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

Gilles Hanquet*, Xavier Salom-Roig, and Steve Lanners

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. Besides 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. Since the isolation of a first member of pama- mycin family with a molecular formula of C_{36}H_{58}NO_{3} and a molecular weight of 621 Da from Streptomyces alboniger by McCann and Pogell in 1979,[4] 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).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 macrodiolides 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[6] and recombinant microorganisms have been developed for the production of pamamycins, in particular pamamycins 607 (1b) and 621A (1e).[8] 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...
in 1988. 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.

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. However, most studies were directed toward the formation of the two diastereomers or pure the two diastereomers of the OH by a phenylthio group elimination and alkoxide-directed 1,6-addition, the bicyclic compounds 4, 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. 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). The latter was reacted with two equiv. allylithium 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 Apel reaction afforded the iodides 11a, b.

Halogen-lithium exchange of iodides 11 and subsequent addition of 2-acetyl furan 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 alkylfurans 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 alkylithium led to the syn-selective introduction of a methyl or ethyl group (15a, b) via the previously described elimination/alkoxide-directed 1,6-addition and ozonolysis followed by treatment of the resulting mixture under eliminative conditions delivering the expected hemiketals 15a, b as single stereoisomers. Treat-
ment of hemiketals 15a,b with thiophenol and trifluoroborane led to the formation of a thiolactol 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 hydrozaic 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 trans-esterification 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 24e,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
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 retrom 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 enantioselectively pure sulfoxide 30 as chiral auxiliary.

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 Claissen-type condensation with the anion of (–)-(S)-methyl-p-tolysulfoxide (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 Krarpos-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 hydrog enation 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 acylmimidazole 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,BCl/ Et,N[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 regioselective.[19] 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 hydroxy 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(II) 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 PhH/DEAD/ (PhO)2P(O)NCl, and the resulting azides

**Scheme 6.** Hanquet preparation of C(8)–C(18) and C(1′)–C(11′) fragments 25 and 25 of pamamycin 607 (1b) and 621D (1f).

Reagents and conditions: a: i) Ethylene glycol, TMSCI, CH3Cl, 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% from 32 (de > 95%); c: MeNBH/OAc, AcOH, 97% 34; d: i) t-BuBr, CHCl3, 50 °C; (ii) Me2OSi, CHCl3, 20 °C; (iii) K2CO3, 75% 35; e: i) TBDPSi, imidazole, DMF, (ii) ethyl malonate, EtoNa, THF, –78 °C; (iii) MgCl2,EtOH, DMF, reflux, 73% 36; f: t-butylicarbonate, LDA, THF, –78 °C, (ii) oxalic acid, CH3Cl, 75% 29; g: LDA 2 equiv, LiCl 3 equiv, THF, then EtoH, 70% 37; h: (i) TBAF, THF, (ii) H2, MeOH, Rh, Al2O3, 4 bars, 73% 38 and 67% 25; i: (i) TBSiCl, imidazole, DMF, (ii) LAH, ether, (iii) SO2-pyridine, Hunig base, CH2Cl2, 89% 28. LDA = lithium diisopropylamide, TBAF: tetra-butyl ammonium fluoride.

**Scheme 7.** Preparation of ethyl ketones 27 and aldozation key-step. Reagents and conditions: a: CDI, THF, rt, then RCH2CO2Bu (R = Me, Et), LDA, –78 °C, 91% 40a, 96% 40b; b: (i) CH3OH, TEOS, CSA, rt, (ii) m-CPBA, CH2Cl2, rt, 58% 41a, 60% 41b; c: (i) Me2CuL2, Et2O, 0 °C (ii) PDC, DMF, rt, 92% 27a,b over two steps; d: 27a,b, Chx2BBr, Et2N, solvent, 0 °C, then 28, –78 to –23 °C, 52% 42a, 61% 42b. Chx2BBr = diethyleneoxyboron chloride, CDI = carbonyl diimidazole, TEOS = triethyloxyorthoformate, m-CPBA = m-chloroperbenzoic acid, PDC = pyridinium dichromate.
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 macrocyclization precursor, which was cyclized under Fleming conditions\(^{[11]}\) to 1b as pure macrodilide.

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 pentaenitrole 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 2-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\(_3\) and benzyl butanoate 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 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.\(^{[12]}\)

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One more time, the macrodiolide core of 1f 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 prepared in 60% yield (Scheme 9).
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 AlMe3/MeNH2/OMe/HCl and n-PrMgBr sequentially to afford ketone 65. Treatment of 65 with MeNBH(OAc)2 installed the C(8’) stereogenic center and subsequent deprotection of the silyl ether and acetamide 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 MgCl2 were present. Thus, MgCl2 (2 equiv.) / Et3N (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 acetamide, 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 59 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-glucalactonea through a convenient route developed previously by Wu and coworkers.252 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 acetamide using CF3COOH in dichlormethane 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 DBHAL-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 oxazolidinone 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 was prepared according to Scheme 11 starting from the dendrimeric epoxide precursor 1c.

Scheme 10. Wu’s retrosynthetic analysis of 1c.

Scheme 11. Wu’s preparation of the smaller fragment 58 of pamamyacin 621A (1d). Reagents and conditions: a: 61, TiCl4, TMEDA, 84% 64 dr 23:1; b: (i) MeNH2/OMe/AICl3, (ii) n-PrMgBr, 90% 65; c: (i) MeNBH(OAc)2, MeCN/AcOH, –20 °C, (ii) TBAA/THF, (iii) MeCr(OMe)2 CSA, (iv) 70% AcOH, 75% 66 from 65; d: (i) Dess-Martin periodinane, (ii) 61, MgCl2 (2 equiv.), Et3N (3 equiv.), TMSCI (3 equiv.), (iii) DDO, THF/water, 56% 67; e: (i) H+/Pd-C, Et3N, EtOAc, (ii) Et3N, MeSiCl, CH2Cl2, 1N HCl, THF-MeOH 89% 68; f: pyridine 110 °C, 2 h, 62% 58 dr 95:5. TMEDA: tetramethyl ethylene diamine, DQ: 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 pamamyacin 621A (1c). Reagents and conditions: a: (i) EtMgBr/CuCN, THF, –10 °C, (ii) MOMCI, Hüng base, 94% 71 over two steps; b: TFA/water, CH2Cl2, (i) NaIO4/silica gel/CH2Cl2-water, (ii) PhOP/Br(CH2Cl2), (iii) n-BuLi, THF, –78 °C, 81% 59 from 71; c: TiCl4, TMEDA, 84% 72 (dr 20:1); d: NaBH4, THF/water 0 °C to rt; e: PhCHO/OMe2, CS, 85% 73; e: (i) DiBAl-H, CH2Cl2, 0 °C to rt, (ii) Dess-Martin periodinane, CH2Cl2, NaHCO3, 89% 74 over two steps; f: TiCl4, CH2Cl2, (-)-sparteine, –78 °C, 2h, 94% 76, (dr 95:5); g: (i) TMSCI, 2,6-lutidine, DMF, (ii) DiBAl-H, CH2Cl2, 95% 60 over two steps. MOMCl = methoxymethyl chloride.
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 acetone, (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 \( \text{H}_2\text{O} / \text{LIOH} \) to remove the chiral auxiliary gave compound 81 (Scheme 14). The macrondiolide was then closed using another Yamaguchi esterification at higher temperature to give 82. The acetol 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 azido-phosphonate 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).

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

Ten years after a preliminary communication on the first total synthesis of pamamycin 607 (1b), \[29\] E. J. Thomas reported a full description of the same synthesis along with methyl nonactate using an intramolecular selenoetherification of (Z)-homoolylic alcohols. The desired 1,5-anti isomers of the latter were prepared stereoselectively by a SnCl\(_2\)-promoted addition of 4-methyl-5-allyloxy-2-pentenyl-stannanes 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 macrondiolide 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 \( S. \)
Pamamycin 607 enhanced 2.6 fold the production of the antibiotic virginiamycin M, 1.7 and 1.9 fold. Finally, pamamycin 607’s mode of action in this context has not yet been elucidated.

The structure–activity relationship of pamamycins has primarily 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. HK1-0118: homopamamycin-621A (R = R = R = R = R = R = 1b, n = 3, Fig. 2) and bishomopamamycin-635A (R = R = R = R = 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 substitution of methyl group R 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 [15N] glutamic acid or valine, or [14C]-acetate or –propionate, [13C]acetate or –propionate, or [15N] glutamic acid or valine. Results show that the carbon skeleton of pamamycin 607 is derived from six acetate, four propionate and three succinate units. By MS analyses of [15N]-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.[31] They concluded that the amination and methylation occur before the closure of the macrodiolide ring.

Very recently Metz, Petzke and Luzhetsky elucidated the biosynthesis of the pamamycins (1) by studying the enzyme(s) that incorporate succinate.[32] 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

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**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 KR’s 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 macrodilides 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 these 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).14

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1 [a] P.A. McCann, B. M. Pogell, J. Antibiot. 1979, 32, 673; b) S. Kondo, K. Yasui, M. Natsume, M. Katayama, S. Marumo, J. Antibiot. 1988, 41, 1196; c) P. Lefèvre, P. Peirs, M. Brabant, M. Fauville-Dufaux, R. Vanhoof, K. Haygen, X. M. Wang, B. Pogell, Y. Wang, P. Fischer, P. Metz, J. Content, J. Antimicrob. Chemother. 2004, 54, 824.
2 [a] M. Natsume, K. Yasui, S. Kondo, S. Marumo, Tetrahedron Lett. 1991, 32, 3087; b) M. Natsume, J. Tazawa, K. Yagi, H. Abe, S. Kondo, S. Marumo, J. Antibiot. 1995, 48, 1159; c) I. Kozono, N. Chikamoto, H. Abe, M. Natsume, J. Antibiot. 1999, 52, 329.
3 [a] Y. Rebets, E. Brötz, N. Manderscheid, B. Tokovenko, M. Myronovskij, P. Metz, L. Petzke, A. Luhertzsky, Angew. Chem. Int. Ed. 2015, 54, 2280.
4 [a] L. Petzke, A. Herold, C. Fleck, K. Treier-Marxen, P. Odman, J. Dickhaut, M. Weingarten, A. Luhertzsky, Y. Rebets, E. Brötz, N. Manderscheid, M. Myronovskiy, 2015, WO2015/092575 A1.
5 [a] For reviews covering the subject prior to 2005, see: a) P. Metz, Top. Curr. Chem. 2005, 244, 215; b) E. J. Kang, E. Lee, Chem. Rev. 2005, 105, 4348.
6 [a] C(1′)–C(11′) fragment of pamamycin 635A: A. Miura, H. Kiyota, S. Kuwahara, Tetrahedron 2005, 61, 1061; b) (C6–C10) domain of pamamycin 607: B.H. Fraser, R.J. Mulder, P. Perlmutter, Tetrahedron 2006, 62, 2857; c) (C1′–C18) fragment of Pamamycin 593 and de-N-methylpamamycin 579: A. Miura, S. Takigawa, Y. Furuya, Y. Yokoo, S. Kuwahara, H. Kiyota, Eur. J. Org. Chem. 2008, 4955.
7 [a] R. D. Walkup, G. Park, Tetrahedron Lett. 1988, 29, 5805.
8 [a] O. Germay, N. Kumar, E. J. Thomas, Tetrahedron Lett. 2001, 42, 4969.
9 [a] P. Fischer, A. B. García Segovia, M. Gruner, P. Metz, Angew. Chem. Int. Ed. 2005, 44, 6231.
10 [a] P. Fischer, M. Gruner, A. Jager, O. Kataeva, P. Metz, Chem. Eur. J. 2011, 17, 13334.
11 [a] Y. Wang, H. Bernsmann, M. Gruner, P. Metz, Tetrahedron Lett. 2001, 42, 7801; b) I. Fleming, S. K. Ghosh, J. Chem. Soc. Perkin Trans. 1 1998, 2733.
12 [a] S. Lanners, H. Norouzi-Arasi, X. J. Salom-Roig, G. Hanquet, Angew. Chem. Int. Ed. 2007, 46, 7086.
13 [a] S. Lanners, H. Norouzi-Arasi, X. J. Salom-Roig, G. Hanquet, to be published.
14 [a] G. Hanquet, F. Colobert, S. Lanners, G. Solladié, ARKIVOC 2003, vii, 328.
15 [a] G. Hanquet, X. J. Salom-Roig, L. Gressot-Kempf, S. Lanners, G. Solladié, Tetrahedron: Asymmetry, 2014, 14, 1291.
16 [a] D. A. Evans, K. T. Chapman, E. M. Carreira, J. Am. Chem. Soc. 1988, 110, 3560.
17 [a] G. Hanquet, X. J. Salom-Roig, S. Lemeitour, G. Solladié, Eur. J. Org. Chem. 2002, 2112.
18 [a] H. C. Brown, K. Ganesan, R. K. Dhar, J. Org. Chem. 1993, 58, 147; b) H. C. Brown, K. Ganesan, J. Org. Chem. 1993, 58, 7162.
19 [a] S. Lanners, H. Norouzi-Arasi, N. Khiri, G. Hanquet, Eur. J. Org. Chem. 2007, 4065.
20 [a] D. A. Evans, A. H. Hoveryda, J. Am. Chem. Soc. 1999, 120, 6447.
21 [a] P. A. Bartlett, J. D. Meadowes, E. Ottow, J. Am. Chem. Soc. 1984, 106, 5304.
22 [a] G. B. Ren, Y. K. Wu, Org. Lett. 2009, 11, 5638; b) G. B. Ren, Y. X. Huang, Y. P. Sun, Z. H. Li, Y. K. Wu, J. Org. Chem. 2010, 75, 5048; c) G. Ren, Y. K. Wu, Chin. J. Chem. 2010, 28, 1651.
23 [a] Y. K. Wu, Y. P. Sun, Org. Lett. 2006, 8, 2831.
24 [a] Y. K. Wu, Synlett 2013, 24, 1623.
25 [a] J. Mulzer, C. Pietschman, B. Schollhorn, J. Buschmann, P. Luger, Liebigs Ann. 1995, 1433.
26 [a] Z. J. Wu, J. Gao, G. B. Ren, Z. B. Zhen, Y. H. Zhang, Y. K. Wu, Tetrahedron 2009, 65, 289.
27 [a] O. Germay, N. Kumar, C. G. Moore, E. J. Thomas, Org. Biomol. Chem. 2012, 10, 9709.
28 [a] M. Hashimoto, H. Katsura, R. Kato, H. Kawaide, M. Natsume, Biosci. Biotechnol. Biochem. 2011, 75, 1722.
29 [a] I. Kozono, M. Hashimoto, U. Gräfe, H. Kawaide, H. Abe, M. Natsume, J. Antibiot. 2008, 61, 98.
30 [a] R. Manzi, S. Belaidi, A. Kerassa, T. Lanez, Int. Lett. Chem. Phys. Astronomy 2014, 33, 146.
31 [a] M. Hashimoto, H. Koma, I. Kozono, H. Kawaide, H. Abe, M. Natsume, Biosci. Biotechnol. Biochem. 2005, 69, 315.
32 [a] M. Hashimoto, I. Kozono, H. Kawaide, H. Abe, M. Natsume, J. Antibiot. 2005, 58, 722.