DNA–carbohydrate interactions. Design and synthesis of a head-to-tail dimer of the calicheamicin oligosaccharide

K. C. Nicolaou,* Keiichi Ajito, Hironori Komatsu, Brian M. Smith, Peter Bertinato and Luigi Gomez-Paloma

Department of Chemistry and Skaggs Institute of Chemical Biology, The Scripps Research Institute, 10666 North Torrey Pines Road, La Jolla, California 92037 USA and Department of Chemistry and Biochemistry, University of California, San Diego, 9500 Gilman Drive, La Jolla, California 92093 USA

The chemical synthesis of the head-to-tail dimer 4 of the calicheamicin oligosaccharide from building blocks 5, 6 and 12 is described.

Sequence-specific binding of small molecules to DNA is of considerable interest owing to its potential value in chemistry, biology and medicine.[1–6] The design and synthesis of molecules exhibiting such interactions with DNA is, therefore, an important goal in contemporary chemistry. As part of our program in this area we have recently designed, synthesized and studied[7–13] a series of DNA-binding oligosaccharides including a head-to-head dimer of the calicheamicin γ_1^I **1** oligosaccharide domain **2**, compound **3**,[12,13] Fig. 1. Here we report the design and synthesis of the potentially more useful head-to-tail dimer **4** of the calicheamicin γ_1^I oligosaccharide **2** Fig. 1. The reported compound (**4**) exhibits high affinity for specific duplex DNA sequences[14] and could serve as a prototype for a series of sequence-specific DNA-binding molecules.

As with the head-to-head dimer **3**,[12] the design of the head-to-tail dimer **4** was aided by computer modelling and was based on the precise DNA binding of the monomeric calicheamicin γ_1^I oligosaccharide **2** to one of its preferred sequences, 5′-TCCT·AGGA-3′, as determined by ^{1}H NMR spectroscopy[10,15] and footprinting[8,11,16] experiments. Thus computer-aided docking of various candidate structures into the minor groove of duplex DNA along a 5′-TCCTTCCT·AGGAAGGA-3′ track revealed a four-carbon chain as the appropriate tether to link the two oligosaccharide units at the indicated positions as shown in structure **4** (see Fig. 2 in ref. 14). The connectivity of the two oligosaccharide units distinguishes this head-to-tail dimer **4** from the head-to-head dimer **3** in that the former is, in principle, extendible to higher oligomers that may exhibit even higher specificity towards selected DNA sequences. Below we describe a solution to the synthetic challenge posed by these head-to-tail structures and report the synthesis of the first designed member of this series of oligomers, compound **4**.

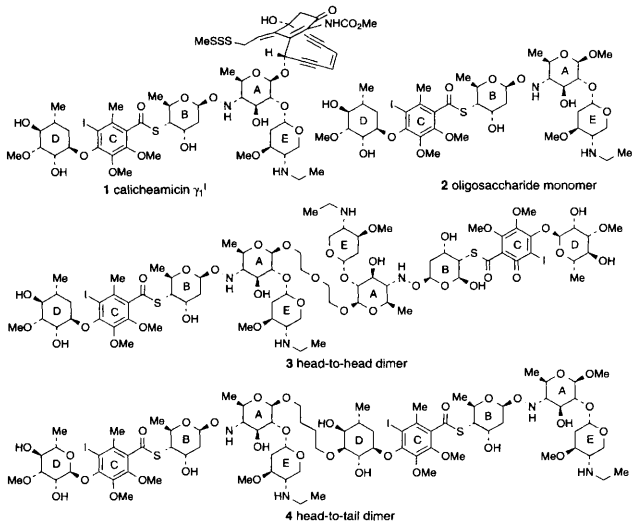

Fig. 1 Structures of calicheamicin γ_1^I **1** and calicheamicin γ_1^I oligosaccharides **2–4**

Conversion of carboxylic acid **5**† into the corresponding acid chloride with oxalyl chloride followed by coupling with thiol **6**[9] in the presence of 4-DMAP led to thioester **7** in 63% yield. Chemoselective removal of the *tert*-butyldimethylsilyl (TBS) group from the enol ether on ring-B was achieved with Bu_4NF–AcOH at −23 °C, furnishing ketone **8** in high yield. Stereoselective reduction of the carbonyl group in **8** with the bulky reducing agent K-Selectride (85% yield from **7**), followed by protection of the generated α-hydroxy compound **9** as a triethylsilyl (TES) ether allows the formation of compound **10** in 93% yield. A second chemoselective desilylation with Bu_4NF–AcOH, this time at 0 °C, led to primary alcohol **11** (78% yield, plus 9% recovered **10**). This appropriately functionalized oligosaccharide unit was now ready for coupling with the activated calicheamicin γ_1^I oligosaccharide domain **12**.[9]

Coupling of **11** (1.8 equiv.) and trichloroacetimidate **12**[9] under the influence of $BF_3{\cdot}Et_2O$ at −78 to −40 °C furnished the β-glycoside **13** as the major product in 51% yield (plus 45% recovered **11**). The *o*-nitrobenzyloxy group at the terminal A-ring of the dimer was then replaced with a methoxy group to afford compound **16** *via* intermediates **14** and **15** using the following sequence: (*a*) photolytic cleavage to give **14** (70%, mixture of anomers); (*b*) trichloroacetimidate formation to give **15** (mixture of anomers); (*c*) glycosidation with excess methanol to give **16** (49% from **14**, plus 18% α-anomer, plus 17% lactol **14**). Sequential removal of the silicon and Fmoc protecting groups with HF·py and Et_2NH, respectively, afforded the bisoxime **18**, *via* intermediate **17**, in 81% overall yield from **16**. Finally, reduction of both C=N bonds in **18** with $NaCNBH_3$ in the presence of $BF_3{\cdot}Et_2O$ gave the targeted head-to-tail dimer **4**‡ as the major product in 41% yield (plus other isomers). The final product **4** was purified by HPLC [reverse phase C-18 Vydac 210TP510 column, MeOH–H_2O–conc. NH_4OH (150 : 49 : 1), 5 ml min^{-1}, retention time 12.8 min].

The described chemistry delivers the head-to-tail dimer **4** and opens the way, through reiteration of the sequence, for the synthesis of higher oligomers of this series of compounds for molecular recognition studies with DNA and other chemical and biological investigations, including the synthesis of highly specific DNA cleaving agents and inhibition of transcription factor binding to DNA.[13] The binding of oligosaccharides **3** and **4** to their specific DNA binding sites is reported elsewhere.[14]§

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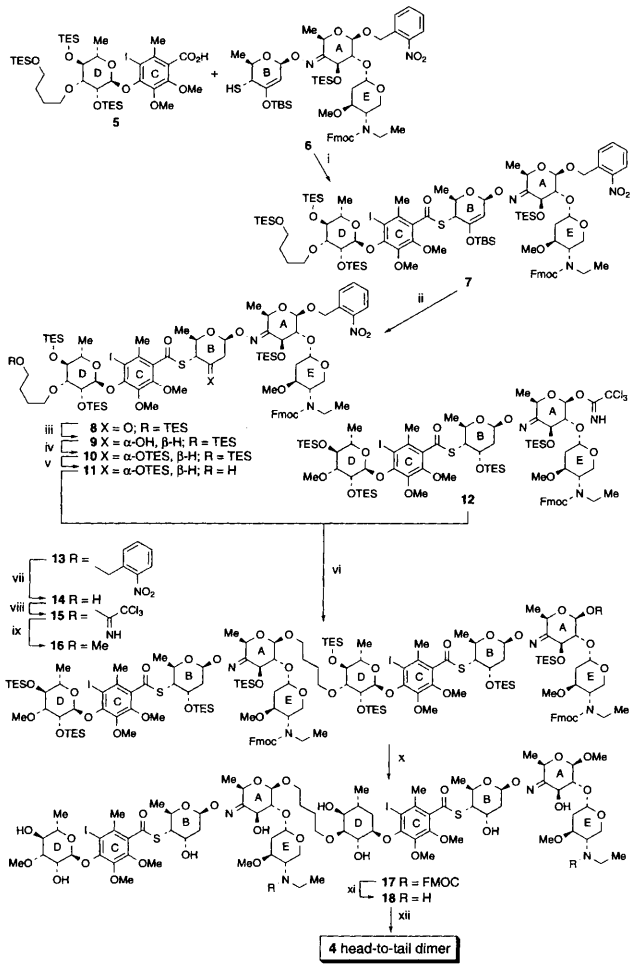

Scheme 1 Synthesis of head-to-tail dimer **4**. *Reagents and conditions*: i, 1.0 equiv. **5**, 5.0 equiv. $(COCl)_2$, 15 equiv. pyridine, PhH, 25 °C, 1 h; then 1.0 equiv. **6**, 13 equiv. of 4-DMAP, CH_2Cl_2, 0 °C, 2.5 h, 49%; ii, 1.05 equiv. Bu_4NF, 5.0 equiv. AcOH, THF, −23 °C, 0.5 h; iii, 3.0 equiv. K-Selectride, THF–DME (1 : 10), −78 °C, 2.5 h, 85% for 2 steps; iv 6.0 equiv. TESOTf, 9.0 equiv. Pr^i_2NEt, CH_2Cl_2, 25 °C, 2 h, 93%; v, 3.3 equiv. Bu_4NF, 7.2 equiv. AcOH, THF, 0 °C, 3 h, 78% of **11**, plus 9% recovered **10**; vi, 1.0 equiv. **12**, 1.8 equiv. **11**, 0.5 equiv. $BF_3{\cdot}Et_2O$, 4 Å molecular sieves, CH_2Cl_2, −78 → −40 °C, 3 h, 51%, plus 45% recovered **11**; vii, hν (Hanovia mercury lamp), THF–H_2O (10 : 1), 15 min, 0 °C, 70% of anomeric mixture; viii, 30 equiv. CCl_3CN, NaH (cat.), CH_2Cl_2, 25 °C, 1 h; ix, 1.0 equiv. $BF_3{\cdot}Et_2O$, 4 Å molecular sieves, MeOH–CH_2Cl_2 (1 : 100), −78 → −40 °C, 2 h, 49% of **16**, plus 18% of α-anomer and 17% recovered lactol **14**; x, excess HF·pyridine, CH_2Cl_2, pyridine, −40 → 0 °C, 20 h; xi, Et_2NH–THF (1 : 1), 25 °C, 3 h, 81% for 2 steps; xii, 60 equiv. $NaCNBH_3$, 26 equiv. $BF_3{\cdot}Et_2O$, CH_2Cl_2, −60 → −40 °C, 2.5 h, 41% of **4**, plus other isomers. TES = triethylsilyl; TBS = *tert*-butyldimethylsilyl; Fmoc = 9-fluoromethoxycarbonyl.

Footnotes

† The synthesis of this compound will be described in the full account of this work.

‡ *Selected physical data* for **4**: colourless solid: R_f = 0.27 (silica, benzene–EtOAc–MeOH (1 : 2 : 2); $[\alpha]^{23}_D$ −39 (*c* 0.7, MeOH); IR (neat) ν_{max}/cm^{-1} 3600–3200, 2933, 1682, 1558, 1456, 1416, 1392, 1240, 1155, 1067 and 960; ^1H NMR (500 MHz, CD_3OD) δ 5.62 (bs, 2 H, D-1, D-1), 5.41 (bs, 1 H, E-1), 5.40 (bs, 1 H, E-1), 5.09 (bd, *J* 10 Hz, 2 H, B-1, B-1), 4.49 (bm, 1 H, D-2), 4.45 (bm, 1 H, D-2), 4.34 (d, *J* 8.0 Hz, 1 H, A-1), 4.24 (bm, 2 H, B-3, B-3), 4.23 (d, *J* 8.0 Hz, 1 H, A-1), 4.19 (m, 2 H, D-5, D-5), 4.06 (m, 2 H, B-5, B-5), 3.95 (dd, *J* 10, 10 Hz, 2 H, A-3, A-3), 3.93 (s, 6 H, $2CH_3O$), 3.87 (s, 6 H, $2CH_3O$), 3.78 (dd, *J* 9.5, 3.0 Hz, 1 H, D-3), 3.63 (dd, *J* 9.5, 9.5 Hz, 1 H, D-4), 3.62 (dd, *J* 9.5, 9.5 Hz, 1 H, D-4), 3.57 (s, 3 H, CH_3O), 3.53 (s, 3 H, CH_3O), 3.41 (s, 6 H, $2CH_3O$), 2.83–2.68 (m, 6 H, $2NCH_2$, E-4, E-4), 2.45 (m, 2 H, E-2_{eq}, E-2_{eq}), 2.39 (s, 6 H, $2ArCH_3$), 2.282 (dd, *J* 10, 10 Hz, 1 H, A-4), 2.276 (dd, *J* 10, 10 Hz, 1 H, A-4), 2.00 (m, 2 H, B-2_{eq}, B-2_{eq}), 1.85–1.74 (m, 6 H, $OCH_2CH_2CH_2CH_2O$, B-2_{ax}, B-2_{ax}), 1.49 (m, 2 H, E-2_{ax}, E-2_{ax}), 1.41 (d, *J* 6.0 Hz, 6 H, B-6, B-6), 1.38 (bd, *J* 6.5 Hz, 6 H, A-6, A-6), 1.29 (d, *J* 6.0 Hz, 3 H, D-6), 1.28 (d, *J* 6.0 Hz, 3 H, D-6), 1.19 (bt, *J* 7.0 Hz, 3 H, NCH_2CH_3) and 1.18 (bt, *J* 7.0 Hz, 3 H, NCH_2CH_3); ^{13}C NMR (125 MHz, CD_3OD) δ 194.1, 153.2, 153.1, 152.0, 144.6, 144.5, 134.6, 131.9, 131.8, 105.1, 105.1, 103.9, 102.8, 101.5, 100.1, 99.8, 94.3, 94.2, 81.7, 80.9, 80.3, 80.3, 77.6, 77.4, 72.7, 72.6, 72.3, 72.3, 72.1, 72.1, 71.3, 70.7, 70.5, 70.4, 70.3, 69.3, 69.3, 69.1, 68.2, 62.4, 62.3, 62.0, 61.5, 61.5, 60.2, 60.1, 57.5, 56.9, 56.2, 52.5, 42.8, 42.8, 39.0, 35.0, 27.8, 27.5, 25.7, 19.4, 18.7, 18.6, 18.1, 18.0, 15.0 and 14.7; FAB HRMS (NBA/CsI) *m/z* 2111.4815 (M + Cs^+); calcd for $C_{78}H_{124}I_2N_4O_{34}S_2$: 2111.4682.

§ All new compounds exhibited satisfactory spectral and exact mass data. Yields refer to spectroscopically and chromatographically homogeneous materials.

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