A unified approach to polyene macrolides: Synthesis of candidin and nystatin polyols

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Polyene macrolide antibiotics are naturally occurring antifungal agents. Members of this class include amphotericin B, which has been used widely to treat systemic fungal infections. A general synthetic strategy has been devised to prepare polyol chains associated with the polyene macrolides. Cyanohydrin acetonide alkylations were used to assemble the carbon skeleton, and a simple modification of the strategy allowed an advanced intermediate to be converted to either the candidin polyol or the nystatin polyol. The candidin polyol was further elaborated to a protected candidin aglycone. This strategy will be applicable to other members of the polyene macrolide natural products.

The mycosamine-containing polyene macrolides are clinically important antifungal agents. Amphotericin B (1) is the most prominent member of this class (2, 3) which includes rimocidin (**1**) (4), nystatin (**2**) (5), candidin (**3**) (6), and others (7). The synthesis of amphotericin B has been the subject of extensive investigation (8–12). In general, the antifungal activity of these polyenes has been attributed to their assembly into ion channels in the presence of sterol-containing membranes (2, 3). A flexible synthetic route into these compounds would allow the structural basis of this interesting self-assembly phenomenon to be explored systematically.

An obvious stereochemical relationship exists between these polyene macrolides (Fig. 1). The substitution and configuration of the hemiacetal ring and of the adjacent stereogenic centers are conserved throughout the members of the class. We set out to develop a unified synthetic strategy that is flexible enough to be applied to any member of the class. Polyene macrolides are of interest to synthetic chemists (13–15), and we recently reported the synthesis of the rimocidin aglycone (16). Herein is described a generalization of the strategy that is illustrated with syntheses of nystatin and candidin polyols and of the protected candidin aglycon **34**.

The hemiacetal ring found in each of these polyenes would arise from the protected segment **4**, where the C13 ketone is masked as a cyanohydrin. The cyanohydrin group enables the key bond disconnection between cyanohydrin acetonide **6** and alkylating agent **5**, which incorporates all the stereogenic centers in the hemiacetal ring. The R group in **4** would include part or all of the remaining polyol chain. Cyanohydrins are well established as acyl anion equivalents (17, 18), as are dithiane anions (19–22). However, cyanohydrin acetonides have several important advantages over simple cyanohydrins or dithianes. We have shown that they alkylate to give the axial nitrile (e.g., **4**) with high diastereoselectivity (23–25), rather than the mixtures commonly found with simple cyanohydrins. They are also easier to deprotonate than dithianes, the anions are excellent nucleophiles, and they can be deprotected under very mild conditions (25). These features make a cyanohydrin acetonide disconnection a very powerful strategy for convergent synthesis.

Materials and Methods

Starting materials were purchased from Aldrich unless otherwise noted. Reactions were carried out in accord with safe laboratory practices (26). New compounds were characterized by ^{1}H NMR, ^{13}C NMR, and IR spectroscopy and by MS or elemental analysis.

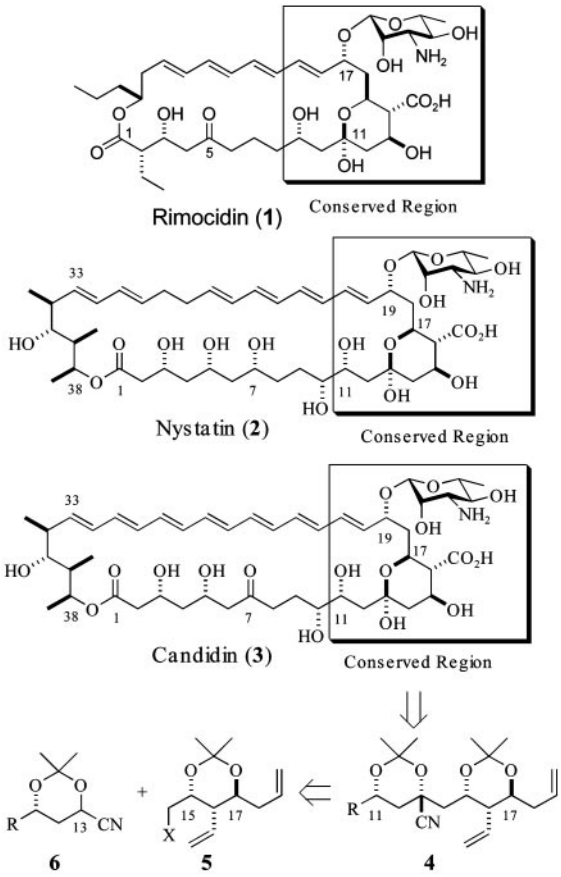

Fig. 1. The antifungal agent candidin and a strategic bond disconnection of the conserved region common to this class of polyene macrolides.

Experimental procedures and compound characterization for all new compounds are presented in the supporting information, which is published on the PNAS web site.

Results and Discussion

Syntheses of the candidin fragments **8**, **12**, and **16** are outlined in Fig. 2. The synthesis of iodide **8** from Evans aldol adduct **7** has been reported (16). Synthesis of cyanohydrin acetonide **12** began with a Sharpless asymmetric dihydroxylation of alkene **9**, which was prepared by silylation of the corresponding diol, itself

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Abbreviations: THF, tetrahydrofuran; DMPU, 1,3-dimethyl- 3,4,5,6-tetrahydro-2-(1H)-pyrimidinone; LDA, lithium diisopropylamide; PPTS, pyridinium tosylate; DIBAL-H, diisobutylaluminum hydride.

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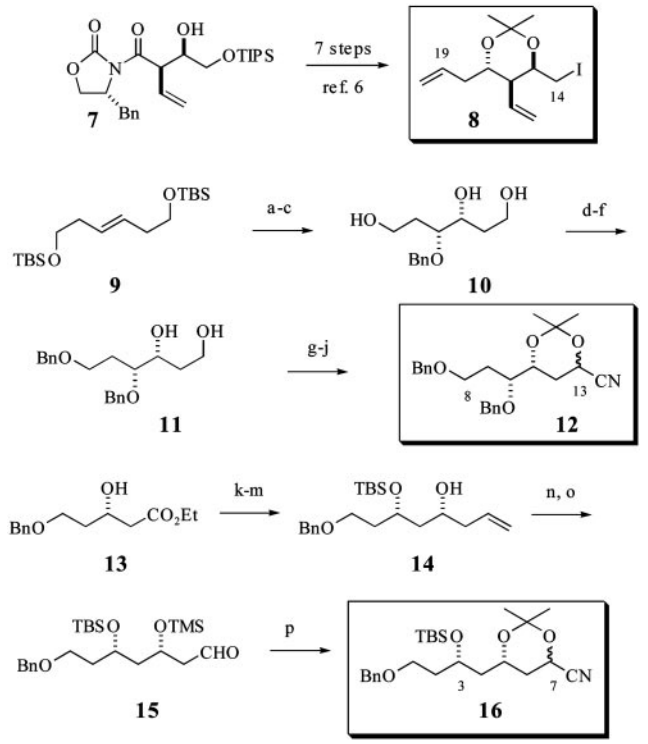

Fig. 2. Synthesis of the candidin fragments **8**, **12**, and **16**. Reagents and conditions: (a) AD-mix-β, 92%; (b) $PhCH(OMe)_2$, PPTS, 81%; (c) $LiAlH_4$, $AlCl_3$, 91%; (d) 2,2-dimethoxypropane PPTS, 90%; (e) BnBr, KH, Bu_4NI, 97%; (f) Dowex-H^+, MeOH, 99%; (g) 2,2,6,6-tetramethylpiperidine-*N*-oxyl (1%), NaOCl, CH_2Cl_2; (h) trimethylsilyl cyanide, KCN·18-crown-6; (i) Dowex-H^+, MeOH; 83% from **11**; (j) 2,2-dimethoxypropane PPTS, PhH, 90%; (k) TBSOTf, 2,6-lutidine, 100%; (l) diisobutylaluminum hydride (DIBAL-H), 98%; (m) *l*-$(ipc)_2B$-allyl, 98%; (n) trimethylsilyl chloride, 4-dimethylaminopyridine, imidazole, 99%; (o) (*i*) OsO_4, *N*-methylmorpholine-*N*-oxide; (*ii*) $NaIO_4$; (p) (*i*) trimethylsilyl cyanide, KCN·18-crown-6; (*ii*) 2,2-dimethylpropane-1,3-diol, camphorsulfonic acid, 94%.

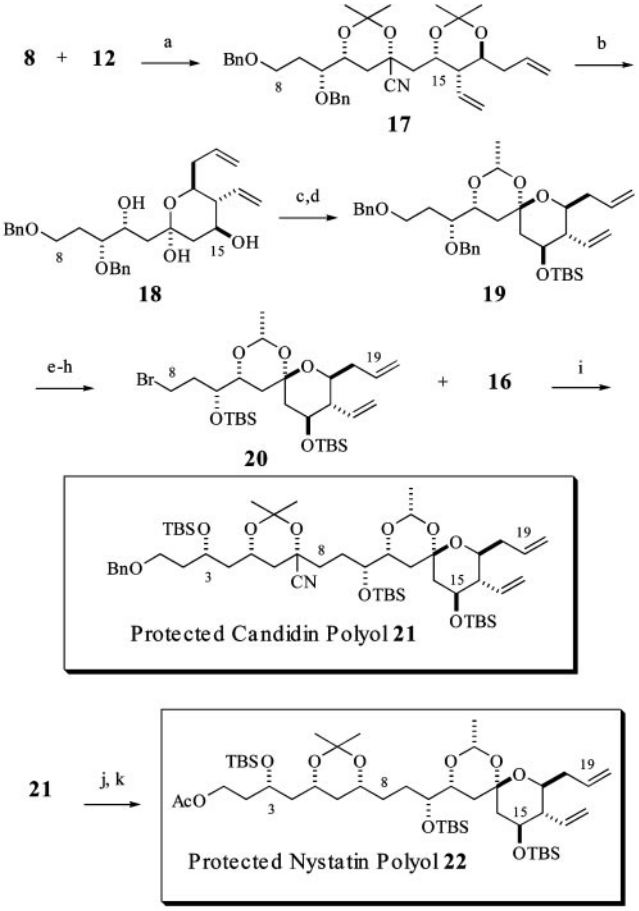

Fig. 3. Synthesis of the conserved hemiacetal segment and of the candidin polyol chain. Reagents and conditions: (a) LDA, DMPU (5 eq), −40°C, 1.5 h, 89%; (b) (*i*) 6 N HCl, MeOH; (*ii*) Et_3N, 89%; (c) CH_3CHO, PPTS, 85%; (d) TBSOTf, 2,6-lutidine, 81% (α-Me), 10% (β-Me); (e) Li, NH_3 (liq), 99%; (f) MsCl, *i*-Pr_2NEt, 99%; (g) TBSOTf, 2,6-lutidine, 97%; (h) Bu_4NBr, 100%; (i) LDA (1.5 eq), DMPU, THF, −78°C, 30 min (MeOH quench), 95%; (j) Li, NH_3 (liq), 86%; (k) Ac_2O, Et_3N dimethylaminophenol, 97%.

available by reduction of (*E*)-dihydromuconic acid (27). Desymmetrization of the C_2-symmetric diol was accomplished by reductive cleavage of the corresponding benzylidene acetal. Reprotection gave 1,3-diol **11**. Conversion to the cyanohydrin acetonide **12** was accomplished by selective 2,2,6,6-tetramethylpiperidine-*N*-oxyl oxidation of the primary alcohol (28), followed directly by cyanohydrin formation and acetonide protection. In most cases, we consider this sequence to be the preferred route to cyanohydrin acetonides. Synthesis of **16** began with ester **13** (29, 30). Protection, reduction, and enantioselective allylboration (31) gave **14** as a single diastereomer. Conversion to **15** was uneventful, and cyanohydrin acetonide formation was accomplished under standard conditions (23). Chiral building blocks **8**, **12**, and **16** were each prepared on a multigram scale by using these synthetic routes.

Assembly of the polyol chain is outlined in Fig. 3. Iodide **8** and cyanohydrin **12** were combined in a 1:1.3 ratio in tetrahydrofuran (THF). 1,3-Dimethyl-3,4,5,6-tetrahydro-2-(1H)-pyrimidinone (DMPU) and lithium diisopropylamide (LDA) were added, and the reaction was stirred at −40°C to give coupled product **17** in 89% yield. Premixing the electrophile and the cyanohydrin simplifies the reaction and improves the reliability of the coupling step (25). Hydrolysis of the acetonide groups followed by treatment with Et_3N liberated the ketone, which spontaneously cyclized to produce hemiacetal **18** in excellent yield. The hemiacetal **18** was protected as an acetaldehyde acetal to give a separable mixture of the major α-methyl epimer **19** and β-methyl epimer in 81% and 10% yield after silylation. Standard refunctionalization gave bromide **20**. Bromide **20** and cyanohydrin **16** were combined in a 1:1.2 ratio and coupled by addition of LDA and DMPU at −78°C to give **21** in 95% yield. Cyanohydrin **21** includes the complete C1–C19 polyol segment of candidin.

Nystatin and candidin have similar structures and only differ in the presence of a C7 ketone and a C28–C29 alkene in candidin. The advanced synthetic intermediate **21** has the C7 candidin ketone masked as a cyanohydrin. Reductive decyanation with Li in ammonia stereoselectively reduces the masked ketone to a protected alcohol (23, 24). The product, compound **22**, incorporates all the atoms in the appropriate stereochemical arrangement for the C1–C19 segment nystatin. Thus, slight modifications in the synthetic route to candidin polyol **21** leads to the nystatin polyol **22**.

Synthesis of the polyene segment of candidin (which is identical with the corresponding segment of amphotericin B) is outlined in Fig. 4. Ester **23** was prepared by Noyori reduction of ethyl acetoacetate. Frater–Seebach alkylation and refunctionalization provided the aldehyde (32, 33). Evans aldol reaction between **24** and **25**, followed by Weinreb amide formation, produced the adduct **26**. Protection and reduction gave the aldehyde **27**, precursor to the hexaene. Initial attempts to use Wollenberg's strategy led to poor yields and mixtures of alkene

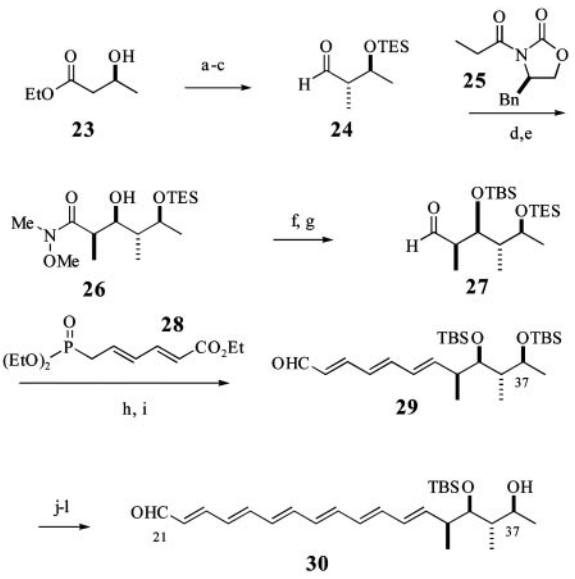

Fig. 4. Synthesis of the polyene segment for candidin. Reagents and conditions: (a) LDA, THF, then MeI; 83% (96:4 dr); (b) TESCl, imidazole, dimethylaminophenol, 90%; (c) DIBAL-H, Et_2O, −78°C, 90%; (d) Bu_2BOTf, Et_3N, **25**, then **24**, 75%; (e) MeN(OMe)H, $AlMe_3$, CH_2Cl_2, 72%; (f) TBSOTf, 2,6-lutidine, 97%; (g) DIBAL-H, 75%; (h) **28**, LDA, THF, −78°C, 70% (and 10% Z); (i) (*i*) DIBAL-H; (*ii*) MnO_2, 96%; (j) **28**, sodium hexamethyldisilazane, THF, −78 to 0°C, 54%; (k) PPTS, MeOH, 98%; (l) (*i*) DIBAL-H; (*ii*) MnO_2, 66%.

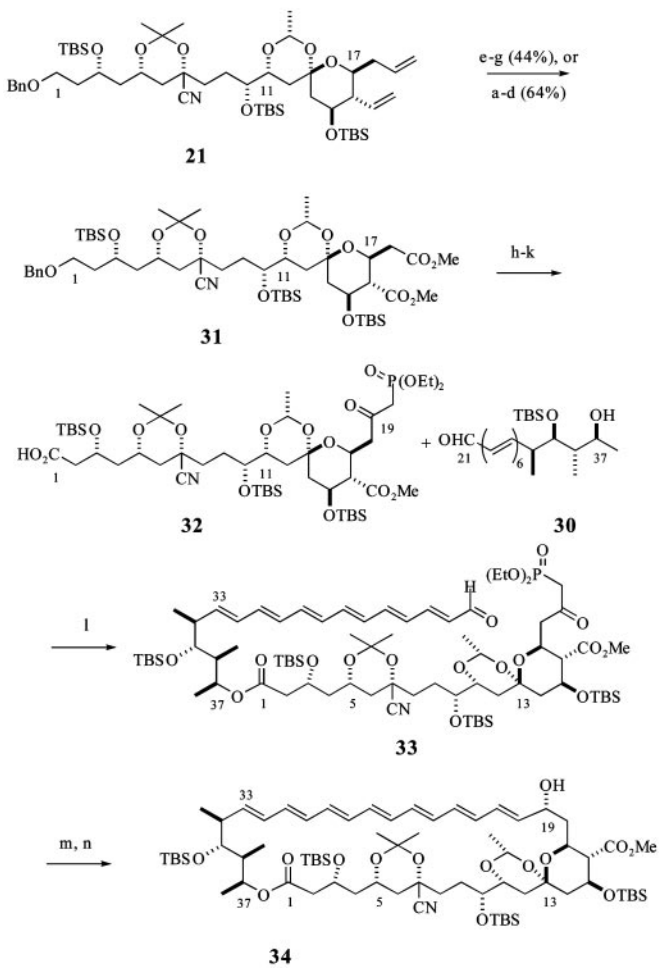

Fig. 5. Synthesis of the protected candidin macrolide **34**. Reagents and conditions: (a) OsO_4, *N*-methylmorpholine-*N*-oxide; (b) $Pb(OAc)_4$; (c) $NaClO_2$; (d) CH_2N_2; 64% from **21**; (e) O_3, −78°C; $P(OEt)_3$; (f) $NaClO_2$; (g) CH_2N_2; 44% from **21**; (h) $CH_3P(O)(OEt)_2$ and *n*-BuLi, −78°C, 67%; (i) H_2, $Pd(OH)_2/C$, 91%; (j) Dess–Martin periodinane; (k) $NaClO_2$; (l) **32** and $Cl_3C_6H_2COCl$, Et_3N; then **30**, 78% for three steps; (m) K_2CO_3, 18-crown-6, $PhCH_3$, (0.001 M substrate) 72%; (n) $NaBH_4$, $CeCl_3{\cdot}7H_2O$, MeOH, 85%.

isomers (34, 35). A Horner–Emmons homologation proved more reliable. Reaction of aldehyde **27** with phosphonate **28** (36) in the presence of LDA produced the desired *E* alkene along with a small amount of *Z* alkene that could be separated and isomerized with I_2. Reduction of the ester and oxidation with MnO_2 produced the aldehyde **29**. The second Horner–Emmons reaction with **28** was more effective with sodium hexamethyldisilazane as a base rather than LDA. The final steps follow Nicolaou's route (37). Deprotection of the triethylsilyl group with pyridinium tosylate (PPTS), followed by reduction and oxidation, produced the sensitive polyene aldehyde **30**.

Conversion of polyol **21** to the protected candidin aglycon **34** is illustrated in Fig. 5. Oxidation of the diene **21** to the diester **31** was surprisingly difficult. Ozonolysis, $NaClO_2$ oxidation, and treatment with diazomethane produced the diester **31** in 44% optimized yield. The intermediate bisozonide in this sequence forms intractable mixtures of cyclic acetals. Stepwise oxidation of the two alkenes, the first by osmylation and the second by ozonolysis, produced **31** in a 64% overall yield but requires more steps. The anion from diethyl methylphosphonate added selectively to the less hindered ester of **31**. Deprotection and oxidation of the C1 benzyl ether gave acid **32**, which was coupled with alcohol **30** under Yamaguchi conditions (38). Macrolide formation was realized by using K_2CO_3 and 18-crown-6 in $PhCH_3$ at 23°C (39, 40). Higher temperatures led to partial epimerization at C16, and LiCl/DBU conditions (41) led to decomposition. Reduction of the resulting polyene ketone with $NaBH_4$ and $CeCl_3\ 7H_2O$ (42, 43) produced a single stereoisomer of the alcohol **34** in 85% yield, in good agreement with reductions in the amphotericin B (8) and rimocidin (16) structures. The configuration at C19 was confirmed by Mosher ester analysis (44). Candidin aglycon **34** was prepared from the polyol **21** in ten steps.

Conclusion

A general route to the mycosamine family of polyene macrolide aglycones has been developed. The conserved hemiacetal ring arises from the fragment **8**, and different cyanohydrin acetonides may be coupled to **8** to produce a variety of polyol chains. The polyol segments of rimocidin, nystatin, and candidin each have been produced by using this strategy, and both candidin and rimocidin macrolides have been prepared from their respective polyol segments. Investigation of the synthesis of these natural products continues with the study of efficient methods to introduce the mycosamine saccharide into these sensitive molecules (45).

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