(–)-Sandramycin: Total Synthesis and Preliminary DNA Binding Properties

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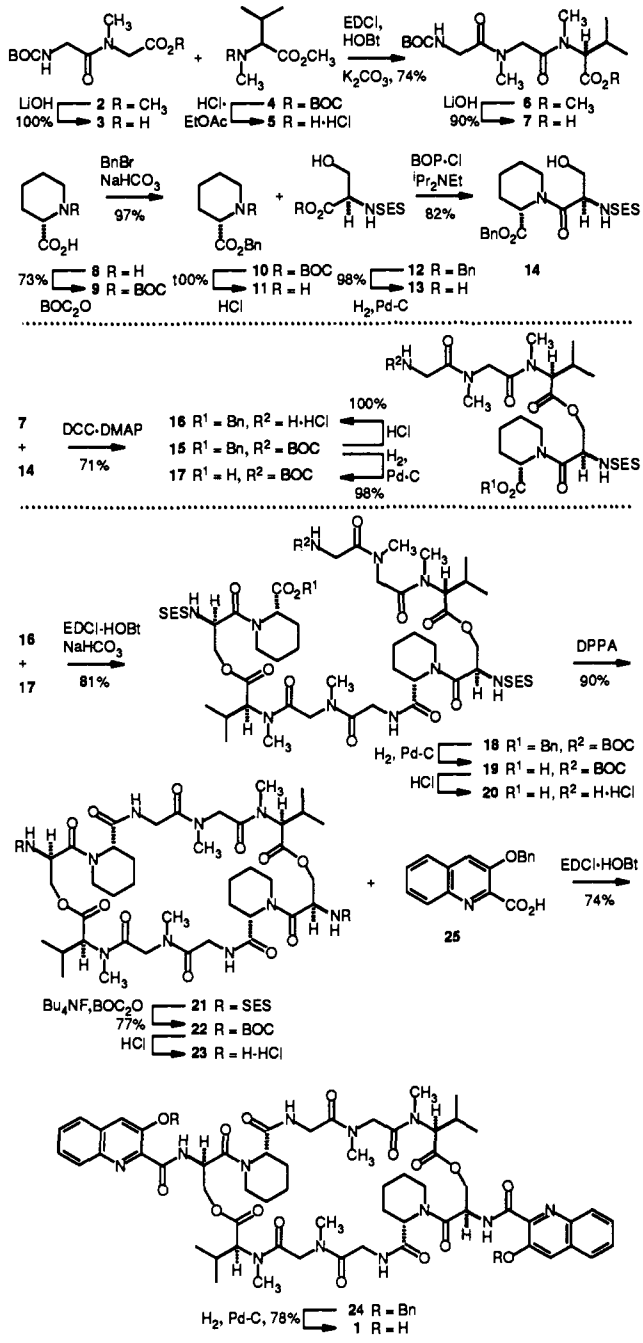
Sandramycin (**1**), a potent antitumor antibiotic[1] isolated from a *Norcardioides* sp. (ATCC 39419) and characterized in spectroscopic and chemical degradation studies,[2] constitutes one member of a growing class of cyclic decadepsipeptides including luzopeptins A-E[3] and quinaldopeptin[4] which possess potent antitumor, antiviral, and antimicrobial activity.[3-5] Like the additional members of this class of agents, sandramycin possesses a two-fold axis of symmetry and two pendant heteroaromatic chromophores that could be expected to provide sequence-selective DNA bifunctional intercalation similar to that described for the luzopeptins.[6-8] In this respect, the agents are functionally related to the bicyclic octadepsipeptide quinoxaline antitumor antibiotics[9] including echinomycin and triostin A.[10,11] Herein, we detail the total synthesis of (–)-sandramycin (**1**), which constitutes the first total synthesis[12] of a member of this class of naturally occurring agents, confirming the structural and absolute stereochemical assignment, and describe the first report of its high-affinity, bifunctional intercalation.

Key elements of the approach include the late stage introduction of the chromophore, providing simple access to analogs possessing modified intercalation capabilities, symmetrical pentadepsipeptide coupling, and macrocyclization of the 32-membered decadepsipeptide conducted at the single secondary amide site and a convergent assemblage of the pentadepsipeptide with introduction of the labile ester linkage in the final coupling reaction.

Coupling of BOC-Gly-Sar-OH (**3**)[13] with L-*N*-Me-Val-OCH_3[14] (**5**; 1 equiv of DCC, Et_3N, catalytic DMAP, CH_2Cl_2, 25 °C, 24 h, 74%) followed by methyl ester hydrolysis of **6** (3 equiv of LiOH, 3:1:1 THF–MeOH–H_2O, 25 °C, 3 h, 90%) provided **7**. Coupling of L-pipecolinic acid benzyl ester (**11**, Scheme I, derived from L-pipecolinic acid[15]) with D-*N*-SES-Ser-OH[16] (**13**, 1.3 equiv of BOP-Cl,[17] Et_3N, CH_2Cl_2, 0 °C, 10 h, 82%) provided the dipeptide **14**. This coupling to provide a tertiary amide could be conducted without deliberate protection of the D-serine hydroxyl group, competitive racemization,[17] or β-elimination and provided **14** suitably protected for incorporation into **15**. Esterification of **7** with **14** provided **15** and was accomplished with use of DCC–DMAP[18] (1 equiv of DCC, 1.0 equiv of DMAP, CH_2Cl_2, 0 °C, 24 h, 71%). Competitive racemization of the L-valine center proved problematic when this reaction was conducted under

Scheme I



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conventional conditions, and the use of increasing amounts of DMAP was found to suppress epimerization (8, 11, 24, and 32% with 1.0, 0.5, 0.2, and 0.1 equiv of DMAP). Linear decadepsipeptide formation was accomplished by deprotection of the amine (3 M HCl–EtOAc, 25 °C, 30 min, 100%) and carboxylic acid (H_2, 10% Pd–C, CH_3OH, 25 °C, 12 h, 98%) of **15** to provide **16** and **17**, respectively, which were coupled with formation of the secondary amide (1 equiv of EDCI, 1 equiv of HOBt, 2.2 equiv of $NaHCO_3$, CH_2Cl_2, 25 °C, 24 h, 81%) to provide **18**. Cyclization of **18** to provide the 32-membered cyclic decadepsipeptide **21**, $[\alpha]^{23}_D$ –88 (*c* 0.85, $CHCl_3$), with ring closure conducted at the single secondary amide site was accomplished by sequential benzyl ester (H_2, 10% Pd–C, CH_3OH, 25 °C, 12 h) and BOC deprotection (3 M HCl–EtOAc, 25 °C, 30 min) followed by treatment of **20** with diphenyl phosphorazidate (4 equiv of DPPA, 10 equiv of $NaHCO_3$, 0.003 M DMF, 0 °C, 48 h, 90% overall).[19] Upon cyclization, the cyclic decadepsipeptide adopts the rigid solution conformation of **1**. Removal of the SES protecting group was accomplished under mild conditions (10 equiv of Bu_4NF, 29 equiv of $(BOC)_2O$, THF, 25 °C, 48 h, 77%) but required in situ trap of the liberated amine as its BOC derivative since the linking ester proved to be unstable to the free amine under the reaction conditions. An X-ray structure determination of **22**[20] confirmed the structural and stereochemical assignments and revealed a cyclic decadepsipeptide conformation essentially identical to that found in the X-ray structure of luzopeptin A.[3c] BOC deprotection of **22** (3 M HCl–EtOAc, 25 °C, 30 min), coupling of the resulting amine **23** with 3-benzyloxyquinoline-2-carboxylic acid[21] (**25**; 4.2 equiv of EDCI, 5.5 equiv of HOBt, Et_3N, DMF, 25 °C, 48 h, 74%) and a final deprotection of **24** ($[\alpha]^{23}_D$ –107 (*c* 0.3, $CHCl_3$); H_2, 10% Pd–C, EtOAc, 25 °C, 12 h, 78%) provided (–)-**1**, $[\alpha]^{23}_D$ –153 (*c* 0.17, $CHCl_3$), identical with a sample of authentic material.

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(20) The author has deposited the atomic coordinates for this structure with the Cambridge Crystallographic Data Centre. The coordinates may be obtained upon request from the Director, Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB2 1EZ, UK.

(21) Full details of the synthesis of **25** are provided in the supplementary material.

Table I. Comparative Biological and DNA Binding Properties

property	sandramycin (**1**)	luzopeptin A
K_B[a] (M^{-1})	9.2×10^6 (1:10)	2.9×10^7 (1:5.5)
(–)-unwinding [c][b]	0.022	0.044
(+)-winding [c][c]	0.033	0.11
IC_{50}, Molt-4 (nM)	0.7	0.8
IC_{50}, L1210 (nM)	0.8	0.2
IC_{50}, B16 (nM)	0.4	0.07

[a] Calf thymus DNA, K_B = absolute binding constant determined by flourescence quenching. The value in parentheses is the agent:base pair ratio at saturating high-affinity binding. [b] Agent/base pair ratio required to unwind negatively supercoiled ϕX174 DNA. [c] Agent/base pair ratio required to induce complete rewinding or positive supercoiling of ϕX174 DNA.

Preliminary studies of the DNA binding properties of **1** revealed that it possesses a binding constant comparable to that of luzopeptin A, binds with a higher selectivity than luzopeptin A (saturated binding at a 1:10 vs 1:5 agent:base pair ratio), and induces the unwinding of negatively supercoiled ϕX174 DNA and its rewinding or positive supercoiling characteristic of bisintercalation at lower agent concentrations than luzopeptin A, Table I. Full details of these and related studies and their extension to structural analogs will be detailed in due course.

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Supplementary Material Available: Full physical and spectroscopic characterization of **1** and **6**, **7**, **14**, **15**, **18**, **21**, **22**, **24**, and **25**; crystallographic data for **22** (20 pages); listing of observed and calculated structure factors (6 pages). This material is contained in many libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.