component **7** to give **24**, and with subsequent cycloreversion to the conjugated diene **8**. The fact that coupling is limited to terminal alkynes may have a steric origin. Formation of the more stable and thus less reactive conjugated carbene complex **23** might explain why the yne–ene metathesis requires longer reaction times than conventional cross-metathesis between alkenes. Volatile ethylene is formed in the cross-metathesis of terminal alkenes, whereas the yne–ene metathesis takes place with atom economy. The driving force in yne–ene metathesis may be the formation of a conjugated diene.

The type of reaction described here is, to our knowledge, the first selective crossed yne–ene metathesis. Application of this cross-metathesis between terminal alkynes and alkenes has been demonstrated by the synthesis of variously functionalized dienes. The reaction opens the way to interesting structural elements: thus, conjugated allylsilanes have found a variety of applications, for example in Sakurai reactions.[18] The metathesis products are also of interest with respect to Diels–Alder reactions and cycloadditions. We are currently investigating the applications of yne–ene metathesis in natural product synthesis.

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[14] Representative experimental procedure for yne–ene cross-metathesis: propargyl acetate (280 mg, 2.85 mmol) and allyltrimethylsilane (980 mg, 8.57 mmol) were dissolved in dichloromethane (15 mL). After addition of **[Ru]** (165 mg, 0.19 mmol) the mixture was stirred for 24 h at room temperature. The reaction mixture was concentrated under vacuum and purified chromatographically with *tert*-butyl methyl ether/petroleum ether (1/9) on silica gel. There was obtained 543 mg (90%) of product **10**. (*E*)/(*Z*)-isomers were assigned on the basis of ^{1}H-^{1}H and ^{1}H-^{13}CNMR correlation measurements (400 MHz). Selected spectroscopic data: (E)-**10**: ^{1}H NMR (400 MHz, $CDCl_3$): δ = 5.91 (d, J = 16 Hz, 1H), 5.7 (dd, J = 16 Hz, 8 Hz, 1H), 5.03 (s, 1H), 5.00 (s, 1H), 4.70 (s, 2H), 2.07 (s, 3H), 1.54 (d, J = 8 Hz, 2H), −0.02 (s, 9H); ^{13}C NMR (67.9 MHz, $CDCl_3$): δ = 170.5 (C), 140.1 (C), 128.5 (CH), 127.3 (CH), 113.8 (CH_2), 64.1 (CH_2), 23.6 (CH_2), 20.7 (CH_3), −1.95 (CH_3); (*Z*)-**10**: ^{1}H NMR (400 MHz, $CDCl_3$): δ = 5.65 (d, J = 13 Hz, 1H), 5.63 (dd, J = 13 Hz, 8 Hz, 1H), 5.22 (d, J = 2 Hz, 1H), 5.10 (s, 1H), 4.66 (s, 2H), 2.07 (s, 3H), 1.73 (d, J = 8 Hz, 2H), 0.04 (s, 9H); ^{13}C NMR (67.9 MHz, $CDCl_3$): δ = 170.4 (C), 139.8 (C), 130.4 (CH), 123.7 (CH), 114.5 (CH_2), 66.8 (CH_2), 20.7 (CH_3), 20.0 (CH_2), −2.2 (CH_3); MS: *m/z*(%): 212 (5) [M^+], 197 (3), 169 (5) [$M^+ - C_2H_3O$], 117 (25), 79 (100), 73 (91), 43 (18); HR-MS: calcd for $C_{11}H_{20}O_2Si$ [M^+]: 212.1233, found: 212.122.

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Total Synthesis of Eleutherobin**

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Eleutherobin[1, 2] **1** (Figure 1) is a newly discovered antitumor agent which has a mechanism of action[3] like Taxol (**2**) and a novel molecular architecture. Its structure was based on spectroscopic results,[1, 2] although no absolute configuration was assigned. Isolated from an *Eleutherobia* species of marine soft corals (possibly *E. albiflora* Alcynacea, Alcyoniidea) collected in the Indian Ocean near Bennett's Shoal in Western Australia, this substance is extremely scarce, and yet its tubulin polymerization and microtubule-stabilizing properties and 100-fold higher potency (over the mean cytotoxicity of an NIH cell line panel) against selected breast, renal, ovarian, and lung cancer cells[2] make it (along with epothilones[4–9] A (**3**) and B (**4**), Figure 1), one of the most promising antitumor agents isolated from nature in recent years. Here we report the first total synthesis of eleutherobin (**1**) which renders the natural product (**1**) and two biologically active analogues (**33** and **34**, see Scheme 3) readily available for further studies. Furthermore, the described chemical synthesis allows assignment of the absolute stereochemistry of eleutherobin (**1**) and opens an avenue for the generation of combinatorial libraries for biological screening purposes.

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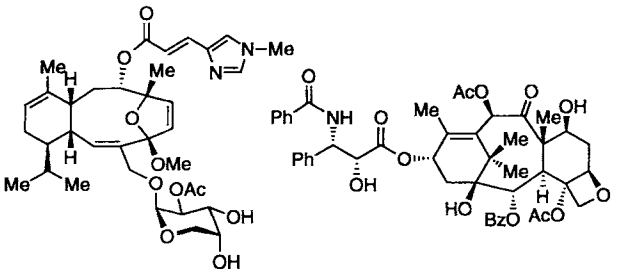
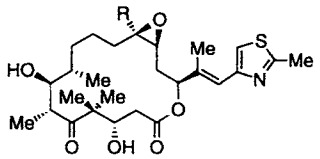


1: eleutherobin

2: Taxol



3: R = H : epothilone A
4: R = Me : epothilone B

Figure 1. Molecular structures of eleutherobin (**1**), Taxol (**2**), and epothilones A (**3**) and B (**4**) (Ac = acetyl, Bz = benzoyl).

The structure of eleutherobin (**1**), which is similar to those of eleuthosides[10] A and B, sarcodictyins[11–14] A and B, and valdivones[15], includes a synthetically challenging tricyclic skeleton (**ABC**, Figure 2) and two side chains, one containing an

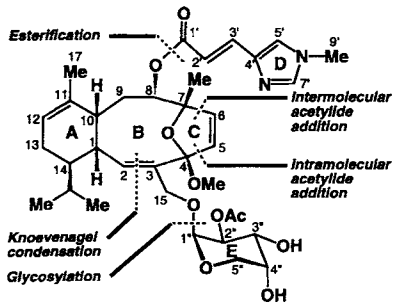


Figure 2. Numbering and strategic bond disconnections of eleutherobin (**1**) (Ac = acetyl).

(*E*)-*N*(6′)-methylurocanic acid residue (**D**) and the other a β-linked 2″-*O*-acetyl-D-arabinopyranose unit (**E**). The strategy for the total synthesis of this naturally occurring substance was designed based on a retrosynthetic analysis featuring the strategic bond disconnections highlighted in Figure 2. Thus, a properly functionalized ring **A** was to be derived from (+)-carvone and elaborated to a more advanced intermediate by an intermolecular acetylide addition to a ketone and a Knoevenagel condensation with an aldehyde functionality to form bonds C6–C7 and C2–C3, respectively. A glycosylation with a suitably functionalized D-arabinose derivative was then to follow, in order to introduce the carbohydrate moiety, whereas an intramolecular acetylide-aldehyde condensation was expected to form the 10-membered ring of the target molecule (bond C4–C5). Oxidation to an ynone and protecting group manipulations, followed by selective reduction of the triple bond (C5–C6) was expected to allow formation of the **BC** framework by intramolecular attack of the C7 hydroxyl function on the C4 carbonyl group. Finally, the (*E*)-*N*(6′)-methylurocanic acid moiety was to be introduced by esterification, following which eleutherobin (**1**) was expected to emerge upon deprotection.

Scheme 1 summarizes the construction of the initially targeted key ynal precursor **18**. Thus, the (+)-carvone-derived,[14, 16] TBS-protected (for abbreviations see legends) aldehyde **5** was converted to a C8 mixture (about 1.25:1) of hydroxyketones **6** by reaction with 1-ethoxyvinyllithium followed by acid hydrolysis (82% overall yield). Addition of ethynylmagnesium bromide to hydroxyketones **6** led, stereospecifically,[17] to a mixture of two acetylenic diols (C7, C8) **7** in 76% overall yield. Desilylation of **7** with TBAF afforded, after flash chromatography (silica gel, ether/hexane 3:1), pure triol **8** (52% yield) along with its C7, C8 diastereoisomer (40% yield). Triol **8** (identified by correlation with a previously synthesized sample)[14] was then converted to the primary alcohol **10** by persilylation (TES-OTf) and selective monodesilylation (PPTS-MeOH) in 98% overall yield. Oxidation of **10** with TPAP-NMO[18] then produced aldehyde **11** (98% yield) which underwent smooth Knoevenagel condensation with ethyl cyanoacetate in the presence of β-alanine[19] to afford, stereoselectively, the desired (*E*)-α,β-unsaturated cyanoester **12** in 95% yield. The reduction of **12** with DIBAL at −78 to −10 °C (hexanes) proceeded in high yield and with remarkable selectivity to afford the desired glycosylation acceptor, hydroxyaldehyde **13** in 75% yield.

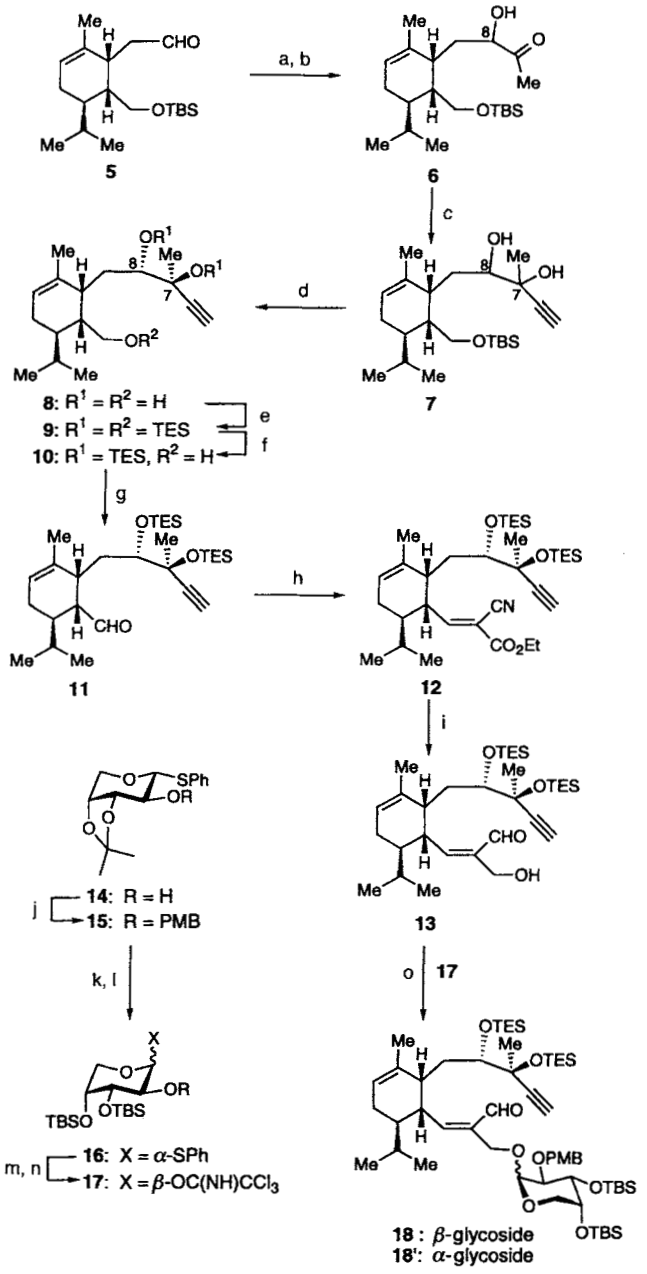
The D-arabinose trichloroacetimidate derivative **17** required for coupling was prepared from the previously synthesized[20] phenyl thioglycoside **14**: The reaction of **14** with PMB-Cl in the presence of NaH afforded **15** (93% yield), and the acid-induced removal of the acetonide group gave the expected diol (84% yield). Subsequent silylation with TBS-OTf led to **16** (97% yield), whose reaction with NBS in acetone:H_2O (93:7) gave the corresponding lactol (80% yield), which underwent a reaction with CCl_3CN in the presence of NaH to give **17** (93% yield)[21].

Coupling of **13** with **17** (2.0 equiv) in ether proceeded smoothly in the presence of TMS-OTf at 0 °C to afford predominantly the β-glycoside **18** along with its α-anomer (**18′**) in high yield. Flash column chromatography (silica gel, Et_2O in hexanes, 1:20) led to pure β-glycoside **18** (54% yield) and α-glycoside **18′** (28% yield) (for spectroscopic data see Table 1).

The conversion of ynal **18** to eleutherobin (**1**) followed the path shown in Scheme 2. Ring closure of **18** was facilitated by the action of LiHMDS in THF at −20 °C, which furnished the 10-membered ring alcohol **19** (mixture of C4 diastereoisomers). Oxidation of **19** with Dess–Martin reagent[22] then led to the bis-conjugated ketone **20** (80% overall yield from **18**). At this stage, the acetyl group was installed on the carbohydrate ring of the molecule by removing the PMB group (DDQ, 90% yield) and acetylating the resulting secondary alcohol **21** (Ac_2O, Et_3N, 4-DMAP, 90% yield). The acetate **22** was then exposed to $Et_3N \cdot 3HF$ in THF at 25 °C, conditions which allowed the selective removal of both TES groups leading to diol **23** in 86% yield. Compound **23** was then subjected to Lindlar hydrogenation conditions, which gave crude lactol **25** in high yield. Although the postulated intermediate, bis-enone **24**, was not detected, its intermediacy in this sequence is logical and indeed required. Treatment of lactol **25** with PPTS in MeOH at 25 °C led to the isolation of its methoxy derivative **26** in 78% overall yield from **23**, as a single stereoisomer. The β-stereochemistry of the methoxy group in **26** was assumed on the basis of molecular models, and was confirmed by its conversion to eleutherobin (**1**).

For the purposes of esterification, the known[23] ethyl (*E*)-*N*(6′)-methylurocanate **27** (Scheme 2) was sequentially converted to sodium salt **28** (NaOH, THF, H_2O) and *tert*-butyl dride **29** [*t*BuC(O)Cl, THF, about 90% overall yield]. The at-

  0570-0833/97/3622-2521 $ 17.50+.50/0



Scheme 1. Construction of the glycosylated ynal **18**: a) CH_2=CH(OEt) (2.0 equiv), *t*BuLi (1.8 equiv, 1.7 M in THF), THF, $-78 \rightarrow 0$ °C, 1 h; then cool to -78 °C and add **5** (1.0 equiv); then slowly warm to -40 °C; b) conc. H_2SO_4, Et_2O, 25 °C, 2 min, 82% for two steps; c) HC≡CMgBr (5.0 equiv, 0.5 M in THF), THF, $-78 \rightarrow -20$ °C, 14 h, 76% as an approximate 1.25:1 mixture of diastereoisomers; d) TBAF (2.0 equiv, 1 M in THF), THF, $0 \rightarrow 25$ °C, 1 h, **8** (52%), plus its C7–C8 diastereoisomer (40%); e) TES-OTf (5.0 equiv), Et_3N (10.0 equiv), CH_2Cl_2, 25 °C, 100%; f) PPTS (0.1 equiv), MeOH:CH_2Cl_2 (3:1), 25 °C, 45 min, 98%; g) TPAP (0.05 equiv), NMO (1.5 equiv), CH_2Cl_2, 4 Å MS, 1.5 h, 98%; h) $NCCH_2CO_2Et$ (30.0 equiv), β-alanine (4.0 equiv), 95% EtOH, 72 h, 50 °C, 95%; i) DIBAL (10.0 equiv), hexanes, $-78 \rightarrow -10$ °C, 75%; j) NaH (1.1 equiv), DMF, 0 °C, 30 min; then PMB-Cl (1.2 equiv), 2 h, 93%; k) TsOH·H_2O (0.1 equiv, 7.2 mM), ethylene glycol-MeOH (1:10), 25 °C, 6 h, 84%; l) TBDMS-OTf (4.0 equiv), Et_3N (10.0 equiv), CH_2Cl_2, 0 °C, 2 h, 97%; m) NBS (3.4 equiv), pyridine (11.0 equiv), acetone-H_2O (93:7), 80%; n) NaH (0.1 equiv), Cl_3CCN (5.0 equiv), CH_2Cl_2, 25 °C, 3.5 h, 93%; o) **17** (2.0 equiv), TMS-OTf (0.1 equiv), Et_2O, 0 °C **18** (54%), plus its α-anomer **18'** (28%). TBAF = tetra-*n*-butylammonium fluoride, TES-OTf = triethylsilyl trifluoromethanesulfonate, PPTS = pyridinium *p*-toluenesulfonate, TPAP = tetra-*n*-propylammonium perruthenate, NMO = 4-methylmorpholine *N*-oxide, DIBAL = diisobutylaluminum hydride, DMF = *N*,*N*-dimethylformamide, PMB-Cl = *p*-methoxybenzylchloride, TsOH·H_2O = *p*-toluenesulfonic acid monohydrate, TBDMS-OTf = *tert*-butyldimethylsilyl trifluoromethanesulfonate, NBS = *N*-bromosuccinimide, TMS-OTf = trimethylsilyl trifluoromethanesulfonate.

Table 1. Selected physical properties of compounds **18**, **30**, **33**, and **34**.

18: $R_f = 0.8$ [silica gel, hexane:ether (3:1)]; $[\alpha]_{25}^{D} = -28.3$ (c = 1.0 in chloroform). IR (film): $\tilde{\nu}_{max}$ 2954, 1676, 1612, 1512, 1461, 1364, 1250, 1114, 1038, 835, 740 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$, eleutherobin numbering): δ = 10.16 (s, 1H, H-4), 7.21 (d, J = 8.5 Hz, 2H), 6.94 (d, J = 11.5 Hz, H-2), 6.82 (d, J = 8.5 Hz, 2H), 5.38 (bs, 1H, H-12), 4.74 (d, J = 2.0 Hz, 1H), 4.62 (d, J = 11.9 Hz, 1H), 4.42 (d, J = 11.9 Hz, 1H), 4.21 (m, 2H), 3.92 (bs, 1H), 3.78 (s, 3H), 3.72 (m, 1H), 3.50 (m, 4H), 2.25 (m, 1H), 2.01 (m, 3H), 1.89 (m, 2H), 1.75 (m, 1H), 1.66 (s, 3H), 1.57 (m, 2H), 1.42 (m, 2H), 1.31 (s, 3H), 1.23 (m, 2H), 0.89 (m, 35H), 0.66 (m, 16H), −0.03 (m, 12H); ^{13}C NMR (125 MHz, $CDCl_3$): δ = 189.6, 159.1, 155.5, 136.3, 136.2, 130.8, 129.5, 121.0, 113.6, 99.3, 86.8, 78.8, 76.4, 75.5, 73.0, 72.7, 71.4, 67.1, 64.0, 55.2, 39.0, 37.2, 34.2, 29.7, 28.4, 26.0, 25.9, 24.4, 23.6, 21.9, 21.2, 18.2, 18.1, 17.0, 7.1, 7.0, 6.0, 5.6, −4.5, −4.6, −4.7, −4.9; HRMS (FAB): calcd for $C_{57}H_{102}O_9Si_4Na$ (M + Na^+): 1065.6499, found 1065.6543.

30: $R_f = 0.4$ [silica gel, ethyl acetate:hexane (4:1)]; $[\alpha]_{25}^{D}$ −61.3 (c = 0.6 in chloroform); IR (film): $\tilde{\nu}_{max}$ 2929, 2856, 2362, 2338, 1746, 1707, 1639, 1468, 1367, 1296, 1253, 1150, 1062, 1038, 1001, 886, 837, 775 cm^{-1}; UV(CH_2Cl_2) λ_{max} (log ε) 286 nm (0.924); ^1H NMR (600 MHz, $CDCl_3$, eleutherobin numbering): δ = 7.52 (d, J = 15.6 Hz, 1H, H-3'), 7.44 (s, 1H, H-7'), 7.07 (s, 1H, H-5), 6.55 (d, J = 15.6 Hz, 1H, H-2'), 6.08–6.05 (m, 2H, H-5, H-6), 5.55 (d, J = 9.5 Hz, 1H), 5.24 (bs, 1H, H-12), 4.99–4.96 (m, 1H), 4.88 (s, 1H), 4.79 (d, J = 7.4 Hz, 1H), 4.25 (d, J = 12.4 Hz, 1H), 3.97 (dd, J = 8.3, 2.3 Hz, 1H), 3.95–3.92 (m, 1H), 3.88–3.84 (m, 2H), 3.72–3.64 (m, 1H), 3.69 (s, 3H, H-21), 3.47 (dd, J = 11.6, 4.4 Hz, 1H), 2.58 (d, J = 11.2 Hz, 1H), 2.32–2.28 (m, 1H), 2.05–1.94 (m, 2H), 2.02 (s, 3H, C(O)CH_3), 1.61–1.51 (m, 5H), 1.51 (s, 3H, H-17), 1.42 (s, 3H), 1.25–1.22 (m, 4H), 0.95 (d, J = 6.6 Hz, 3H), 0.90 (d, J = 6.6 Hz, 3H), 0.87 (s, 9H), 0.86 (s, 9H), 0.08 (s, 3H), 0.05 (s, 3H), 0.04 (s, 3H), 0.04 (s, 3H); ^{13}C NMR (150 MHz, $CDCl_3$): δ = 170.2, 166.7, 139.2, 138.4, 136.3, 134.2, 133.9, 133.3, 130.8, 122.7, 121.2, 115.9, 95.0, 89.8, 81.5, 71.5, 69.5, 69.3, 64.0, 60.4, 49.6, 42.5, 38.6, 34.0, 33.6, 29.6, 29.0, 25.8, 24.4, 24.3, 22.2, 22.0, 21.1, 21.0, 20.6, 18.2, 18.1, 14.2, −4.2, −4.5, −4.7, −4.9; HRMS (FAB): calcd for $C_{47}H_{76}N_2O_{10}Si_2Cs$ (M + Cs^+): 1017.4093, found 1017.4139.

33: $R_f = 0.3$ [silica gel, ethyl acetate:hexane (2:1); $[\alpha]_{25}^{D}$ −61.6 (c = 0.3 in chloroform); IR (film): $\tilde{\nu}_{max}$ 3428, 2924, 2854, 1732, 1712, 1638, 1463, 1371, 1265, 1158, 1060, 997 cm^{-1}; ^1H NMR (600 MHz, $CDCl_3$, eleutherobin numbering): δ = 7.50 (d, J = 15.5 Hz, 1H, H-3'), 7.29 (s, 1H, H-5'), 6.65 (d, J = 15.5 Hz, 1H, H-2'), 6.09 (dd, J = 19.0, 5.9 Hz, 1H), 5.54 (d, J = 9.4 Hz, 1H), 5.26 (bs, 1H, H-12), 4.96 (dd, J = 9.8, 3.5 Hz, 1H), 4.90 (d, J = 3.6 Hz, 1H), 4.81 (d, J = 7.4 Hz, 1H), 4.28 (d, J = 12.2 Hz, 1H), 4.03–3.90 (m, 3H), 3.86 (d, J = 12.5 Hz, 1H), 3.81 (dd, J = 11.2, 2.0 Hz, 1H), 3.69 (dd, J = 12.6, 2.0 Hz, 1H), 3.20 (s, 3H, H-21), 2.72 (s, 3H, H-2''), 2.64–2.53 (m, 1H), 2.38–2.24 (m, 1H), 2.10 (s, 3H, C(O)CH_3), 1.55 (s, 3H, H-17), 1.50–0.70 (m, 8H), 1.50 (s, 3H, H-16), 0.97 (d, J = 6.5 Hz, 3H), 0.92 (d, J = 6.5 Hz, 3H); ^{13}C NMR (150 MHz, $CDCl_3$): δ = 171.2, 167.0, 166.3, 151.1, 137.2, 136.6, 133.9, 133.4, 132.6, 131.0, 121.8, 121.2, 119.9, 115.7, 93.4, 89.7, 81.7, 71.7, 69.3, 69.0, 68.0, 62.0, 49.6, 45.5, 42.3, 38.6, 34.1, 31.4, 29.6, 28.9, 24.4, 24.1, 22.1, 21.8, 20.9, 20.4, 19.3; HRMS (FAB): calcd for $C_{35}H_{47}NNaO_{10}S$ (M + Na^+) 696.2818, found 696.2802.

34: $R_f = 0.25$ [silica gel, methanol:dichloromethane (5%)]; IR (film): $\tilde{\nu}_{max}$ 3390, 2924, 2852, 2354, 1734, 1708, 1639, 1630, 1453, 1367, 1245, 1161, 1038, 999 cm^{-1}; UV(CH_2Cl_2) λ_{max} (log ε) 290 nm (0.828); ^1H NMR data (500 MHz, $CDCl_3$, eleutherobin numbering): δ 7.54 (d, J = 15.6 Hz, 1H, H-2'), 7.47 (s, 1H, H-7'), 7.11 (s, 1H, H-5'), 6.57 (d, J = 15.6 Hz, 1H, H-3'), 6.21 (d, J = 5.9 Hz, 1H, H-6), 6.00 (d, J = 5.9 Hz, 1H, H-5), 5.62 (d, J = 9.5 Hz, 1H), 5.30–5.26 (m, 1H), 4.97 (dd, J = 3.8, 2.0 Hz, 1H), 4.82 (d, J = 7.5 Hz, 1H), 4.72–4.69 (m, 1H), 4.17 (d, J = 10.8 Hz, 1H), 3.96 (d, J = 10.9 Hz, 1H), 3.72 (s, 3H, H-21), 3.65–3.62 (m, 2H), 3.55–3.43 (m, 5H), 3.23 (s, 3H, H-9'), 3.02–2.94 (m, 1H), 2.71–2.53 (m, 1H), 2.11 (s, 3H, C(O)CH_3), 2.01–1.96 (m, 9H), 1.56–1.21 (m, 3H), 1.53 (s, 3H, H-17), 1.46 (s, 3H, H-16), 1.00 (d, J = 6.6 Hz, 3H), 0.94 (d, J = 6.6 Hz, 3H); HRMS (FAB): calcd for $C_{35}H_{47}N_2O_{10}Cs$ (M + Cs^+): 789.2363, found 789.2334.

tachment of the (*E*)-*N*(6')-methylurocanic acid residue onto the main framework of eleutherobin was accomplished by reacting alcohol **26** with mixed anhydride **29** in the presence of Et_3N and 4-DMAP in CH_2Cl_2 at 25 °C, furnishing ester **30** in 75% yield (for spectroscopic data see Table 1). Finally, exposure of **30** to TBAF in THF generated eleutherobin (**1**) in 88% yield. Synthetic eleutherobin (**1**) exhibited identical properties to those reported[1, 2] for the natural substance (^1H and ^{13}C NMR, MS, $[\alpha]_{25}^{D}$, IR, and UV). The synthetic correlation of eleutherobin (**1**) ($[\alpha]_{25}^{D}$ = 67 (c = 0.2, MeOH)) to (+)-carvone and D-arabinose establishes its absolute stereochemistry as that shown in structure **1**.

In order to explore the structure–activity relationships of eleutherobin, two novel analogues were designed and targeted

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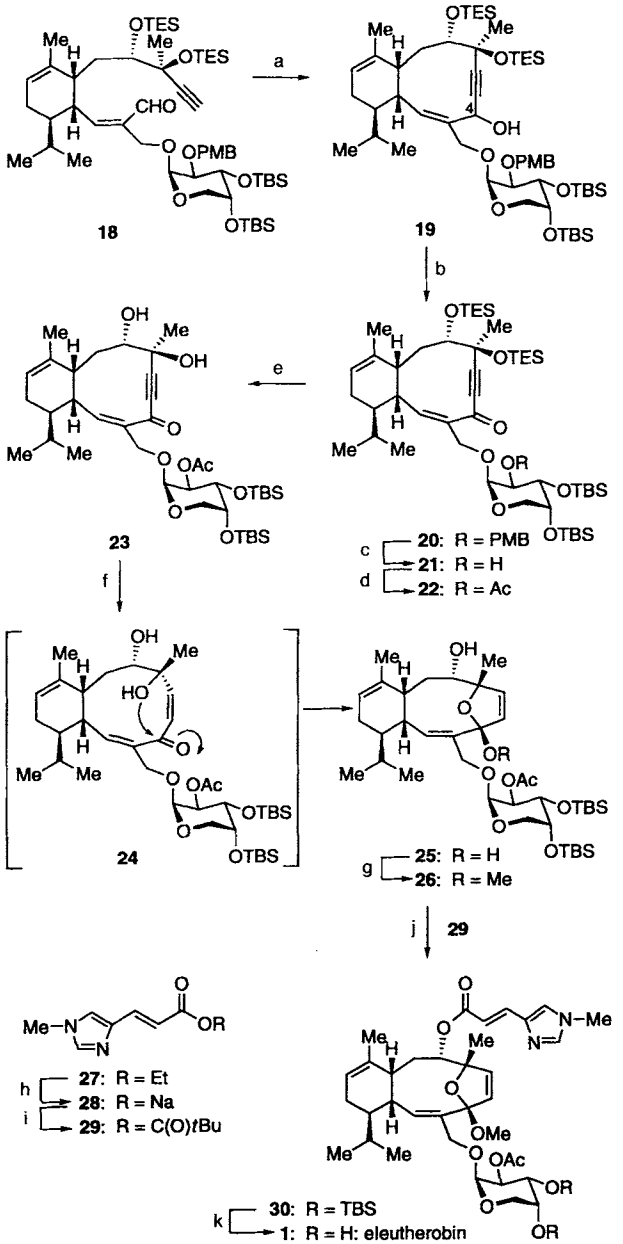
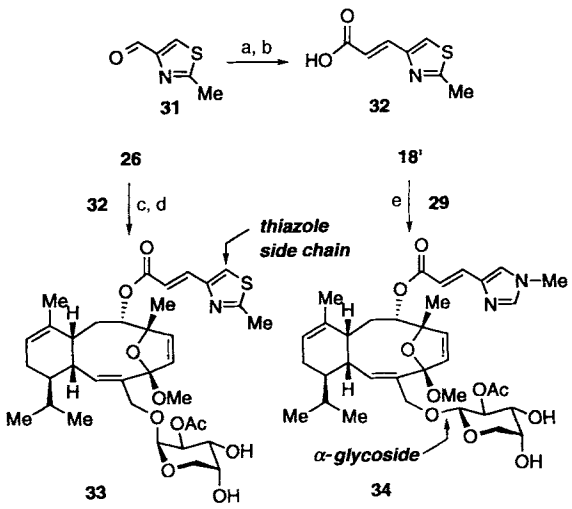


Scheme 2. Total synthesis of eleutherobin (**1**): a) LiHMDS (2.0 equiv), THF, −20 °C, 10 min; b) Dess-Martin periodinane (2.0 equiv), $NaHCO_3$ (10.0 equiv), pyridine (10.0 equiv), CH_2Cl_2, 0 °C, 20 min, 80% for two steps; c) DDQ (2.0 equiv), CH_2Cl_2:H_2O (19:1), 25 °C, 2 h, 90%; d) Ac_2O (10.0 equiv), Et_3N (10.0 equiv), 4-DMAP (3.0 equiv), 25 °C, 1.5 h, 90%; e) $Et_3N \cdot 3HF$, THF (1:5), 0 → 25 °C, 3 h, 86%; f) Lindlar's catalyst (0.5 equiv), H_2, toluene, 25 °C, 30 min; g) PPTS (0.5 equiv), MeOH, 25 °C, 10 min, 78% for two steps; h) NaOH (1.05 equiv), THF:H_2O, 1:1, 25 °C, 8 h, 100%; i) *t*BuC(O)Cl (1.1 equiv), THF, 25 °C, 5 h; j) **29** (5.0 equiv), Et_3N (10.0 equiv), 4-DMAP (2.0 equiv), CH_2Cl_2, 25 °C, 30 h, 75%; k) TBAF (3.0 equiv), THF, 25 °C, 4 h, 88%. LiHMDS = lithium hexamethyldisilazide, DDQ = 2,3-dichloro-5,6-dicyano-1,4-benzoquinone, Ac = acetyl, 4-DMAP = 4-dimethylaminopyridine, PPTS = pyridinium *p*-toluenesulfonate, TBAF = tetra-*n*-butylammonium fluoride.

for synthesis (Scheme 3). The epothilone-inspired analogue **33**, which carries a thiazole-containing side chain instead of the *N*(6′)-methylurocanic acid residue, was constructed by esterification of alcohol **26** (DCC, 4-DMAP, 70% yield) with the corresponding carboxylic acid (**32**, prepared from aldehyde **31**[24] as shown in Scheme 3), followed by TBAF-induced desilylation (85% yield). The α-glycoside analogue of eleutherobin, compound **34**, was constructed from the α-glycoside **18′** (Scheme 3)



Scheme 3. Structure and synthesis of eleutherobin analogues **33** and **34**: a) $Ph_3P{=}CHCO_2Me$ (1.5 equiv), benzene, 80 °C, 85%; b) LiOH (3.0 equiv), THF:H_2O (1:1), 97%; c) DCC (1.8 equiv), 4-DMAP (0.5 equiv), **32** (2.0 equiv), CH_2Cl_2, 25 °C, 6 h, 70%; d) TBAF (5.0 equiv, 1 M in THF), THF, 25 °C, 3 h, 85% e) see legend to Scheme 2. DCC = 1,3-dicyclohexylcarbodiimide, 4-DMAP = 4-dimethylaminopyridine, TBAF = tetra-*n*-butylammonium fluoride.

by the same sequence and in similar yields as described for eleutherobin (**1**).

In the filtration-colorimetric tubulin polymerization assay[5] using MAP-rich tubulin, synthetic eleutherobin (**1**) and its analogues **33** and **34** exhibited activity comparable to that of Taxol (**2**) (**2**: 49%; **1**: 70%; **33**: 56%; **34**: 54%). The data for eleutherobin (**1**) are consistent with those obtained with the natural substance (**1**),[2,25] whereas those for **33** and **34** establish the first important and path-pointing structure–activity relationships within the eleutherobin family.

The described chemistry opens a pathway for a practical synthesis of eleutherobin (**1**) and its analogues. Furthermore, the reported chemical synthesis allowed the assignment of the absolute stereochemistry of the natural product, and the preparation of its first biologically active analogues (**33** and **34**). Applications to the large-scale production of eleutherobin (**1**) and the generation of combinatorial libraries of this and related structures for chemical biology studies are now possible.

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