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New Insights into the Synthesis and Biological Activity of the Pamamycin Macrodiolides

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Abstract: After a brief account of the biological properties of pamamycins, this review highlights the latest developments on the total synthesis and the biosynthesis of these macrodiolides.

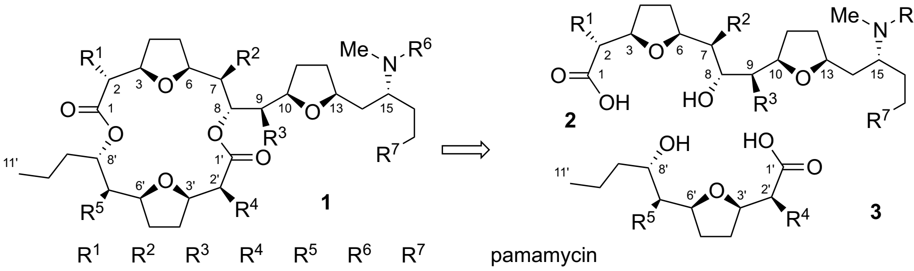
Keywords: Biosynthesis · Pamamycins · Total synthesis

The pamamycin family of macrodiolides, isolated from a variety of *Streptomyces* species, have attracted considerable attention from the scientific community over the last two decades due to their pronounced bioactivities and challenging molecular structures. Beside their aerial *mycelium*-inducing activity, they also enhance the production of secondary metabolites in several *Streptomyces* species and display antibacterial (active against Gram-positive bacteria) and antifungal activities.[1] Since the isolation of a first member of pamamycin family with a molecular formula of $C_{36}H_{63}NO_7$ and a molecular weight of 621 Da from *Streptomyces alboniger* by McCann and Pogell in 1979,[1a] a re-examination of extracts by Marumo and co-workers in 1987 showed a large number of homologs with various substituents in certain positions (Fig. 1).[2]

Structurally, the interesting features include a 16-membered macrodiolide and three *cis*-2,5-disubstituted tetrahydrofurans with adjacent methyl-substituted stereogenic centers, composed of two hydroxy acids, commonly called the 'larger' (C(1)–C(18), **2**) and 'smaller' (C(1')–C(11') **3**) fragments (Fig. 1). The attractive features and potential uses of pamamycins have spurred attempts to provide sufficient amounts of macrolides to enable pharmacological application. Thus, numerous methods to chemically synthesize pamamycins as well as to use natural producers have been developed during the last decades. However, on the one side synthesis methods are complex and lengthy and on the other fermentation methods are limited by the low production level of the microorganisms used, and apart from the most abundant congener pamamycin 607 (**1b**), other homologues are obtained as mixtures from culture media. Recently, a pamamycin biosynthesis gene cluster has been identified[3] and recombinant microorganisms have been developed for the production of pamamycins, in particular pamamycins 607 (**1b**) and 621A (**1c**).[4] This account will cover synthetic work described since 2005 as well as recent findings on the biological activity of the pamamycins and the studies on their biosynthesis.[5] Additional synthetic approaches to the constituent hydroxycarboxylic acids[6] will not be detailed in this account.

Total Synthesis

The first efforts related to the total synthesis of the pamamycins were disclosed



	R1	R2	R3	R4	R5	R6	R7	pamamycin
1a	Me	H	Me	Me	H	Me	Me	593
1b	Me	Me	Me	Me	H	Me	Me	607
1c	Me	Me	Me	Me	Me	Me	Me	621A
1d	Et	H	Me	Me	Me	Me	Me	621B
1e	Me	Me	Et	Me	H	Me	Me	621C
1f	Et	Me	Me	Me	H	Me	Me	621D
1g	Me	Me	Et	Me	Me	Me	Me	635A
1h	Me	Me	Me	Et	Me	Me	Me	635B
1i	Et	Me	Me	Me	Me	Me	Me	635C
1j	Et	Me	Me	Et	H	Me	Me	635D
1k	Me	Me	Et	Et	H	Me	Me	635E
1l	Et	Me	Et	Me	H	Me	Me	635F
1m	Et	Me	Et	Et	H	Me	Me	649A
1n	Et	Me	Et	Me	Me	Me	Me	649B
1o	Me	H	Me	Me	H	H	Me	De-*N*-methyl-579
1p	Me	Me	Me	Me	H	H	Me	De-*N*-methyl-593A
1q	Et	H	Me	Me	H	H	Me	De-*N*-methyl-593B
1r	Me	Me	Me	Me	H	Me	Pr	MS-282a
1s	Me	Me	Et	Me	H	Me	Et	MS-282b

Fig. 1. The pamamycin macrodiolides and their hydroxy acid constituents **2** ('larger fragment') and **3** ('smaller fragment')

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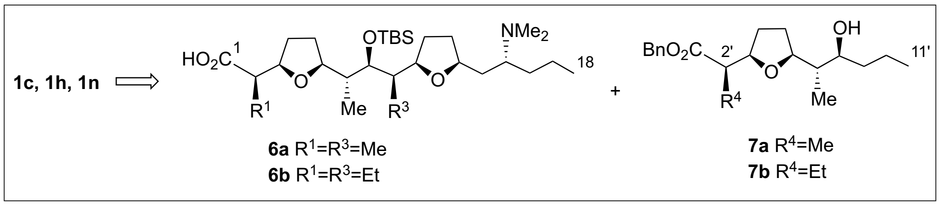
in 1988.[7] However, even though several groups embarked on studies towards a total synthesis, none was described during the 1990s. Stereocontrol of the tetrahydrofuran rings and the adjacent stereogenic centers in the fragments **2** and **3** proved quite difficult and the first total synthesis was described by E. J. Thomas *et al.* in 2001.[8] Then, three other elegant total syntheses along with various synthetic approaches to the hydroxy acid constituents **2** ('larger fragment') and **3** ('smaller fragment') of the pamamycins emerged and have been reviewed in two accounts in 2005.[5] However, most studies were directed toward pamamycin 607 (**1b**), the major homolog extracted from the natural source, and total synthesis towards some of the other molecules only appeared after 2005.

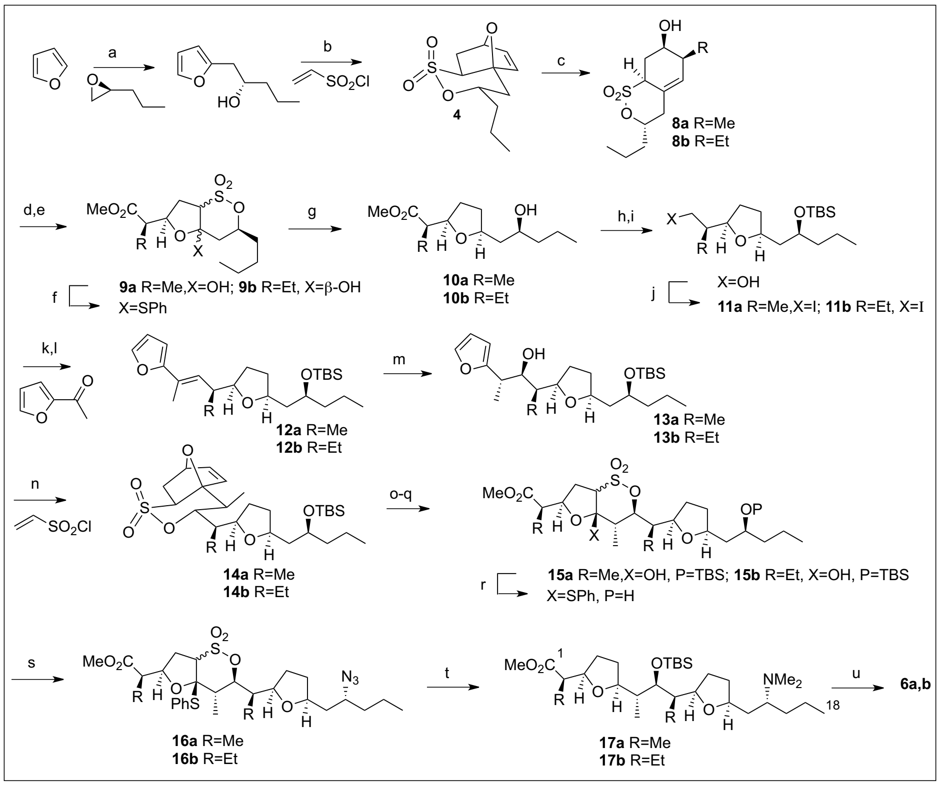
Metz Total Synthesis of Pamamycins 621A (1c), 635B (1h) and 649B (1n)[9,10]

P. Metz slightly modified his elegant sultone route developed for the synthesis of pamamycin 607 (**1b**),[5] to prepare homologs 621A (**1c**) and 635B (**1h**) differing only in terms of the smaller fragment **3** (R^4 = Me, Et),[9] and 649B (**1n**) differing only in terms of the larger fragment **2** (R^1 = R^3 = Et),[10] This approach was based on the stereoselective intramolecular Diels–Alder reaction of vinylsulfonates providing, *via* the pivotal sultones **4** and **5**, silyl ethers **6a**,**b** of the hydroxy acids **2** and benzyl esters **7a**,**b** of the smaller fragments **3** epimeric at C(2') (Scheme 1). In the course of the total synthesis of **1b**, Metz had demonstrated that a complete C(2') epimerization of **7a** occurred during the final Yamaguchi macrocyclization using Fleming conditions.[11] Since fragment **7a** was more easily accessible compared to its epimer **3**, these macrocyclization conditions had been implemented successfully in a previous shortened total synthesis of pamamycin **1b** and proved to be applicable to corresponding simplified routes towards other pamamycins.

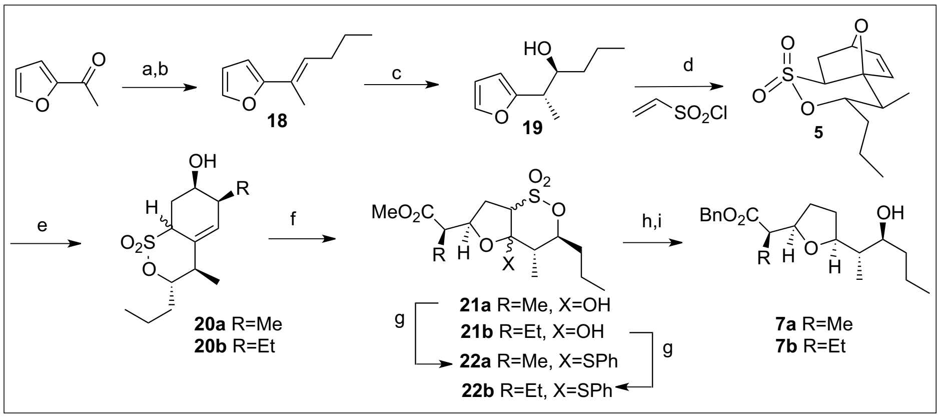
The synthesis of the larger fragments **6a**,**b** started with sultone **4** that had already been used as a precursor for pamamycin 607 (**1b**).[5] The latter was reacted with two equiv. alkyllithium providing, *via* a domino elimination and alkoxide-directed 1,6 addition, the bicyclic compounds **8a** as mixture of diastereoisomers and pure **8b** (Scheme 2). Ozonolysis of the mixture **8a** or pure **8b** followed by eliminative workup, led to the formation of the two diastereoisomers **9a** or pure **9b**. Lewis acid-catalyzed substitution of the OH by a phenylthio group followed by treatment with Raney nickel in the presence of hydrogen gave **10a**,**b** as single tetrahydrofurans. TBS protection followed by ester reduction and Appel reaction afforded the iodides **11a**,**b**. Halogen-lithium exchange of iodides **11** and subsequent addition of 2-acetylfuran to the resultant organolithium intermediate yielded two diastereomeric tertiary alcohols, which were converted to (*E*)-olefins **12a**,**b** with complete diastereoselectivity upon brief exposure to substoichiometric amounts of concentrated hydrochloric acid solution. Diastereoselective hydroboration/oxidation controlled by 1,3-allylic strain of **12a**,**b** gave largely the desired stereoisomers **13a**,**b**. The hydroxy alkyl furans **13a**,**b** were submitted to an additional iteration of the sultone route based on the formation of a vinylsulfonate and an intramolecular Diels-Alder reaction to afford sultones **14a**,**b** as single diastereomers. Treatment of the latter with 2 equiv. of the appropriate alkyllithium led to the *syn*-selective introduction of a methyl or ethyl group *via* the previously described elimination/alkoxide-directed 1,6-addition and ozonolysis followed by treatment of the resulting mixture under eliminative conditions delivered the expected hemiketals **15a**,**b** as single stereoisomers. Treat-



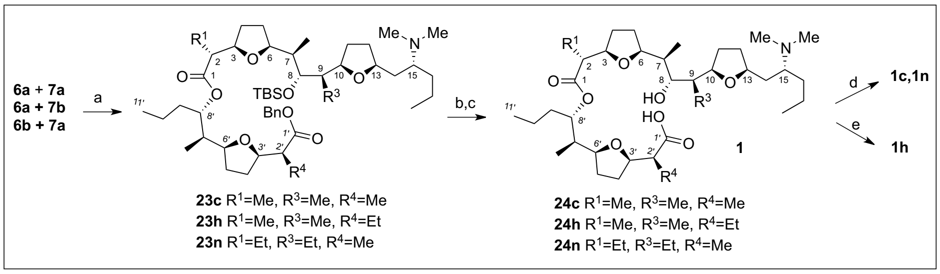
Scheme 1. Metz retrosynthetic analysis of **1c**, **1h**, **1n**. TBS = *tert*-butyl-dimethylsilyl



Scheme 2. Metz synthesis of the larger fragments **6a**,**b** of **1b**,**c**,**h**,**n**. *Reagents and conditions*: a: (i) BuLi, THF, –78 to –15 °C, (ii) (S)1,2-epoxypentane, –15 °C to rt, 84%; b: vinylsulfonyl chloride, Et_3N, THF, 0 °C to rt, 88%; c: (i) MeLi (for **8a**) or EtLi (for **8b**) 2 equiv., THF, –78 to 0 °C; (ii) NH_4Cl,H_2O, –78°C to rt, 66% **8a**, 36% **8b**; d: O_3, $NaHCO_3$, CH_2Cl_2, MeOH, –78 °C; e: Ac_2O, pyridine, CH_2Cl_2, rt for **9a** or rt to reflux for **9b**, 83% **9a**, 87% **9b**; f: PhSH, $BF_3{\cdot}Et_2O$, CH_2Cl_2, rt; g: Raney Ni (W2), 50 bar H_2, EtOH, rt, 44% from **9a** for **10a**, 33% from **9b** for **10b**; h: TBSCl, imidazole, DMAP, DMF, rt; i: $LiAlH_4$, Et_2O, 0 °C to rt; j: I_2, Ph_3P, imidazole, Et_2O,MeCN, rt, 95% from **10a** for **11a**, and 94% from **10b** for **11b**. k: (i) *t*-BuLi, Et_2O, hexane, –78 °C, (ii) 2-acetylfuran, MS 4A, –78 °C to rt; l: conc. aq. HCl, $CHCl_3$, rt, 69% **12a** from **11a**, 71% **12b** from **11b**; m: (i) $BH_3{\cdot}THF$, THF, 0 °C to rt, (ii) 30% aq. H_2O_2, NaOH, 0 °C to rt, 65% **13a** from **12a**, 51% **13b** from **12b**; n: vinylsulfonyl chloride, Et_3N, THF, 0 °C to rt, 93% **14a**, 88% **14b**; o: (i) MeLi 2 equiv., THF (for **14a**) or EtLi 2 equiv., THF, Et_2O, hexane (for **14b**), –78 to rt; (ii) NH_4Cl, H_2O, –78 °C to rt; p: O_3,$NaHCO_3$,CH_2Cl_2,MeOH, –78 °C; q: Ac_2O, pyridine, CH_2Cl_2, rt to reflux, 53% **15a** from **14a** and 45% **15b** from **14b**; r: PhSH,$BF_3 \bullet Et_2O$,CH_2Cl_2, rt; s: HN_3, DEAD or DIAD (for **16b**), Ph_3P, toluene, 0 °C to rt, 76% **16a** from **15a**, 75% **16b** from **15b**; t: (i) Raney Ni (W2), 50 bar H_2, EtOH, rt, (ii) aq. CH_2O, (iii) TBSOTf, 2,6-lutidine, CH_2Cl_2, rt, 50% **17a** from **16a**, 47% **17b** from **16b**; u: LiOH, THF, MeOH, rt, 97% **6a**, 41% **6b**. DMAP = 4-(N,N-dimethylamino)pyridine.



Scheme 3. Metz synthesis of the smaller fragments **7a**,**b** of **1b**,**c**,**h**,**n**. *Reagents and conditions*: a: BuMgBr, Et_2O, reflux; b: cat. HCl, CH_2Cl_2, reflux, 58% **18** from 2-acetylfuran, *E/Z* = 93:7; c: (i) $IpcBH_2$ (derived from (+)-α-pinene), THF, –25 °C, (ii) H_2O_2, NaOH, 70 °C, 76% **19** (81% *ee*) + *syn* isomer; d: (i) Vinylsulfone chloride, NEt_3, THF, 0 °C to rt, (ii) recrystallization, 74% **5** (99% *ee*); e: (i) MeLi, 2 equiv. for **20a**, EtLi, 2 equiv. for **20b**, THF, –78 °C to rt, (ii) NH_4Cl, H_2O, –78 °C to rt, 53% **20a**, 68% **20b**; f: (i) O_3, $NaHCO_3$, CH_2Cl_2, MeOH, –78 °C, (ii) Ac_2O, pyridine, CH_2Cl_2, rt to reflux, 87% **21a**, 86% **21b**; g: PhSH, $BF_3{\cdot}Et_2O$, CH_2Cl_2, rt, 79% **22a**, 80% **22b**; h: Raney Ni (W2), 50 bar H_2, EtOH, rt; i: BnOH, 10 mol% Bu_2SnO, 160 °C, 55% **7a** from **22a** and 58% **7b** from **22b**. $IpcBH_2$ = monoisopinocampheylborane.



Scheme 4. Completion of Metz synthesis of pamamycins **1c**,**h**,**n**. *Reagents and conditions*: a: (i) 2,4,6-trichlorobenzoyl chloride, Et_3N, THF, 0 °C to rt, (ii) **7a** or **7b**, DMAP, toluene, rt, 88% **23c**, 78% **23h** and 80% **23n**; b: aq. HF, MeCN, rt; c: H_2, 10% Pd/C, THF, rt, 97% **24c**, 80% **24h** and 95% **24n**; d: 2,4,6-trichlorobenzoyl chloride, DMAP, MS 4 Å, CH_2Cl_2, rt, 64% **1c** from **24c**, 53% **1n** from **24n**; e: (i) 2,4,6-trichlorobenzoyl chloride, Et_3N, THF, 0 °C to rt, (ii) DMAP, toluene, reflux, 53% **1h** from **24h**. MS = molecular sieves.

ment of hemiketals **15a**,**b** with thiophenol and trifluoroborane led to the formation of a thioketal and concomitant removal of the TBS protecting group to afford the corresponding alcohol. The latter was converted into azides **16a**,**b** under Mitsunobu conditions using hydrazoic acid. Subsequent treatment of **16a**,**b** with Raney nickel under hydrogen pressure followed by addition of an aqueous formaldehyde solution effected the desired desulfurization, while also allowing the reduction of the azide into a primary amine, double methylation of the latter. The resulting alcohols were subsequently protected as TBS-ethers **17a**,**b**. Finally, mild saponification of methyl esters **17a**,**b** yielded the larger fragment coupling partners **6a**,**b**.

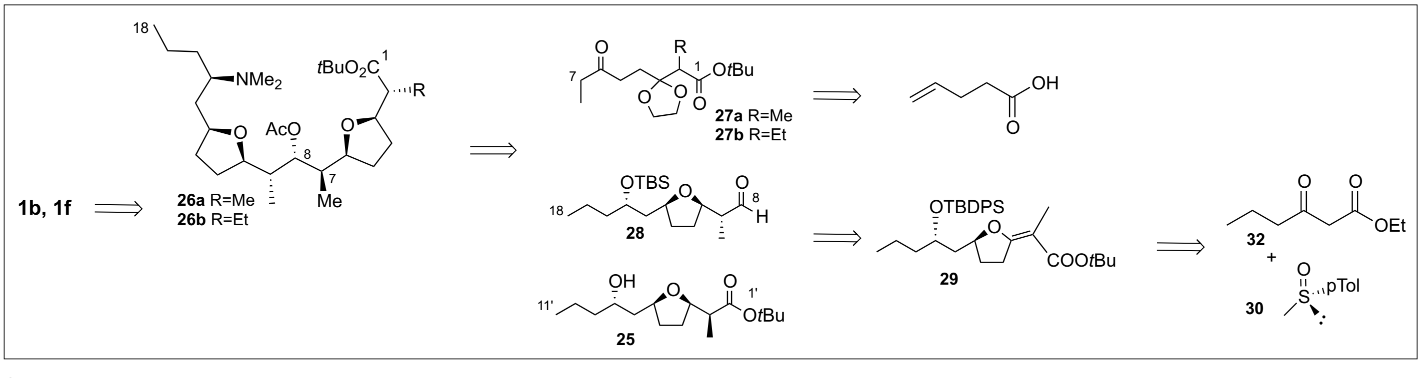
A similar sultone-based strategy was applied to the synthesis of the C(2') epimeric smaller fragment benzyl esters of **1c**,**h**,**n** as well (Scheme 3). Asymmetric hydroboration of the *E/Z* (97/3) mixture of olefin **18**, itself resulting from butyl Grignard addition/acid catalyzed elimination on 2-acetylfuran, led, after oxidative work up, to the *anti*-alcohol **19** (81% *ee*) along with small amounts of the *syn* isomer. A subsequent reaction with vinylsulfonyl chloride induced a domino esterification/intramolecular Diels-Alder reaction to give the pure *exo*-sultone **5** (99% *ee*) in 74% yield. Application of the domino reaction sequence of elimination/alkoxide-directed 1,6-addition, followed by sequential ozonolysis/cyclization, Lewis acid-catalyzed hydroxy/phenylthio exchange converted sultone **5** to thioethers **22a**,**b**, which after a domino reductive elimination/hydrogenation and a dibutyltin oxide-catalyzed transesterification were transformed into benzyl esters **7a**,**b**.

Having observed complete C(2') epimerization during their first total synthesis of pamamycin **1b**,[11a] the authors completed the total synthesis of pamamycins **1c**,**h**,**n** using first an intermolecular Yamaguchi esterification of silyloxy acids **6a** with benzyl esters **7a**,**b** leading to *seco*-acids **23c**,**h** and silyloxy acid **6b** with benzyl ester **7a** leading to *seco*-acid **23n**. Finally, after deprotection of the C(8) hydroxy group and C(1') carboxylic acid, modified Yamaguchi cyclization of **24c**,**n** under Fleming conditions afforded pamamycin 621A (**1c**)[9] and 649B (**1n**)[10] as single diastereoisomers in good yields (Scheme 4).

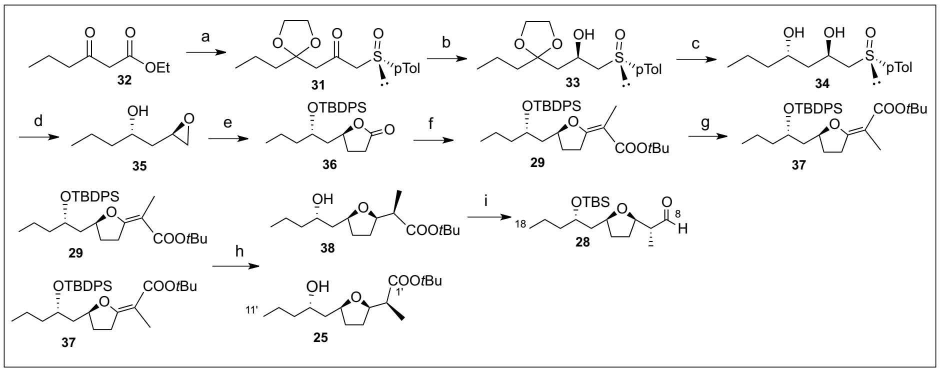
Probably due to the greater steric hindrance caused by the C(2') ethyl group of *seco*-acid **24h**, Yamaguchi lactonization was found to require a prior activation as a mixed anhydride followed by refluxing in toluene under high dilution conditions in the presence of DMAP.[9] Using these conditions, pamamycin 635D (**1h**) was obtained in 53% isolated yield.

Hanquet Total Synthesis of Pamamycins 607 (1b) and 621D (1f)[12,13]

A new total synthesis of pamamycin 607 (**1b**) was reported in 2007[12] by our own group and has been recently extended to pamamycin 621D (**1f**).[13] Our approach



Scheme 5. Hanquet retrosynthetic analysis of **1b** and **1f**.



Scheme 6. Hanquet preparation of C(8)–C(18) and C(1')–C(11') fragments **28** and **25** of pamamycin 607 (**1b**) and 621D (**1f**). *Reagents and conditions;* a: (i) Ethylene glycol, TMSCl, CH_2Cl_2, rt; (ii) **31**, LDA 2 equiv., THF, –78 °C to rt, 60% for two steps; b: (i) Dibal-H, THF, –78°C, (ii) oxalic acid, THF/water, 64% **33** from **32** (*de*>95%); c: $Me_4NBH(OAc)_3$, AcOH, 97% **34**; d: (i) *t*-BuBr, $CHCl_3$, 50 °C, (ii) Me_3OBF_4, CH_2Cl_2, 20 °C; (iii) K_2CO_3, 75% **35**; e: (i) TBDPSCl, imidazole, DMF, (ii) ethyl malonate, EtONa, THF, –78 °C, (iii) $MgCl_2$-$6H_2O$, DMF, reflux, 73% **36**, f: (i) *t*-butyl propionate, LDA, THF, –78 °C, (ii) oxalic acid, CH_2Cl_2, 75% **29**; g: LDA 2 equiv., LiCl 3 equiv., THF, then EtOH, 70% **37**; h: (i) TBAF, THF, (ii) H_2, MeOH, Rh, Al_2O_3, 4 bars, 73% **38** and 67% **25**; i: (i) TBSCl, imidazole, DMF, (ii) LAH, ether, (iii) SO_3-pyridine, Hunig base, CH_2Cl_2, 89% **28**. LDA = lithium diisopropylamide, TBAF: tetra-butyl ammonium fluoride.

relied on the obvious disconnection of the two lactone linkages to afford the C(1')–C(11') fragment **25** and the C(1)–C(18) fragments **26a**,**b** (Scheme 5). The C(7)–C(8) bond was identified as an aldol retron and disconnected to obtain precursors **27a**,**b** and **28**.

This retrosynthetic analysis led to the proposal of a convergent approach in which three fragments of similar molecular weight, **25**, **27** and **28**, were to be joined at a late stage. Additionally, the observation that **25** and **28** were diastereoisomers differing by the configuration at C(2) and C(2') triggered the hypothesis of a common intermediate **29**. An *E-Z* isomerization followed by a *cis*-hydrogenation of the tetrahydrofuran-alkylidene double bond should lead to both fragments in a diastereodivergent manner. Common intermediate **29** was accessible using enantiomerically pure sulfoxide **30** as chiral auxiliary.[14]

Synthesis of the common intermediate **29** started with the β-ketosulfoxide **31** obtained from ethyl butyryl acetate (**32**) after formation of the dioxolane and a Claisen-type condensation with the anion of (–)-(*S*)-methyl-*p*-tolylsulfoxide (**30**, Scheme 6).[15] DIBAL-H reduction and subsequent hydrolysis afforded the corresponding [S(*S*),2(*R*)]-β-hydroxysulfoxide **33** in 80% yield (de > 95%) which was subjected to an Evans reduction[16] giving *anti*-[S(*S*),2(*R*),4(*S*)]-β,δ-dihydroxy-sulfoxide **34** (de>95%), in 97% yield by crystallization. The reduction of the sulfoxide auxiliary, methylation at sulfur and intramolecular displacement of the sulfenium leaving group afforded the [2(*R*),4(*S*)]-β-hydroxy epoxide **35** in 75% yield. Protection of the alcohol as TBDPS ether followed by a ring-opening with ethyl malonate anion and subsequent Krapcho-type decarboxylation afforded the lactone **36**. Addition with the lithium enolate of *t*-butyl propionate to **36** produced a hemiketal which underwent an acid-catalyzed dehydration to afford the desired common intermediate **29** in 75% yield as a single thermodynamically preferred *E* isomer.

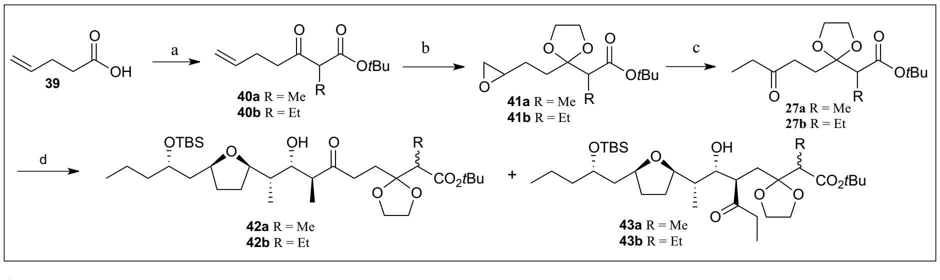
E-Z isomerization of **29**[17] was performed using LDA in the presence of LiCl and gave **37** in high yield.

Deprotection of either double bond isomer with TBAF and stereoselective hydrogenation on the more accessible face of the alkene using a Rh/alumina catalyst afforded the corresponding 2,5-*cis*-disubstituted tetrahydrofurans **38** and **25**. Finally, protection of the hydroxy groups as TBS ethers and sequential transformation of *t*-butyl ester into an aldehyde led to fragment **28**.

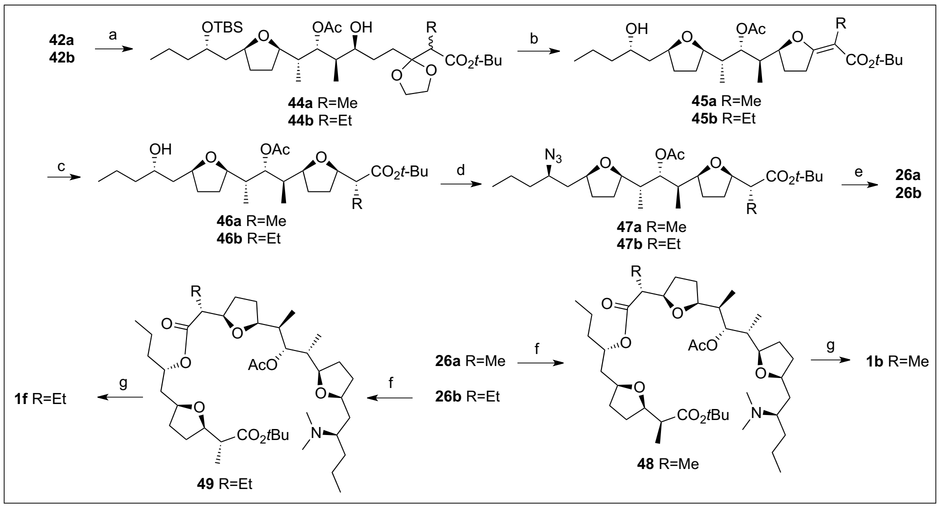
Preparation of the second key step aldol addition partners **27a**,**b** started from commercially available 4-pentenoic acid (**39**) which was submitted to Claisen condensation, *via* its acylimidazole derivative, with *t*-butyl propionate or *t*-butyl butanoate enolate to give ketoesters **40a**,**b** (Scheme 7). Protection of the ketone followed by epoxidation of the terminal double bond led to epoxides **41a**,**b**. Finally ethyl ketones **27a**,**b** were obtained from the latter by ring-opening of epoxides and oxidation of the resulting alcohols.

With the larger fragment precursors **27a**,**b** and **28** in hand, a regio- and diastereoselective aldol addition using Chx_2BCl/Et_3N[18] was performed. An unusual behavior of ketones such as **27a**,**b** was observed, in that their enolization under boron-mediated conditions was shown to be strongly solvent-dependent.[19] Whereas the use of pentane resulted in the formation of the undesired regioisomer **43a**,**b** as the major product, the selectivity was reversed when diethyl ether was used as the solvent and, in these conditions, **42a** and **42b** were isolated as pure products in 52% and 61% yield respectively.

The preparation of the C(1)–C(18) fragments **26a**,**b** (Scheme 8) relied on an *anti*-selective reduction of the β-hydroxyketone motif with concomitant differentiation of the two secondary hydroxyl groups; the use of the Tishchenko reduction using samarium(II) iodide as described by Evans[20] allowed this transformation to give acetates **44a**,**b**. Whereas the hydrolysis of the dioxolane under acidic conditions and the acid-mediated cyclization followed by TBS deprotection using Amberlyst 15 sulfonic acid resin afforded the unsaturated intermediate **45a** efficiently, harsher conditions were required in the case of intermediate **44b**. In this case, hydrated iron(III) chloride adsorbed on silica gel was particularly efficient under microwave heating and cleanly cleaved the dioxolane group and triggered a cyclization-dehydration cascade with concomitant TBS deprotection to form the five-membered ring of intermediate **45b**. A 2,5-*cis*-selective hydrogenation using the conditions described by Bartlett[21] afforded intermediates **46a**,**b**. The amine functional group was introduced using a Mitsunobu inversion of the secondary alcohol using hydrazoic acid or a safer alternative such as PPh_3/DEAD/$(PhO)_2P(O)N_3$, and the resulting azides



Scheme 7. Preparation of ethyl ketones **27** and aldolization key-step. *Reagents and conditions:* a: CDI, THF, rt, then RCH_2CO_2tBu (R = Me, Et), LDA, –78 °C, 91% **40a**, 96% **40b**; b: (i) $(CH_2OH)_2$, TEOF, CSA, rt, (ii) *m*-CPBA, CH_2Cl_2, rt, 58% **41a**, 60% **41b**; c: (i) Me_2CuLi, Et_2O, 0 °C (ii) PDC, DMF, rt, 92 % **27a**,**b** over two steps; d: **27a**,**b**, Chx_2BCl, Et_3N, solvent, 0 °C, then **28**, –78 to –23 °C, 52% **42a**, 61% **42b** Chx_2BCl = dicyclohexylboron chloride. CDI = carbonyl diimidazole, TEOF = triethylorthoformate, *m*-CPBA = *m*-chloroperbenzoic acid, PDC = pyridinium dichromate.



Scheme 8. Final steps completing the synthesis of pamamycin 607 (**1b**) and pamamycin 621D (**1f**). *Reagents and conditions:* a: SmI_2 cat., MeCHO, THF, –10 °C, 59% **44a**, 61% **44b**; b: (i) oxalic acid, CH_2Cl_2, H_2O, (ii) TBAF, THF, rt, 78% **45a** or $FeCl_3.6H_2O/SiO_2$, $CHCl_3$, microwave, 10min, 71% **45b**; c: H_2, 4 bar, Rh/Al_2O_3, MeOH, rt, 90% **46a**, 86% **46b**; d: HN_3, PPh_3, DIAD, toluene, rt, 72% **47a**, or $(PhO)_2P(O)N_3$, DIAD, PPh_3, THF, rt, 5h 86% **47b**; e: H_2, Pd/C, HCHO, AcOH, MeOH, rt, 85% **26a**,**b**. f: (i) TFA, CH_2Cl_2, rt for **26a**, or $MgBr_2$, CH_2Cl_2 for **26b**; (ii) 2,4,6-$Cl_3PhCOCl$, Et_3N, THF, then **25**, DMAP, toluene, 65% **48**, or 2,4,6-$Cl_3PhCOCl$, Et_3N, THF, then **38**, DMAP, toluene, 75% **49** g: (i) Lipase type VII, DMF, H_2O; (ii) $MgBr_2$, CH_2Cl_2, (iii) 2,4,6-$Cl_3PhCOCl$, DMAP, CH_2Cl_2, 53% **1b**, 60% **1f** over three steps. TFA = trifluoroacetic acid, DIAD = diisopropyl azodicarboxylate.

47a,**b** were reduced by hydrogenation in the presence of Pd/C. The addition of formaldehyde to the hydrogenation reaction led to concomitant reductive amination to afford the fully functionalized C(1)–C(18) fragments **26a**,**b**.

The final sequence of steps towards pamamycin 607 (**1b**) followed the order established by the Metz group to avoid epimerization at C(2) during the Yamaguchi esterifications.[11] Deprotection of *tert*-butyl ester **26a** using trifluoroacetic acid and a first Yamaguchi reaction between the resulting acid and the small fragment **25** led to the *seco*-ester **48** in good yield (Scheme 8). The delicate removal of the acetate protecting group was performed using mild enzymatic conditions and saponification of the remaining *tert*-butyl ester led to the macrolactonization precursor, which was cyclized under Fleming conditions[11] to **1b** as pure macrodiolide.

In the course of our recent approach to pamamycin 621D (**1f**), we tried to avoid the lengthy and tedious column chromatography purification needed to separate the aldol addition product **42b** from its regioisomers **43b** by preparing an unsaturated analogue of ketone **27b**, which should deliver only external aldol product (Scheme 9). The sequence started by the treatment of pentanenitrile under Pinner conditions, followed by reaction of the imidate intermediate with ethylene glycol to afford the orthoester **50**. Bromination and subsequent base-promoted elimination afforded the desired orthoester **51** which was reacted with the silyl ketene acetal **53** derived from *tert*-butyl butanoate in the presence of zinc(II) bromide (1 equiv.) to give the protected β-ketoesters **52**. The latter was converted into the more reactive terminal double bond *via* cross metathesis with ethylene. After the reaction was complete, ethylene was removed using several vacuum/argon cycles and ethyl vinyl ketone, carbene **56** and copper iodide were added to the reaction mixture leading to **54**. Using previously published conditions for the enolization of **27a**,**b**, aldol adduct **55** was obtained as pure enantiomer in 66% isolated yield, and subsequently reduced to the known fragment **42b**.[12]

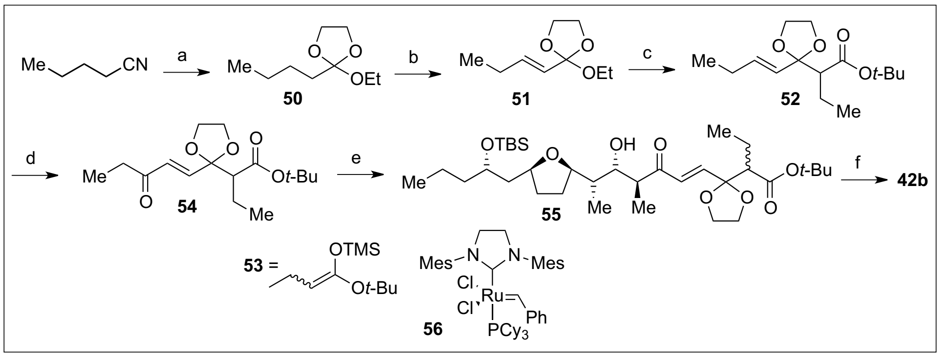
Finally, a slightly modified sequence was applied to the synthesis of **42b** (iron-catalyzed deprotection/cyclization/dehydration of **44b** to prepare **45b** and a Mitsunobu displacement from **46b** using PPh_3/DEAD/$(PhO)_2P(O)N_3$ instead of HN_3) compared to that used for **26a** (Scheme 8)[12] and the C(1)–C(18) precursor of pamamycin 621D (**26b**) was isolated as pure product.

Deprotection of the *tert*-butyl ester in **26b** and subsequent reaction with hydroxyester **38** was achieved leading to the formation of the *seco*-ester **49** in 75% yield (Scheme 8). It is noteworthy that the use of fragment **38** leads to the formation of a *seco*-acid **49** that is epimeric to the natural product at C(2'). However, it considerably shortens the overall length of the synthesis in that both the C(1)–C(18) and C(1')–C(11') fragments can be synthesized from the same precursors. The delicate deprotection of the acetate protecting group required the previously described use of mild enzymatic conditions to avoid side-reactions.[12] This hydrolysis proceeded cleanly in 91% yield, while the subsequent removal of the *tert*-butyl group was quantitative. As previously described by Metz,[5,9,10] macrolactonization under Fleming conditions leads to complete epimerization at C(2') and afforded pamamycin 621D (**1f**) in 60% yield.

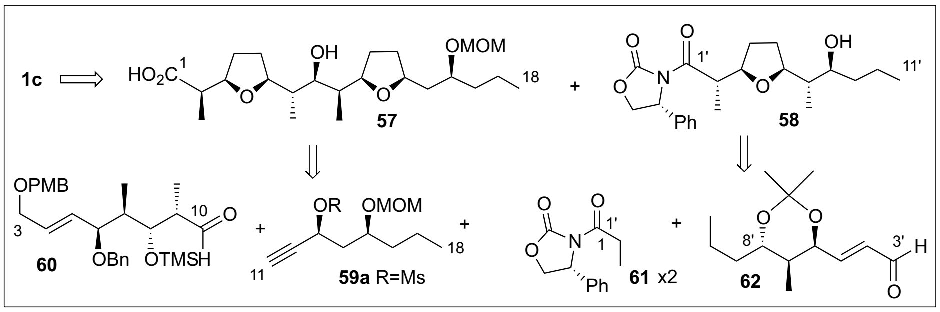
Wu's Total Synthesis of Pamamycin 621A (1c)[22]

Based on previous work on the synthesis of nonactic acid,[23] Wu and coworkers developed a convergent strategy towards the total synthesis of pamamycin 621A, using an Evans/Crimmins aldolization as key step, and intramolecular O-alkylation for the construction of the tetrahydrofuran rings.[24]

One more time, the macrodiolide core of **1c** was first disconnected into the C(1)–C(18) (**57**) and C(1')–C(11') (**58**) hydroxyacids. Fragment **57** was then further simplified into three key fragments, alkyne **59a**, aldehyde **60** and enantiomerically pure acyloxazolidinone **61** (Scheme 10). Similarly, the smaller fragment **58** was



Scheme 9. Preparation of the unsaturated ethyl ketone **54** and its aldol condensation with aldehyde **28**. *Reagents and conditions:* a: (i) HCl, EtOH, i-Pr_2O, –20 °C, overnight, (ii) ethylene glycol, HCl, 24 h, 76% **50**; b: (i) Br_2 (0.5 equiv.), pyridine (1.1 equiv.), CH_2Cl_2, 0 °C to rt, 24 h, (ii) *t*-BuOK (1.2 equiv.), DMSO, 0 °C to rt, overnight, 72% **51**; c: **53**, $ZnBr_2$, CH_2Cl_2, rt, 76% **52**; d: C_2H_4, **56** (3 mol%), toluene then (i) degas, (ii) ethyl vinyl ketone, **56** (5 mol%), CuI (10 mol%), 84% **54**; e: Chx_2BCl, Et_3N, Et_2O, 0 °C, then **28**, –78 to 0 °C, 66% **55**; f: H_2, Pd/C (5%), NH_4OH, MeOH, 88% **42b**.



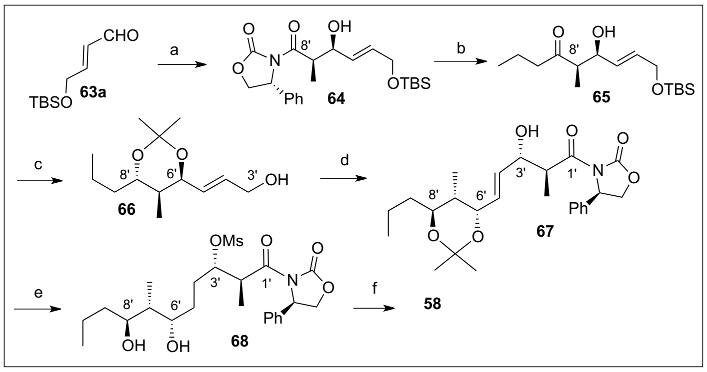
Scheme 10. Wu's retrosynthetic analysis of **1c**.

retrosynthetically simplified *via* C–O disconnection at the THF ring and the resulting polypropionate disconnected between C(2')–C(3') into aldehyde **62** and the same acyloxazolidinone **61**.

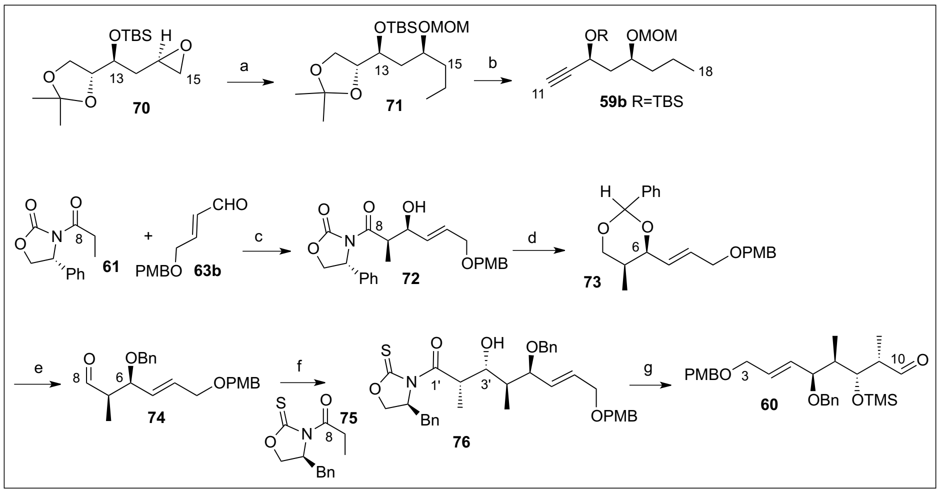
The preparation of **58** began with a Crimmins aldol addition between enal **63a** and enantiomerically pure acyloxazolidinone **61** (Scheme 11). The resulting aldol **64** (dr 23:1) was reacted with AlMe$_3$/MeNH(OMe)·HCl and *n*-PrMgBr sequentially to afford ketone **65**. Treatment of **65** with Me$_4$NBH(OAc)$_3$ installed the C(8') stereogenic center and subsequent deprotection of the silyl ether and acetonide formation from the diol provided alcohol **66**, which upon oxidation using the Dess-Martin periodinane produced aldehyde **62**. In sharp contrast with literature, Evans aldol addition using aldehyde **62** was found to occur only if stoichiometric amounts of MgCl$_2$ were present. Thus, MgCl$_2$ (2 equiv.) / Et$_3$N (5 equiv.) in the presence of 3 equiv. of TMSCl and subsequent treatment with DDQ led to **67** in 56% overall yield. The alcohol **67** was converted into **68** by hydrogenation of the alkene, mesylation of the alcohol at C(3'), and removal of the acetonide, affording the correct functionalization for the formation of the THF ring by intramolecular nucleophilic displacement with inversion at C(3'). Thus, by heating **68** in neat pyridine at 110 °C for 3 h, **58** was obtained in 63% yield as a single isomer.

According to the synthetic plan (Scheme 10), the more complex fragment **57** came from the double intramolecular O-alkylation of the linear intermediate **69** which was obtained from its precursors **59** (the C(11)–C(18) fragment) and **60** (the C(3)–C(10) fragment). Alkyne **59** was prepared according to Scheme 12 starting from the enantiomerically pure epoxide precursor **70**. The latter[25] was readily prepared from inexpensive D-gluconolactone through a convenient route developed previously by Wu and coworkers.[26] Ring opening of **70** with EtMgBr/CuCN in THF followed by protection of the alcohol as MOM ether led to fully protected polyol **71**. Selective removal of the acetonide using CF$_3$COOH in dichloromethane at ambient temperature afforded the desired alkyne **59** after oxidative cleavage of the resulting diol and Corey-Fuchs alkynylation.

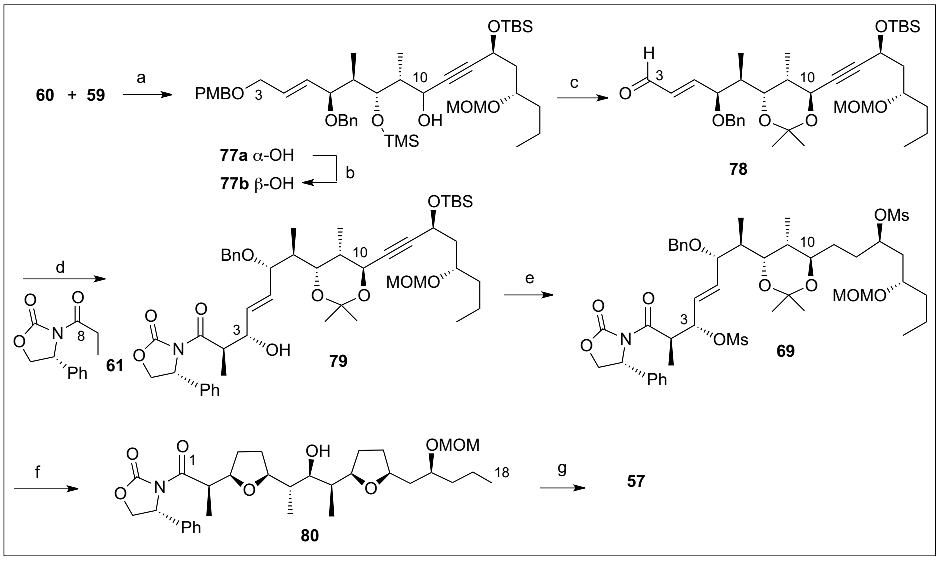
Fragment **60** was prepared using two asymmetric Evans/Crimmins aldol reactions to control the absolute configurations of the C(6)–C(9) stereotetrad (Scheme 12). The first aldol condensation with enal **63b** gave β-hydroxy carbonyl compound **72** and reductive cleavage of the chiral auxiliary followed by protection with benzaldehyde dimethyl acetal afforded **73** in 91% yield. Reduction of the acetal using DIBAL-H and oxidation of the resulting alcohol by the Dess-Martin periodinane afforded aldehyde **74**. The chain was further extended by two carbons by an aldol addition of **74** with acyl-substituted oxazolidinethione **75** under Crimmins' conditions using (–)-sparteine as base. Protection of the alcohol in the aldol product **76** as a trimethylsilyl (TMS) ether (facilitating purification at a later stage) preceded the removal of the chiral auxiliary which



Scheme 11. Wu's preparation of the smaller fragment **58** of pamamycin 621A (**1c**). *Reagents and conditions:* a: **61**, TiCl$_4$, TMEDA, 84% **64** dr 23:1, b: (i) MeNH(OMe)/AlCl$_3$, (ii) *n*-PrMgBr, 90% **65**, c: (i) Me$_4$NBH(OAc)$_3$, MeCN/AcOH, –20 °C, (ii) TBAF/THF, (iii) MeC(OMe)$_3$ CSA, (iv) 70% AcOH, 75% **66** from **65**, d: (i) Dess-Martin periodinane, (ii) **61**, MgCl$_2$ (2 equiv.), Et$_3$N (3 equiv.), TMSCl (3 equiv.), (iii) DDQ, THF/water, 56% **67**, e: (i) H$_2$/Pd-C, Et$_3$N, EtOAc, (ii) Et$_3$N, MsCl, CH$_2$Cl$_2$, 1N HCl, THF-MeOH 89% **68**, f: pyridine 110 °C, 2 h, 62% **58** dr 95:5. TMEDA: tetramethyl ethylene diamine, DDQ: 2,3-dichloro-5,6-dicyano-1,4-benzoquinone.



Scheme 12. Wu's preparation of subunits **59** and **60** of the larger fragment **57** of pamamycin 621A (**1c**). *Reagents and conditions:* a: (i) EtMgBr/CuCN, THF, –10 °C, (ii) MOMCl, Hünig base, 94% **71** over two steps; b: (i) TFA/water, CH$_2$Cl$_2$, (ii) NaIO$_4$/silica gel/CH$_2$Cl$_2$-water, (iii) Ph$_3$P/CBr$_4$, CH$_2$Cl$_2$, (iv) *n*-BuLi, THF, –10 °C, 81% **59** from **71**; c: TiCl$_4$, TMEDA, 84% **72** (dr 20:1); d: (i) NaBH$_4$, THF/water 0 °C to rt, (ii) PhCH(OMe)$_2$, CSA, 85% **73**; e: (i) DIBAL-H, CH$_2$Cl$_2$ 0 °C to rt, (ii) Dess-Martin periodinane, CH$_2$Cl$_2$, NaHCO$_3$ 89% **74** over two steps; f: TiCl$_4$, CH$_2$Cl$_2$, (–)-sparteine, –78 °C, 2h, 94% **76**, (dr 95.5); g: (i) TMSCl, 2,6-lutidine, DMF, (ii) DIBAL-H, CH$_2$Cl$_2$, 95% **60** over two steps. MOMCl = methoxymethyl chloride.

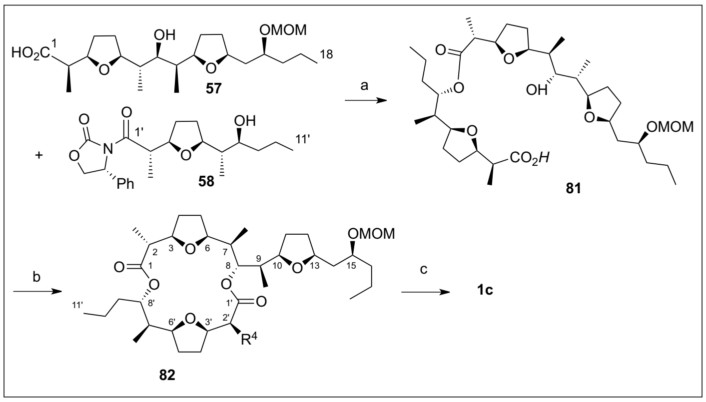


Scheme 13. Wu's preparation of the larger fragment **57** of pamamycin 621A (**1c**) . *Reagents and conditions:* a: *n*-BuLi, THF, –20 °C, 100% **77a/77b** (2:3); b: Dess-Martin periodinane, (ii) L-selectride, THF, –78 °C, 88% **77b** over two steps; c: (i) TFA/CH_2Cl_2 50%, rt, (ii) $Me_2C(OMe)_2$/PPTS, CH_2Cl_2, (iii) CH_2Cl_2 buffer (*p*H 7) 10:1, DDQ, (iv) Dess-Martin periodinane, 85% **78** from **77b**; d: $TiCl_4$/TMEDA, CH_2Cl_2, rt, 90% **79** (dr 23:1); e: (i) HF-pyr, THF, rt, (ii) H_2/Pd-C, EtOAc/Et_3N, (iii) MsCl, Et_3N, CH_2Cl_2, rt 84% **69** over three steps; f: (i) TFA/CH_2Cl_2 50%, rt, (ii) H_2/Pd-C, THF, rt, (iii) 2,6-lutidine, 120 °C, 2h, 93% **80** over three steps, g: H_2O_2/LiOH, THF, rt, 94% **57**. PPTS = *p*-tolylsulfonic acid.

afforded the desired aldehyde **60**. The latter was quite instable and was immediately reacted with the lithium alkynylide of **59b** to produce a separable mixture (2:3) of **77a** and **77b** (Scheme 13).

The undesired isomer **77a** could be converted into **77b** by an oxidation-reduction sequence. Aldehyde **78**, needed to control the C(2) and C(3) stereogenic centers in the backbone of the large fragment *via* Crimmins aldolization, was prepared from **77b** by sequentially (i) removing the TMS protecting group, (ii) protecting the 1,3-diol as an acetonide, (iii) cleaving the *para*-methoxybenzyl ether, and (iv) oxidizing the resulting primary alcohol using Dess-Martin periodinane. Treatment of **78** with the titanium enolate of oxazolidinone **61** in the presence of TMEDA gave adduct **79** with a good isolated yield. After removal of the silyl protecting group on the homopropargylic alcohol with HF-pyr, the alkene and alkyne were hydrogenated and the two secondary alcohols were mesylated to afford intermediate **69**. Cleavage of the ketal protecting group in **69** using 50% aqueous TFA followed by hydrogenolysis of the benzyl protecting group gave, after heating with 2,6-lutidine and subsequent treatment with hydrogen peroxide and lithium, the larger fragment of pamamycin 621A (**57**) as pure compound.

The coupling of **57** and **58** under Yamaguchi conditions followed by treatment of the resulting *seco*-oxazolidinone with H_2O_2/LiOH to remove the chiral auxiliary gave compound **81** (Scheme 14). The macrodiolide was then closed using another Yamaguchi esterification at higher temperature to give **82**. The acetal protecting group was then removed with 10% of HBr solution and the deprotected alcohol at C(15) was converted into an azide with inversion of configuration using triphenylphosphine, DEAD, and diphenyl azidophosphonate to introduce an azido group at C(15) with concurrent inversion of the configuration. The unexpectedly difficult azide reduction was finally achieved using tributyltin hydride in refluxing toluene in the absence of any added radical initiators. The resulting amine was methylated using standard reductive amination conditions to give pamamycin 621A (**1c**).



Scheme 14. Endgame of Wu's total synthesis of pamamycin 621A (**1c**). *Reagents and conditions:* a: (i) 2,4,6-trichlorobenzoyl chloride, DMAP, MS 4 A, CH_2Cl_2, rt, (ii) H_2O_2/LiOH, THF, rt, 80% **81**; b: 2,4,6-trichlorobenzoyl chloride (20 equiv.), DMAP (20 equiv.)/NEt_3 (20 equiv.), toluene, 110 °C, 66% **82**; c: (i) 10% HBr in CH_3CN, (ii) PPh_3/DEAD, $(PhO)_2P(O)N_3$, THF, rt, (iii) *n*-Bu_3SnH, toluene, reflux, 2h, (iv) $NaBH_3CN$, AcOH, 37% HCHO, CH_3CN, 82% **1c**.

Thomas' Total Synthesis of Pamamycin 607 (1b)[27]

Ten years after a preliminary communication on the first total synthesis of pamamycin 607 (**1b**),[7,9] E. J. Thomas reported a full description of the same synthesis along with methyl nonactate using an intramolecular selenoetherification of (*Z*)-homoallylic alcohols. The desired 1,5-*anti* isomers of the latter were prepared stereoselectively by a $SnCl_4$-promoted addition of 4-methyl-5-alkoxy-2-pentenylstannanes to the requisite aldehydes. This method displayed a high level of remote asymmetric induction.[27]

Biological Activity and Structure–Activity Relationships

As mentioned previously, in addition to their complex structures, the synthetic efforts towards the pamamycins have also been fuelled by their biological activity. The more recent results (after 2005) are summarized below. In this context, Natsume and coworkers[28] recently examined the effect of pamamycin 607 (**1b**) on antibiotic production by several *Streptomyces* spp. They observed that this macrodiolide increased the puromycin production by 2.7 fold in the pamamycin producer, *S. alboniger* NBRC 12738, and also increased the synthesis of streptomycin by 1.5 fold in *S.*

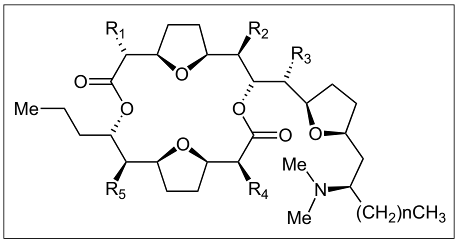


Fig. 2. Pamamycin analogues used in the structure–activity relationship.

griseus NBRC 12875 and that of cinerubins A and B by *S. tauricus* JCM 4837 by 1.7 and 1.9 fold. Finally, pamamycin 607 enhanced 2.6 fold the production of the antibiotic virginiamycin M$_1$, synthesized by *Streptomyces* sp. 91-a. Pamamycin 607's mode of action in this context has not yet been elucidated.

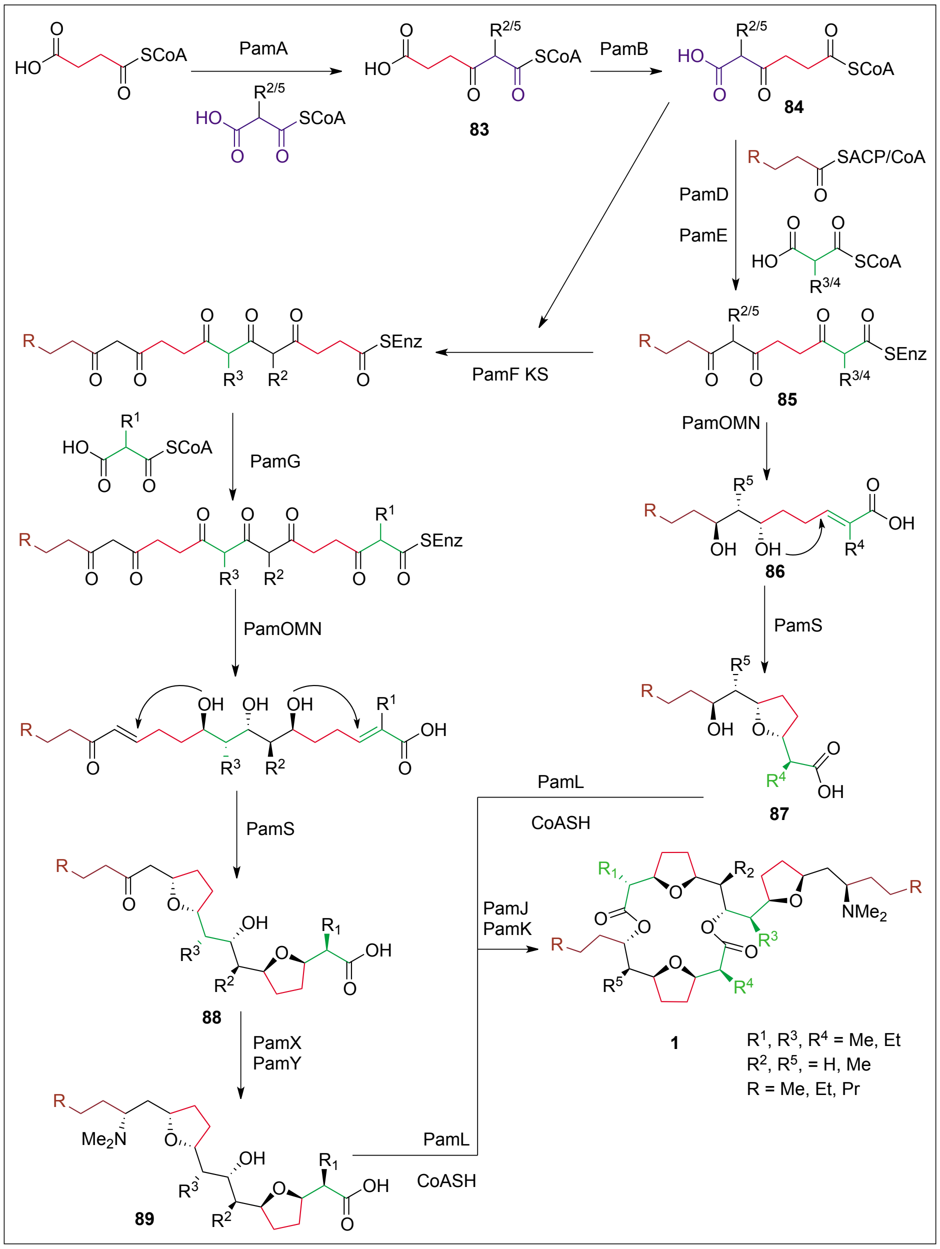
The structure–activity relationship of pamamycins has mainly been studied by Natsume *et al.* In a recent report,[29] they determined the effect of side chain length on aerial mycelium-inducing activity by isolating two pamamycin side chain homologues from *Streptomyces* sp HKI-0118: homopamamycin-621A (R$_1$–R$_5$ identical to **1b**, n = 3, Fig. 2) and bishomopamamycin-635A (R$_1$–R$_5$ identical to **1b**, n = 4, Fig. 2). Taking into account this study, they summarized the structure–activity relationship of pamamycins published by them and other authors in the recent years. Thus, opening of the macrodiolide ring or scission into two constituent hydroxyl acids results in a marked drop in activity. The amine group is indispensable to aerial mycelium-inducing activity and *N*-demethylation increases this activity 1.5 times. Substitution of methyl group R$_1$ with an ethyl group or hydrogens R$_2$ and R$_5$ by a methyl group decreased the activity by *ca.* 1/10. Substitution of the methyl group at R$_3$ or R$_4$ with an ethyl group resulted in a sharp decrease in activity. Finally, the side chain homologue homopamamycin-621A (n=3) exhibited only 1/10th of the original activity and bishomopamamycin-635A (n=4) was found to be inactive.

Even if in the current state of things pamamycins cannot be considered as potential drugs, the oral bioavailability of two pamamycin analogues has been discussed in the literature. Indeed, Lipinski's rule of five is a rule of thumb to evaluate drug-likeness or determine a chemical compound with a certain pharmacological or biological activity that would be likely to be an orally active drug in humans. Belaidi's team[30] has shown that pamamycins 607 and 621 do not obey the Lipinski rule. Thus, even if they don't have any hydrogen bond donors and less than 10 hydrogen bond acceptors, their molecular mass is superior to 500 Da (607.87 and 621.90) and their calculated octanol-water partition coefficient (log P) is greater than 5 (5.36 and 5.86). In general, orally active drugs have no more than one violation of Lipinski's rule. The two violations for pamamycins 607 suggest that these compounds theoretically would have problems with oral bioavailability.

Biosynthesis of Pamamycins

Natsume *et al.* studied the biosynthetic origins of the carbon skeleton and nitrogen atom of pamamycin 607 by feeding experiments with ^{13}C-acetate or –propionate, L-[^{15}N] glutamic acid or valine, or ^{15}NH$_4$Cl in *Streptomyces alboniger* in 2005.[31] Results show that the carbon skeleton of pamamycin 607 is derived from six acetate, four propionate and three succinate units. By MS analyses of ^{15}N-labeled pamamycin 607 it was demonstrated that the nitrogen atom of the dimethylamino group present in pamamycins is derived from the α-amino group of an amino acid which has been introduced into the pamamycin structure by transamination, followed by N-methylation. The same authors have investigated the nitrogen incorporation in the biosynthetic pathway of pamamycin using blocked mutants in *Streptomyces* alboniger.[32] They concluded that the amination and methylation occur before the closure of the macrodiolide ring.

Very recently Metz, Petzke and Luzhetskyy elucidated the biosynthesis of the pamamycins (**1**) by studying the enzyme(s) that incorporate succinate.[3] They identified a pamamycin biosynthesis gene cluster by aligning genomes of two pamamycin-producing strains. This unique cluster contains polyketide synthase (PKS) genes encoding seven discrete ketosynthase (KS) enzymes and one acyl-carrier protein (ACP)-encoding gene. A cosmid containing the entire set of genes required for pamamycin biosynthesis was successfully expressed in a heterologous host. Genetic and biochemical studies allowed complete delineation of pamamycin biosynthesis. Thus, as depicted in Scheme 15, the pathway commences with the formation of the key compounds 4-oxoadipyl-CoA and



Scheme 15. Proposed biosynthetic pathway of pamamycins (**1**).

5-methyl-4-oxoadipyl-CoA **83** in a condensation reaction that involves either malonyl- or methylmalonyl-CoA reacting with succinyl-CoA and is catalyzed by a PamA. The subsequent rotation of compounds **83** by PamB acyltransferase afforded **84**. The carbon chain of intermediates **84** are then extended twice: first with a short-chain acyl, supplied probably as ACP-ester, under PamD catalysis and then by the addition of malonyl-CoA under PamE catalysis by a Claisen-type condensation leading to intermediate **85**. Then, from compound **85**, the biosynthetic pathway splits into two parallel routes. On the one hand, intermediate **85** is transformed by KRs PamO, M, and N into the unsaturated diol **86**. Intramolecular cyclization to the corresponding THF ring catalyzed by PamS, leads to the production of **87**. On the other hand, the addition of an additional adipate **84** catalyzed by PamF to intermediate **85** precedes the completion of the carbon chain *via* condensation of malonate catalyzed by PamG. After ketone group reductions, two dehydrations and two 1,4 intramolecular additions catalyzed by PamO, M, N, and S, compound **88** bearing two tetrahydrofuran rings is obtained. Finally, **88** undergoes further reductive amination and methylation, respectively by Pam X and PamY, to afford **89**. Hydroxy acids **87** and **89** are activated an additional time by the acyl-CoA ligase PamL. Completion of the biosynthesis of **1** is achieved by PamJ and PamK which catalyze the final cyclization involving an uncommon C–O condensation reaction.

In addition to their unique structures, the potential uses of pamamycins have attracted a great deal of interest in the synthetic community and several total synthesis of some members of this family of macrodiolides have been published in recent years as we have detailed in the first part of this report. Nevertheless, in order to enable technical applications, methods that provide sufficient amounts of pamamycins are still necessary. In this context, since very recently, polypeptides and polynucleotides as well as gene clusters, expression cassettes and vectors comprising one or several of those polynucleotides for the production of pamamycins are accessible. These tools can thus be used to construct, identify and improve microorganisms having the capacity to produce one or several pamamycins, in particular pamamycin 607 (**1b**) and/or pamamycin 621A (**1c**).[4]

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