Total Synthesis of Luzopeptins A−C

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The luzopeptins (**1**−**3**, Figure 1) are potent antitumor antibiotics that were isolated from *Actinomadura luzonensis*[1] and identified through a single-crystal X-ray structure determination of **1**.[2] They constitute the initial members of a growing class of C2-symmetric cyclic decadepsipeptides which now include the quinoxapeptins (**4**−**5**),[3] quinaldopeptin,[4] and sandramycin (**6**)[5−7] that bind to DNA with bisintercalation.[6−11] In addition to their potent cytotoxic and antitumor activity,[1,8,12,13] they are potent inhibitors of HIV reverse transcriptase (RT)[7,14] including single and double mutants[3] responsible for the emerging clinical resistance to recently introduced RT inhibitors. Moreover, the cytotoxic potency of the luzopeptins (A > B ≫ C) and their antiviral potency/HIV RT inhibition (C > B > A) are reversed, with luzopeptin C exhibiting suppression of HIV replication in infected MT-4 cells at non-cytotoxic concentrations,[14] and we have observed similar divergent structure activity relationships with a recent series of sandramycin analogues.[7]

Despite their importance as prototypical DNA bisintercalators with potent biological properties, they have been the subject of only limited synthetic efforts.[15−18] Herein, we report the first total synthesis of luzopeptins A−C. The luzopeptins and quinoxapeptins contain the identical cyclic decadepsipeptide and differ only in the attached chromophore and in the acyl substituents found on the unusual L-(4*S*)-hydroxy-2,3,4,5-tetrahydropyridazine-3-carboxylic acid (L-Htp) subunit. Consequently, key elements of the approach include the late-stage introduction of the chromophore potentially providing access to both the luzopeptins and quinoxapeptins, the late-stage L-Htp alcohol acylation permitting the divergent synthesis of the luzopeptins and quinoxapeptins, symmetrical pentadepsipeptide coupling and macrocyclization of the 32-membered depsipeptide conducted at the single secondary amide site, and a convergent assembly of the pentadepsipeptide with introduction of the hindered and labile ester linkage in the final coupling reaction under near racemization free conditions.

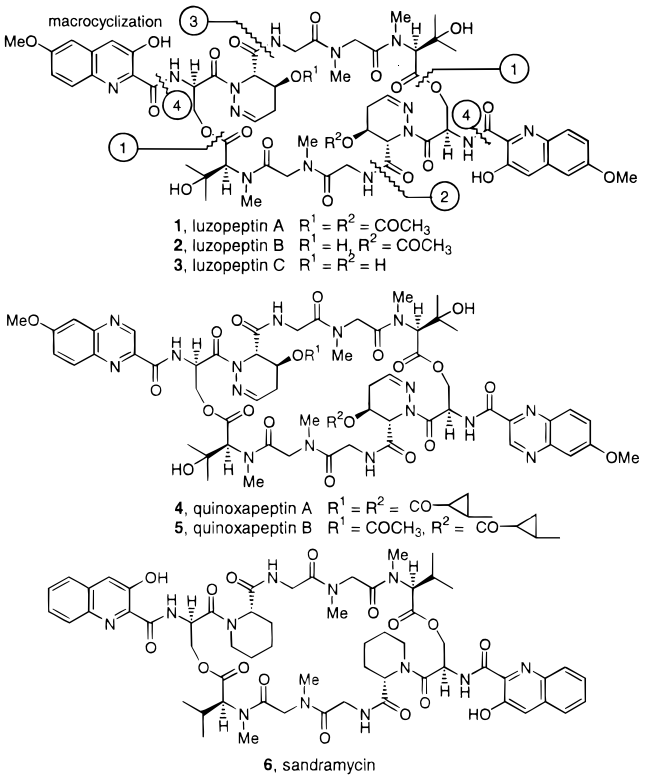

Figure 1.

The convergent assemblage of the key pentadepsipeptide **19** from the tripeptide **17** and protected dipeptide **18**[18] is summarized in Scheme 1. The protected *N*-methyl (3*R*)-hydroxyvalinol **12** for incorporation into **17** was derived from 3-methyl-2-buten-1-ol by Sharpless epoxidation with (+)-L-DIPT providing the known (2*S*)-epoxide **7**.[19] Formation of the carbamate **8** upon reaction with methyl isocyanate (1.5 equiv, CH_2Cl_2, 23 °C, 2 h, 94%) followed by base-catalyzed intramolecular epoxide opening provided the **9** that cleanly rearranged to the more stable cyclic carbamate **10** (>25:1) under the reaction conditions (5.0 equiv of NaH, THF, 25 °C, 24−72 h, 66−85%).[20] Protection of the primary alcohol (1.6 equiv of DHP, 0.06 equiv of PPTs, CH_2Cl_2, 23 °C, 17 h, 99%), hydrolysis of the cyclic carbamate (4 equiv of KOH, $(CH_2OH)_2$−H_2O, 150 °C, 25 h, 92−94%), and coupling with BOC-Gly-Sar-OH (**13**,[6] 1.05 equiv of EDCI[21] and HOAt,[21] DMF, 23 °C, 83%) provided **14**. Subsequent deprotection of the primary

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(21) EDCI = 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride; HOAt = 1-hydroxy-7-azabenzotriazole; DCC = 1,3-dicyclohexylcarbodiimide.

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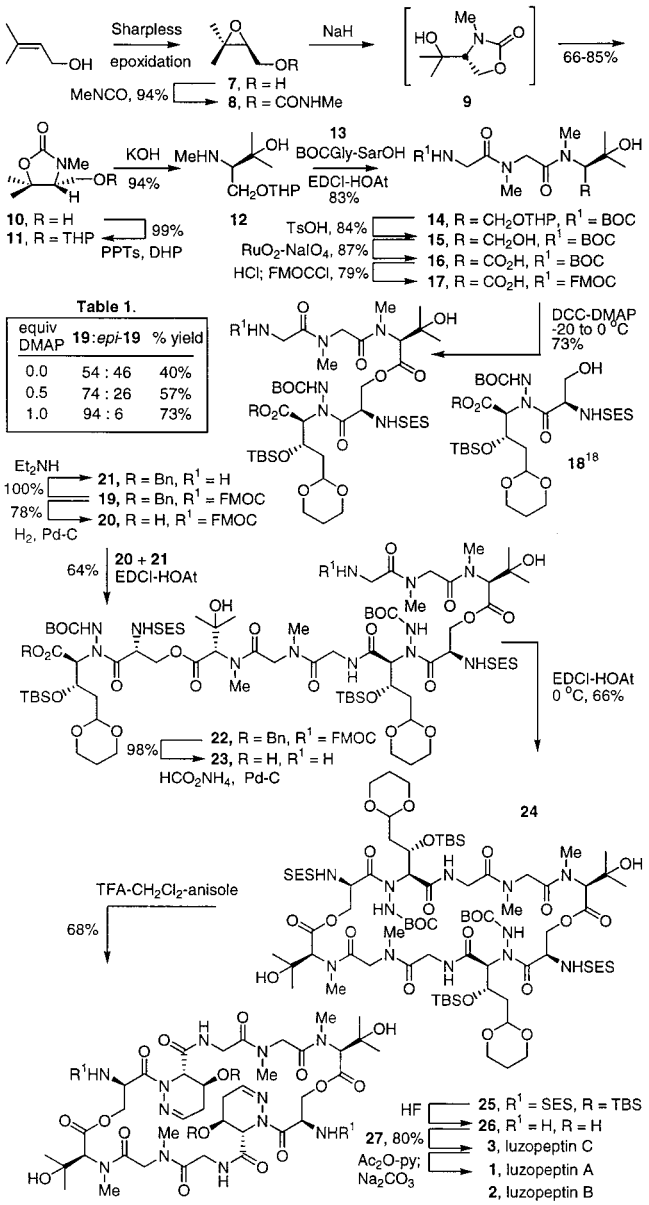
Scheme 1



alcohol (0.05−0.1 equiv of TsOH, CH_3OH, 24 h, 23 °C, 84%), oxidation of **15** to the carboxylic acid **16** that was most effectively accomplished with RuO_2−$NaIO_4$[22] (0.03 equiv/3 equiv, $CCl_4/CH_3CN/H_2O$ 2/2/3, 23 °C, 24 h, 87%), and FMOC/BOC exchange of the amine protecting group provided **17** (4 M HCl−dioxane, 23 °C, 30 min; 1.05 equiv of FMOCCl, 10% aq $NaHCO_3$−dioxane, 23 °C, 8 h, 79%). The key esterification reaction linking the tripeptide **17** (2.0 equiv) with **18**,[18] suitably functionalized for closure to the unusual L-Htp subunit, was accomplished in a surprisingly effective manner upon treatment with DCC-DMAP[6,21] (3.0 equiv/2.0 equiv, CH_2Cl_2, −20 to 0 °C, 17 h, 73%). Much lower conversions and near complete racemization of the hindered *N*-methyl L-*β*-hydroxyvaline were observed with a wide range of alternative reagents or when the reaction was conducted in the absence of DMAP, and the use of increasing amounts of DMAP was found to suppress racemization and improve the overall conversion (Table 1 in Scheme 1). Hydrogenolysis of the benzyl ester deliberately conducted at 10−12 °C to minimize a slow and competitive FMOC deprotection which was observed at 20−25 °C (H_2, 10% Pd−C, CH_3OH, 3 h, 76−78%) and complementary FMOC deprotection of **19** (Et_2NH−CH_3CN 1/2, 23 °C, 20 min, ca. 100%) provided **20** and **21**, respectively.

Linear decadepsipeptide **22** formation was accomplished by coupling **20** with **21** in a reaction mediated by EDCI−HOAt (3 equiv/3 equiv, CH_2Cl_2, 0 °C, 2 h, 64% overall from **19**). Single-step deprotection of both the benzyl ester and FMOC by transfer hydrogenolysis (25% aqueous HCO_2NH_4, 10% Pd−C, EtOH−H_2O, 23 °C, 4 h, 98%) followed by macrocyclization of the crude amino acid **23** with ring closure at the single secondary amide site provided the 32-membered cyclic decadepsipeptide **24** (EDCI−HOAt, 5.0 equiv/5.5 equiv, CH_2Cl_2, 0 °C, 16 h, 63% overall from **22**). Following macrocyclization, the decadepsipeptide exhibited a well-defined ^1H NMR spectrum indicative of a rigid conformation and material that was free of contaminate diastereomers. The use of CH_2Cl_2 versus DMF as solvent and the deliberate exclusion of added bases enhanced the coupling and macrocyclization conversions presumably by minimizing competitive *β*-elimination or retro aldol reactions of the substrates and products. Treatment of **24** with TFA−CH_2Cl_2 in the presence of anisole (1/1/0.4, 0 °C for 2 h, 0 to 23 °C, 1 h, 68%) smoothly provided the fully functionalized cyclic decadepsipeptide **25** incorporating the protected L-Htp residue. Notably, the conversion of **24** to **25** requires six steps involving BOC deprotection, acetal cleavage, and imine cyclization within each L-Htp subunit and proceeded cleanly without competitive OTBS deprotection.[23] Completion of the synthesis required SES deprotection followed by chromophore introduction. The former proved more challenging than related efforts,[6] and treatment of **25** with Bu_4NF or CsF under a variety of conditions ($\pm BOC_2O$)[6] led to deprotection of the OTBS groups without removal of the NSES or, under forcing conditions, to substrate degradation. Gratifyingly, both the NSES and OTBS groups were removed effectively upon treatment with anhydrous HF (neat, anisole, 0 °C, 1.5 h) providing **26** and defining a new, acidic set of conditions that may find widespread applicability to peptide NSES deprotections.[24] Coupling of **26** with 3-hydroxy-6-methoxyquinoline-2-carboxylic acid (5 equiv **27**,[25,26] 5 equiv of EDCI−HOBt, 10 equiv of $NaHCO_3$, DMF, 23 °C, 11 h, 80% overall from **25**) without protection of the chromophore phenol provided luzopeptin C identical in all respects (^1H and ^{13}C NMR, IR, MS, $[\alpha]_D$) with that reported for natural material. Following procedures detailed in the structure elucidation studies,[1] peracetylation of luzopeptin C followed by mild basic hydrolysis of the phenol acetates provided luzopeptin A (Ac_2O-py 1/1, 23 °C, 7 h; 0.1 M Na_2CO_3, THF/CH_3OH/H_2O 3/1/1, 23 °C, 50 min, 50%)[27] and smaller amounts of luzopeptin B (20%) identical in all respects with natural material.

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Supporting Information Available: Characterization of **1**−**3**, **7**, **8**, **10**−**12**, **15**−**17**, **19**, *epi*-**19**, **22**, **24**, and **25** (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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