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Synthesis of a transition-state analog for the hydrolysis of the zearalenone lactone

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Abstract—The development of a catalytic antibody for the hydrolysis of the lactone functionality in zearalenone (**1**) is viewed as a potential solution to animal fertility problems associated with the estrogenic mycotoxin. A phosphonomacrolactone is proposed as a hapten for the generation of such antibodies. A suitably functionalized aryl phosphonic acid **4** was condensed with the racemic aliphatic fragment **5** via a Mitsunobu reaction. Macrocyclic formation was achieved via RCM to give advanced intermediate **23**, a phosphonate analog of zearalenone, ready for deprotection and conjugation to a carrier protein.

1. Introduction

Zearalenone (**1**) is a mycotoxin, first isolated by Stob and coworkers in 1962,[1] which is produced by various species of *Fusarium*.[2] Zearalenone is estrogenic and affects a variety of animals. In New Zealand, sheep ingests zearalenone when grazing on fungi-infested pastures.[3] Levels of the toxin peak in the warm, dry conditions of late summer, and early autumn and unfortunately coincide with the sheep mating season. This results in reduced fertility: more barren ewes, fewer twins, later lambs, and associated productivity losses.

This toxin represents a considerable problem to the global agricultural and horticultural industries. It is not surprising that a number of methods have been considered to counter its effects. Degradation of the toxin to harmless byproducts has been demonstrated by microorganisms.[4] Androvax® is a steroid-based vaccine that stimulates ovulation and thereby counteracts the detrimental effects of toxins such as zearalenone.[5] A novel approach would be the use of catalytic antibodies to degrade the toxin in vivo—either by inoculation with an antibody preparation or by stimulating the animals to produce their own therapeutic antibodies.

We proposed that a transition-state analog for hydrolysis of the macrolactone would be a suitable hapten for the production of such antibodies (Fig. 1). The byproducts of the seco-acid (**2**) are known to be harmless (Scheme 1). Ester

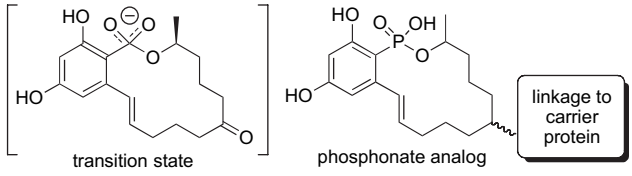
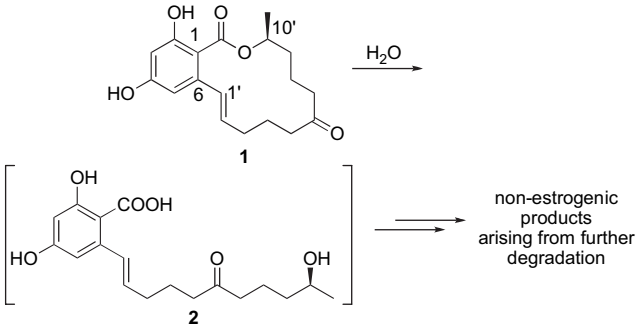


Figure 1. A transition-state analog.

hydrolysis by catalytic antibodies is well established.[6] While lactone hydrolysis has not been reported, the reverse reaction—lactone formation—has been demonstrated.[7]



Scheme 1. Hydrolysis of zearalenone (**1**).

Retrosynthetic analysis of the hapten, a phosphonomacrolactone,[8] is illustrated in Scheme 2. We decided to pursue a racemic synthesis, since the geometry in the lactone region of the molecule was already significantly perturbed by the phosphonate. We felt that fidelity to the 10′*S* configuration

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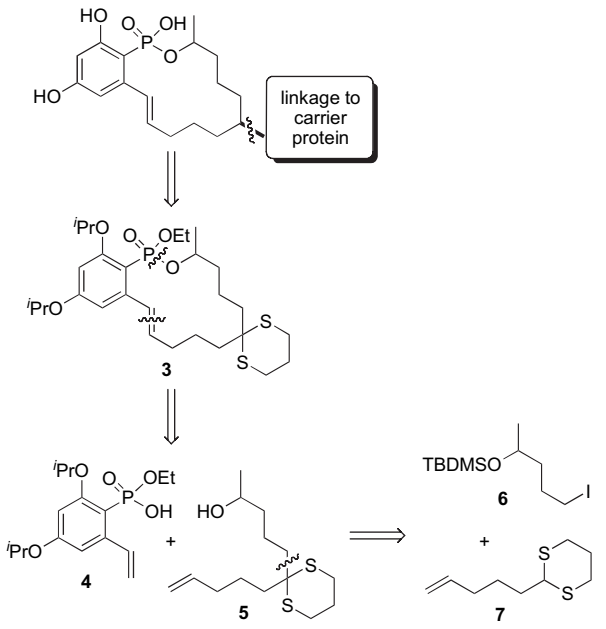
in zearalenone would serve little purpose. Indeed, the use of both enantiomers, in antibody generation, would double our chances of inducing an effective complementary binding site.

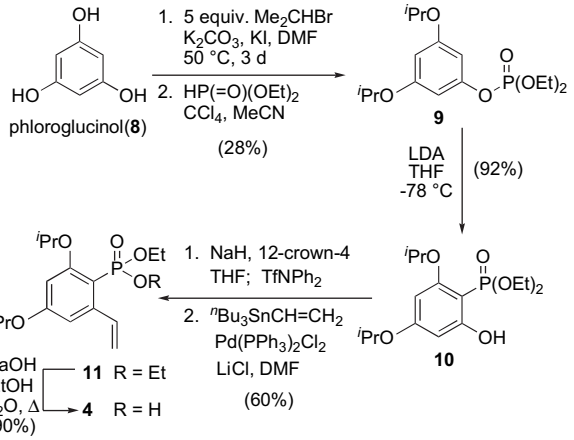


Scheme 2. Retrosynthetic analaysis.

Compound **3** represents an advanced intermediate that requires deprotection, followed by conjugation of the ketone to a carrier protein.[9] Compound **3** is conveniently broken down into an aromatic fragment **4** and a aliphatic fragment **5**. It was envisaged that formation of a phosphonate ester would precede formation of the 14-membered ring via an RCM reaction. The latter has precedent in a recent synthesis of zearalenone itself by Fürstner et al.[10] We considered a number of approaches to the synthesis of the aliphatic fragment **5**. A key building block was an alkyl halide typified by iodide **6**. Displacement of the halide in **6**, with the anion derived from dithiane **7**,[11] could be expected to assemble the carbon skeleton of the aliphatic fragment. The aryl phosphonate presented two significant challenges: formation of the Ar–P bond and formation of the phosphonomacrolactone. We have recently reported the trials and tribulations associated with the synthesis of **4**.[12] In this paper, we describe our synthesis of **5** and the amalgamation of the two fragments.

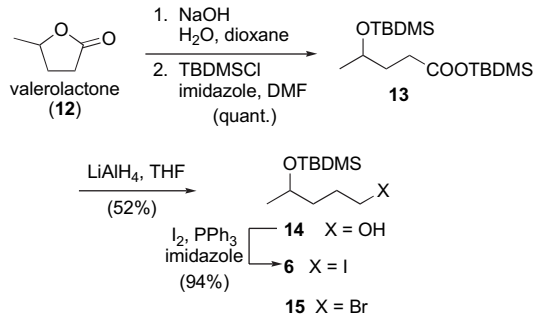
2. Results and discussion

The synthesis of diethyl phosphonate **11** was reported elsewhere[12] and is summarized in Scheme 3. The only low-yielding step was the conversion of C3-symmetric phloroglucinol (**8**) to its diisopropyl ether derivative. Phosphorylation of the remaining phenol was conducted under Atherton–Todd conditions.[13] An anionic phospho-Fries rearrangement[14] led to compound **10**. The phenol in compound **10** was converted to the corresponding triflate and then to the styrene **11**, under Stille conditions. Partial hydrolysis of the phosphonate ester gave compound **4**.



Scheme 3. Synthesis of the aromatic fragment.

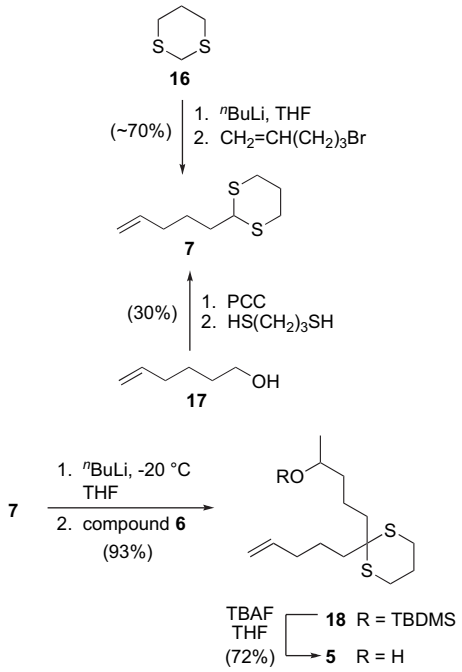
We prepared iodide **6**, and the corresponding bromide **15**, in racemic form, according to Scheme 4. Hydrolysis of the lactone in γ-valerolactone (**12**) and protection of the sodium salt gave the bis-TBDMS derivative **13**. The ester was reduced to the primary alcohol **14** by analogy to a procedure reported by Amino et al.[15] The bromide **15** had been prepared and utilized previously,[16] but in our hands it was unstable and difficult to purify. Fortunately, iodide **6** was readily obtained; although it was best to prepare this compound immediately prior to coupling.



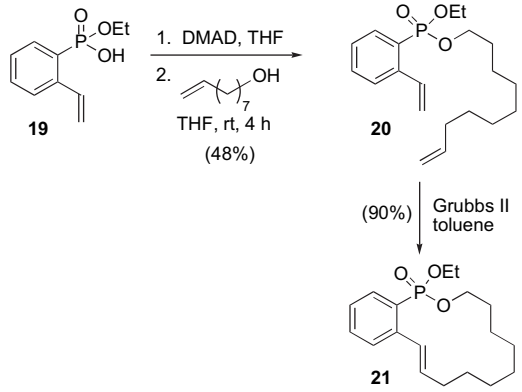
Scheme 4.

Dithiane **7** has been prepared previously by reaction of the anion of 1,3-dithiane (**16**) with 5-bromopent-1-ene (Scheme 5).[17] Careful optimization of reaction conditions led to the formation of **7** in ~70% yield, but it was incredibly difficult to separate **7** from residual **16** and byproducts of the butyllithium. An alternative synthesis, via hex-5-enal,[18] gave a lower yield of dithiane **7**, but it could be produced on gram scale in high purity. Condensation of the anion of **7** with iodide **6** gave compound **18**[19] in excellent yield. The secondary alcohol was liberated under standard conditions to give **5**, ready for coupling to the aromatic fragment.

Prior to committing our valuable intermediates **4** and **5**, we investigated the assembly of a phosphonomacrolactone in a model system (Scheme 6). There are many methods for phosphonate ester formation, but recent reports encouraged us to use Mitsunobu conditions.[8b] Thus, condensation of the simplified phosphonate **19**[12] and dec-9-en-1-ol via a Mitsunobu reaction[20] gave **20** in moderate yield. Closure of the



Scheme 5. Assembly of the aliphatic fragment.

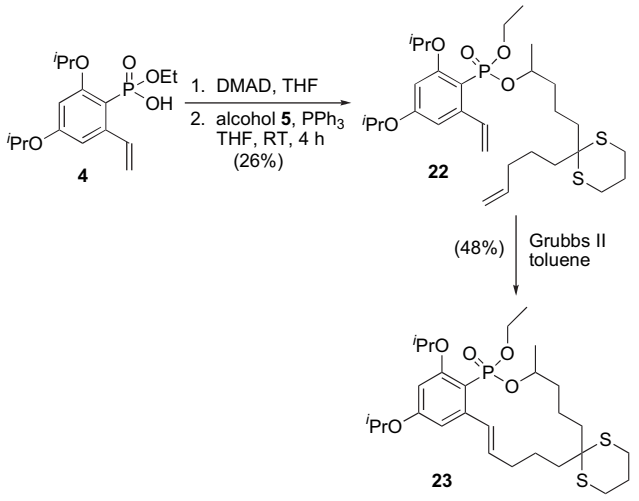


Scheme 6. Model system for macrocycle formation.

14-membered ring to give **21** was accomplished in excellent yield via standard RCM conditions.

Turning our attention to the more complex system, we encountered considerable difficulties (Scheme 7). The best yield obtained for the Mitsunobu reaction was 26% and this required the use of 2 equiv of the acid (so-called alcohol-limiting conditions). Previous experience[21] encouraged us to prepare the acid chloride of **4**, but this failed to condense at all with alcohol **5**, even in the presence of silver cyanide.

The RCM step was also much lower yielding than in the model system. We suspected that the dithiane functionality may be the cause of problems in one or both of these crucial steps. We therefore removed the dithiane from alcohol **5** (HCl, DMSO) and performed the esterification and RCM reactions with no improvements in yields.



Scheme 7. Formation of the hapten macrocycle.

3. Conclusion

We have prepared compound **23**, an advanced intermediate *en route* to a transition-state analog for the hydrolysis of the lactone functionality in zearalenone. The phosphonic acid was introduced onto the aromatic ring via an anionic phospho-Fries rearrangement. Phosphonate ester formation, via a Mitsunobu reaction, gave a disappointing yield in the formation of model system **20** and this deteriorated in the real system **22**. Ring closing metathesis was an effective means of forming the macrocycle in zearalenone itself[10] and the model system **21** (Scheme 6). The yield was low in the real system. Nevertheless, sufficient material can be produced in a convergent manner, to investigate the generation of antibodies that might catalyze the degradation of zearalenone.

4. Experimental

4.1. General details

All reactions were conducted under a dry nitrogen atmosphere unless otherwise noted. Reagents were obtained from commercial suppliers and used directly with the following exceptions. Tetrahydrofuran was dried over sodium/benzophenone and distilled prior to use. Diisopropylethylamine, triethylamine, and pyridine were dried and distilled from CaH_2 and stored over KOH pellets. Flash chromatography was performed using Scharlau 60 silica gel (230–400 mesh) with the indicated solvents. Thin layer chromatography (TLC) was carried out on precoated silica plates (Merck Kieselgel 60 F_{254}) and compounds were visualized by UV fluorescence or by staining with anisaldehyde or phosphomolybdic acid. ^1H and ^{13}C NMR spectra were obtained using either a JEOL JNM-GX270W or a Bruker Avance 400 spectrometer. Chemical shifts for spectra in $CDCl_3$ are given in parts per million (ppm) downfield from tetramethylsilane as internal standard (^1H) or relative to residual solvent (^{13}C). High resolution mass spectra were recorded using a VG7070 mass spectrometer operating at nominal accelerating voltage of 70 eV.

4.1.1. (2,4-Diisopropoxy-6-vinyl-phenyl)phosphonic acid monoethyl ester (4). A solution of diester derivative **11** (100 mg, 0.8 mmol, 1 equiv) in EtOH (2 mL) and 2 M NaOH (2 mL) was heated at reflux for 3 h. The mixture was concentrated to remove the ethanol, diluted with water (8 mL), neutralized with concd HCl, and extracted with EtOAc (2×15 mL). The combined organic extracts were washed with water (20 mL), dried over $MgSO_4$, filtered, and concentrated to give **4** as a colorless oil (191 mg, 90%). This crude product was used without further purification. R_f 0.25 (10:1 CH_2Cl_2–MeOH). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.28 (3H, t, J 7.0 Hz, OCH_2CH_3), 1.35 (6H, d, J 6.0 Hz, $CHMe_2$), 1.39 (6H, d, J 6.0 Hz, $CHMe_2$), 4.04 (2H, app.p, J 7.0 Hz, $POCH_2CH_3$), 4.62 (2H, hept., J 6.0 Hz, 2×$CHMe_2$×2), 5.29 (1H, d, J 10.8 Hz, CH=CH_2 cis), 5.51 (1H, dd, J 17.2, 1.4 Hz, CH=CH_2 trans), 6.39 (1H, dd, J 5.5, 2.3 Hz, ArH), 6.66 (1H, dd, J 5.5, 2.3 Hz, Ar*H*), 7.68 (1H, dd, J 17.2, 10.8 Hz, CH=CH_2); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 16.2 (d, J 7 Hz), 21.8, 22.0, 61.2 (d, J 6 Hz), 69.9, 71.6, 101.2 (d, J 10 Hz), 106.6 (d, J 15 Hz), 107.7 (d, J 193 Hz), 116.0, 137.7 (d, J 4 Hz), 146.2 (d, J 10 Hz), 161.7, 161.7 (d, J 6 Hz); HRMS (EI$^+$): M$^+$, found 328.14442. $C_{16}H_{25}O_5P$ requires 328.14396.

4.1.2. (±)-4-*tert*-Butyldimethylsilyloxypentanoic acid *tert*-butyldimethylsilyl ester (13). γ-Valerolactone (**12**) (2.60 g, 26 mmol, 1.0 equiv) was dissolved in a mixture of water (15 mL) and dioxane (15 mL). Sodium hydroxide (1.038 g, 26 mmol, 1.0 equiv) was added and the solution stirred at rt for 30 min. The mixture was concentrated and dried over P_2O_5 to give the sodium salt of 4-hydroxypentanoic acid as a colorless solid. This was suspended in DMF (60 mL). *tert*-Butyldimethylsilylchloride (11.75 g, 78 mmol, 3.0 equiv), imidazole (7.08 g, 104 mmol, 4.0 equiv), and DMAP (1.59 g, 13 mmol, 0.50 equiv) were added. The mixture was stirred at rt overnight and then at 50 °C for 1 h. Water (60 mL) was added and the mixture extracted with diethyl ether (3×35 mL). The combined organic layers were washed with 10% citric acid (20 mL) and brine (20 mL), dried over $MgSO_4$, filtered, and concentrated. The residue was purified by distillation to give **13** as a colorless oil (6.84 g, 91%). R_f 0.72 (3:1 hexanes–EtOAc); bp 110 °C (0.1 mmHg). ^{1}H NMR ($CDCl_3$, 270 MHz) δ 0.04 (6H, s, $SiMe_2$), 0.26 (6H, s, $SiMe_2$), 0.88 (9H, s, Si^tBu), 0.93 (9H, s, Si^tBu), 1.13 (3H, d, J 6.2 Hz, *Me*CHOTBDMS), 1.68 (2H, m, CH_2CH_2COOTBDMS), 2.38 (2H, t, J 7.5 Hz, CH_2COOTBDMS), 3.86 (1H, m, C*H*OTBDMS); ^{13}C NMR ($CDCl_3$, 67.5 MHz) δ −4.8, −4.4, 17.6, 18.1, 23.8, 25.6, 25.9, 32.0, 34.6, 67.4, 174.1; HRMS (FAB$^+$): MH$^+$, found 347.2428451. $C_{17}H_{39}O_3Si_2$ requires 347.243777.

4.1.3. (±)-4-*tert*-Butyldimethylsilyloxy-pentan-1-ol (14). A solution of **13** (910 mg, 2.6 mmol, 1.0 equiv) in diethyl ether (3 mL) was added dropwise over 10 min to a suspension of $LiAlH_4$ (220 mg, 5.8 mmol, 2.2 equiv) in diethyl ether (7.4 mL) at 0 °C. The mixture was stirred at 0 °C for 1 h 45 min and then at rt for 10 min. The reaction was quenched by the addition of a few drops of methanol. The mixture was diluted with EtOAc (70 mL) and washed with brine (35 mL). The combined aqueous layers were extracted with EtOAc (20 mL). The combined organic extracts were dried over $MgSO_4$, filtered, and concentrated. The residue was purified by flash chromatography, eluting with 10:1 hexanes–EtOAc to give **14** (620 mg, 52%). R_f 0.33 (3:1 hexanes–EtOAc). ^{1}H NMR ($CDCl_3$, 270 MHz) δ 0.07 (6H, s, $SiMe_2$), 0.89 (9H, s, Si^tBu), 1.16 (3H, d, J 6.2 Hz, *Me*-CHOTBDMS), 1.5–1.7 (4H, m, $CH_2CH_2CH_2OH$), 3.63 (2H, m, CH_2OH), 3.89 (1H, m, C*H*OTBDMS); ^{13}C NMR ($CDCl_3$, 100 MHz) δ −4.8, −4.5, 18.1, 23.2, 25.8, 28.5, 36.0, 63.0, 68.4; HRMS (FAB$^+$): MH$^+$, found 219.1777. $C_{11}H_{27}O_2Si$ requires 219.1780.

4.1.4. (±)-2-*tert*-Butyldimethylsilyloxy-5-iodopentane (6). Triphenylphosphine (528 mg, 2 mmol, 2 equiv), imidazole (204 mg, 3 mmol, 3 equiv), and iodine (764 mg, 3 mmol, 3 equiv) were added sequentially to a solution of primary alcohol **14** (218 mg, 1 mmol, 1 equiv) in THF (10 mL) at 0 °C under nitrogen. The mixture was warmed to rt and stirred for 3 h. The mixture was filtered through a pad of silica gel and the filtrate was concentrated. The residue was purified by flash chromatography, eluting with 10:1 hexanes–EtOAc to give the iodide derivative **6** as a colorless oil (308 mg, 94%). R_f 0.60 (10:1 Hex–EtOAc). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 0.03 (6H, s, $SiMe_2$), 0.87 (9H, s, $SiBu$), 1.11 (3H, d, J 6.0 Hz, *Me*CHOTBDMS), 1.46–1.50 (2H, m, $CH_2CH_2CH_2I$), 1.79–1.93 (2H, m, CH_2CH_2I), 3.17 (2H, t, J 7.0 Hz, CH_2I), 3.80 (1H, sext., J 6.0 Hz, C*H*OTBDMS); ^{13}C NMR ($CDCl_3$, 100 MHz) δ −4.8, −4.4, 7.3, 18.0, 23.8, 25.8, 29.8, 40.3, 67.6; HRMS (EI+): MH$^+$, found 329.07945. $C_{11}H_{26}IOSi$ requires 329.07977.

4.1.5. Hex-5-enal.[22] Molecular sieves (2.5 g, 4 Å) were added to a solution of 5-hexen-1-ol (1.00 g, 10 mmol, 1.0 equiv) in CH_2Cl_2 (100 mL) at 0 °C. The mixture was stirred for 5 min. Pyridinium chlorochromate (3.23 g, 15 mmol, 1.5 equiv) was added in portions over a period of 5 min, and the mixture was stirred at rt for 4 h. The reaction mixture was diluted with diethyl ether (100 mL) and filtered through a mixture of silica gel, Celite™, and activated charcoal. The dark brown residue was washed with diethyl ether (40 mL). The greenish yellow solution was concentrated to ~10 mL and transferred to a round bottomed flask. Short-path distillation led to the isolation of hex-5-enal as a colorless liquid (325 mg, 32%). Bp 38 °C (1 atm). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.72 (2H, pent., J 7.3 Hz, $CH_2CH_2CH_2$), 2.07 (2H, q, J 7.3 Hz, CH_2CH=CH_2), 2.45 (2H, td, J 7.3, 1.6 Hz, CH_2CH=O), 4.90–5.09 (2H, m, CH=CH_2), 5.74 (1H, ddt, J 17.0, 10.7, 7.3 Hz, CH=CH_2), 9.74 (1H, d, J 1.6 Hz, CH=O); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 21.1, 32.9, 43.0, 115.5, 137.5, 202.5.

4.1.6. 2-(4-Pentenyl)-1,3-dithiane (7). Propane-1,3-dithiol (101 μL, 108 mg, 1 mmol, 1 equiv) was added to a solution of hex-5-en-al (100 mg, 1 mmol, 1 equiv) in $CHCl_3$ (2 mL) at rt, stirred for 1 h, and then cooled to −10 °C. $BF_3{\cdot}OEt_2$ (128 μL, 144 mg, 1 mmol, 1 equiv) was added dropwise and the resulting solution was allowed to warm to rt and stirred for 17 h. The mixture was washed with water (3×5 mL), 10% aqueous NaOH (5 mL), and water (5 mL), dried over $MgSO_4$, filtered, and concentrated. The residue was purified by flash chromatography, eluting with 10:1 hexanes–EtOAc to give the dithiane derivative **7** as a colorless oil (177 mg, 94%). R_f 0.43 (10:1 Hex–EtOAc). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.55–1.63 (2H, m, CH_2CH_2CH=CH_2), 1.71–1.79 (2H, m, CH_2CH_2S), 1.80–1.88 (2H, m, CH_2CHS), 2.03–2.12 (2H, m, CH_2CH=CH_2), 2.77–2.90 (4H, m,

$CH_2S\times2$), 4.02 (1H, t, *J* 7.0 Hz, SC*H*S), 4.93–5.03 (2H, m, CH=CH_2), 5.72–5.82 (1H, m, C*H*=CH_2); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 25.7, 26.0, 30.4 (2C), 33.2, 34.8, 47.4, 114.9, 138.0; HRMS (EI): M^+, found 188.06936. $C_9H_{16}S_2$ requires 188.06934.

4.1.7. 2-*tert*-Butyldimethylsilyloxy-10-undecen-6-1,3-dithiane (18).[19] ^{n}BuLi (350 μL of 1.5 M solution in hexane, 33.6 mg, 0.525 mmol, 1.05 equiv) was added dropwise to a stirred solution of dithiane **7** (94 mg, 0.5 mmol, 1.00 equiv) in THF (3 mL) at −20 °C under nitrogen. The mixture was stirred at −20 °C for 2 h. The temperature was decreased to −78 °C and a solution of freshly prepared iodide **6** (180 mg, 0.55 mmol, 1.10 equiv) in THF (2 mL) was added dropwise. The temperature was gradually warmed to −20 °C and stirred for 3 h. The reaction was quenched with aq $NaHCO_3$ (2 mL) and extracted with diethyl ether (2×15 mL). The combined organic layers were washed with water (15 mL) and brine (15 mL), dried over $MgSO_4$, filtered, and concentrated. The residue was purified by flash chromatography, eluting with 20:1 hexanes–EtOAc to give dithiane derivative **18** as a colorless oil (181 mg, 93%). R_f 0.48 (10:1 Hex–EtOAc). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 0.06 (3H, s, Si*Me*), 0.07 (3H, s, Si*Me*), 0.90 (9H, s, Si^t*Bu*), 1.14 (3H, d, *J* 6.1 Hz, *Me*CHOTBDMS), 1.22–1.52 (6H, m), 1.83–1.99 (6H, m), 2.08 (2H, q, *J* 7.0 Hz, CH_2CH=CH_2), 2.79–2.82 (4H, m, CH_2S×2), 3.81 (1H, app. sext., *J* 6.1 Hz, C*H*OTBDMS), 4.97–5.07 (2H, m, CH=CH_2), 5.80 (1H, ddt, *J* 17.2, 10.2, 7.0 Hz, C*H*=CH_2); ^{13}C NMR ($CDCl_3$, 100 MHz) δ −4.7, −4.4, 18.1, 20.4, 23.4, 23.8, 25.6, 25.9, 33.7, 37.4, 38.4, 39.8, 53.2, 68.5, 115.0, 138.3; HRMS (EI): M^+, found 388.23008. $C_{20}H_{40}OS_2Si$ requires 388.22899.

4.1.8. 2-(4-Hydroxypentyl)-2-(4-pentenyl)-1,3-dithiane (5). TBAF (220 μL, 1 M solution in THF, 0.22 mmol, 1.1 equiv) was added to a solution of silyl ether **18** (78 mg, 0.2 mmol, 1.0 equiv) in THF (1 mL) at rt under nitrogen. The mixture was stirred overnight, and then filtered through a pad of silica gel. The filtrate was concentrated and the residue purified by flash column chromatography, eluting with 5:1 hexanes–EtOAc to give alcohol **5** as a colorless oil (40 mg, 72%). R_f 0.13 (5:1 Hex–EtOAc). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.16 (3H, d, *J* 6.2 Hz, *Me*CHOH), 1.39–1.53 (6H, m), 1.83–1.92 (6H, m), 2.06 (2H, q, *J* 7.0 Hz, CH_2CH=CH_2), 2.77–2.78 (4H, m, CH_2S×2), 3.78 (1H, sext., *J* 6.2 Hz, C*H*OH), 4.96 (1H, dd, *J* 10.2, 2.0 Hz, CH=CH_2 cis), 5.00 (1H, dd, *J* 17.2, 2.0 Hz, CH=CH_2 trans), 5.78 (1H, ddt, *J* 17.2, 10.2, 7.0 Hz, C*H*=CH_2); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 20.4, 23.3, 23.5, 25.4, 25.9 (2C), 33.6, 37.5, 38.1, 39.2, 53.1, 67.8, 115.0, 138.2; HRMS (EI): M^+, found 274.14275. $C_{14}H_{26}OS_2$ requires 274.14251.

4.1.9. Ethyl non-8-enyl (2-vinylphenyl)phosphonate (20). A solution of dec-9-en-1-ol (156 μL, 137 mg, 0.88 mmol, 1.1 equiv) and triphenylphosphine (231 mg, 0.88 mmol, 1.1 equiv) in THF (8 mL) was added dropwise to a solution of acid **19** (170 mg, 0.8 mmol, 1.0 equiv) and dimethylazodicarboxylate (174 μL, 128 mg, 0.88 mmol, 1.1 equiv) in THF (8 mL) at rt. The mixture was stirred under nitrogen for 4 h, concentrated, and the residue purified by flash chromatography, eluting with hexanes–EtOAc, with solvent gradient from 10:1 to 5:1 to give the ester derivative **20** as a colorless oil (135 mg, 48%). R_f 0.25 (2:1 Hex–EtOAc). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.18–1.36 (13H, m, CH_2×5 and OCH_2CH_3), 1.58–1.66 (2H, m, $POCH_2CH_2$), 1.96–2.06 (2H, m, CH_2CH=CH_2), 3.92–4.16 (4H, m, POCH_2×2), 4.86–5.02 (2H, m, CH_2CH=CH_2), 5.35 (1H, d, *J* 10.8 Hz, ArCH=CH_2 cis), 5.70 (1H, d, *J* 17.4 Hz, ArCH=CH_2 trans), 5.74–5.82 (1H, m, CH_2C*H*=CH_2), 7.29–7.44 (2H, m, ArH), 7.49 (1H, t, *J* 7.0 Hz, ArH), 7.63 (1H, t, *J* 7.0 Hz, ArH), 7.3 (1H, dd, *J* 17.4, 10.8 Hz, ArC*H*=CH_2); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 16.2 (d, *J* 7 Hz), 25.4, 28.8, 28.9, 29.0, 29.2, 30.3 (d, *J* 7 Hz), 33.7, 62.0 (d, *J* 5 Hz), 66.0 (d, *J* 5 Hz), 114.0, 116.7, 125.6 (d, *J* 181 Hz), 126.1 (d, *J* 15 Hz), 127.1 (d, *J* 15 Hz), 134.0 (d, *J* 3 Hz), 134.1 (d, *J* 10 Hz), 135.3 (d, *J* 5 Hz), 139.1, 141.2 (d, *J* 10 Hz); HRMS (EI): M^+, found 350.20067. $C_{20}H_{31}O_3P$ requires 350.20108.

4.1.10. 2-Ethoxy-(2,3-benzo-1-oxa-2-phosphacyclotetradec-5-ene) 2-oxide (21). Grubbs' second generation catalyst (12 mg, 0.014 mmol, 5 mol %) was added to a solution of ester **20** (100 mg, 0.28 mmol, 1 equiv) in toluene (70 mL). The mixture was stirred at 80 °C under nitrogen for 23 h, concentrated, and the resulting residue purified by flash chromatography, eluting with 2:1 hexanes–EtOAc to give **21** as a colorless oil (82 mg, 90%). R_f 0.13 (2:1 Hex–EtOAc). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.23–1.63 (12H, m, CH_2×6), 1.25 (3H, t, *J* 6.8 Hz, $POCH_2CH_3$), 2.24 (2H, q, *J* 6.5 Hz, CH=CHCH_2), 3.91–4.16 (4H, m, POC*H*×2), 6.12 (1H, dt, *J* 15.7, 6.5 Hz, ArCH=C*H*), 7.03 (1H, d, *J* 15.7 Hz, ArC*H*=CH), 7.18–7.23 (1H, m, ArH), 7.40 (1H, t, *J* 7.3 Hz, ArH), 7.56 (1H, t, *J* 7.3 Hz, ArH), 7.91 (1H, dd, *J* 14.8, 7.3 Hz, ArH); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 16.3 (d, *J* 6 Hz), 22.9, 24.5, 25.7, 26.1, 26.2, 29.1 (d, *J* 7 Hz), 31.0, 62.3 (d, *J* 5 Hz), 64.9 (d, *J* 7 Hz), 124.8 (d, *J* 185 Hz), 125.8 (d, *J* 14 Hz), 126.1 (d, *J* 14 Hz), 128.4 (d, *J* 5 Hz), 132.5 (d, *J* 3 Hz), 133.7, 134.2 (d, *J* 10 Hz), 141.3 (d, *J* 9 Hz); HRMS (EI): M^+, found 322.16973. $C_{18}H_{27}O_3P$ requires 322.16978.

4.1.11. Ethyl 2-[6-(1,3-dithiane)undec-10-enyl] (4,6-diisopropoxy-2-vinylphenyl)phosphonate (22). A solution of alcohol **5** (60 mg, 0.22 mmol, 1.0 equiv) and triphenylphosphine (58 mg, 0.22 mmol, 1.0 equiv) in THF (2 mL) was added dropwise to a solution of acid **4** (144 mg, 0.44 mmol, 2.0 equiv) and dimethylazodicarboxylate (32 mg, 0.22 mmol, 1.0 equiv) in THF (3 mL) at rt. The mixture was stirred under an atmosphere of nitrogen for 3 h, concentrated, and the residue purified by flash chromatography, eluting with hexanes–EtOAc with a gradient from 10:1 to 5:1 to give the ester derivative **22** as a colorless oil (33 mg, 26%). R_f 0.28 (2:1 Hex–EtOAc). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.16 (3H, d, *J* 6.2 Hz, CH(OR)*Me*), 1.25 (3H, td, *J* 7.0, 4.4 Hz, $POCH_2CH_3$), 1.31 (12H, d, *J* 6.0 Hz, $OCHMe_2$×2), 1.37–2.06 (14H, m), 2.65–2.77 (4H, m, CH_2S×2), 3.90–4.13 (2H, m, POCH_2), 4.48–4.63 (3H, m, C*H*Me_2×2 and C*H*(OR)Me), 4.90–5.05 (2H, m, CH_2CH=CH_2), 5.23 (1H, d, *J* 10.8 Hz, ArCH=CH_2 cis), 5.44 (1H, dd, *J* 17.2, 1.6 Hz, ArCH=CH_2 trans), 5.73 (1H, ddt, *J* 17.2, 10.8, 6.8 Hz, CH_2C*H*=CH_2), 6.31 (1H, d, *J* 4.0 Hz, ArH), 6.52 (1H, dd, *J* 4.0, 2.4 Hz, ArH), 7.81 (1H, dd, *J* 17.2, 10.8 Hz, ArC*H*=CH_2); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 16.2, 20.0, 21.5, 22.0 (2C), 22.1 (3C), 23.3, 25.6, 26.0, 33.8, 37.7,

38.2, 53.0, 60.9, 61.0, 69.7, 70.4, 73.0, 100.5, 105.7, 107.2 (*J* 154 Hz), 114.9, 115.5, 138.1, 138.2, 147.0, 161.6, 162.0; HRMS (EI): M^+, found 584.27636. $C_{30}H_{49}O_5PS_2$ requires 584.27591.

4.1.12. 2-Ethoxy-2,3-(1′,5′,-diisopropoxy)benzo-10-(1,3-dithiane)-14-methyl-1-oxa-2-phosphacyclotetradec-5-ene 2-oxide (23). Grubbs' second generation catalyst (8.5 mg, 0.01 mmol, 10 mol %) was added to a solution of ester **22** (58.5 mg, 0.1 mmol, 1 equiv) in toluene (50 mL). The reaction mixture was stirred at 80 °C under nitrogen for 23 h, concentrated, and the resulting residue purified by flash chromatography, eluting with 2:1 hexanes–EtOAc to give macrolactone **23** as a colorless oil (27 mg, 48%). R_f 0.30 (2:1 Hex–EtOAc). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.28–1.36 (18H, m, *Me*CHO, $POCH_2CH_3$, $OCHMe_2\times2$), 1.47–2.37 (14H, m), 2.73–2.83 (4H, m, $CH_2S\times2$), 3.84–3.99 (1H, $POCH_2$), 4.02–4.15 (1H, m, $POCH_2$), 4.50 (1H, pent, *J* 6.0 Hz, $OCHMe_2$), 4.58 (1H, pent, *J* 6.0 Hz, $OCHMe_2$), 4.72–4.81 (1H, m, MeCHO), 5.94 (1H, dt, *J* 15.8, 7.1 Hz, ArCH=CH), 6.28 (1H, dd, *J* 5.0, 2.2 Hz, ArH), 6.49 (1H, dd, *J* 5.0, 2.2 Hz, ArH), 7.56 (1H d, *J* 15.8 Hz, ArCH=CH); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 12.7, 16.4, 21.9 (2C), 22.0 (4C), 23.9, 25.7, 26.0, 30.2, 35.6, 37.2, 37.5, 53.4, 61.2, 61.3, 69.7, 70.8, 71.3, 100.6, 107.3 (d, *J* 193 Hz), 115.1, 132.5, 147.5, 161.5, 161.6; HRMS (EI): M^+, found 556.24517. $C_{28}H_{45}O_5PS_2$ requires 556.24461.

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Supplementary data

^{1}H and ^{13}C NMR spectra for compounds **4**, **5**, **6**, **7**, **13**, **14**, **18**, **20**, **21**, **22**, and **23**. Supplementary data associated with this article can be found in the online version, at doi:10.1016/j.tet.2007.07.042.

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