Letter

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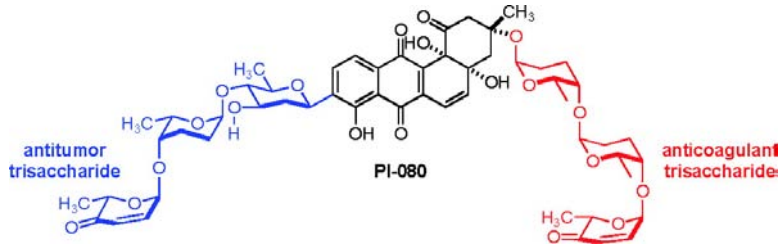

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De Novo Asymmetric Synthesis and Biological Evaluation of the Trisaccharide Portion of PI-080 and Vineomycin B2

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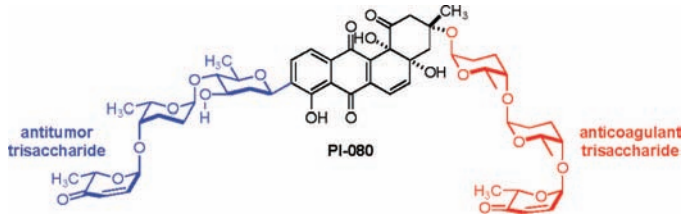
ABSTRACT



A highly enantio- and diastereoselective synthesis of an α-L-aculose, α-L-rhodinose, and β-D-olivose trisaccharide is described. The key transformations include the palladium-catalyzed glycosylation, Myers' reductive rearrangement, diastereoselective dihydroxylation, and regioselective Mitsunobu inversion. Significant apoptotic antitumor activity was found for this trisaccharide, which has implication for vineomycin B2 and PI-080 structure−activity relationship.

Of the anthracycline antibiotics, the angucycline family of antibiotics has been of particular interest to both the synthetic and biological communities due to their unique structures and potent antitumor and antibacterial activities.[1] The structural diversity of this class of natural products is typified by two different *C*-glycoside natural products: PI-080 (**1**)[2] and vineomycin B2 (**2**).[3] Both PI-080 and vineomycin B2 share a β-*C*-glycoside linked α-L-aculose, α-L-rhodinose (**4**), and β-D-olivose trisaccharide subunit, whereas PI-080 has an additional α-L-aculose-bis-α-L-rhodinose trisaccharide (**3**).

In addition to representing a range of structures (Figure 1), the angucycline family of antibiotics possesses a range of biological activities. The antibiotic vineomycin B2 displays potent antitumor activity,[3,4] whereas PI-080 strongly inhibits blood coagulation.[2]

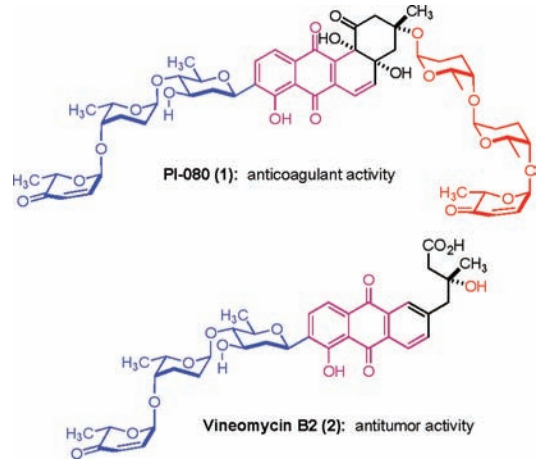


Figure 1. Structures of PI-080 and vineomycin B2.

While the antitumor activity of the angucyclines has been attributed to the anthraquinone portion of natural products,[4] the

(1) (a) Rohr, J.; Thiericke, R. *Nat. Prod. Rep.* **1992**, *9*, 103–137. (b) Krohn, K.; Rohr, J. *Top. Curr. Chem.* **1997**, *188*, 127–195. (c) Andriy, L.; Andreas, V.; Andreas, B. *Mol. BioSyst.* **2005**, *1*, 117–126.

(2) Two natural products have been isolated with the same structure as PI-080. For PI-080, see: (a) Kawashima, A.; Kishimura, Y.; Tamai, M.; Hanada, K. *Chem. Pharm. Bull.* **1989**, *37*, 3429–3431. For PI-6621, see: (b) Kawashima, A.; Yoshimura, Y.; Kamigori, K.; Yagishi, M.; Mizutani, T. *Chem. Abstr.* **1989**, *114*, 22464d.

(3) For the isolation of vineomycin B2, see: (a) Omura, S.; Tanaka, H.; Oiwa, R.; Awaya, L.; Masuma, R.; Tanaka, K. *J. Antibiot.* **1977**, *30*, 908–916. (b) Imamura, N.; Nakinuma, K.; Ikekawa, N.; Tanaka, H.; Omura, S. *J. Antibiot.* **1981**, *34*, 1517–1518.

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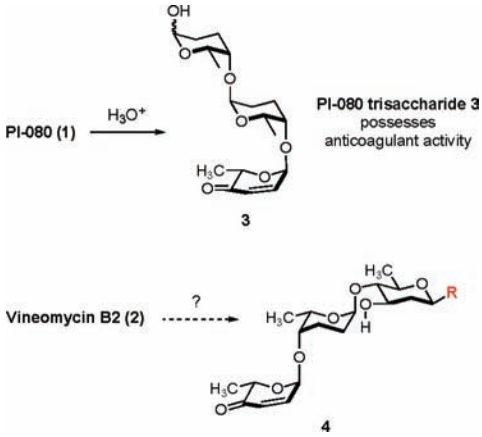
anticoagulant activity of PI-080 appears to be associated with one of the trisaccharide portions of the molecule (i.e., the trisaccharide **3**).[2] As part of a larger effort to use the de novo synthesis of carbohydrates for medicinal chemistry, we became interested in elucidating the structural origins of the anticancer activity of vineomycin B2 (Scheme 1). In particular, we were interested in determining whether any of the antitumor activity associated with vineomycin B2 could be assigned to the trisaccharide subunit **4** (R = OPMB).

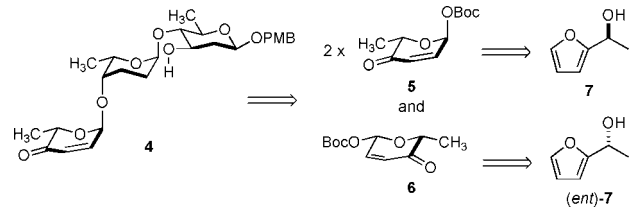
There are no reports of the total synthesis of PI-080 and vineomycin B2,[5] however the synthesis of the trisaccharide portion **3** has been completed by Sulikowski.[6] While the trisaccharide **3** could be obtained from natural sources, access to the trisaccharide subunit **4** (R = OPMB) required synthesis. Recently, we had some success using our Pd-catalyzed glycosylation and post-glycosylation reactions for the synthesis of several naturally occurring rare sugar structural motifs.[7] Herein we describe the de novo synthesis of trisaccharide subunit **4** from commercially available acylfuran. In addition, we report its antiproliferative activity toward several tumor cells lines and show this activity occurs via apoptosis at 50 μM and necrosis at higher concentration.

Scheme 1. Structures of PI-080 and Vineomycin B2 and Their Trisaccharide Motifs **3** and **4**



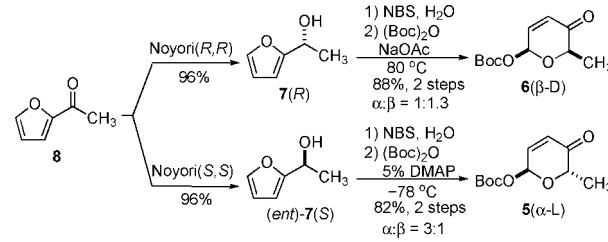
Retrosynthetically, we envisioned the α-L-aculose and α-L-rhodinose subunits of trisaccharide **4** as coming from two α-L-pyranones (**5**). Similarly, we conceived the β-D-olivose ring of **4** as coming from β-D-pyranone **6** (Scheme 2). The two pyranones could be prepared from the corresponding enantiomeric furyl alcohols, which in turn could be prepared by a Noyori reduction of 2-acylfuran (Scheme 2).

Scheme 2. Retrosynthetic Analysis of the Vineomycin B2 Trisaccharide



Previously, we have shown that the required Pd glycosyl donors α-L-pyranones **5** and β-D-pyranones **6**, as well as their enantiomers, could be prepared from acyl furan **8** (Scheme 3). The stereodivergent route employed an enantioselective Noyori reduction (**8** to **7**/*ent*-**7**),[8,9] an Achmatowicz oxidation, and diastereoselective *tert*-butyl carbonate formation (Scheme 3).[7,10] The β-pyranone **6** could be isolated in 50% when the Boc protection was performed at elevated temperature ($(Boc)_2O$/NaOAc in benzene at 80 °C). Alternatively, α-pyranone **5** could be selectively prepared in 62% (α:β = 3:1) at low temperature (−78 °C).

Scheme 3. Approach to D/L-, α/β-Boc-Pyranones **5** and **6**



With a practical route to the desired pyranone glycosyl donors, we next turned our attention toward the preparation of 2-deoxy-β-L-olivose ring of the trisaccharide **4**. Previously, we reported an approach to 2-deoxy-β-glycoside associated with *allo*- and *gluco*-stereochemistry.[7,11] Key to the successful implementation of this strategy is the selective Mitsunobu-like inversion of the *allo*-stereochemistry of the digitoxose sugar **12** into olivose sugar **13** (Scheme 4).

In practice, we began with the palladium-catalyzed glycosylation (5 mol % of $Pd_2(dba)_3$·$CHCl_3$/10% PPh_3) of PMBOH

(4) Both vineomycin B2 and the related monosaccharide angucycline, vineomycinone B2 (**2** sans α-L-aculose-α-L-rhodinose), possess antitumor activity against S-180 solid tumors in mice, thus associating the antitumor activity with the common anthraquinone portion of the molecules; see ref 3.

(5) For the synthesis of vineomycinone B2, see: (a) Danishefsky, S. J.; Uang, B. J.; Quallich, G. *J. Am. Chem. Soc.* **1985**, *107*, 1285–1293. (b) Bolitt, V.; Mioskowski, C.; Kollah, R. O.; Manna, S.; Rajapaksa, D.; Falck, J. R. *J. Am. Chem. Soc.* **1991**, *113*, 6320–6321. (c) Tius, M. A.; Gomez-Galeno, J.; Gu, X.-q.; Zaidi, J. H. *J. Am. Chem. Soc.* **1991**, *113*, 5775–5783. (d) Matsumoto, T.; Katsuki, M.; Jona, H.; Suzuki, K. *J. Am. Chem. Soc.* **1991**, *113*, 6982–6992.

(6) (a) Sobti, A.; Kyungjin Kim, K.; Sulikowski, G. A. *J. Org. Chem.* **1996**, *61*, 6–7. For related approaches, see: (b) Sasaki, K.; Matsumura, S.; Toshima, K. *Tetrahedron Lett.* **2007**, *48*, 6982–6986.

(7) (a) Zhou, M.; O'Doherty, G. A. *Org. Lett.* **2008**, *10*, 2283–2286. (b) Guo, H.; O'Doherty, G. A. *J. Org. Chem.* **2008**, *73*, 5211–5220.

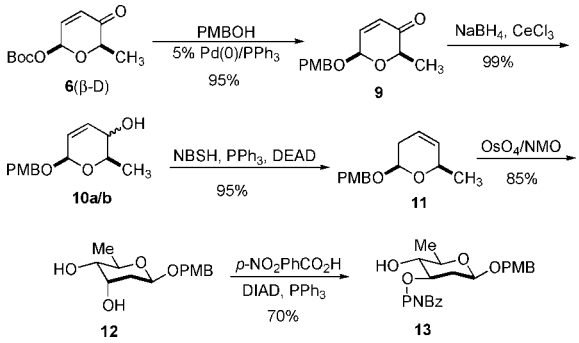
(8) Fujii, A.; Hashiguchi, S.; Uematsu, N.; Ikariya, T.; Noyori, R. *J. Am. Chem. Soc.* **1996**, *118*, 2521–2522.

(9) (a) Li, M.; Scott, J. G.; O'Doherty, G. A. *Tetrahedron Lett.* **2004**, *45*, 1005–1009. (b) Li, M.; O'Doherty, G. A. *Tetrahedron Lett.* **2004**, *45*, 6407–6411.

(10) (a) Babu, R. S.; O'Doherty, G. A. *J. Carbohydr. Chem.* **2005**, *24*, 169–177. (b) Guo, H.; O'Doherty, G. A. *Org. Lett.* **2005**, *7*, 3921–3924.

(11) Zhou, M.; O'Doherty, G. A. *J. Org. Chem.* **2007**, *72*, 2485–2493.

Scheme 4. Synthesis of a Protected β-D-Olivose **13**



with the β-D-pyranone **6** to form the β-benzyloxy pyranone **9** in 95% yield as a single diastereomer (Scheme 4). Ketone reduction of pyranone **9** with $NaBH_4/CeCl_3$ gave a mixture of allylic alcohols **10a/b** in 99% yield (dr ~ 1.5 to 1). Fortunately, both diastereomers **10a/b** could be used in the next reaction. Applying the Myers' reductive rearrangement conditions (NBSH, PPh_3/DEAD, NMM, −30 °C to rt)[12] to the mixture of allylic alcohols **10a/b** cleanly provided olefin **11** in 95% yield. Finally, exposing olefin **11** to the Upjohn conditions (OsO_4/NMO)[13] gave exclusively the diol **12** with *allo*-stereochemistry in 85% yield. We then turned to our selective diol inversion. Thus, subjecting **12** to the typical Mitsunobu conditions (*p*-NO_2PhCO_2H/PPh_3/DIAD) cleanly converted it into the protected β-D-olivose **13** in 70% yield. A nice feature of this approach is that the nitrobenzoate group also serves as a temporary protecting group.

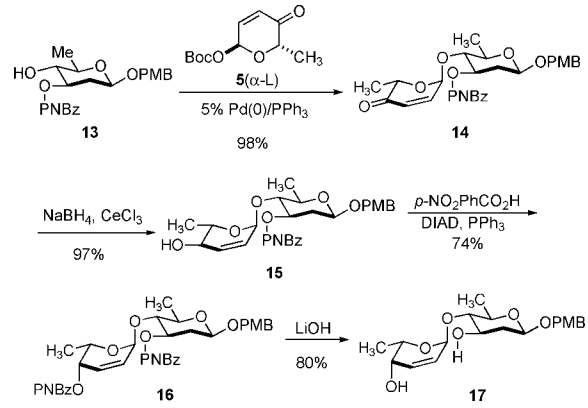
With a viable route to the protected β-D-*olivo*-sugar **13** in hand, our efforts turned to the installation of the α-L-rhodinose portion (Scheme 5). Thus, the *olivo*-sugar **13** and α-L-pyranone **5** were coupled using our typical palladium-catalyzed glycosylation conditions (5 mol % of $Pd_2(dba)_3$·$CHCl_3$/10% PPh_3, 98% yield) to form **14** with complete stereocontrol at the anomeric center. Luche reduction of the keto group in pyranone **14** stereoselectively provided allylic alcohol **15** (97%). At this stage, a Mitsunobu reaction (PPh_3/DIAD/*p*-NO_2PhCO_2H, 74%) was used to afford bis-nitrobenzoate **16** with inverted stereochemistry at C-4 of the newly introduced sugar. Unfortunately, all efforts to differentiate the two *p*-nitrobenzoate groups in **16** were unsuccessful. As a result, we opted for hydrolyses of both benzoates with excess LiOH which afford diol **17** (80%) and then turned to the selective introduction of the α-L-aculose at C-4 of the rhodinose sugar.

Our investigations of the selective introduction of the α-L-aculose at C-4 were performed on both diol **17** and diol **18** (Scheme 6), which could easily be prepared by hydrogenation with excess diimide (NBSH/Et_3N, 80%). The Pd-catalyzed glycosylation (5 mol % of $Pd_2(dba)_3$·$CHCl_3$/10% PPh_3) of alcohol **18** with α-L-pyranone **5** proved to be the most selective.

(12) (a) Myers, A. G.; Zheng, B. *J. Am. Chem. Soc.* **1996**, *118*, 4492–4493. (b) Myers, A. G.; Zheng, B.; Movassaghi, M. *J. Org. Chem.* **1997**, *62*, 7507–7507. (c) Haukaas, M. H.; O'Doherty, G. A. *Org. Lett.* **2002**, *4*, 1771–1774.

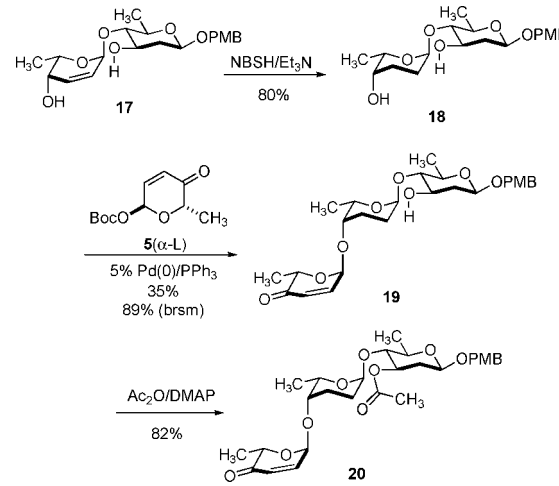
(13) VanRheenen, V.; Kelly, R. C.; Cha, D. Y. *Tetrahedron Lett.* **1976**, *17*, 1973–1976.

Scheme 5. Synthesis of a β-D-Olivose/α-L-Rhodinose Disaccharide



This was most conveniently performed at low conversion using a slight excess of α-L-pyranone **5** under dilute conditions to provide 35% yield of the desired trisaccharide **19**, along with 61% recovered starting material **18** (89% yield, based on recovered starting material). To confirm the regioselectivity of glycosylation, **19** was acylated with Ac_2O/DMAP to form **20** (82%). Analysis of the one- and two-dimensional NMR data of acetate **20** indicated that the glycosylation of **18** occurred selectively at the C-4 position of the α-L-rhodinose sugar. Overall, the trisaccharide **19** was prepared from the achiral acylfuran **8** in 14 steps and 17% overall yield.

Scheme 6. Synthesis of the Vineomycin B2/PI-080 Trisaccharide **19**



With significant quantities of trisaccharide **19** in hand, we decided to screen it against two types of cancer cell lines from nine different tissue types (Table 1). While we were motivated to test **19** primarily as a control for future studies, we were delighted to find it had wide ranging cytotoxicity, displaying significant growth inhibition (GI_{50} from 0.1 to 11 μM) and cytotoxicity (LC_{50} from 5.1 to 100 μM) against these various cell lines. In fact, at 50 μM, the trisaccharide **19** showed greater

Table 1. Cytotoxicity Evaluation of Trisaccharide **19**

cell type	panel/cell line	**19** (μM) GI_{50}[a]	TGI[b]	LC_{50}[c]
leukemia	RPMI-8226	0.12	2.80	>100
	SR	0.60	19.0	>100
non-small cell lung	NCI-H23	1.98	4.59	12.0
	NCI-H460	11.0	23.7	51.0
colon	COLO-205	1.66	3.02	5.50
	SW-620	1.35	2.64	5.13
CNS	SF-268	3.09	9.54	31.5
	U251	1.82	4.10	9.26
melanoma	LOX IMVI	1.53	2.87	5.40
	SK-MEL-28	1.82	3.22	5.71
ovarian	IGROV1	1.62	3.32	6.81
	OVCAR-3	1.60	2.95	5.43
renal	TK-10	2.32	6.46	23.1
	UO-31	1.49	2.81	5.30
prostate	PC-3	1.74	3.99	9.16
	DU-145	1.81	3.20	5.66
breast	MCF7	1.63	3.38	7.04
	T-47D	1.42	2.77	54.1

[a] GI_{50}: the drug concentration resulting in a 50% reduction in the net protein increase in control cells. [b] TGI: the drug concentration resulting in no increase in the measured protein as compared to control cells. [c] LC_{50}: the drug concentration resulting in a 50% reduction in the measured protein as compared to control cells.

cytotoxicity than cisplatin at 300 μM (Figure 2). Interestingly, this level of cytotoxicity is in the range of that found for other glycosylated angucycline antibiotics.[14]

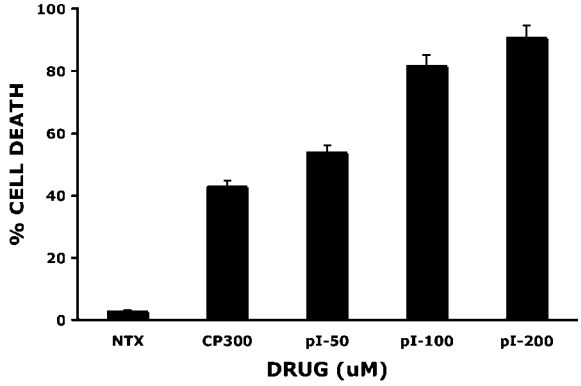


Figure 2. Cytotoxicity of **19**/cisplatin toward H460. Graph denotes total cell death (apoptosis + necrosis) observed upon treatment of H460 cells with either no drug (NTX), 300 μM cisplatin (CP300), 50 μM of **19** (PI-50), 100 μM of **19** (PI-100), 200 μM of **19** (PI-200).

We decided to further study the mechanism of cytotoxicity of **19** toward human H460 lung epithelial cancer cells (Figure 2). Thus, H460 human lung epithelial cells were treated with trisaccharide **19** at a range of concentrations (50, 100, and 200 μM) and cisplatin (300 μM) for comparison. To ascertain the mechanism of cell death (Figure 3), the samples were then stained with propidium iodide (PI, 30 μg/mL) and 1 μg/mL Hoechst (HT, 1 μg/mL). Apoptotic cells appeared shrunken with condensed nuclei and stained only for Hoechst, whereas cells that have underwent apoptosis-dependent or apoptosis-independent necrosis stained for both PI and Hoechst.

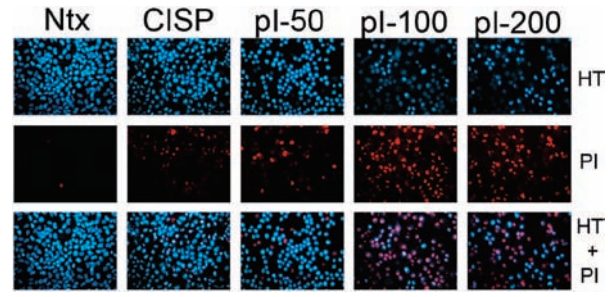


Figure 3. Mechanism of cell death apoptosis/necrosis. H460 lung epithelial cancer cells were treated with either cisplatin (300 μM) or **19** (50, 100, and 200 μM) for 8 h in serum-free medium, following which each well was incubated in 30 μg/mL propidium iodide (PI) and 1 μg/mL Hoechst (HT) for 30 min. Images show cells stained for Hoechst alone (top panel, blue), PI alone (middle panel, red), or both.

At 50 μM of trisaccharide **19**, the H460 cells stained mainly for Hoechst and displayed condensed nuclear bodies without significant uptake of propidium iodide, suggesting that cell death was due primarily to apoptosis. However at higher concentrations of **19**, cell death predominantly occurred via necrosis, which was indicated by PI staining without nuclear bodies.

In conclusion, a highly enantioselective de novo route to vineomycin B2/PI-080 trisaccharide **19** has been developed. The trisaccharide portion of these natural products was demonstrated to suppress the proliferation of several human cancer cell lines via an apoptotic mechanism at 50 μM. However, when **19** was tested at higher concentrations (100 and 200 μM), a significant amount of necrosis was also detected. This synthetic/biological study establishes for the first time the importance of the trisaccharide portion to the SAR for these molecules. The preparation and further biological investigation of other analogues are ongoing.

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Supporting Information Available: Complete experimental procedures and spectral data for all new compounds can be found in the Supporting Information. This material is available free of charge via the Internet at http://pubs.acs.org.

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(14) Abdelfattah, M. S.; Kharel, M. K.; Hitron, J. A.; Baig, I.; Rohr, J. *J. Nat. Prod.* **2008**, ASAP 10.1021/np800281f.