_6$ (10 mol%), $BnMe_2SiH$, CH_2Cl_2, 0°C → RT; 82%; b) i) TBAF·3H_2O, aq. THF, 0°C; ii) PhI, $[Pd_2(dba)_3]\cdot CHCl_3$ (5 mol%), THF, 0°C → RT, 54%; c) i) LDA, THF, −78 → 0°C; ii) PhSeBr, −78 → 0°C; d) *m*CPBA, *i*PrNEt_2, CH_2Cl_2, −78°C → RT, 38% (over both steps); e) Dess–Martin periodinane, CH_2Cl_2, 74%; f) TMSCl, Et_3N, THF, then LiHMDS, −78°C; g) i) **41b**, MS 4 Å, propionitrile, then **42**, −78°C; ii) HF·pyridine, THF/pyridine, 0°C → RT, 65%; dba = dibenzylideneacetone; *m*CPBA = *meta*-chloroperbenzoic acid.

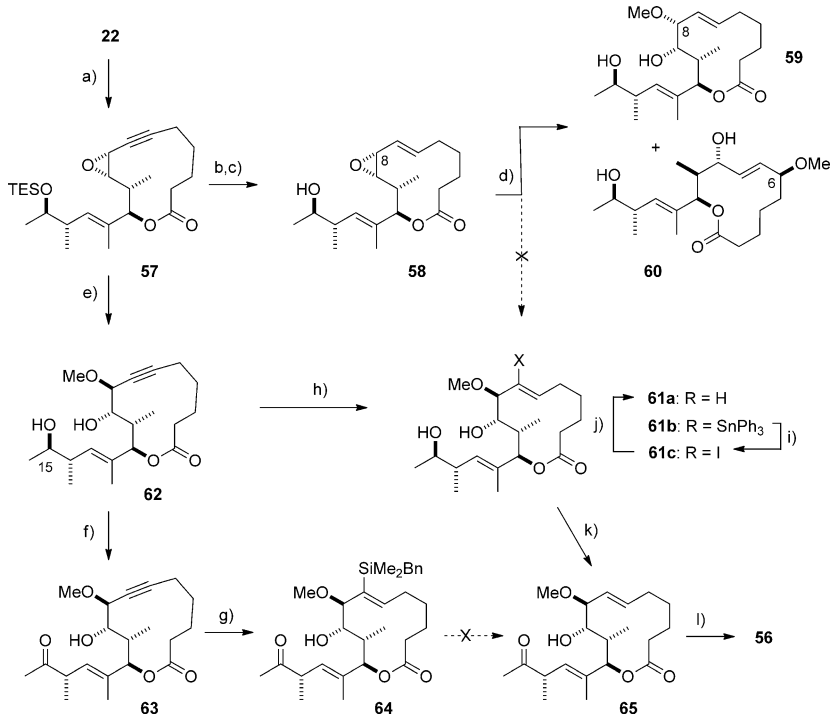


Scheme 12. Cycloalkynes of type **A** as a possible link between lactimidomycin (**1**), isomigrastatin (**2**) and the hybrid structure **56**.

calibration point for the projected biological evaluation, because it had previously been described as a very potent cell migration inhibitor as well.[19]

To set the additional chiral centers at C8 and C9 of isomigrastatin's head group, enyne **22** was subjected to a Jacobsen epoxidation[66] to give product **57** as the only detectable isomer (Scheme 13). A two-step *trans*-reduction of the alkyne unit via a ruthenium-catalyzed hydrosilylation followed by treatment with TBAF delivered diene **58** with concomitant cleavage of the TES-ether. Unfortunately, however, all attempts at an acid catalyzed opening of the vinyl epoxide with MeOH failed to afford the required product **61a** under a variety of experimental conditions. The 1,4-adduct **60** was invariably formed as the major compound, accompanied by the incorrect stereoisomer **59** generated by epoxide opening with formal retention of configuration.[67] Even the use of $[Rh(CO)_2Cl]_2$ did not change the outcome, although this catalyst is known for excellent 1,2-selectivity in the opening of alkenyl epoxides with various nucleophiles.[68]

Gratifyingly, however, the epoxide opening by MeOH took the expected regio- and stereoselective course when performed with the propargylic substrate **57**, although the silyl ether protecting group was concomitantly cleaved. This minor issue needed no further at-



Scheme 13. Initial foray into the isomigrastatin series and preparation of the structural hybrid **56**: a) (*R*,*R*)-(−)-[1,2-Cyclohexanediamino-*N*,*N*′-bis(3,5-di-*tert*-butylsalicylidene]manganese chloride (10 mol%), aq. NaOCl, Na_2HPO_4, CH_2Cl_2, 4°C, 66%; b) $[Cp^*Ru(MeCN)_3]PF_6$ (15 mol%), $BnMe_2SiH$, CH_2Cl_2, 0°C → RT; 82%; c) TBAF, THF, 50°C; 91% (over both steps); d) MeOH, 0°C, TsOH (5 mol%) (**59**/**60** 40:60), see text; e) MeOH, TsOH (50 mol%), 60°C, 94%; f) Dess–Martin periodinane, CH_2Cl_2, 0°C, 73%; g) $[Cp^*Ru(MeCN)_3]PF_6$ (50 mol%), $BnMe_2SiH$, CH_2Cl_2, 35% (**64**) + 14% of regioisomer; h) Ph_3SnH, AIBN, toluene, 80°C, 65% (**61b**); i) I_2, CH_2Cl_2, −78°C → RT, 82%; j) Bu_3SnH, AIBN, toluene, 65°C, 63%; k) Dess–Martin periodinane, CH_2Cl_2, 0°C, 68%; l) i) TMSCl, Et_3N, THF, then LiHMDS, −78°C; ii) **41b**, MS 4 Å, propionitrile, then **42**, −78°C; iii) HF·pyridine, THF/pyridine, 0 °C, 30% (unoptimized, over three steps).

tention, since the subsequent oxidation of diol **62** with Dess–Martin periodinane[69] was perfectly regioselective, converting only the more accessible C15-OH group to the corresponding ketone. The constitution and stereostructure of compound **63** thus formed was confirmed by single crystal X-ray diffraction (Figure 1).

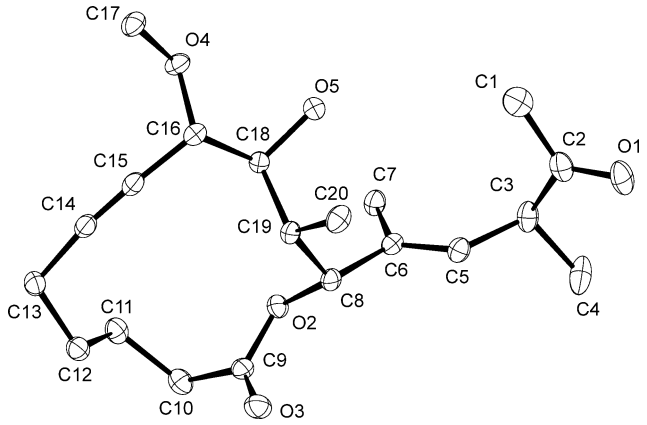
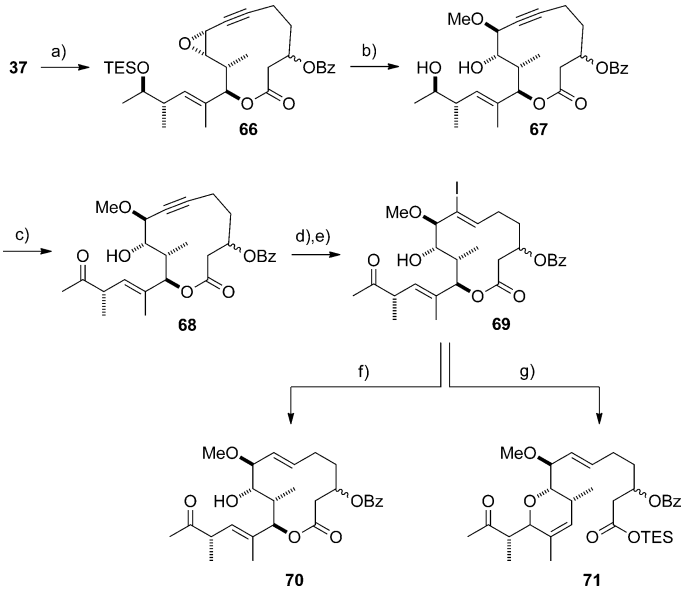


Figure 1. Structure of cycloalkyne **63** in the solid state.[70]

Unfortunately though, the projected *trans*-reduction of the propargylic alkyne entity failed with both **62** and **63**, even though for different reasons: **62** was totally inert toward ruthenium-catalyzed hydrosilylation, whereas ketone **63** could be forced to react in modest yield upon increasing the amount of $[Cp^*Ru(MeCN)_3]PF_6$ to 50 mol%; the subsequent protodesilylation of the resulting alkenylsilane **64** (and its regioisomer, not shown) to product **65**, however, led to instantaneous decomposition of the material and no attempts were made to change this fate. Rather, a radical *trans*-hydrostannation of propargylic derivatives, as described by Hale and co-workers,[71] seemed more promising. In fact, reaction of **62** with Ph_3SnH in the presence of AIBN gave product **61b** as a single regio- and stereoisomer in appreciable yield. The subsequent proto-destannation could be achieved in one step using an excess of trifluoroacetic acid, but the reaction was found erratic. Therefore, we preferred a two-step sequence, comprising a high yielding iodo-destannation[72] followed by a radical deiodination, which afforded product **61a** as a single isomer.

In line with the results outlined above, oxidation of this diol with Dess–Martin periodinane was also fully regioselective. Treatment of the resulting product **65** with TMSCl/Et_3N and LiHMDS in THF at low temperature afforded the necessary kinetic silyl enol ether, which was immediately reacted with the glutarimide-based aldehyde **41b** in the presence of the boron-based Lewis acid **42** to give the corresponding Mukaiyama aldol product. A desilylative work up with HF·pyridine in buffered medium furnished the targeted screening candidate **56** in a non-optimized 30% yield over the final three steps.

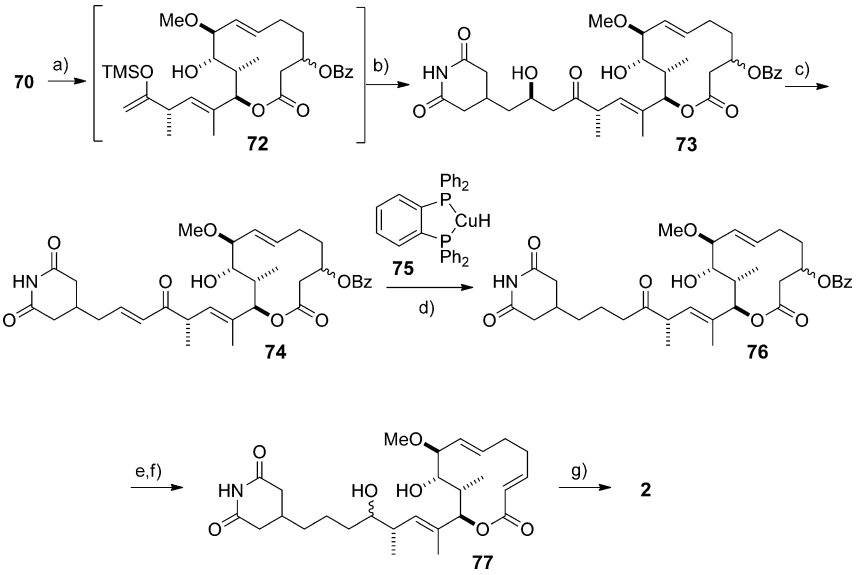
With the "diverted synthesis" of the hybrid structure **56** completed, the way to isomigrastatin seemed paved (Scheme 14). All it took was to repeat the sequence using the well available cycloalkyne **37** as the starting material. In line with our expectations, all steps worked well, including the *trans*-hydrostannation, iodine-for-tin exchange and radical deiodination with formation of the key intermediate **70**.[73] In contrast, an attempted reduction of the C–I bond in **69** with Et_3SiH under palladium catalysis was accompanied by an unusual allylic substitution process, in which the lactone ring of **69** gets opened as the pyran ring of **71** is closed.



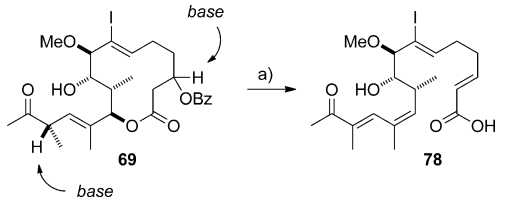
Scheme 14. a) (*R*,*R*)-(−)-[1,2-Cyclohexanediamino-*N*,*N*′-bis(3,5-di-*tert*-butylsalicylidene]manganese chloride (10 mol%), aq. NaOCl, Na_2HPO_4, CH_2Cl_2, 4°C, 63%; b) MeOH, TsOH (50 mol%), 60°C, 73%; c) Dess–Martin periodinane, CH_2Cl_2, 0°C, 87%; d) Ph_3SnH, AIBN, toluene, 80°C; e) I_2, CH_2Cl_2, 0°C → RT, 55% (over both steps); f) Ph_3SnH, AIBN, toluene, 70°C, quant.; g) Et_3SiH, $[Pd(PPh_3)_4]$ (20 mol%), toluene, 80°C, 69% (mixture of diastereomers).

Compound **70** could be elaborated with ease to the aldol product **73** by oxidation and Mukaiyama reaction of the intermediate silyl enol ether **72** under the conditions outlined above (Scheme 15). Since isomigrastatin (**2**)—in contrast to lactimidomycin (**1**)—lacks the C15-OH group on the side chain, a route had to be found to reduce this functionality to a methylene group. After some experimentation, we opted for the treatment of **73** with I_2 and imidazole in the presence of resin-bound PPh_3,[74] which furnished enone **74** in a single operation. This product could then be chemoselectively reduced to ketone **76** with the aid of the soluble copper hydride complex **75**,[75] although a large excess of this reagent was necessary for complete conversion.

The only remaining task at this point was the elimination of the benzoate substituent in the lactone headgroup of **76**. Whereas the analogous step in our approach to lactimidomycin (**1**) (see Scheme 7) had been high yielding, this reaction failed in the present case, despite the similarity of the two compounds; **76** was merely decomposed. To shed light



Scheme 15. a) TMSCl, Et_3N, THF, then LiHMDS, −78 °C; b) i) **41b**, MS 4 Å, propionitrile, then **42**, −78 °C; ii) HF·pyridine, THF/pyridine, 0 °C → RT, 57% (over both steps), c) I_2, imidazole, polystyrene-resin supported PPh_3, CH_2Cl_2, 0 °C → RT, 60%; d) **75** (excess), toluene, 59%; e) $NaBH_4$, THF, 0 °C → RT; f) DBU, THF, 73% (over both steps); g) Dess–Martin periodinane, CH_2Cl_2, 0 °C, 64%; DBU = 1,8-diazabicyclo[5.4.0]undec-5-ene.



Scheme 16. a) DBU, THF, MS 4 Å, then aq. HCl, 71%.

into this unexpected late-stage complication, ketone **69** was used as a supposedly valid yet less "expensive" model. In so doing, it was found that the lactone ring is readily opened with formation of acid **78** (Scheme 16).

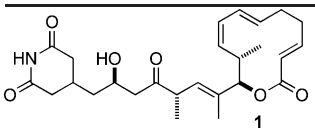
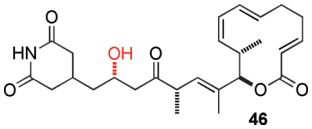
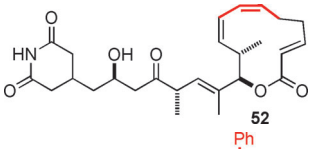
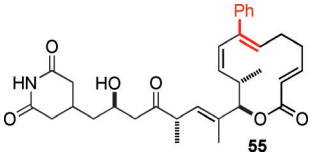
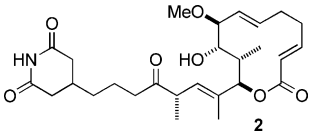
This result implies that the proton next to the lateral ketone is kinetically as acidic as the protons of the endocyclic aldol subunit. An extensive screening of various bases of different steric demand did not provide any satisfactory hit, and the vinylogous ring opening **69** → **78** could not be avoided. Therefore, the total synthesis of isomigrastatin (**2**) was completed by a short detour (Scheme 15): after reduction of the ketone in **76** with $NaBH_4$, the elimination of the benzoate worked well using DBU as the base. The resulting product **77** was then swiftly reoxidized to furnish isomigrastatin (**2**), the analytical and spectral data of which were in full accord with those reported in the literature.[2,29]

Biological assessment: An assortment of samples prepared during this investigation was subjected to biological testing. First, the cytotoxicity was determined using the highly invasive human breast cancer cell line MDA-MB-231, the human colon carcinoma cell line LoVo, and the mouse mammary adenocarcinoma cell line 4T1. The IC_{50} values were determined in two or three (4T1) rounds, and were found well reproducible within and across the independent experiments.[76] Different batches of material were used and the integrity of the samples was confirmed by HPLC after testing. Only the data of the compounds with IC_{50} values ≤ 1 μM are compiled in Table 1, whereas all other tested substrates (**19**, **23**, **44**, **45**, **46**, **47**, **48**, **51**, **54**, **56**, **61a**, **65**) showed no noticeable activity in this concentration range, including the bipartite compound **46**, in which the macrolactone and the glutarimide portions are tethered via an ester linkage. Furthermore, none of the compounds lacking the glutarimide moiety showed noticeable activity at concentrations ≤ 1 μM.

As expected, lactimidomycin (**1**) was found highly cytotoxic, even though an order of magnitude less active against the MDA-MB-231 cell line than previously reported,[19] whereas the opposite trend was observed with the murine 4T1 cell line, which was found particularly sensitive. It is tempting to speculate that the particular sensitivity of the 4T1 cells under the conditions of this assay may be related to their extremely rapid proliferation rate compared with the other cell lines tested, potentially rendering them more dependent upon protein translation.[77] Inversion of the lateral hydroxyl group at C17, as materialized in compound **46**, reduces the activity by a factor of ten. Somewhat unexpectedly, our data suggest that the *E*-configured C6–C7 double bond within the macrolactone is a rather permissive site for structural variations, since derivative **55** with a large phenyl substituent attached to C7 and product **52** comprising a *Z*-configured C6–C7 olefin both retain appreciable activity; in fact, the cytotoxicity of the geometric isomers **1** and **52** is almost identical. In contrast, isomigrastatin (**2**) is much less potent than **1**, despite considerable structural homology; this finding is congruent with the literature.[19] The comparison of the one day and four day MTS endpoints shows the very rapid onset of action. The more potent cytotoxicity in 4T1 cells under the conditions of this assay may be related to the very fast proliferation rate of these cells compared with the other cell lines tested, potentially rendering them more dependent upon protein translation.

Very much to our surprise, however, none of the tested compounds showed any appreciable specific effect on cell migration, neither for 4T1 nor for MDA-MB-231 cells. Since this result stands in marked contrast to previous literature reports, utmost care was taken to ensure the validity and

Table 1. Four day (4 d) and one day (1 d) cytotoxicity (IC_{50}, nM) of lactimidomycin and congeners against three different cancer cell lines (MTS endpoints);[a] comparison with literature data (ref. [19]), where available.

Compound	4T1 4 d[b]/1 d[b]	Lit.	MDA-MB-231 4 d/1 d	Lit.	LoVo 4 d/1 d
 **1**	7.0/2.9	110	28/52	4.3	15/18
 **46**	69.1/36.1		239/327		138/138
 **52**	15.8/7.6		26/41		30/30
 **55**	56.8/21.7		74/114		91/119
 **2**	282.3/135.5	170	935/>1000	130	400/407
cytochalasin D	525/>10000				

[a] MTS = 5-(3-Carboxymethoxyphenyl)-2-(4,5-dimethylthiazolyl)-3-(4-sulfophenyl)tetrazolium inner salt. [b] Mean of two independent experiments.

consistency of our data. To this end, a full dose-response study was carried out for the compounds shown in the Table using the 4T1 cell line, with cytochalasin D as a positive control to calibrate the assay. Furthermore, the testing was performed in the classical scratch wound healing (SWH) format (Figure 2) as well as with the more modern 2D Platypus migration assay and a 3D transwell migration assay (data not shown). Different batches of compounds were used in the two (LoVo, MDA-MB-231) or three (4T1) independent rounds of testing.

As expected, the positive control cytochalasin D (a well-documented attenuator of actin polymerization) inhibited cell migration in the SWH assay by 40% at 100 nM in 4T1 cells (represented in Figure 2B) and 30% in MDA-MB-231 cells at 100 nM (not shown). Migratory inhibition was more pronounced at 64 and 78% in these cell lines, respectively, when the concentration of cytochalasin D was increased to 300 nM (not shown), which is still far lower than its 1 day cytotoxicity IC_{50} of >10000 nM. Nearly 92% inhibition was observed for cytochalasin at 400 nM in 4T1 cells, clearly indicative of the ability of migration to be attenuated in this cell line at non-toxic doses. In contrast, no significant anti-migratory effect was observed for lactimidomycin, isomigrastatin and derivatives thereof. A full dose response was conducted with these compounds in the SWH assay with 4T1 cells, and the following minimal inhibition of migration was observed at concentrations just below their one day cytotoxicity IC_{50}: **1**, 10% inhibition at 1.6 nM; **2**, 20% inhibition at 100 nM; **46**, 14% inhibition at 25 nM; **52**, 35% inhibition at 6.2 nM; **55**, 3% inhibition at 12.5 nM.

Hence, no appreciable effect of these compounds on migration was found when tested at sub-toxic doses: while this is true for all compounds shown in Table 1, the outcome is particularly evident in the case of isomigrastatin (**2**), which is the least cytotoxic agent (IC_{50} = 135.5 nM after 1 d, 4T1 cells) and hence the most likely candidate for specific cell migration inhibition to be observable after this period of time. As can be seen from Figure 2D, however, the scratch wound was largely closed by migrating 4T1 cells after incubation with **2** (100 nM) for 16 h (only ≈16% of the clearing remains compared with time zero) and similar closure was observed at 11 concentrations in a two-fold dose response from 0.2 to 200 nM. The literature IC_{50} for migration inhibition of the same cell line is 23 nM,[19,78] which we cannot reconcile with our data. It had been claimed in the literature that these compounds are extremely potent inhibitors of cell migration; their cytotoxicity was stated to set in only at concentrations well above their effective doses.[19] Our findings are different, if not the opposite: the compounds turned out to be acutely cytotoxic after one day, thus making it essentially impossible to assess migration activity since the vast majority of cells are dead at this point. We propose that cytotoxic activity should always be assessed for the same duration as the short-term migration assay (i.e., 1 d) in order to best distinguish any potentially specific anti-migratory effects of compounds from general cell death. Therefore, with the cell lines and conditions used for these assays, as detailed in the experimental section, these agents are cytotoxic in human and mouse cell lines but fail to inhibit migration at sub-toxic doses.

Conclusion

Whereas previous synthesis-driven explorations of the chemical space around the 14-membered bipartite glutarimide-macrolide migrastatin (**3**) yielded compounds **6**–**10** with spe-

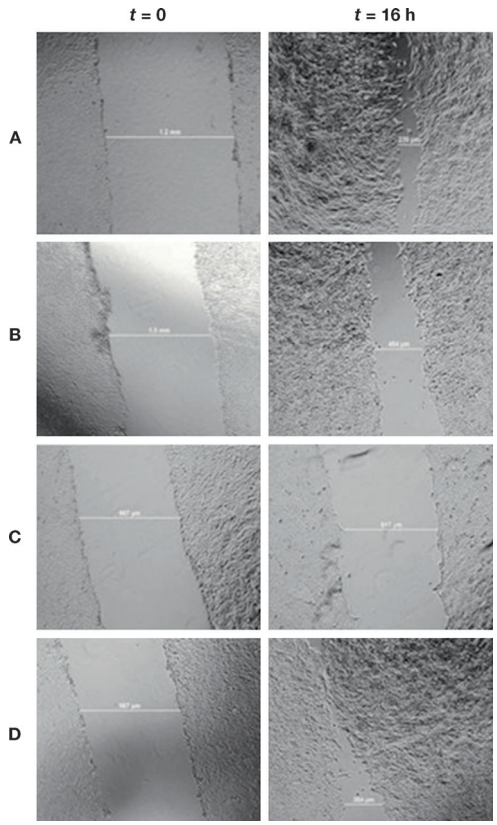


Figure 2. Representative results of a scratch wound healing cell migration inhibition assay performed with the 4T1 mouse carcinoma cell line; left panels, time zero (start of treatments); right panels, 16 h after start of drug treatment. **A**, DMSO-treated control; **B**, cytochalasin D, 100 nM; **C**, lactimidomycin (**1**), 100 nM; **D**, isomigrastatin (**2**), 100 nM. It is emphasized that the apparent inhibition of cell migration by **1** (~98% of the time zero clearing still remains after 16 h) is largely or exclusively due to cell death (cytotoxicity IC_{50} = 2.9 nM, 1 d MTS endpoint). As evident in panel **D**, the less cytotoxic **2** (IC_{50} = 135 nM, 1 d MTS endpoint) shows no significant cell migration inhibition.

cific cell migration inhibition properties, which currently undergo preclinical testing,[5,7] studies into the smaller homologues lactimidomycin (**1**) and isomigrastatin (**2**) had been much less prolific. It was only after the fermentation titer of these compounds had been improved and additional minor co-metabolites were isolated from the broth,[20,79] that these highly strained 12-membered macrolides could be evaluated in some detail. This led to the somewhat contradictory conclusions that **1** and **2** are supposedly more potent cell migration inhibitors than **3** and progeny,[19] although they were shown to primarily target the eukaryotic ribosome and, in so doing, bring protein biosynthesis to a halt at the translation stage.[21] Translation inhibition, however, has no immediate functional link to cell motility or active cell movement.

We took these somewhat difficult-to-reconcile literature reports as an incentive to develop a de novo chemical approach to lactimidomycin (**1**) as the allegedly most active member of the entire series. This challenge has been met by the route described herein, which is short, productive and scalable, and could therefore easily fuel a detailed preclinical evaluation of this natural product. This success is innately linked to the power of the latest generation molybdenum alkylidyne catalysts for ring-closing alkyne metathesis,[39,40,44] which allowed the highly strained macrolide head group of **1** to be forged with remarkable efficiency. Moreover, deliberate digression from the underlying synthesis blueprint also brought the sister compound isomigrastatin (**2**) as well as various non-natural designer analogues into reach. This compound collection allowed the biological activity of 12-membered glutarimide-macrolides to be re-assessed. It is striking that our data do not allow us to confirm any significant cell migration inhibition properties for any of the tested compounds; rather, lactimidomycin and several non-natural isomers were found acutely cytotoxic, able to effectively kill the different tested cancer cells before a specific migratory effect could set in.

Arguably, the high cytotoxicity of **1** can be explained if the ribosome constitutes the major biological target, as previously suggested.[21] **1** and **2** might therefore serve as tools for the chemical interrogation of this essential biological machinery.[80] Whether or not ribosome inhibitors can also qualify as leads in a medicinal chemistry context remains to be seen.[81] It has been argued that a sufficient therapeutic window could arise from the fact that transformed cells, by virtue of their higher translation rates, are inherently more sensitive. Furthermore, several proteins with important regulatory functions in cancer progression are known to be short-lived. As a consequence, their level is strongly dependent on ongoing protein synthesis and the rapid loss of such survival factors might engender clinically useful therapeutic effects. Finally, the argument has been raised that metastasis as well as chemoresistance are developmental processes and as such translation-dependent. Potent translation inhibitors might therefore help sensitize resistant tumors toward the standard chemotherapeutic agents. In fact, a recent investigation demonstrated that even the moderately potent glutarimide-based ribosome binder cycloheximide (**11**) leads to remarkable synergistic effects when administered with standard antitumor drugs.[82] These data advocate for similar studies on the chemosensitizing effect of the much more potent translation-elongation inhibitor lactimidomycin (**1**) and progeny.[83]

Experimental Section

All experimental details can be found in the Supporting Information. The material includes compound characterization, a crystallographic abstract for the X-ray structure of compound **63**, a description of the bioassays, and copies of the NMR spectra of new compounds.

Acknowledgements

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is gratefully acknowledged. We thank the analytical departments of the MPI for excellent support.

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