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Synthesis and stereochemistry of the terminal spiroketal domain of the phosphatase inhibitor dinophysistoxin-2

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Abstract—An expedient synthesis of both axially and equatorially C35 methyl substituted spiroketals representing the C28–C38 domain of the potent and selective protein serine/threonine phosphatase inhibitor dinophysistoxin-2 (DTX-2) was developed to enable comparative stereochemical analyses and a stereochemically correct total synthesis of DTX-2. Comparison of proton and carbon NMR data of the synthetic diastereomers with those published for DTX-2 indicates that DTX-2 possesses the (30*S**,34*R**,35*S**)-relative configuration with an axial C35 methyl substituent.

Diarrhetic shellfish poisoning (DSP) continues to afflict humans worldwide via the consumption of contaminated shellfish.[1] Marine dinoflagellates of the genus *Dinophysis* are among the primary producers of DSP toxins,[2] which include okadaic acid (OA, **1**),[3] dinophysistoxin-1 (**2**, DTX-1), and dinophysistoxin-2 (**3**, DTX-2, Fig. 1).[4–6] We have previously documented the importance of the terminal spiroketal domain of **1** for the differential inhibition of serine/threonine protein phosphatases 1 and 2A, the primary targets of DSP toxins.[7] Accordingly, the recent stereochemical clarification of the terminal spiroketal domain of DTX-2 and an interpretation of its selective phosphatase binding reported by Larsen et al.[8] prompted us to independently examine the stereochemical assignments, as a prelude to total syntheses of DTX-2 and its congeners. For this purpose the syntheses and analyses of potential DTX-2 terminal spiroketal domain diastereomers are summarized here.

The structure of DTX-1 was originally published by Murata et al. in 1982, wherein the carbon skeleton of DTX-1 was found to match that of OA, but with one additional methyl substituent at C35 (Fig. 1).[4] According to the original ^{1}H NMR vicinal coupling constant

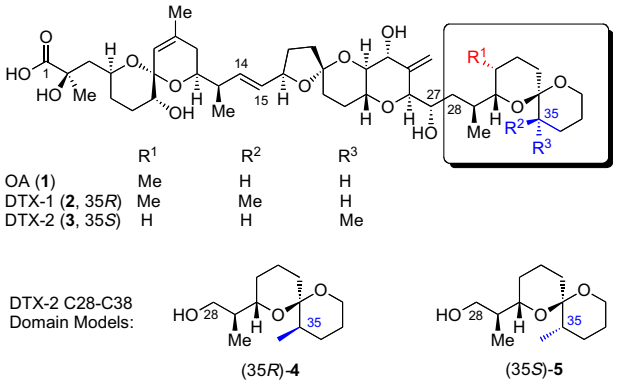

Figure 1. Structures of major diarrhetic shellfish toxins and DTX-2 synthetic spiroketal models.

data, the C35 methyl substituent of DTX-1 is equatorially oriented.[4]

A decade later DTX-2 was isolated and characterized by the Wright group.[6] Their published structural assignment mirrored that of DTX-1, but without the methyl substituent at C31 that is resident in both **1** and **2**. Based on NOE correlations, they assigned the DTX-2 C35 methyl group as being axially oriented. However, they indicated that the *same* NOE data regarding the C35 methyl group of DTX-2 were obtained with DTX-1.[4] The assumption that **2** and **3** bore the same C35 axial substitution was propagated in the primary and secondary literature. In 1998, Sasaki et al. assigned the absolute

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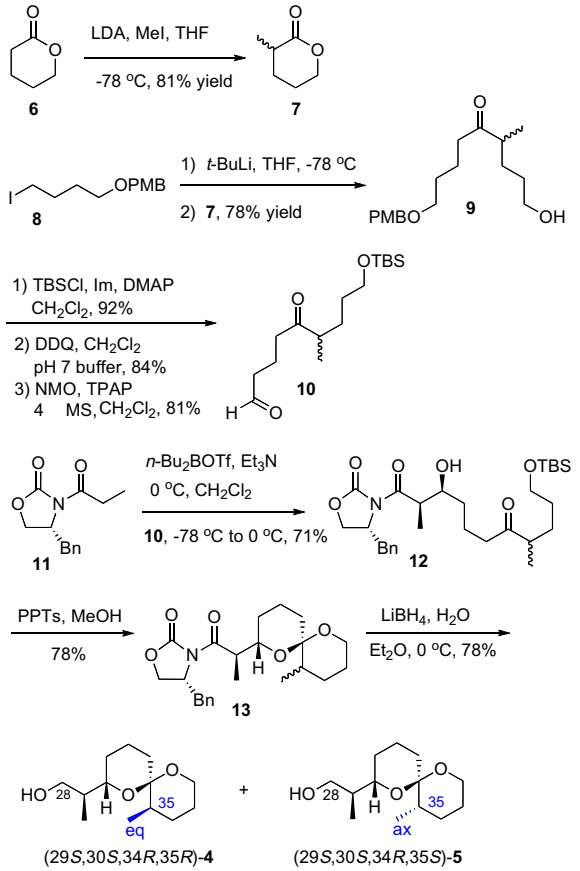
configuration of DTX-1, designating C35 as 35*R*, which corresponds to equatorial substitution at C35.[9] Most recently, the published reports that DTX-1 and DTX-2 possess the same relative configuration at C35 have been re-evaluated by Larsen et al. based upon comprehensive de novo NMR spectroscopic analyses of the natural products.[8] Larsen and coworkers concluded that DTX-1 and DTX-2 have equatorial and axial methyl substituents at C35, respectively.[8]

To independently examine the relative stereochemistry about the C29–C38 domain of DTX-2 and provide stereochemically correct intermediates for projected total syntheses, synthetic fragments representing C28–C38 of DTX-2 with both equatorially (**4**) and axially (**5**) oriented methyl-bearing stereogenic centers at C35 (Fig. 1) were synthesized and analyzed. This approach allows a direct spectroscopic comparison between synthetic probes of known relative stereochemical configurations and the terminal spiroketal domains of DTX-1 and DTX-2 that have been in question. Moreover, the C28 hydroxyl functionalization of the stereochemical probes **4** and **5** parallels that of the C28–C8 intermediates used in total syntheses of **1**[10] and 7-deoxy-**1**.[11]

The synthesis of **4** and **5** commenced with δ-valerolactone **6** (Scheme 1). α-Methylation followed by nucleophilic opening of the lactone with the organolithium derived from primary iodide **8** generated racemic ketone **9**. Protecting group manipulations and subsequent oxidation provided aldehyde **10**. This aldehyde was coupled with the boron enolate of *N*-propionyl oxazolidinone **11** to yield the *syn* aldol product **12** in 78% yield.[12] Treatment of **12** with pyridinium *p*-toluenesulfonate in methanol initiated both deprotection of the latent primary alcohol and subsequent thermodynamic dehydrative cyclization to form spiroketals **13**, epimeric at C35 (DTX-2 numbering). Attempts to chromatographically separate the two C35 epimers at this stage were unsatisfactory. However, after reductive excision of the oxazolidinone auxiliary, the resultant diastereomeric primary alcohols were separated successfully to provide the equatorially C35-methyl-substituted spiroketal **4** and the axially C35-methyl-substituted **5**.



Scheme 1. Synthesis of DTX-2 terminal spiroketal models.

The relative configurations at C34 and C35 of **4** and **5** were assigned on the basis of NOE data (Fig. 2). Irradiation of the C35 methyl group of **4** gave rise to NOE enhancements of the resonances of both geminal C36 protons, as well as of the C33 axial proton. Upon irradiation of the C35 methyl group of **5**, NOE enhancements of the resonance intensities of both protons at C33, the equatorial proton at C36, and the axial proton at C37 were observed. Combined, these experimental results indicate that the C35 methyl group of **4** is equatorially disposed on the thermodynamically defined (34*R*)-spiroketal, whereas that of **5** is axially oriented. Given the established aldol chemistry[12] used to access **4** and **5** via **12**, the absolute configurations of our stereochemical probes are (29*S*,30*S*,34*R*,35*R*)-**4** and (29*S*,30*S*,34*R*,35*S*)-**5**.

The NMR spectral data[8] of the terminal spiroketal domain of DTX-1 and DTX-2 were then compared with those of **4** and **5**. First, the ^{1}H NMR chemical shifts were compared among these four compounds (Fig. 3), which revealed some striking correlations. The chemical shifts of the C36 axial protons of **5** and DTX-2 resonate at 2.09 and 2.12 ppm, respectively, whereas the C36 axial protons of **4** and DTX-1 resonate at 1.50 and 1.52 ppm, respectively ($CDCl_3$). Similarly, chemical shifts of the C37 methylene protons of **5** (1.32 and 1.79 ppm, $\Delta\delta = 0.47$ ppm) and **4** (1.50 and 1.63 ppm, $\Delta\delta = 0.13$ ppm) correlate best with those of DTX-2 (1.25 and 1.78 ppm, $\Delta\delta = 0.53$ ppm) and DTX-1 (1.42 and 1.61 ppm, $\Delta\delta = 0.19$ ppm), respectively. The third set of diagnostic ^{1}H NMR correlations involved the

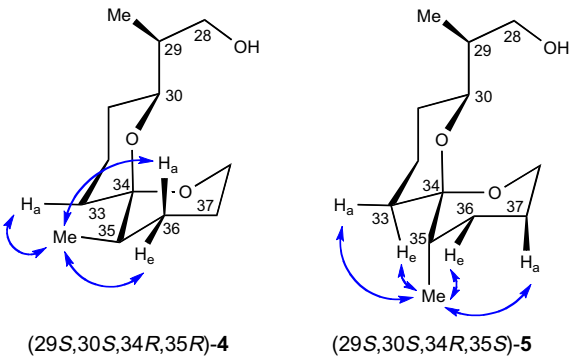
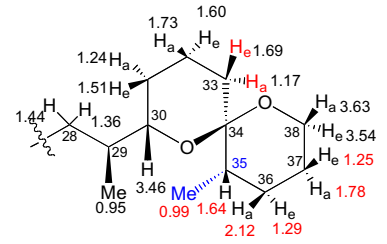
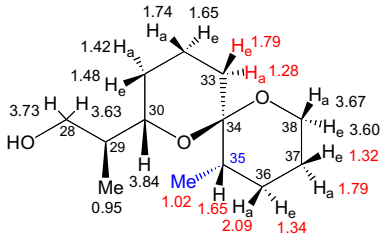
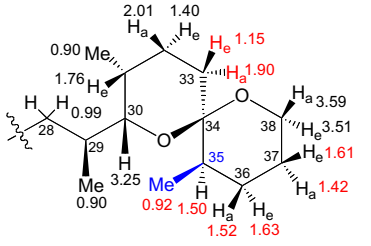


Figure 2. Observed NOEs involving the C35 methyl group of **4** and **5**.







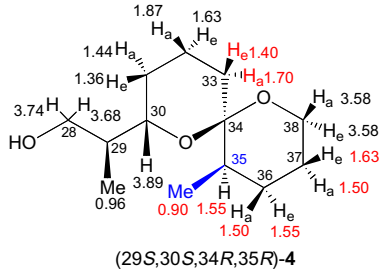
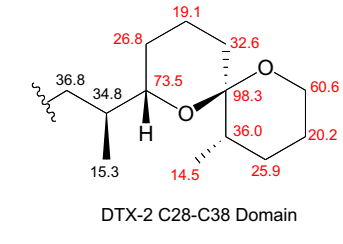
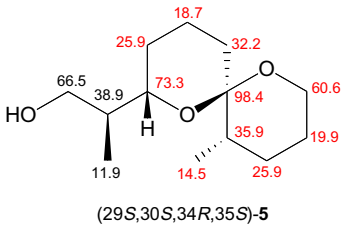
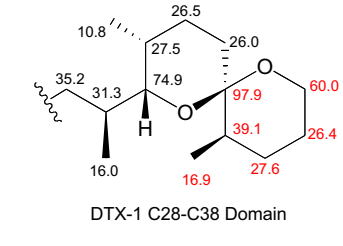


Figure 3. Comparative ^{1}H NMR data of probes **4** and **5** with natural DTX-1[8] and DTX-2[8] ($CDCl_3$).

methylene protons at C33. The relative chemical shifts of the C33 axial and equatorial protons are inverted between **4** (C33-H_{axial} at 1.70 ppm, and C33-$H_{equatorial}$ at 1.40 ppm, $\Delta\delta = 0.30$ ppm) and **5** (C33-H_{axial} at 1.28 ppm, and C33-$H_{equatorial}$ at 1.79 ppm, $\Delta\delta = -0.47$ ppm). In the chair conformation of cyclohexanes the axial protons generally resonate downfield of the geminal equatorial protons by ca 0.5 ppm.[13] However, in both DTX-2 (C33-H_{axial} at 1.17 ppm, and C33-$H_{equatorial}$ at 1.69 ppm, $\Delta\delta = -0.52$ ppm) and **5**, the C33 equatorial proton resonates downfield of the C33 axial proton resonance, reflecting a strong differential stereoelectronic effect of the C35 axial methyl substituent on the C33 protons.[14] The absence of a C35 axial methyl substituent in both **4** and DTX-1 is associated with the C33 methylene protons resonating at the typical differential chemical environments of axial versus equatorial geminal protons. The relative stereochemistry in the C29–C38 region of the natural product DTX-2 corresponds best with that of **5**, whereas the relative configuration at C35 of DTX-1 best matches that of **4** based upon these ^{1}H NMR correlations.







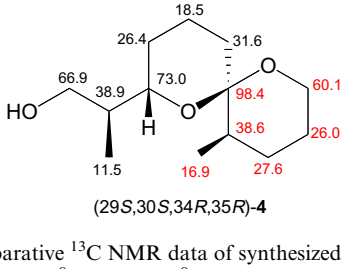


Figure 4. Comparative ^{13}C NMR data of synthesized fragments **4** and **5** with natural DTX-1[8] and DTX-2[8] ($CDCl_3$).

The ^{13}C NMR data of **4**, **5**, DTX-1, and DTX-2 (Fig. 4) were found to be fully in accord with the above analyses of the ^{1}H NMR data. The ^{13}C NMR chemical shifts of **5** map very well upon those of DTX-2, whereas the ^{13}C NMR data for **4** correspond to those of DTX-1 at C35-38, including the C35 methyl substituent. The differences in ^{13}C NMR chemical shifts between DTX-2 and **5** among C30–C38 are less than 0.5 ppm. Similarly, the differences between the ^{13}C NMR chemical shifts of C34–C38 of DTX-1 and **4** are $\leqslant$0.5 ppm. In contrast, there are clearly distinctive differences between the ^{13}C NMR chemical shift data of **5**/DTX-2 versus **4**/DTX-1.

Combined, the spectral correlation data among **2**–**5** fully support the relative steroechemical assignments of (29*S*,30*S*,34*R*,35*S*) to **3** and **5**, and of (29*S*,30*S*, 34*R*,35*R*) to **2** and **4**. It is generally accepted that **1**–**3** share the same overall absolute configurations,[3,6] beyond the C35 epimers of **2** and **3**. These stereochemical results are in agreement with those of Larsen et al., who arrived at the same conclusions based upon spectroscopic and computational analyses of the natural products without the aid of diastereomeric probes of known configurations.[8]

PP2A is less sensitive to DTX-2 than to OA.[15] These differential sensitivities have been ascribed to the orientation of the C35 axial methyl substituent in DTX-2 which incurs less favorable steric interactions in the PP2A hydrophobic groove.[8] On-going efforts in our laboratories toward total syntheses of **2** and non-natural structural variants thereof may allow the further exploitation of differential contacts about the terminal spiroketal domain to enhance selective phosphatase binding.

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