Retrosynthetic and Synthetic Chemistry on Amphotericin B. Synthesis of C(1)—C(20) and C(21)—C(38) Fragments and Construction of the 38-Membered Macrocycle

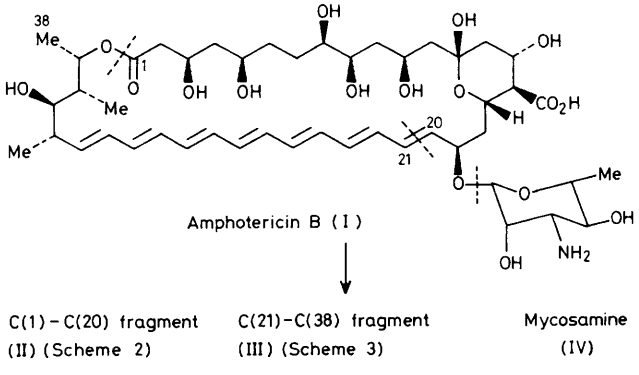
K. C. Nicolaou,*† T. K. Chakraborty, R. A. Daines, and N. S. Simpkins

Department of Chemistry, University of Pennsylvania, Philadelphia, PA 19104, U.S.A.

For the first time, amphotericin B (I) has been successfully derivatized and degraded to intermediates that have been converted into compounds (II) [C(1)—C(20) fragment] and (III) [C(21)—C(38) fragment], projected as major key intermediates for a total synthesis; methods have been developed for the coupling of fragments (II) and (III) to give the ketophosphonate–aldehyde (**28**) and for the cyclization of this precursor to the 38-membered macrocyclic heptaenone (**29**) in 70—80% yield.

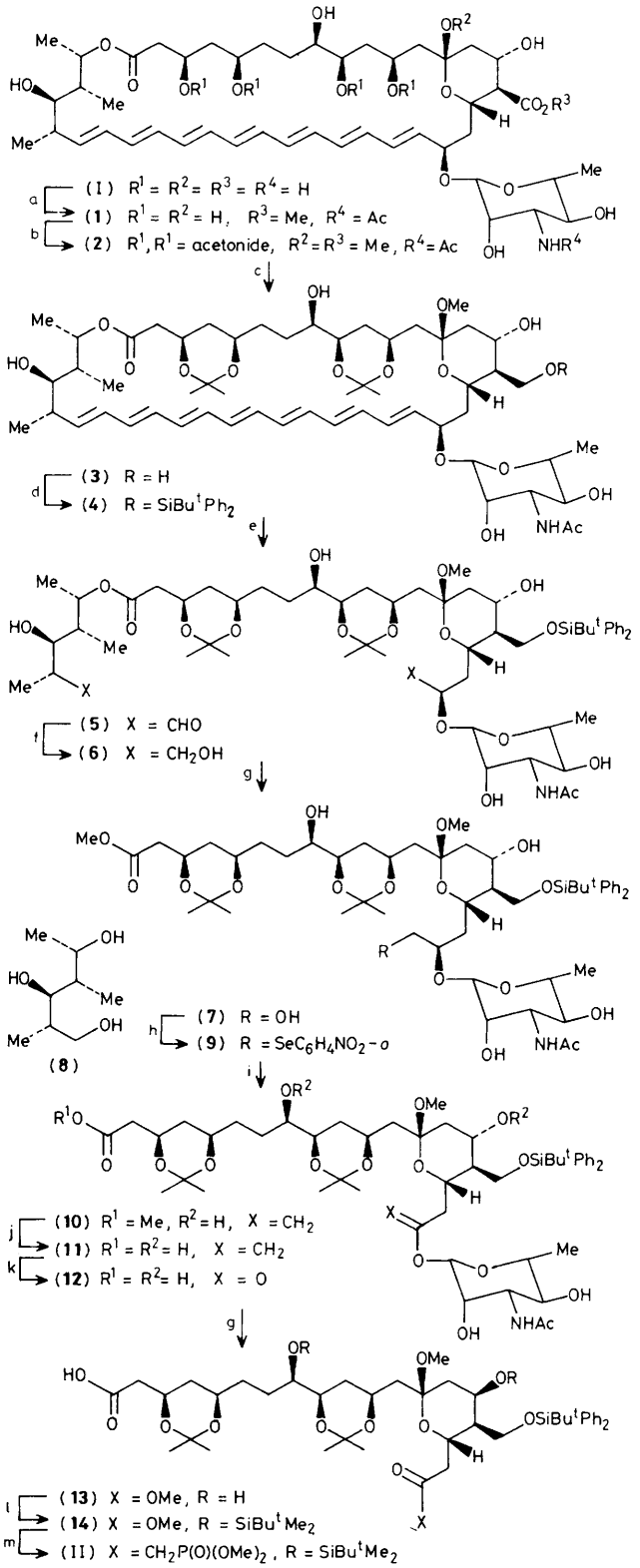
The polyene macrolides are an increasingly important and rather large class of bioactive compounds.[1] Despite their biological, clinical, and commercial importance, however, their chemistry remains relatively unexplored primarily owing to their complex structures and the lack of suitable synthetic technology. Amphotericin B (I),[2] produced by *Streptomyces nodusus*, is one of the most widely used members of this family as an effective antifungal agent and the only complex polyene macrolide whose molecular structure and absolute configuration are firmly established by *X*-ray crystallographic analysis.[3] Chemical and synthetic studies[4] in this area are, therefore, essential for developments regarding total synthesis, structural determinations, and synthesis of analogues. In this communication we describe a series of useful chemical transformations and retrosynthetic studies on amphotericin B (I) as well as efficient synthetic technology for the construction of the 38-membered macrocycle of this complex biomolecule.

Our original strategy for the total synthesis of amphotericin B (I) and related structures was derived from the retrosynthetic analysis shown in Scheme 1 in which the target was disconnected at the lactone, glycoside, and Δ^{20} bonds, defining the C(1)—C(20) and C(21)—C(38) fragments (II) and (III) as the major key intermediates. Furthermore, the plan called for an intramolecular ketophosphonate–aldehyde condensation as a key macrocyclic ring forming reaction[5] to construct the skeleton of amphotericin B (I). Our initial retrosynthetic and synthetic studies were, therefore, directed towards generating fragments (II) and (III) and securing the final stages of the synthesis including the crucial technology for the construction of the 38-membered ring.



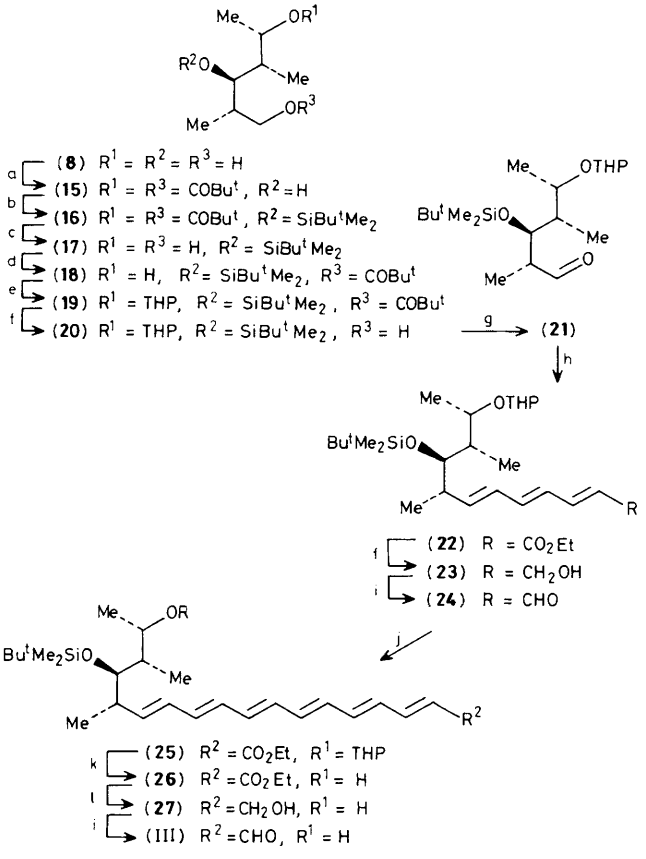
Scheme 1. Retrosynthetic analysis of amphotericin B (I).

† Recipient of a Camille and Henry Dreyfus Teacher–Scholar Award, 1980—1985; J. S. Guggenheim Fellow 1984—1985.

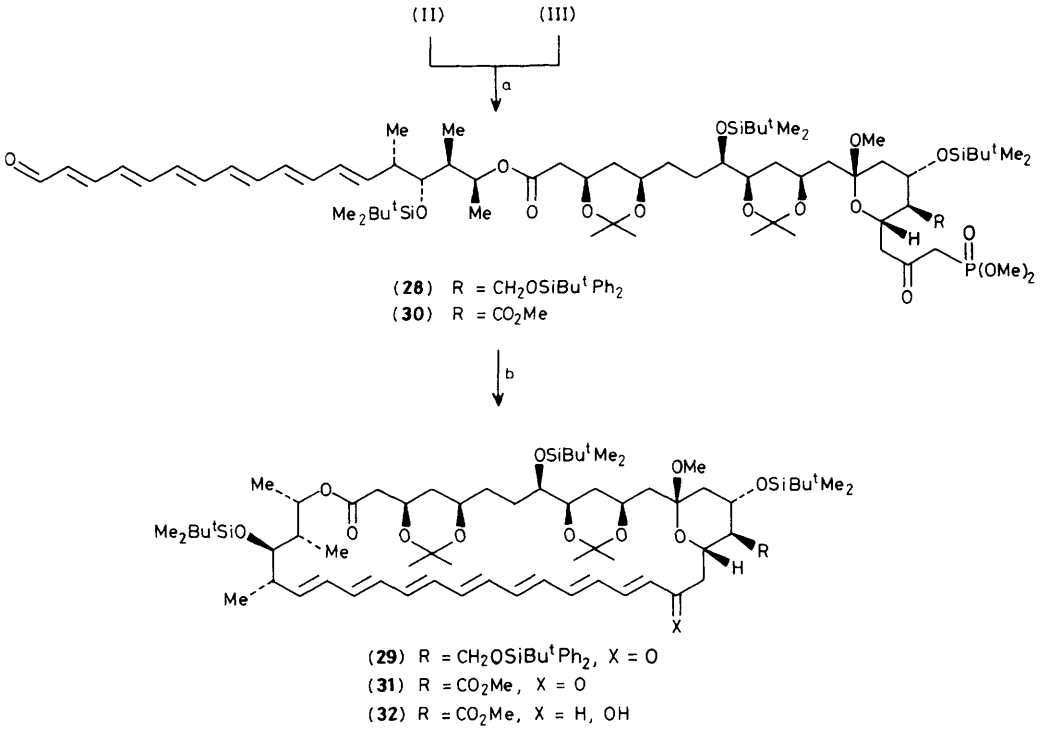


Scheme 2. Synthesis of the C(1)—C(20) fragment (II). Reagents: a, Ac_2O (1.1 equiv.), DMSO–MeOH (1:1), 0 °C, then CH_2N_2–Et_2O, 0 °C; b, camphorsulphonic acid catalysis, $Me_2C(OMe)_2$–MeOH (1:3), 25 °C, 66% overall from (I); c, $NaBH_4$ (15.0 equiv.), MeOH, 40 °C, 85%; d, Bu^tPh_2SiCl (3.0 equiv.), imidazole (5.0 equiv.), DMF, 0 °C, 80%; e, O_3, MeOH–CH_2Cl_2 (1:4), −40 °C, then PPh_3 (10.0 equiv.), 0 to 25 °C, 83%; f, $NaBH_4$ (15.0 equiv.), 25 °C, 85%; g, K_2CO_3 (1.0 equiv.), MeOH, 25 °C, (**7**): 79%, (**8**): 74%; h, *o*-$O_2NC_6H_4SeCN$ (10.0 equiv.), Bu^n_3P (10.0 equiv.), THF–pyridine (2:1), 0 °C, 85%; i, O_3, CH_2Cl_2, −78 °C then Pr^i_2NH (10.0 equiv.), benzene, reflux, 70%; j, LiOH (1.5 equiv.), THF–H_2O (2:1), 0 to 25 °C; k, O_3, CH_2Cl_2, −78 °C, then PPh_3 (2.0 equiv.), 0 to 25 °C, 57% overall from (**10**); l, Bu^tMe_2SiOTf (4.5 equiv.), 2,6-lutidine (6.0 equiv.), CH_2Cl_2, 0°C, then 1 M-NaOH (1.1 equiv.), THF–H_2O (1:1), 0 to 25 °C, 89%; m, $(MeO)_2P(O)Me$–Bu^nLi (each 6.0 equiv.), −78 °C, 76%.

Abbreviations used here and in Schemes 3 and 4: DMSO = dimethyl sulphoxide; DMF = dimethylformamide; THF = tetrahydrofuran; Tf = trifluromethyl sulphonyl; DIBAL = di-isobutylaluminium hydride; PCC = pyridinium chlorochromate; LDA = lithium di-isopropylamide; DCC = dicyclohexylcarbodiimide; DMAP = 4-*N*,*N*-dimethylaminopyridine; DBU = 1,8-diazabicyclo[5.4.0]undec-7-ene; THP = tetrahydropyran-2-yl.



Scheme 3. Synthesis of the C(21)—C(38) fragment (III). Reagents: a, Bu^tCOCl (2.2 equiv.), pyridine, 25 °C, 83%; b, Bu^tMe_2SiOTf (1.3 equiv.), 2,6-lutidine (1.5 equiv.), CH_2Cl_2, 0 to 25 °C, 91%; c, DIBAL (5.0 equiv.), CH_2Cl_2, −78 °C, 100%; d, Bu^tCOCl, pyridine, 25 °C, 92%; e, dihydropyran (1.1 equiv.), camphorsulphonic acid catalysis, CH_2Cl_2, 0 °C, 96%; f, DIBAL (2.5 equiv.), CH_2Cl_2, −78 °C, 100%; g, PCC (2.0 equiv.), 4 Å molecular sieve, NaOAc, CH_2Cl_2, 25 °C, 75%; h, *E*,*E*-$(EtO)_2P(O)CH_2CH{=}CHCH{=}CHCO_2Et$–LDA (each 1.3 equiv.), THF, −78 to 0 °C, 90%; i, MnO_2 (10.0 equiv.), CH_2Cl_2, 25 °C, 98%; j, *E*,*E*-$(EtO)_2P(O)CH_2CH{=}CHCH{=}CHCO_2Et$–LDA (each 2.0 equiv.). THF, −78 to 0 °C, 60%; k, pyridinium toluene-*p*-sulphonate catalysis, MeOH, 45 °C, 82%; l, DIBAL (3.5 equiv.), CH_2Cl_2, −78 °C, 100%.



Scheme 4. Synthesis of the macrocyclic heptaenones (**29**) and (**31**). Reagents: a, (II) (1.0 equiv.), (III) (1.2 equiv.), DCC (1.2 equiv.), DMAP (0.3 equiv.), CH_2Cl_2 (1.0 M solution), 25 °C, 65%; b, K_2CO_3 (6.0 equiv.), 18-crown-6 (13.0 equiv.), toluene (0.001 M solution), 65 °C, or LiCl, (5.0 equiv.) DBU (5.5 equiv.), MeCN (0.01 M solution), 25 °C, 70—80%.

The amino group of amphotericin B (I) was acetylated with Ac_2O and the carboxylic acid group was methylated with CH_2N_2 to afford the acetamide methyl ester derivative (**1**) (Scheme 2).[6] This compound was then treated with MeOH–$Me_2C(OMe)_2$ under acid catalysis furnishing the bis(acetonide) methyl ether derivative (**2**)‡ in 66% overall yield from (I).[7] Selective reduction, presumably assisted by the neighbouring hydroxy group, of the ester functionality in (**2**) was readily achieved with $NaBH_4$ leading to the primary alcohol (**3**) (85%) which was then protected with Hanessian's silyl ether reagent[8] to afford compound (**4**) in 80% yield. Attempts to remove the carbohydrate fragment from amphotericin B (I) at this or at several other stages, in order to produce the aglycone, proved fruitless. It was, however, possible to degrade compound (**4**) by ozonolysis followed by mild reduction (PPh_3) to furnish the dialdehyde (**5**)§ in 83% yield. This dialdehyde was then reduced with $NaBH_4$ to give the corresponding diol (**6**) (85%) which was then cleaved at the ester linkage with K_2CO_3–MeOH to afford fragments (**7**) (79%) and (**8**) (74%)[9] utilized for the construction of (II) and (III) respectively.

Since the sugar unit was still resisting acid hydrolysis without decomposition, the following strategy was devised for its removal as dictated by our retrosynthetic studies directed towards (III). The primary hydroxy group of (**7**) was utilized to form selectively the *o*-nitro selenide (**9**) by Grieco's method[10] (85%). Oxidation by O_3, followed by *syn*-elimination of the resulting selenoxide, led to the enol ether derivative (**10**) in 70% overall yield. Basic hydrolysis of the methyl ester of this derivative afforded the carboxylic acid (**11**) which was then subjected to ozonolysis and mild reduction (PPh_3) to furnish the sugar ester (**12**) [57% overall from (**10**)]. Transesterification of (**12**) with methanol under base catalysis led to the methyl ester (**13**) (79%) which was then converted into the tris(silyl ether) (**14**) by persilylation and mild base hydrolysis (89% overall yield). Finally, excess of $LiCH_2P(O)(OMe)_2$ converted (**14**) into the target, C(1)—C(2) fragment, the ketophosphonate (II) in 76% yield.

The C(21)—C(38) fragment was constructed from the triol (**8**) as detailed in Scheme 3. Thus, (**8**) was transformed into its bis(pivalate) ester (**15**) (83%) with excess of pivaloyl chloride, silylated under forcing conditions to give (**16**) (91%), and then reductively (DIBAL) converted into the diol (**17**). Monoesterification with stoicheiometric amounts of pivaloyl chloride then led to (**18**) which was tetrahydropyranylated leading to (**19**) (96%). Liberation of the primary hydroxy group of (**19**) followed by oxidation furnished the aldehyde (**21**) *via* the alcohol (**20**) in 75% overall yield. Conversion of (**21**) into the *E,E,E*-trienal (**24**) was then achieved by (i) condensation of (**21**) with the lithio derivative of *E,E*-$(EtO)_2P(O)CH_2CH{=}CHCH{=}CHCO_2Et$ leading to the *E,E,E*-triene ester (**22**) (90%);¶ (ii) reduction with DIBAL furnishing (**23**) (100%); and (iii) oxidation with MnO_2 (98%). Repetition of step (i) on (**24**) gave the heptaene ester (**25**) (60%)¶ which suffered acid-catalysed depyranylation leading to the hydroxy ester (**26**) (82%). Finally, DIBAL reduction of

‡ This compound consisted of two methoxy anomers (*ca.* 3:1 by ^1H n.m.r.) the major one being tentatively assigned the designated structure by analogy with the amphotericin B (I) *X*-ray crystal structure.[3] Subsequent reactions were carried out with this mixture.

§ The ^1H n.m.r. spectrum of this compound exhibited only one aldehydic signal (δ 9.68, s, 1H) indicating that a hydroxy group is engaging one of the aldehyde groups in a lactol (presumed to be a δ-lactol, mycosamine C-2-OH).

¶ In addition small quantities of the *Z* isomer of the newly generated double bond were formed and were removed chromatographically.

(**26**) followed by MnO_2 oxidation led to the desired C(21)—C(38) fragment, the all-*trans*-hexaenal (III) *via* alcohol (**27**), in 98% overall yield.

Having constructed the requisite C(1)—C(20) and C(21)—C(38) fragments (II) and (III) from amphotericin B (I) we then proceeded to test the feasibility of the key macrocyclization step of the projected synthesis, utilizing the intramolecular ketophosphonate–aldehyde condensation reaction.[5] To this end, fragments (II) and (III) were coupled (Scheme 4) with the aid of DCC and DMAP[11] to afford the precursor (**28**)** (65%) which was then subjected to cyclization experiments. Under the influence of a mild base such as K_2CO_3–18-crown-6[5] or DBU–LiCl,[12] the ketophosphonate aldehyde (**28**) cyclized smoothly forming the 38-membered ring heptaenone (**29**), in 70—80% yield. The conformational restrictions imposed onto the open-chain precursor (**28**) by its several sp^2 centres, rings, and substituents are undoubtedly responsible for this facile and efficient ring closure. Thus, it appears that this intramolecular ketophosphonate–aldehyde condensation reaction that proved so useful in the synthesis of the 16-membered ring macrolide antibiotics[5] may also provide a powerful technique for the construction of the larger polyene macrolides such as amphotericin B (I) and nystatin.[12]

Furthermore, compound (**2**), after persilylation (excess of Bu^tMe_2SiOTf, 2,6-lutidine) was converted into the ketophosphonate aldehyde (**30**) by similar chemistry as for (**28**). Cyclization of (**30**) according to Scheme 4 then resulted in the formation of (**31**) as a mixture of two methoxy epimers (*ca.* 2:1 ratio, 75% yield). Reduction of the major isomer of this polyenone with excess of $NaBH_4$ (MeOH, 0 °C) led cleanly to a single hydroxy compound (**32**) (95% yield, unassigned stereochemistry) representing a protected form of the aglycone (or its epimer) of amphotericin B.

It is expected that the chemistry described above for the derivatization and degradation of amphotericin B (I) and the facile macrocyclization technology for the construction of the 38-membered ring of this complex antifungal agent will greatly facilitate research in this hitherto difficult-to-explore area. Among further goals, now within reach, are the total synthesis of amphotericin B (I) and the related and clinically useful antifungal agent nystatin,[13] the complete stereochemical elucidation of nystatin and other related polyene antibiotics,[1] and the synthesis of various analogues of these therapeutic agents as part of a search for more effective and less toxic drugs.

All key intermediates described in this paper showed satisfactory spectral and or analytical and/or exact mass data. Yields refer to spectroscopically and chromatographically homogeneous materials.

We thank Dr. C. Cimarusti, Squibb & Sons, for generous samples of amphotericin B, Drs. George Furst and John Dykins of this department, for n.m.r. and mass spectroscopic assistance and comments, and the National Institutes of Health, the Camille and Henry Dreyfus Foundation and Merck Sharp and Dohme, U.S.A., for financial support.

Received, 11th November 1985; Com. 1593

** Data for (**28**): R_f 0.42 (silica, Et_2O); i.r. ($CHCl_3$) $\nu_{max.}$ 1725 and 1675 cm^{-1} (CO); u.v. ($CHCl_3$) $\lambda_{max.}$ 408 nm; ^1H n.m.r. (250 MHz, $CDCl_3$) δ 9.59 and 9.53 (each d, *J* 8.0 Hz, *ca.* 3:1 ratio, 1H total, CHO), 7.59 (m, 4H, ArH), 7.36 (m, 6H, ArH), 7.11 (dd, $J_{23,24}$ 11.1, $J_{23,22}$ 15.0 Hz, 1H, H-23), 6.69 (dd, *J* 11.1 and 14.5 Hz, 1H, olefinic), 6.51—6.01 (m, 9H, olefinic), 5.71 (dd, $J_{33,34}$ 3.1, $J_{33,32}$ 15.0 Hz, 1H, H-33), 5.05 (dq, *J* 6.4 Hz, 1H, H-37), 4.6—3.4 (m, 10H, CH–O, CH_2–O), 3.75—3.69 [m, 6H, $P(OCH_3)_2$], 3.10 and 3.01 (each s, *ca.* 3:1 ratio, 3H total, anomeric OCH_3), 3.10—2.97 [m, 2H, $PCH_2C(O)$], 2.85—1.13 (m, 18H, CH_2, CH), 1.10 (d, *J* 6.3 Hz, 3H, CH_3), 1.04 and 1.03 (each s, 6H, acetonides), 0.99 (d, *J* 6.7 Hz, 3H, CH_3), 0.88, 0.85, 0.79, and 0.72 (each s, 36H total, $SiBu^t$), 0.77 (d, *J* 6.7 Hz, 3H, CH_3), 0.04, 0.0, −0.02, −0.06, and −0.17 (each s, 18H total, $SiMe_2$). Data for (**29**): R_f 0.23 (silica, 70% ether in light petroleum); i.r. ($CHCl_3$) $\nu_{max.}$ 1730 and 1640 cm^{-1} (CO); u.v. ($CHCl_3$) $\lambda_{max.}$ 422 nm; ^1H n.m.r. (250 MHz, $CDCl_3$) δ 7.60 (m, 4H, ArH), 7.37 (m, 6H, ArH), 7.21 (m, 1H, H-21), 6.8—5.9 (m, 11H, olefinic), 6.15 (d, $J_{20,21}$ 15.0 Hz, 1H, H-20), 5.58 (dd, $J_{33,32}$ 15.0, $J_{33,34}$ 9.0 Hz, 1H, H-33), 5.0 (m, 1H, H-37), 4.6—3.4 (m, 10H, CH–O, CH_2O), 2.92 and 2.85 (each, s, *ca.* 3:1 ratio, 3H total, anomeric OCH_3), 2.40—1.95 [m, 5H, $CH_2C(O)$, CH-allylic], 1.9—1.2 (m, 14H, CH_2, CH), 1.17 (d, *J* 6.1 Hz, 3H, CH_3), 1.05 (s, 12H, acetonides), 0.97 (d, *J* 6.9 Hz, 3H, CH_3), 0.89, 0.88, 0.87, and 0.69 (each s, 36H total, $SiBu^t$), 0.79 (d, *J* 6.7 Hz, 3H, CH_3), 0.05, 0.04, 0.03, −0.045, and −0.25 (each s, 18H total, $SiMe_2$). For ^1H n.m.r. assignments, the following papers were useful: J. M. Brown and P. J. Sidebottom, *Tetrahedron*, 1981, **37**, 1421; P. Sowinski, J. K. Pawlak, E. Borowski, and T. Iwashita, *J. Antibiot.*, 1985, **38**, 175.

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