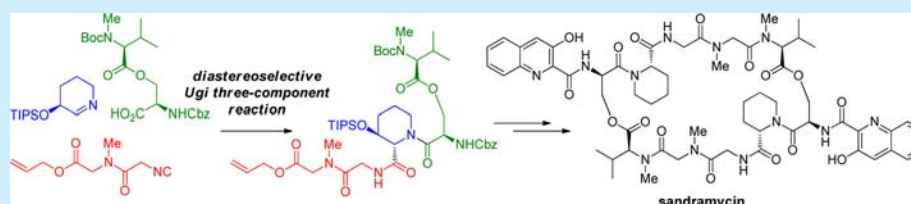


# Total Synthesis of Sandramycin and Its Analogues via a Multicomponent Assemblage

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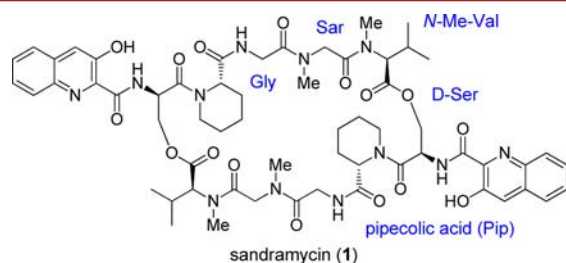
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**S** Supporting Information



**ABSTRACT:** The total synthesis of sandramycin has been accomplished by using a Staudinger/aza-Wittig/diastereoselective Ugi three-component reaction sequence as a key step to obtain a linear pentapeptide. Subsequent [5 + 5] coupling of the pentapeptide, macrolactamization, and introduction of the quinaldin chromophores afforded sandramycin. Dihydroxy and diacetoxy analogues were also prepared, and the cytotoxic activity of these analogues against a range of human cancer cell lines was evaluated.

Sandramycin (**1**),<sup>1</sup> which was isolated from the culture broth of *Norcardioides* sp. (ATCC 39419), constitutes one of the members of a class of C2-symmetric cyclic decadepsipeptides<sup>2</sup> that also include the luzopeptins<sup>3</sup> and quinoxapeptins (Figure 1).<sup>4</sup> Extensive efforts to elucidate the mode of action of **1** by

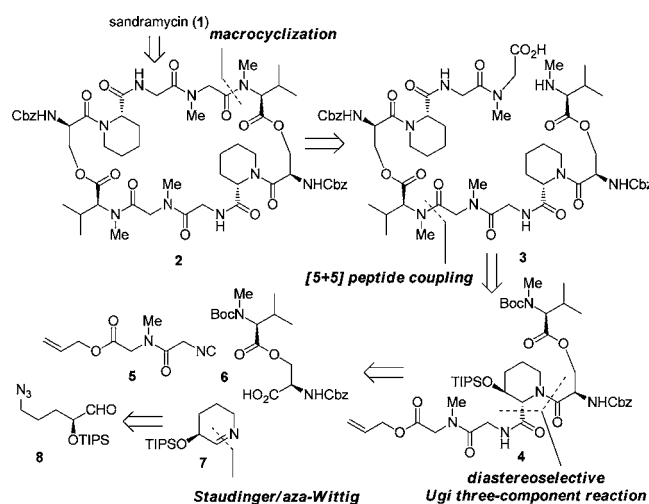


**Figure 1.** Structure of sandramycin.

Boger's group revealed that it binds to the minor groove of double-stranded DNA (dsDNA), preferentially to the regions containing 5'-AT dinucleotides, with bisintercalation of its hydroxyquinaldic acid chromophores.<sup>5</sup> Sandramycin has exceptionally potent activity against mouse leukemia L1210 and mouse melanoma B16 cells *in vitro* with IC<sub>50</sub> values of 0.02 and 0.07 nM, respectively. Furthermore, *in vivo* antitumor activity tested against mouse lymphocytic leukemia P388 in mice is promising. Therefore, sandramycin and its analogues<sup>5</sup> display potential for therapeutic use in anticancer chemotherapy. The cyclic decadepsipeptide framework of **1** constitutes Gly, sarcosine (Sar), *N*-Me-L-Val, L-pipecolic acid (L-Pip), and D-Ser. Total synthesis of **1**<sup>5,7</sup> as well as luzopeptins<sup>8,9</sup> and quinoxapeptins<sup>10</sup> has been accomplished via a sequential peptide coupling approach. Herein we describe the total synthesis of **1** and its analogues via an Ugi three-component

reaction (U3CR) with a cyclic imine,<sup>11,12</sup> which can be applicable to the synthesis of a range of analogues at the peptide framework. We retrosynthetically disconnected the macrocycle **2** and the linear decapeptide **3** at the *N*-methylamide bonds linking Sar and *N*-Me-L-Val residues (Scheme 1). This is advantageous because Sar has no substituent at the  $\alpha$ -position and the peptide coupling is free from racemization in the [5 + 5] coupling and the macrolactamization. Our key step to the approach to the synthesis of the pentapeptide **4** is a sequential Staudinger/

**Scheme 1**



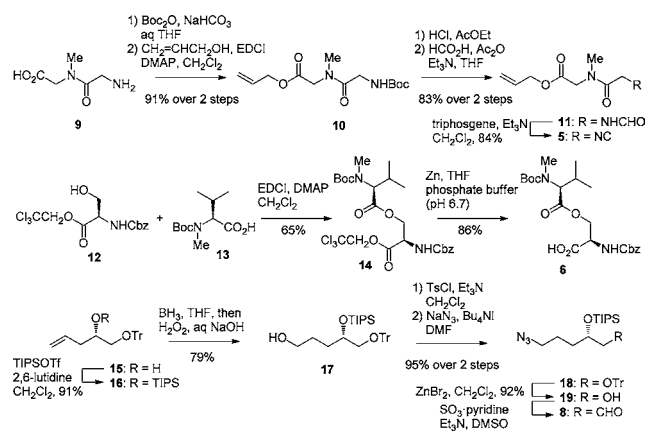
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aza-Wittig/diastereoselective U3CR<sup>13</sup> of the azidoaldehyde **8** with the isonitrile **5** and the carboxylic acid **6** to obtain **4**. This strategy constructs a nonproteinogenic amino acid, L-Pip residue, with simultaneous linking to the two dipeptides **5** and **6** at the C- and N-termini. The silyloxy substituent was introduced at the  $\alpha$ -position to the cyclic imine **7** in order to control the stereoselectivity at the newly formed stereogenic center.<sup>14–25</sup>

Each key component to the U3CR was prepared as shown in Scheme 2. Suitably protected Gly-Sar **11** prepared from **9** was

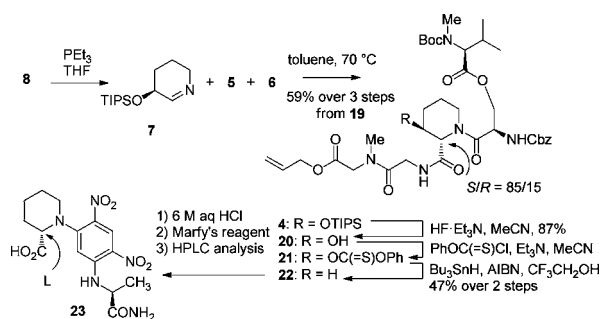
Scheme 2



dehydrated to give the isonitrile **5** (triphosgene, Et<sub>3</sub>N, CH<sub>2</sub>Cl<sub>2</sub>, 84%). The carboxylic acid component **6** was prepared by the coupling of the appropriately protected D-Ser derivative **12**<sup>26</sup> and N-Boc-N-Me-L-Val (**13**)<sup>27</sup> (EDCI, DMAP, CH<sub>2</sub>Cl<sub>2</sub>, 65%) followed by deprotection of the Tce group (Zn, THF, phosphate buffer (pH 6.7), 86%). The known homoallyl alcohol **15**<sup>28</sup> was protected with a TIPS group (TIPSOTf, 2,6-lutidine, CH<sub>2</sub>Cl<sub>2</sub>, 91%) to give **16**, which was converted to the alcohol **17** by hydroboration followed by oxidation (BH<sub>3</sub>, THF, then H<sub>2</sub>O<sub>2</sub>, aq NaOH, 79%). The alcohol **17** was converted to the azide **18**, and deprotection of the Tr group gave **19** (ZnBr<sub>2</sub>, CH<sub>2</sub>Cl<sub>2</sub>, 92%). The resulting primary hydroxyl group was oxidized to afford the aldehyde **8** (SO<sub>3</sub>·pyridine, Et<sub>3</sub>N, DMSO), which was directly used for the following Staudinger/aza-Wittig/U3CR sequence (Scheme 3).

Treatment of **8** with PEt<sub>3</sub> in THF resulted in a clean conversion to the corresponding cyclic imine **7**, which was subsequently reacted with the isonitrile **5** and the carboxylic acid **6** at 70 °C. As a result, the U3CR proceeded in a diastereoselective manner, and the desired pentadepsipeptide **4** was obtained in 68% yield (S/R = 85/15). The major

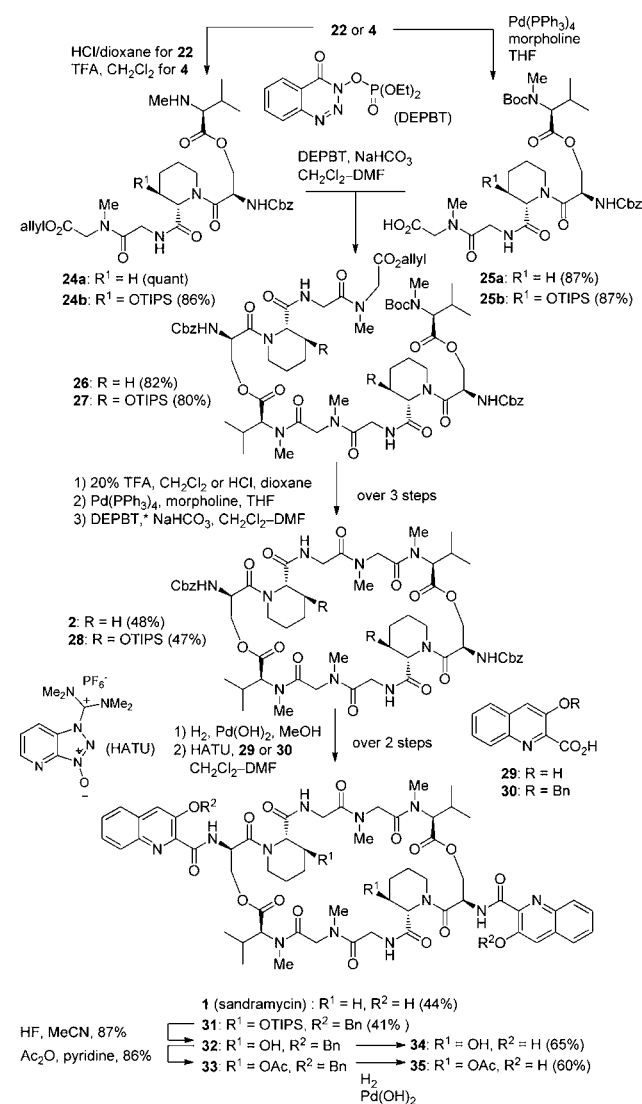
Scheme 3



diastereomer was isolated in 59% yield by silica gel column chromatography. The stereochemistry of the  $\alpha$ -position of the newly constructed Pip residue in the major product was determined by conventional amino acid analysis<sup>29</sup> (see Supporting Information) of the deoxygenated derivative **22**, which was obtained from **4** after deprotection of the TIPS group followed by radical deoxygenation<sup>30</sup> of **20** (PhC(=S)Cl, Et<sub>3</sub>N, CH<sub>2</sub>Cl<sub>2</sub>, then Bu<sub>3</sub>SnH, AIBN, C<sub>6</sub>F<sub>5</sub>OH, CF<sub>3</sub>CH<sub>2</sub>OH, reflux, 47% over two steps). These results unambiguously determined the absolute stereochemistry of the Pip residue and confirmed the C–C bond was formed trans to the protected hydroxyl group of the cyclic imine **7** in the U3CR as expected.

Deprotection of either the Boc group or the allyl group of **22** gave the amine **24a** (25% HCl, dioxane) or the carboxylic acid **25a** (Pd(PPh<sub>3</sub>)<sub>4</sub>, morpholine, THF, 87%) (Scheme 4). The [5

Scheme 4



+ 5] assemblage of **24a** and **25a** afforded the protected decadepsipeptide **26** (3-(diethoxyphosphoryloxy)-1,2,3-benzotriazin-4(3H)-one (DEPBT),<sup>31</sup> NaHCO<sub>3</sub>, CH<sub>2</sub>Cl<sub>2</sub>–DMF, 82%). Deprotection of the Boc and the allyl groups of **26** under the same conditions used for the preparation of **24a** and **25a** gave the free linear decadepsipeptide **4** (Scheme 1), which was then cyclized by treatment with DEPBT in CH<sub>2</sub>Cl<sub>2</sub>–DMF

Table 1. DNA Binding Properties of **1**, **34**, and **35**

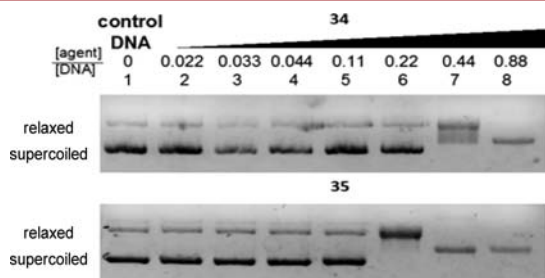
		sequence			
		5'-GCATGC-3' 3'-CGTACG-5'	5'-GCGCGC-3' 3'-CGCGCG-5'	5'-GCTAGC-3' 3'-CGATCG-5'	5'-GCCGCG-3' 3'-CGGCCG-5'
<b>1</b> <sup>a</sup>	$K_b$ ( $10^7$ M <sup>-1</sup> )	23.0	14.5	8.5	8.0
	$\Delta G^\circ$ (kcal/mol)	-11.4	-11.0	-10.8	-10.8
<b>34</b>	$K_b$ ( $10^7$ M <sup>-1</sup> )	3.03	6.07	2.95	5.01
	$\Delta G^\circ$ (kcal/mol)	-8.8	-9.2	-8.8	-9.1
<b>35</b>	$K_b$ ( $10^7$ M <sup>-1</sup> )	4.37	3.33	2.88	3.27
	$\Delta G^\circ$ (kcal/mol)	-9.0	-8.9	-8.8	-8.9

<sup>a</sup>Data from reported.<sup>8b</sup>

to afford the cyclic decapeptide **2** in 48% yield over three steps from **3**. Dimerization/cyclization of the free peptapeptide prepared by deprotection of both *N*- and *C*-terminal protecting groups of the pentapeptide **22** was also investigated to directly obtain the macrocycle **2**. However, those efforts were unsuccessful and only the cyclic pentapeptide was obtained (data not shown).

Finally, the Cbz groups of **2** were removed by hydrogenolysis ( $H_2$ , Pd(OH)<sub>2</sub>, MeOH), and the liberated amines were coupled with 3-hydroxyquinaldic acids **29**<sup>32</sup> to complete the total synthesis of **1** (HATU, *i*Pr<sub>2</sub>NEt, DMF, 44% over two steps). The analytical data of the synthetic **1** were in good agreement with those reported.<sup>5,7</sup> Dihydroxy analogue **34** and diacetoxy analogue **35** were also prepared from **4** and **30** in a manner similar to the synthesis of **1**.

The binding properties of **34** and **35** were investigated (Figure S2), and the results are summarized in Table 1. The binding affinity and sequence selectivity of synthesized cyclic decapeptides were evaluated by fluorescence quenching experiment<sup>5,8</sup> using four octamer double stranded oligodeoxynucleotides (dsODNs). Compared to **1**, analogues **34** and **35** exhibited a reduced binding affinity to dsODNs and their sequence selectivity was subtly altered compared to **1** but still with high DNA binding potency. Treatment of negatively supercoiled  $\phi$ X174 DNA with **34** and **35** successfully caused the unwinding to form relaxed DNA, and a further increase in the amount of agents successfully formed positive supercoils (Figure 2). The cytotoxic activity of **1**, **34**, and **35** was evaluated

Figure 2. DNA unwinding experiments of **34** and **35**.

against human cancer cells (Table 2). Their DNA binding properties and cytotoxic activity are correlated. Namely, synthetic **1** exhibits strong cytotoxic activity against a range of cell lines with IC<sub>50</sub> values in the range 0.8–5.9 nM. On the other hand, the activity of **34** and **35** was reduced by 2–3 orders of magnitude. Introducing the hydroxyl and acetoxy group at the *L*-Pip residue caused a decrease in DNA binding. As a result, the cytotoxic activity was decreased.

Table 2. Cytotoxic Activity of **1** and Its Analogues against Human Cancer Cell Lines<sup>a</sup>

	IC <sub>50</sub> (nM)					
	HCT-118	RPMI8226	A431	RKO	SU-DHL6	SU-DHL10
<b>1</b>	0.8	3.8	3.1	1.3	5.9	3.3
<b>34</b>	1000	2200	1500	1100	1500	1500
<b>35</b>	390	530	630	390	330	370

<sup>a</sup>HCT-118 and RKO: human colon cancer cells. A431: human epidermal cancer cells. RPMI8226: human myeloma cells. SU-DHL6 and SU-DHL10: human diffuse large B-cell lymphoma cells.

Echinomycin,<sup>33</sup> which is another C2-symmetric cyclic octadecapeptide bisintercalator,<sup>34</sup> is known to inhibit the binding of transcription factor HIF-1 $\alpha$  to the *cis*-element in genome DNA.<sup>35</sup> The ability of **1** to inhibit HIF-1 $\alpha$  was preliminarily investigated by using an HIF-1 reporter gene assay in HEK293 cells (Figures S3 and S4). Different from echinomycin, **1** does not inhibit the transcription activity of HIF-1 $\alpha$  and  $\beta$ . These data suggest that the mode of action of **1** is different from that of echinomycin.

In conclusion, the total synthesis of sandramycin (**1**) as well as its analogues **34** and **35** of the macrocycle moiety was accomplished. Central to our approach to the synthesis of the pentadecapeptide **4** is a key Staudinger/aza-Wittig/diastereoselective U3CR sequence and racemization-free [5 + 5] coupling and the macrolactamization. This strategy can be applicable to other congeners of this class of natural products.

## ■ ASSOCIATED CONTENT

### Supporting Information

Complete experimental procedure and characterization data of all the new compounds, amino acid analysis of **22**, DNA binding constant measurement, DNA unwinding experiments, and HIF-1 inhibition assay. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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### Notes

The authors declare no competing financial interest.

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- (29) The pentapeptide **22** was heated under reflux in 6 M aq. HCl for 24 h, and the resulting mixture was treated with Marfey's reagent. The reaction mixture was analyzed by reversed phase HPLC (ODS, 10–60% MeCN–H<sub>2</sub>O linear gradient containing 0.1% TFA) with authentic material **23** derived from L- or D-Pip. For details, see Supporting Information.
- (30) As for the radical reduction step, the use of CF<sub>3</sub>CH<sub>2</sub>OH as a solvent was essential. Extensive β-elimination was observed in toluene, and the desired **22** was not obtained at all.
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