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Synthesis and anti-tumor activity of β-*C*-glycoside analogs of the immunostimulant KRN7000

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Abstract—A ring-closing metathesis approach was employed for the synthesis of a β-*C*-glycoside analog of the immunostimulant KRN7000. The protected *C*-glycosyl amino acid derivative **18** was converted to amino-olefin **20**, and osmylation served to install the diol unit as a mixture of separable *syn* and *anti* isomers. Deprotection to the hydroxy-amine **21** was followed by *N*-acylation and debenzylation to deliver the target compound **5**.

Our laboratory has published several papers that have demonstrated the efficiency of an esterification–ring-closing metathesis strategy (RCM)[1] for the synthesis[2] of a variety of carbohydrate[3] mimetics such as *C*-glycosides,[4] *C*-disaccharides,[5] and recently, *C*-trisaccharides.[6] In this letter, we describe the use of this methodology for the preparation of a stable β-*C*-glycoside analog **5** of the potent immunostimulant KRN7000 (**2**). KRN7000 (**2**) was born out of a structure–function study[7] on the naturally occurring ceramide derivative agelasphin-9b (**1**)[8] discovered by Koezuka and co-workers. It was discovered that **2** possessed potent anti-tumor activity in B16-bearing mice.[7,9] This anti-tumor activity is the result of KRN7000 (**2**) activating the dendritic and natural killer T cells,[10] giving rise to antigen-specific immune stimulation in animals. KRN7000 has also shown promise for the treatment of various autoimmune diseases.[11] The β-gluco derivative AGL-10 (**4**) has also been isolated and demonstrated attenuated anti-tumor activity relative to its α-galactosyl counterparts.[9] Testing of *O*-analogs of both the β- and α-anomers revealed a similar trend.[9] We were curious to determine if a blended β-*C*-glycoside analog,[12] such as **5**, would illicit any biological response since it is known that *C*-glycosides possess other conformations available for binding to the active site compared to their oxygen counterparts[13] (Fig. 1).

Our initial approach involved preparing the optically pure side chain acid **8**.[14] This was accomplished by beginning with ester **6**[15] and relying upon a Wittig-osmylation strategy.[16] Swern oxidation of alcohol **6** followed by a Wittig reaction to provide olefin **7** in 64% overall yield.[17] Osmylation of **7** gave a 1:1 mixture of separable isomers and the desired *erythro*-isomer was protected as an acetonide and saponified to deliver acid **8**[18] (Scheme 1).

DCC-mediated coupling of acid **8** with olefin alcohol **9**[5b] provided ester **10** in excellent yield (Scheme 2). At this point, we anticipated that application of our RCM methodology would afford the protected target structure **13** via the intermediacy of **11** and **12**, however to our surprise, methylenation[19] gave only the products of ester hydrolysis resulting in the quantitative recovery of olefin alcohol **9** and not **11**.[20] Presumably, the Boc group (or the nitrogen atom) was cyclizing onto the Lewis-acid activated ester during methylenation (boxed figure, Scheme 2). Buffering the reaction or installing two Boc

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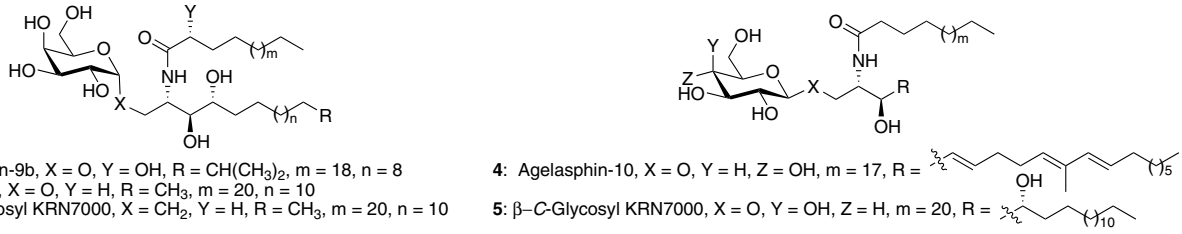
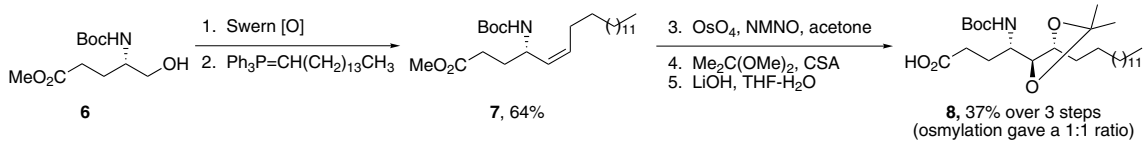
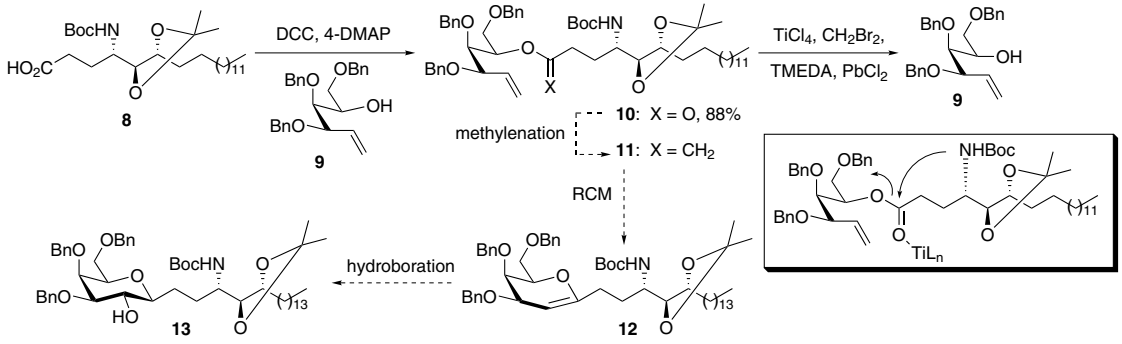


Figure 1. Glycosyl ceramides.



Scheme 1. Synthesis of acid **8**.



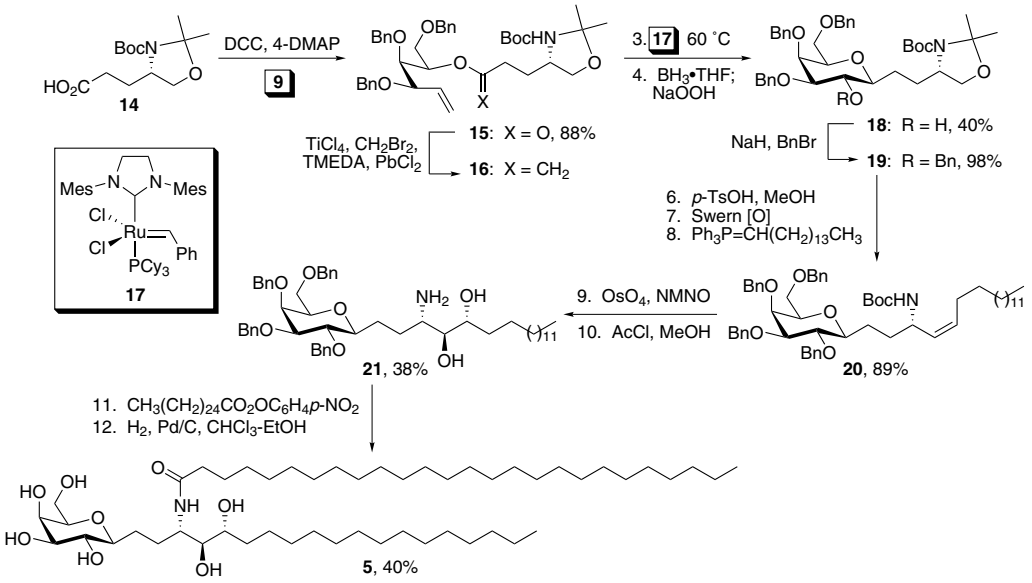
Scheme 2. Attempted RCM-based preparation of precursor **13**.

groups on the amine did nothing to circumvent the problem.

Due to the unfortunate outcome of the Takai methylenation, a modified approach to **5** was developed, and is outlined in Scheme 3.

Ester formation (**14**[21] + **9** → **15**[22]) proceeded smoothly and application of our three-step protocol (Takai methylenation, RCM with 20 mol% of catalyst **17**[23] and hydroboration; oxidative work-up) afforded the target *C*-glycoside **18**[24] in 40% yield over three steps.[25] Benzylation (**18** → **19**, 98%) was followed by acetonide cleavage, oxidation, and Wittig reaction to furnish olefin **20** in 89% yield over three steps. Osmylation (OsO_4, NMNO, THF–H_2O) of olefin **20** proceeded with no selectivity[26] delivering a 1:1 mixture of separable isomers in 92% yield.[27] Osmylation of **20** under anhydrous conditions shifted[28] the ratio in the favor of the undesired *threo*-isomer (4:1).[29] The Boc group on the desired *erythro*-isomer[30] was removed to bring the sequence as far as **21**. Installation of the side chain with *p*-nitrophenyl hexacosanoate[31] followed by reductive debenzylation in a mixed solvent system ($CHCl_3$–EtOH) afforded the target compound **5**[32] in 40% yield over two steps. The corresponding *threo*-isomer-**5** was also generated in an analogous fashion (not shown).

Testing for anti-solid tumor activity in vitro was carried out using the Valeriote disk-diffusion assay.[33] This assay determines differences (Δ) in cytotoxicity between normal or leukemia cells and solid tumor cells. This difference in activity is quantified by zone units. Any zone difference of 250 units or more is considered a hit in the assay, which means that the agent is selectively toxic against solid tumor cells versus either leukemia or normal cells. It was found that compound **5**-*erythro* and **5**-*threo* showed comparable in vitro activity in the assay. Table 1 shows that the **5**-*erythro* derivative exhibited a zone differential of 350 units between colon-38 (C38) solid tumor cells and leukemic cells (L1210) ($_{C38}\Delta S_{L1210} = 350$ units) and no selectivity between C38 and normal murine cells (CFU-GM) ($_{C38}\Delta S_{CFU} = 100$ units). The corresponding *threo*-isomer showed attenuated in vitro data with $_{C38}\Delta S_{CFU} = 250$ zone units (Table 1). Work on the preparation of different analogs and further biological screening (IC_{50} and CI_{90} determination and clonogenic evaluation) of compounds **5**-*erythro* and **5**-*threo* is the next step in these studies.



Scheme 3. Preparation of β-*C*-KRN7000 analog **5**.

Table 1. Disk-diffusion data for **5**

Entry	Compound	μg/disk	$_{C38}\Delta S_{L1210}$	$_{C38}\Delta S_{CFU}$
1	**5**-*erythro*	120	350	100
2	**5**-*threo*	120	250	150

Acknowledgements

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22. Spectral data for ester **15**: $[\alpha]_D = -11.1$ (*c* 1.0, $CHCl_3$); FT-IR (neat) 3063, 3029, 2976, 2930, 2854, 2117, 1738, 1695, 1453, 1389, 1374, 1257, 1166, 1102, 1068, 1027, 735, 697 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ 7.33–7.25 (m, 15H, Ar*H*), 5.91 (ddd, 1H, *J* = 17.5, 10.0, 7.5 Hz, *H*-2), 5.38 (ddd, 1H, *J* = 5.5, 5.5, 3.5 Hz, *H*-5), 5.32 (br s, 1H, *H*-1), 5.29 (s, 1H, *H*-1), 4.73 (d, 1H, *J* = 11.5 Hz, OCH_2Ph), 4.58–4.54 (m, 2H, 2 × OCH_2Ph) 4.48 (d, 1H, *J* = 12.5 Hz, OCH_2Ph), 4.43 (d, 1H, *J* = 11.5 Hz, OCH_2Ph), 4.32 (d, 1H, *J* = 11 Hz, OCH_2Ph), 3.93–3.87 (m, 1H, *H*-3), 3.87–3.85 (m, 1H, 1 × *H*-10), 3.85–3.81 (m, 2H, *H*-9, *H*-4), 3.68–3.63 (m, 1H, 1 × *H*-10), 3.55 (d, 2H, *J* = 5.5 Hz, 2 × *H*-6), 2.35–2.25 (m, 1H, 1 × *H*-7), 2.25–2.15 (m, 1H, 1 × *H*-7), 2.10–1.90 (m, 1H, 1 × *H*-8), 1.88–1.78 (m, 1H, 1 × *H*-8), 1.54 (s, 3H, CH_3), 1.48 (br s, 12H, CH_3, OC(CH_3)$_3$); ^{13}C NMR (125 MHz, $CDCl_3$, data for major rotamer only): δ 172.29, 138.44, 138.08, 135.81, 135.71, 172.70, 172.54, 128.60, 128.56, 128.48, 128.25, 120.09, 119.98, 94.08, 93.56, 79.51, 74.82, 73.33, 71.85, 71.76, 70.40, 68.42, 66.91, 57.02, 56.96, 31.16, 28.88, 28.67, 28.46, 27.83, 23.34, 15.52, 14.46; HRMS (ES): calcd for $C_{40}H_{51}NO_8Na$ $(M)^+$ 696.3507, found 696.3499.
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32. Data for analog **5**: $[\alpha]_D = +44.8$ (*c* 1.0, $CDCl_3$); FT-IR (neat) 3394, 2922, 2852, 2360, 1646, 1465, 1270 cm^{-1}; ^1H NMR (500 MHz, CD_3OD, 10% $CHCl_3$): δ 6.84 (1H, *J* = 8.5 Hz, N*H*), 4.09 (ddd, 1H, *J* = 11.0, 7.0, 5.0 Hz, *H*-9), 3.72 (dd, 1H, *J* = 8.5 Hz, *H*-2), 3.67–3.58, (m, 2H, *H*-3, *H*-4), 3.49–3.37 (m, 5H, 2 × *H*-6, *H*-5, *H*-1, *H*-10), 3.14–3.09 (m, 1H, *H*-11), 2.24–1.10 (m, 78H, 2 × *H*-7, 2 × *H*-8, 37 × CH_2), 0.88 (app t, 6H, *J* = 7.0 Hz, 2 × CH_3); ^{13}C NMR (125 MHz, $CDCl_3$): δ 158.19, 124.60, 123.90, 121.67, 116.53, 116.42, 55.80, 33.51, 31.92, 30.25, 29.60, 29.32, 25.54, 24.89, 22.59, 18.03, 13.40; HRMS (ES): calcd for $C_{51}H_{101}NO_8Na$ $(M)^+$ 878.7419, found 878.7479.
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