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Synthesis and Biological Evaluation of Neopeltolide and Analogs

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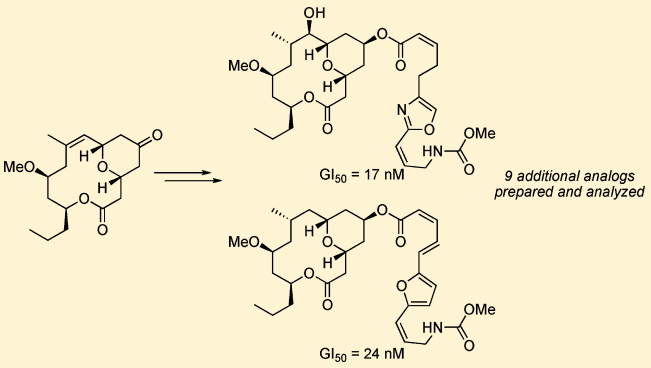
Supporting Information

ABSTRACT: The synthesis of neopeltolide analogues that contain variations in the oxazole-containing side chain and in the macrolide core are reported along with the GI_{50} values for these compounds against MCF-7, HCT-116, and p53 knockout HCT-116 cell lines. Although biological activity is sensitive to changes in the macrocycle and the side chain, several analogues displayed GI_{50} values of <25 nM. Neopeltolide and several of the more potent analogues were significantly less potent against p53 knockout cells, suggesting that p53 plays an auxiliary role in the activity of these compounds.



INTRODUCTION

The isolation of neopeltolide (**1**) from the *Daedalopelta* sponge of the Neopeltidae family off the Jamaican coast[1] quickly sparked a significant research effort[2] that was based on the observation of extremely potent cytotoxic and antifungal activity for a compound of moderate structural complexity (Figure 1). Wright and co-workers reported[1] IC_{50} values of 0.56, 1.2, and 5.1 nM against P388 murine leukemia, A-549 human lung adenosarcoma, and NCI-ADR-RES human ovarian sarcoma cell lines, respectively. Cytostatic activity was postulated for two cell lines that contain p53 mutations. Flow cytometry experiments showed that neopeltolide arrests the cell cycle at the G1 stage. Neopeltolide also showed an MIC (minimum inhibitory concentration) of approximately 1 μM against the pathogenic fungus *Candida albicans*. The correct atomic connectivity for **1** was established by NMR analysis[1] and the absolute and relative stereochemical assignments were determined unambiguously through total synthesis.[3] Several total, formal, and partial syntheses followed the complete structural determination.[4]

Kozmin and co-workers showed[4d] that neopeltolide suppresses ATP synthesis through cytochrome bc_1 inhibition. This activity is also exhibited by the structurally similar but more complex macrolide leucascandrolide A (**2**).[5] Synthetic studies have led to the preparation and biological analysis of several analogues.[6] These studies showed that altering the stereochemical orientations around the macrocycle causes a moderate to large drop in biological activity.[6a−c] Fuwa and co-workers demonstrated[6c] that the C9 methyl and the C11 methoxy groups can be deleted with minimal consequence to biological activity. These observations are significant because shorter synthetic sequences are employed to access the simplified structures compared to the natural product. Replacing the oxazole-containing side chain with commercially available carboxylates such as benzoate or octanoate abrogates activity.[6b] Maier and co-workers reported[6a] that changes to some of the alkenes in the side chain were tolerated and that incorporating an *E*-alkene between C4′ and C5′ provided an approximately 40% increase in potency against one cell line and equivalent potency against two other cell lines. The cytotoxicity of several analogues correlated to their ability to inhibit NADH oxidation in submitochondrial bovine heart particles,[6a] in accord with the hypothesis of biological activity arising from electron transport disruption. Neither the macrocycle or the oxazole subunit showed biological activity, indicating that both components are necessary to effect cytotoxicity. Scheidt, Crews, and co-workers observed cell line-specific cytotoxicity,[6b] signifying that neopeltolide does not act as a general poison.

Prior efforts at modifying the macrocyclic subunit proceeded through pathways in which the point of diversification was introduced at an early stage in the sequence and focused on the synthesis of stereoisomers or structurally simplified analogues. Structural variations of the side chain have been directed toward dramatic simplifications or toward complex acids that require a preparative effort that matches the natural subunit. The objective of our neopeltolide analogue program is to employ a late stage diversification strategy to introduce functional groups to the macrolactone core that enhance hydrophilicity and potentially provide a handle to append to a drug delivery agent and to design side chain analogues that are more accessible than the natural side chain while retaining key elements to effect a potent biological response.

Our synthesis of neopeltolide[4g] proceeded through the DDQ-mediated cyclization[7] of **3** to **4** (Scheme 1). The $\Delta^{8,9}$ alkene group that is required for oxidative carbocation

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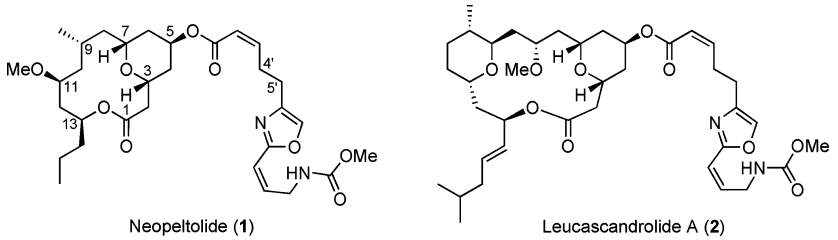
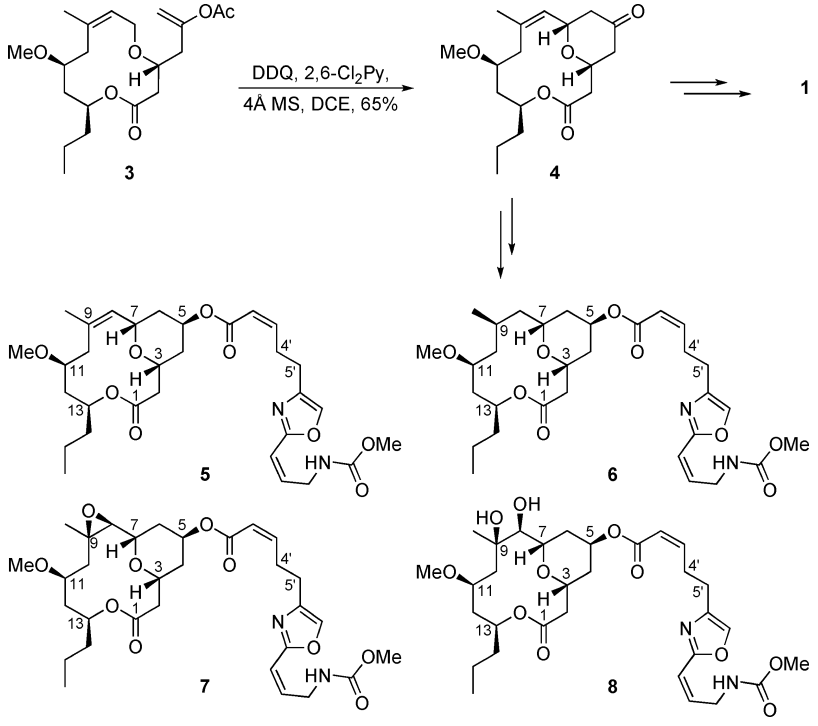


Figure 1. Natural product cytochrome bc_1 inhibitors.

Scheme 1. Late-Stage Diversification for Neopeltolide Analogue Synthesis



formation can be reduced by stereoselective hydrogenation en route to the natural product and can serve as a site for late-stage diverted total synthesis[8] to produce analogues. Given that the potency of the natural product is already sufficiently high, our objectives were directed toward preparing analogues that are easier to prepare, that offer improved physical properties, or that contain a reactive site on the hydrophobic macrocycle for prodrug development. Alkene functionalization allowed the facile synthesis of several analogues from **4**, including 8,9-dehydroneopeltolide (**5**), 9-*epi*-neopeltolide (**6**), 8,9-epoxyneopeltolide (**7**), and 8,9-dihydroxyneopeltolide (**8**).[6d] The brevity of our approach has also allowed us to accrue sufficient quantities of **4** to prepare analogues with alternate side chains that can be prepared more efficiently than the oxazole-containing chain in the natural product. In this paper, we report the synthesis of 8-hydroxyneopeltolide and several side-chain analogues of neopeltolide. The cytotoxic activity of these compounds against MCF7, HCT-116, and a p53 knockout variation of HCT-116 cells is also disclosed.

RESULTS AND DISCUSSION

Hydroboration Studies. The successful formation of diol **8**[6d] led us to consider the preparation of a monohydroxy derivative through hydroboration (Scheme 2). Exposing **9**, prepared through a stereoselective $NaBH_4$-mediated reduction of **4**, to BH_3·THF followed by NaOOH led to diastereomeric alcohols **10** and **11** in an approximately 1:1 ratio. The stereochemical assignments for these compounds were confirmed by NOESY experiments.[9] This was the first alkene functionalization reaction on **9**, which has been shown by crystallography to exist in a cup-shaped conformation that presents the *Re*-face of the alkene for reaction,[4g] whereby no stereocontrol was observed. Previous reactions have employed sterically more demanding alkene functionalization reagents, leading us to attribute this result to the sterically undemanding nature of borane rather than on any conformational mobility in the lactone.[10] Commonly employed bulkier reagents such as thexylborane and dicyclohexylborane were unreactive toward the alkene, but diethylborane[11] reacted with **9** efficiently to form **10** as a single stereoisomer. Coupling **10** and **11** with acid **12**[12] proceeded under Mitsunobu conditions[13] with high regiocontrol at the less hindered C5 center to provide analogues **13** and **14**.

Amide Analogue Synthesis. Converting the ester linkage between the macrolactone and the side chain to an amide linkage provides an additional opportunity for enhancing the polarity of the structure while increasing the expected in vivo stability. To prepare the amide-linked analogue (Scheme 3) we converted **15**, available in two steps from **3**,[6d] to a mesylate under standard conditions and effected a displacement with

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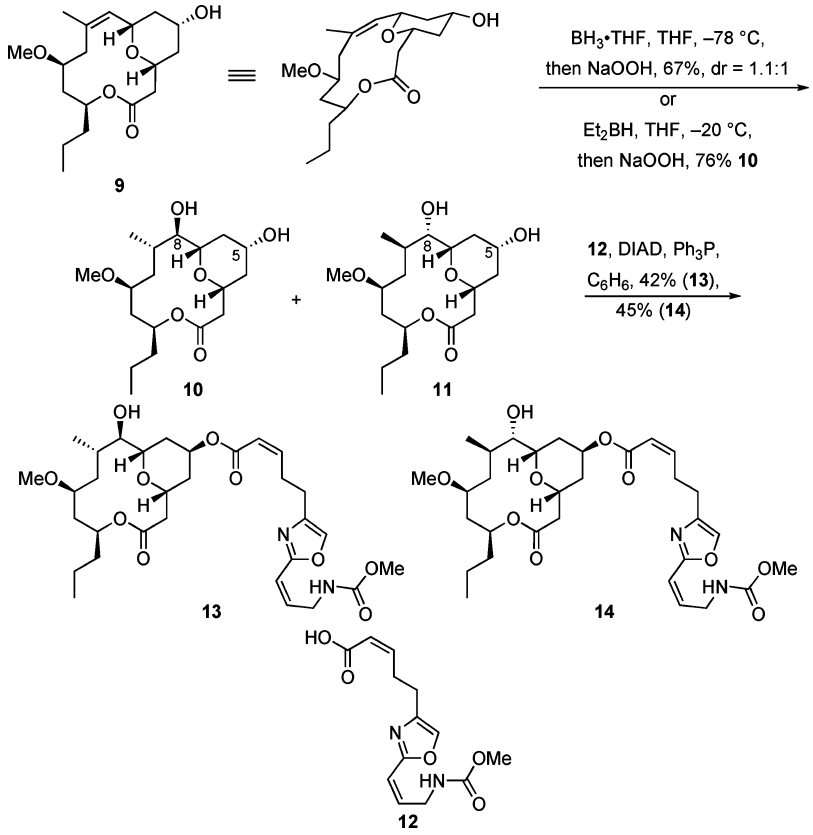
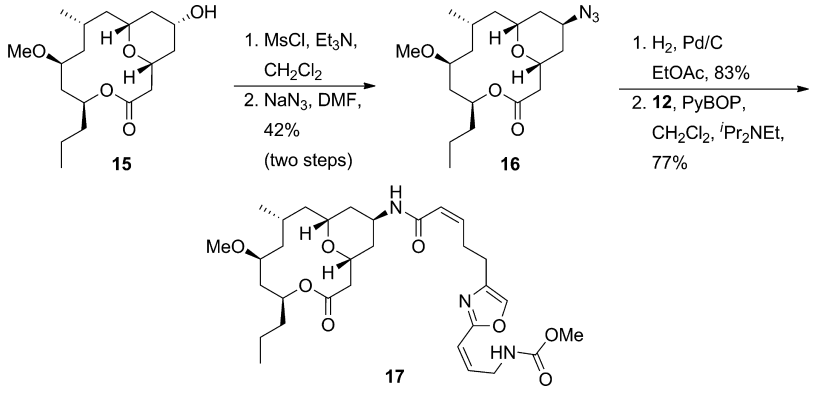
Scheme 2. Synthesis of 8-Hydroxyneopeltolide



Scheme 3. Synthesis of Amide-Linked Neopeltolide



NaN_3 to yield **16**. Reduction of **16** by catalytic hydrogenation provided the amine, which coupled smoothly to **12** to yield amide **17**.

Preparation of Side-Chain Analogues. Many approaches to the neopeltolide/leucascandrolide side chain have been reported,[12] but all require several steps due to the difficulties in preparing the oxazole subunit and the appended *Z*-allylic carbamate. A certain degree of structural complexity in this subunit is required for biological activity, as evidenced by the studies in which the side chain was replaced by benzoate and octanoate groups. This led us to consider whether side chains that contain other aromatic groups or that lacked the carbamate group could show comparable biological activity while being more synthetically accessible.

This study commenced with the synthesis of an analogue in which the oxazole is replaced by a *meta*-substituted benzene ring. The route (Scheme 4) began by the esterification of *m*-bromohydrocinnamic acid (**18**) followed by a Sonogashira coupling[14] with the methyl carbamate of propargyl amine (**19**) to yield **20**. Selective *cis*-reduction of the alkyne was accomplished most effectively with H_2 and P2-nickel,[15] partial reduction of the ester group was achieved with DIBAL-H, and chain extension through the Still–Gennari protocol provided ester **21**.[16] Ester hydrolysis and Mitsunobu coupling to **15** yielded phenyl analogue **22**.

A pyridine-containing side chain was prepared (Scheme 5) from 6-bromo-2-pyridinecarboxaldehyde (**23**) through a variation of the scheme that was developed for the benzene-containing analogue. Exposing **23** to standard Horner–Wadsworth–Emmons conditions[17] provided the expected α,β-unsaturated ester. The alkene group was reduced with $NaBH_4$ and CuCl[18] to yield **24**. These conditions were required to

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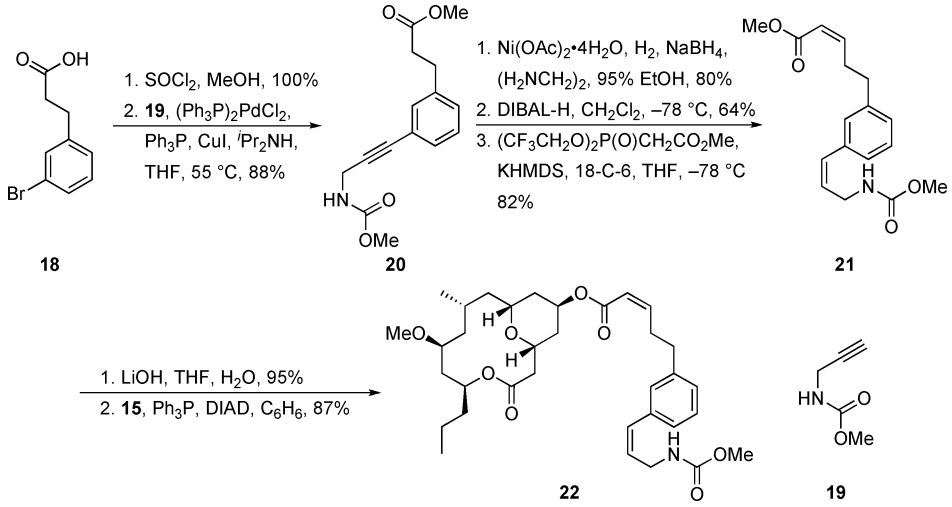
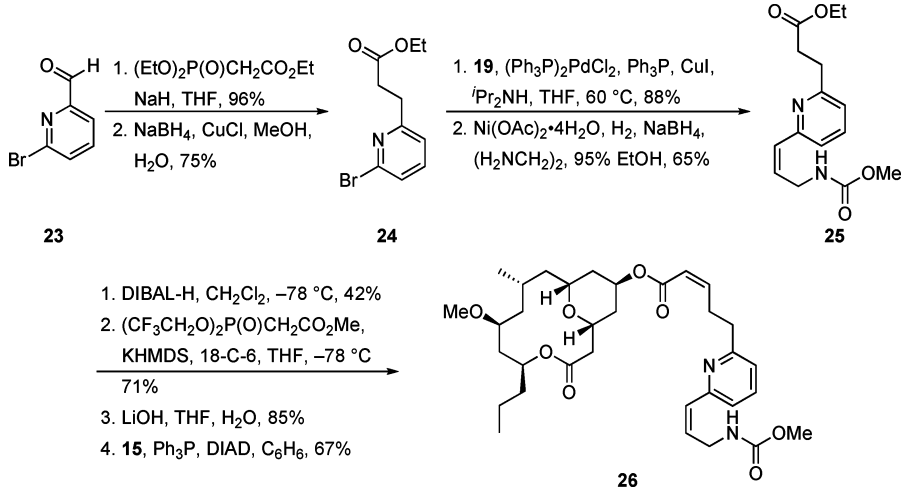
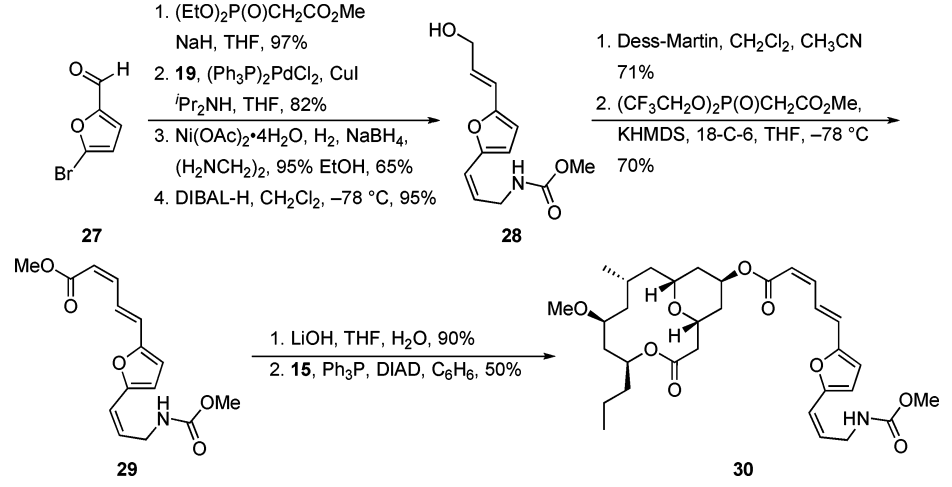
Scheme 4. Synthesis of a Benzene-Containing Side Chain Analogue



Scheme 5. Synthesis of a Pyridine-Containing Analogue



Scheme 6. Synthesis of a Furan-Containing Analogue



circumvent the loss of the bromide group that was observed in catalytic hydrogenation reactions. Coupling **24** with **19** under Sonogashira conditions followed by partial alkyne reduction provided ester **25**. Partial ester reduction, Still–Gennari coupling, ester hydrolysis, and Mitsunobu esterification with **15** yielded analogue **26**.

We selected furan to be a suitable five-membered ring replacement for the oxazole group. The synthesis of this analogue

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Scheme 7. Synthesis of a Des-carbamate Analogue

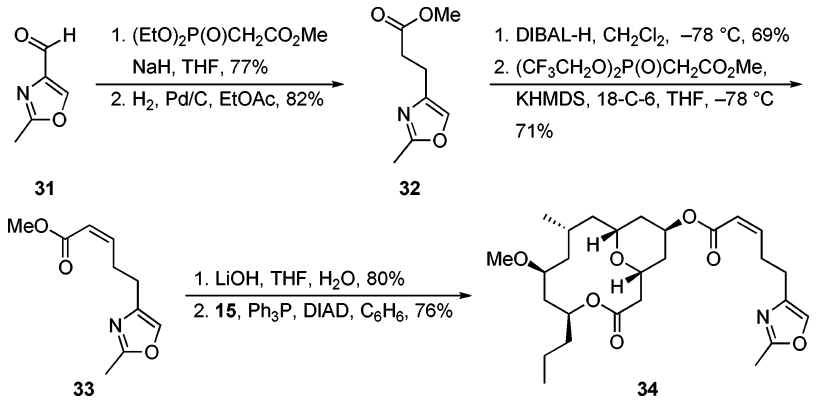

Table 1. GI_{50} Values of Neopeltolide and Analogues

entry	compound	description	MCF-7 GI_{50} (nM)	HCT116 GI_{50} (nM)	HCT116-p53KO GI_{50} (nM)
1	**1**	neopeltolide	6960 ± 260	0.77 ± 0.02	9.9 ± 0.4
2	**5**	8,9-dehydroneopeltolide	30,000 ± 150	5.41 ± 0.79	49.4 ± 0.9
3	**6**	9-*epi*-neopeltolide	1180 ± 100	6.62 ± 0.34	49.8 ± 0.8
4	**7**	8,9-epoxyneopeltolide	>50000	23.8 ± 0.2	47.9 ± 0.2
5	**8**	8,9-dihydroxyneopeltolide	2770 ± 30	4310 ± 40	4290 ± 210
6	**13**	8(*R*)-hydroxyneopeltolide	43610 ± 690	17 ± 1	13 ± 0.1
7	**14**	8(*S*)-hydroxy-9-*epi*-neopeltolide	>50000	48 ± 0.2	49 ± 0.2
8	**17**	amide-linked side chain	>50000	66 ± 0.9	68 ± 0.1
9	**22**	phenyl-containing side chain	>50000	100 ± 8	72 ± 0.3
10	**26**	pyridine-containing side chain	4480 ± 140	2940 ± 40	2260 ± 4
11	**30**	furan-containing side chain	34750 ± 500	24.2 ± 0.1	52.4 ± 0.3
12	**34**	des-carbamoyl side chain	>50000	730 ± 10	550 ± 3
13		paclitaxel	2.8 ± 0.1	4 ± 0.5	6.8 ± 0.01

(Scheme 6) commenced with a Horner–Wadsworth–Emmons extension of 5-bromofurfural (**27**) followed by Sonogashira coupling with **19**. All attempts at selective alkene reduction failed, with alkyne reduction being a significant problem. Reductive debromination was observed when alkene reduction was attempted prior to the Sonogashira reaction. We decided to continue the synthesis with the alkene intact based on Maier's observation[6a] that unsaturation at the 4′-5′ site does not have a negative impact on potency. Alcohol **28** was accessed through a sequence of alkyne partial reduction and ester reduction. Partial ester reduction was not possible on the unsaturated ester. The completion of the synthesis of analogue **30** followed the sequence that was deveoped for the benzene- and pyridine-containing analogues.

The role of the carbamate group was studied by preparing (Scheme 7) an analogue that lacks this group. Commercially available oxazole **31** was converted to ester **32** through a two step sequence. This aldehyde was efficiently converted to analogue **34** by following the previously developed routes.

Biological Results. The potency of these compounds was measured in cell viability studies using MCF-7 breast cancer cells, HCT-116 colon cancer cells, and a p53 knockout variant of the HCT-116 cell line. These cell lines were selected based on our desire to test the specificity of these compounds and to probe the role of p53 in the biological response. Results were obtained through the MTS (3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl-2*H*-tetrazolium) dye reduction assay with PMS (phenozine methosulfate) as an electron acceptor.[19] The assays were conducted over a 3-day incubation period at 37 °C and were run in triplicate or quintuplicate. Growth inhibition (GI) was calculated as defined by the National Cancer Institute ($GI = 100(T - T_0)/(C - T_0)$, where T_0 = cell density at time zero, T = cell density after 72 h with analogue, and C = cell density at 72 h with vehicle). Paclitaxel was used as a positive control to validate the quality of the experimental results. The results are shown in Table 1.

These results showed some very interesting trends. The lack of potency toward MCF-7 cells confirmed that neopeltolide and analogues are not general cell poisons and show some cell line selectivity. While MCF-7 cells have previously been shown to be susceptible to the actions of neopeltolide,[6b] this cell line is known to be heterogeneous, and therefore, variations in biological responses are not uncommon.[20] While no compounds showed enhanced potency compared to neopeltolide, several highly potent agents were identified. Modest changes to the 8- and 9-positions are reasonably well tolerated, although diol analogue **8** showed poor potency. Retaining the alkene at this site led to the greatest potency and removed a step from the synthetic sequence. Introducing a hydroxyl group at C8 provided a reasonably potent analogue that is more polar and contains a handle for the formation of prodrugs.[21] Changes in the structure of the side chain are tolerated far less than changes to the lactone core. The only reasonably potent analogue is **30**, in which a furan serves as a replacement for the oxazole. The lack of potency for pyridyl-containing analogue **26** was surprising in consideration of the basic nitrogen mapping onto the nitrogen atom of the oxazole and suggests that biological responses are quite sensitive to either protonation or to the precise spatial orientation of the alkenyl groups on the oxazole. The carbamate group proved to be essential for potency.

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The results against the p53 knockout cell line provided useful information regarding the growth inhibition mechanism. Neopeltolide and the most potent analogues were less potent against the p53 knockout cells, indicating that p53 plays a supporting role in the biological responses of these compounds. Increasing the polarity in the lactone subunit diminishes the differences in cellular responses, suggesting that p53 plays no role in cellular responses to these compounds. The most potent compound among this group is alcohol **13**. The potency of this compound for the p53 knockout cells is nearly equivalent to the potency of neopeltolide.

■ CONCLUSIONS

We have prepared neopeltolide and several analogues through late-stage alkene functionalization and side-chain variation. These compounds were tested for growth inhibition against MCF-7, HCT-116, and p53 knockout HCT-116 cell lines. The assays showed that (1) neopeltolide and analogues are not general cytotoxins as demonstrated by notable cell line selectivity, (2) changes to the 8- and 9-centers on neopeltolide are usually not highly detrimental to biological potency though no alteration resulted in increased potency, (3) retaining the alkene group that is required for the oxidative cyclization leads to a potent analogue that can be prepared in one fewer step than the natural product, while alkene hydroboration yields an active analogue with increased polarity, (4) variations to the side chain generally lead to significantly diminished potency, with the only active analogue resulting from the replacement of the oxazole group by a furan group, and (5) neopeltolide and the potent analogues are more active toward cells that contain p53, indicating that p53 plays an auxiliary role in biological responses to these compounds. This study illustrates several manners in which the structure of neopeltolide can and cannot be altered in efforts to retain potency while altering physical properties or accessibility and demonstrates the powerful role that synthesis can play in understanding the structure−activity relationships of medicinally interesting natural products.

■ EXPERIMENTAL SECTION

General Procedures. Proton (^{1}H NMR) and carbon (^{13}C NMR) nuclear magnetic resonance spectra were recorded at 300, 400, or 500 MHz and 75, 100, or 125 MHz, respectively. The chemical shifts are given in parts per million (ppm) on the delta (δ) scale. Tetramethylsilane (TMS) or the solvent peak was used as a reference value, for ^{1}H NMR: TMS (in $CDCl_3$) = 0.00 ppm, CD_3OD =3.31, for ^{13}C NMR: TMS (in $CDCl_3$) = 0.00, CD_3OD = 49.00. Data are reported as follows: (s = singlet; d = doublet; t = triplet; q = quartet; dd = doublet of doublets; dt = doublet of triplets; br = broad). Samples for IR were prepared as a thin film on a NaCl plate by dissolving the compound in CH_2Cl_2 and then evaporating the CH_2Cl_2. Analytical TLC was performed on precoated (25 mm) silica gel 60F-254 plates. Visualization was done under UV (254 nm). Flash chromatography was done using 32−63 60 Å silica gel. Methylene chloride was distilled under N_2 from CaH_2. Reagent grade ethyl acetate, diethyl ether, pentane and hexanes (commercial mixture) were used as purchased for chromatography. Benzene was dried with 4 Å molecular sieves. THF was distilled from sodium. Other reagents were obtained from commercial sources without further purification. All reactions were performed in oven or flame-dried glassware with magnetic stirring unless otherwise noted.

Synthesis of Diol 10. A solution of BH_3·Me_2S (2 M in THF, 0.5 mL, 1.0 mmol) was added dropwise at rt to a solution of triethylborane (1 M in THF, 2 mL, 2.0 mmol). After 20 min, 0.78 mL (0.62 mmol) of this freshly prepared diethylborane solution was added dropwise into a solution of alkene **9** (8.3 mg, 0.026 mmol) in 0.2 mL of THF at −20 °C. The reaction was allowed to room temperature while stirring overnight. A 10% NaOH solution (0.86 mL) and a 30% aqueous H_2O_2 solution (0.34 mL) were successively added dropwise at 0 °C. After 3 h at rt, the resulting mixture was extracted with Et_2O, and the combined extracts were washed with brine, dried over $MgSO_4$, filtered, and concentrated under reduced pressure. The residue was purified by flash chromatography (EtOAc/CH_2Cl_2/MeOH 1:1:0.1) to yield **10** (6.4 mg, 71% yield): ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.80−4.88 (m, 1H), 3.69−3.88 (m, 2H), 3.33−3.39 (m, 1H), 3.32 (s, 3H), 3.12 (app t, J = 9.3 Hz, 1H), 2.59 (dd, J = 4.6, 13.6 Hz, 1H), 2.39 (dd, J = 9.8, 13.6 Hz, 1H), 2.22−2.27 (m, 1H), 1.97−2.06 (m, 2H), 1.73−1.83 (m, 2H), 1.48−1.62 (m, 4H), 1.19−1.37 (m, 6H), 0.97 (d, J = 7.0 Hz, 3H), 0.92 (t, J = 7.4 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.8, 79.2, 75.6, 72.1, 68.1, 62.8, 56.3, 42.2, 40.9, 38.6, 38.2, 37.8, 37.0, 34.7, 29.8, 19.0, 16.2, 13.9; IR (film) 3358, 2957, 2925, 2872, 1727, 1454, 1374, 1262, 1186, 1148, 1084, 1027, 799 cm^{-1}; HRMS (ESI) m/z calcd for $C_{18}H_{32}O_6Na$ $[M + Na]^+$ 367.2097, found 367.2084; $[\alpha]^{25}_D$ = +0.7 ($CHCl_3$, c = 0.58).

Synthesis of Diol 11. To a solution of **9** (11.5 mg, 0.035 mmol) in THF (0.4 mL) at −78 °C was added dropwise 1 M BH_3·THF solution (135 μL, 0.135 mmol), and then the reaction was allowed to room temperature overnight. A 10% NaOH solution (60 μL) and a 30% aqueous H_2O_2 solution (150 μL) were successively added dropwise at 0 °C. After 3 h at room temperature, the resulting mixture was extracted with Et_2O and the combined extracts were washed with brine, dried over $MgSO_4$, filtered, and concentrated under reduced pressure. The residue was purified by flash chromatography (5% MeOH in CH_2Cl_2) to afford 4.2 mg (35%) of **10** and 3.9 mg (32%) of **11**: ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.19−5.27 (m, 1H), 3.85−3.94 (m, 2H), 3.35−3.44 (m, 1H), 3.33 (s, 3H), 3.27−3.33 (m, 2H), 2.66 (dd, J = 3.8, 15.2 Hz, 1H), 2.50 (dd, J = 11.2, 15.4 Hz, 1H), 2.18 (s, 1H), 2.14 (dd, J = 3.5, 4.6 Hz, 1H), 2.00 (ddd, J = 2.4, 2.4, 12.4 Hz, 1H), 1.81−1.93 (m, 2H), 1.77 (dd, J = 3.4, 10.7 Hz, 1H), 1.45−1.68 (m, 5H), 1.15−1.45 (m, 4H), 1.08 (d, J = 7.0 Hz, 3H), 0.91 (t, J = 7.3 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.4, 80.0, 78.5, 72.9, 72.8, 67.9, 56.6, 41.9, 40.3, 39.3, 38.8, 37.2, 35.8, 33.8, 24.0, 18.8, 13.9; IR (film) 3400, 2956, 2922, 1727, 1457, 1366, 1263, 1153, 1089 cm^{-1}; HRMS (ESI) m/z calcd for $C_{18}H_{32}O_6Na$ $[M + Na]^+$ 367.2097, found 367.2089; $[\alpha]^{25}_D$ = +2.7 ($CHCl_3$, c = 0.26).

Synthesis of Amide Analogue 17. To a mixture of the macrocyclic alcohol **15** (11 mg, 0.031 mmol), triethylamine (48 μL, 0.34 mmol), and dichloromethane (0.5 mL) at 0 °C was added methanesulfonyl chloride (8.0 μL, 0.10 mmol). The reaction was stirred at 0 °C for 30 min, allowed to warm room temperature, and stirred for 4 h. The reaction mixture was cooled to 0 °C, treated with 1 N HCl, and extracted with dichloromethane (3×). The organic extracts were dried with $MgSO_4$, filtered, and concentrated under vacuum to afford the mesylate: ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.12−5.20 (m, 1H), 4.82 (ddd, J = 5.0, 11.3, 16.3 Hz, 1H), 3.80 (dddd, J = 2.2, 4.3, 11.2, 11.2 Hz, 1H), 3.52 (app t, J = 8.9 Hz, 1H), 3.31 (s, 3H), 3.23 (app t, J = 10.0 Hz, 1H), 3.03 (s, 3H), 2.65 (dd, J = 4.4, 14.5 Hz, 1H), 2.44 (dd, J = 10.7, 14.5 Hz, 1H), 2.12−2.19 (m, 1H), 2.03−2.08 (m, 1H), 1.84 (dd, J = 10.5, 13.4 Hz, 1H), 1.61−1.73 (m, 2H), 1.45−1.59 (m, 5H), 1.10−1.41 (m, 7H), 0.99 (d, J = 6.7 Hz, 3H), 0.91 (t, J = 7.2 Hz, 3H). This crude product was redissolved in DMF (1 mL), treated with sodium azide (16.9 mg, 0.26 mmol), and then stirred at 80 °C overnight. The reaction mixture was dispersed between water and diethyl ether, and the organic layer was concentrated under vacuum. Flash chromatography (30% ethyl acetate in pentane) afforded azide **16** (5 mg, 42% over two steps): ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.19 (ddd, J = 4.5, 10.0, 10.0 Hz, 1H), 4.02−4.09 (m, 2H), 3.54 (app t, J = 9.5 Hz, 2H), 3.30 (s, 3H), 2.61 (dd, J = 4.3, 14.5 Hz, 1H), 2.35 (dd, J = 10.8, 14.6 Hz, 1H), 1.84 (dd, J = 10.9, 14.7 Hz, 1H), 1.63−1.76 (m, 2H), 1.46−1.61 (m, 6H), 1.31−1.43 (m, 5H), 1.11−1.25 (m, 3H), 0.99 (d, J = 6.4 Hz, 3H), 0.92 (t, J = 7.3 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.6, 75.6, 75.3, 73.0, 69.4, 56.2, 44.0, 42.2, 40.1, 37.0, 35.9, 34.8, 31.3, 25.6, 19.0, 13.9; IR (film) 2956, 2922, 2871, 2100, 1731, 1460, 1438, 1382, 1272, 1244, 1197, 1165, 1086, 1032, 992, 797 cm^{-1}; HRMS (ESI) m/z calcd for $C_{18}H_{31}N_3O_4Na$ $[M + Na]^+$ 376.2212, found 376.2239; $[\alpha]^{25}_D$ = +39.1 ($CHCl_3$, c = 0.45).

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A mixture of **16** (5 mg, 0.013 mmol) and 10% Pd/C (5 mg) in ethanol (0.5 mL) was stirred under a hydrogen atmosphere for 5 h. The mixture was filtered through Celite and purified by flash chromatography (10% MeOH in dichloromethane with 1% ammonium hydroxide) to give 4 mg (83%) of the amine: ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.14−5.23 (m, 1H), 4.09−4.21 (m, 1H), 3.63 (m, 2H), 3.44 (app s, 1H), 3.31 (s, 3H), 2.57 (dd, J = 3.8, 14.7 Hz, 1H), 2.35 (dd, J = 10.8, 14.0 Hz, 1H), 2.17 (s, 1H), 1.86 (dd, J = 10.6, 14.6 Hz, 1H), 1.30−1.40 (m, 5H), 1.42−1.75 (m, 8H), 1.14 (t, J = 11.6 Hz, 2H), 0.98 (d, J = 6.1 Hz, 3H), 0.91 (t, J = 7.4 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 171.2, 75.6, 74.7, 72.8, 69.0, 56.2, 44.3, 42.6, 42.3, 40.2, 39.5, 38.3, 37.0, 31.4, 25.7, 19.9, 13.9; IR (film) 3372, 2956, 2923, 2869, 1728, 1460, 1439, 1382, 1341, 1275, 1196, 1157, 1088, 1029, 795 cm^{-1}; HRMS (ESI) m/z calcd for $C_{18}H_{33}NO_4Na$ $[M + Na]^+$ 350.2307, found 350.2320; $[\alpha]^{25}_D$ = +21.8 ($CHCl_3$, c = 0.38).

The macrolactone amine (4 mg, 0.012 mmol), the oxazole acid **12** (8 mg, 0.027 mmol), diisopropylethylamine (9 μL, 0.05 mmol), and CH_2Cl_2 (0.8 mL) were mixed and cooled to 0 °C. PyBOP (19 mg, 0.036 mmol) was added, and the mixture was allowed to warm room temperature overnight. The reaction mixture was then concentrated and purified by flash chromatography (40% pentane in ethyl acetate) to afford **17** (5 mg, 77%): ^{1}H NMR (300 MHz, CD_3OD) δ 7.67 (s, 1H), 6.30 (dt, J = 2.1, 11.9 Hz, 1H), 6.10 (dt, J = 7.1, 11.5 Hz, 1H), 6.06 (d, J = 11.9 Hz, 1H), 5.99 (dt, J = 1.4, 11.5 Hz, 1H), 5.16−5.25 (m, 1H), 4.33 (dd, J = 1.7, 5.8 Hz, 2H), 4.16−4.22 (m, 1H), 4.08 (dddd, J = 1.3, 1.3, 10.9, 10.9 Hz, 1H), 3.71 (app t, J = 8.8 Hz, 1H), 3.68 (s, 3H), 3.56 (app t, J = 9.7 Hz, 1H), 3.31 (s, 3H), 3.01 (dt, J = 7.4, 7.4 Hz, 2H), 2.71 (t, J = 7.4 Hz, 2H), 2.67−2.74 (m, 1H), 2.32 (dd, J = 10.9, 14.8 Hz, 1H), 1.91 (dd, J = 10.9, 14.8 Hz, 1H), 1.69−1.79 (m, 2H), 1.45−1.61 (m, 7H), 1.10−1.43 (m, 6H), 1.02 (d, J = 6.5 Hz, 3H), 0.96 (t, J = 7.3 Hz, 3H); ^{13}C NMR (75 MHz, CD_3OD) δ 173.2, 168.9, 161.9, 145.0, 142.6, 139.2, 136.1, 124.3, 116.0, 77.2, 77.1, 73.9, 56.5, 52.7, 45.5, 44.9, 43.6, 43.5, 41.2, 41.1, 38.1, 173.2, 168.9, 161.9, 145.0, 142.6, 139.2, 136.1, 124.3, 116.0, 77.2, 77.1, 73.9, 56.5, 52.7, 45.5, 44.9, 43.6, 43.5, 41.2, 41.1, 38.1, 37.4, 36.2, 32.7, 28.7, 26.7, 26.1, 20.1, 14.2; IR (film) 3413, 2922, 2851, 1725, 1711, 1660, 1631, 1550, 1533, 1462, 1377, 1260, 1086, 1018, 798 cm^{-1}; HRMS (ESI) m/z calcd for $C_{31}H_{47}N_3O_8Na$ $[M + Na]^+$ 612.3261, found 612.3264; $[\alpha]^{25}_D$ = +10.7 (MeOH, c = 0.53).

Synthesis of Ester 20. To a solution of 3-(3-bromophenyl)-propanoic acid (1.603 g, 7.0 mmol) in methanol (20 mL) was added dropwise $SOCl_2$ (1.03 mL, 14.2 mmol) at 0 °C. The reaction was allowed to room temperature and stirred overnight. Concentration at reduced temperature and flash chromatography (20% ethyl acetate in hexanes) afforded 1.727 g (quantitative yield) of the desired ester: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.35 (s, 1H), 7.34 (d, J = 7.4 Hz, 1H), 7.12−7.19 (m, 2H), 3.68 (s, 3H), 2.92 (t, J = 7.6 Hz, 2H), 2.62 (t, J = 7.9 Hz, 2H).

A flame-dried 50 mL round-bottom flask was charged with the methyl ester (122 mg, 0.50 mmol), $PdCl_2(PPh_3)_2$ (35 mg, 0.050 mmol), PPh_3 (26 mg, 0.10 mmol), CuI (9.5 mg, 0.050 mmol), distilled diisopropylamine (0.64 mL, 4.5 mmol), and distilled THF (2 mL). The reaction was degassed with argon for 15 min, and then methyl prop-2-ynylcarbamate **19** (124 mg, 1.1 mmol) was added dropwise with a syringe. The reaction mixture was heated to 55 °C overnight under argon. Flash chromatography with 40% ethyl acetate in hexanes gave 121 mg (88%) of the desired product: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.14−7.26 (m, 4H), 5.12 (brs, 1H), 4.16 (d, J = 4.9 Hz, 2H), 3.71 (s, 3H), 3.67 (s, 3H), 2.91 (t, J = 7.8 Hz, 2H), 2.61 (t, J = 7.7 Hz, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 173.3, 140.6, 131.4, 129.2, 128.7, 128.5, 128.1, 127.2, 126.7, 51.6, 35.6, 29.7; IR (film) 3428, 2064, 1597, 1531, 1447, 1397, 1310, 1182, 874 cm^{-1}; HRMS (ESI) m/z calcd for $C_{15}H_{17}NO_4Na$ $[M + Na]^+$ 298.1055, found 298.1047.

Synthesis of Benzene-Containing Side-Chain Ester 21. A 25 mL round-bottom flask was charged with $Ni(OAc)_2{\cdot}4H_2O$ (22 mg, 0.087 mmol) and 95% ethanol (1 mL), and a hydrogen atmosphere was applied. A solution of $NaBH_4$ in 95% ethanol (0.11 mL, 1 N with 0.1 N NaOH, 0.11 mmol) was added to the flask within 10 s. After 3 min, ethylenediamine (12 μL, 0.18 mmol) was added, followed by another 3 min of stirring. Alkyne **20** (55 mg, 0.20 mmol) was added. After 1.5 h, the reaction mixture was partitioned between diethyl ether and water. The organic phase was dried with sodium sulfate, filtered, and concentrated under vacuum. Flash chromatography with 50% ethyl acetate in hexanes gave 44 mg (80%) of the desired product: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.27 (t, J = 7.0 Hz, 1H), 7.04−7.11 (m, 3H), 6.54 (d, J = 11.6 Hz, 1H), 5.67 (dt, J = 6.6, 11.5 Hz, 1H), 4.86 (brs, 1H), 4.06 (t, J = 5.4 Hz, 2H), 3.68 (s, 3H), 3.67 (s, 3H), 2.95 (t, J = 7.8 Hz, 2H), 2.64 (t, J = 7.5 Hz, 2H).

A 25 mL round-bottom flask was charged the alkynyl ester (44 mg, 0.16 mmol) and dichloromethane (3 mL). The mixture was cooled to −78 °C, and a 1 M DIBAL-H (0.21 mL, 0.21 mmol) solution in hexanes was added dropwise under nitrogen. After 1 h of stirring at −78 °C, the reaction was quenched with saturated ammonium chloride aqueous solution. Flash chromatography with 40% ethyl acetate in hexanes gave 25 mg (64%) of the desired product: ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.82 (s, 1H), 7.26 (t, J = 4.0 Hz, 1H), 7.02−7.11 (m, 3H), 6.54 (d, J = 11.6 Hz, 1H), 5.67 (dt, J = 6.6, 11.6 Hz, 1H), 4.81 (brs, 1H), 4.07 (t, J = 5.3 Hz, 2H), 3.68 (s, 3H), 2.96 (t, J = 7.4 Hz, 2H), 2.79 (t, J = 7.6 Hz, 2H).

A 25 mL round-bottom flask was charged methyl bis-(trifluoroethyl)phosphonoacetate (27 μL, 0.13 mmol), 18-crown-6 (102 mg, 0.39 mmol), and THF (1 mL). The mixture was cooled to −78 °C, and KHMDS (19 mg, 0.095 mmol) in 2 mL of THF was added dropwise under nitrogen. After 30 min of stirring, the aldehyde (17 mg, 0.068 mmol) in 2 mL of THF was added dropwise, followed by 4 h of stirring at −78 °C. The reaction was quenched with saturated NH_4Cl aqueous solution and extracted with ethyl acetate. Flash chromatography with 30% ethyl acetate in hexanes gave 17 mg (82%) of the desired product: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.27 (t, J = 7.6 Hz, 1H), 7.12 (d, J = 7.7 Hz, 1H), 7.07 (s, 1H), 7.06 (d, J = 8.1 Hz, 1H), 6.54 (d, J = 11.6 Hz, 1H), 6.26 (dt, J = 7.5, 11.5 Hz, 1H), 5.80 (dt, J = 1.6, 11.5 Hz, 1H), 5.67 (dt, J = 6.7, 11.8 Hz, 1H), 4.84 (brs, 1H), 4.08 (t, J = 5.2 Hz, 2H), 3.70 (s, 3H), 3.68 (s, 3H), 2.99 (td, J = 1.3, 7.6 Hz, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 166.7, 149.2, 141.2, 136.4, 131.5, 128.9, 128.4, 127.4, 126.5, 120.0, 52.2, 51.1, 39.3, 35.0, 30.4; IR (film) 3399, 1719, 1643, 1570, 1453, 1251, 1147, 1071, 1023, 819 cm^{-1}; HRMS (ESI) m/z calcd for $C_{17}H_{21}NO_4Na$ $[M + Na]^+$ 326.1368, found 326.1360.

Synthesis of Bromopyridine 24. To a suspension of 60% sodium hydride (135 mg, 3.38 mmol) in THF (10 mL) under nitrogen was added $(EtO)_2P(O)CH_2CO_2Et$ (0.62 mL, 3.1 mmol) dropwise at 0 °C. After 30 min, 6-bromopicolinaldehyde (465 mg, 2.5 mmol) in 2 mL of THF was added, and the reaction mixture was allowed to room temperature. After 1 h the reaction mixture was partitioned between diethyl ether and water. The organic phase was dried, filtered, and concentrated under vacuum. Flash chromatography with 20% diethyl ether in hexanes gave 615 mg (96%) of the desired product: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.57 (t, J = 7.3 Hz, 1H), 7.53 (d, J = 15.5 Hz, 1H), 7.44 (d, J = 7.7 Hz, 1H), 7.46 (d, J = 7.5 Hz, 1H), 6.95 (d, J = 15.6 Hz, 1H), 4.22(q, J = 7.1 Hz, 2H), 1.33 (t, J = 7.1 Hz, 3H).

In a 25 mL round-bottom flask the unsaturated ester (595 mg, 2.32 mmol) and CuCl (241 mg, 2.43 mmol) were dissolved in 80% methanol (13.5 mL) at 0 °C under nitrogen. $NaBH_4$ (92 mg, 2.3 mmol) was added followed 1 h later by another portion of $NaBH_4$ (92 mg, 2.3 mmol). One hour later, a third portion of $NaBH_4$ (46 mg, 1.2 mmol) was added. After 30 min, the reaction mixture was partitioned between diethyl ether and water, and the organic phase was dried, filtered, and concentrated under vacuum. Flash chromatography with 20% diethyl ether in dichloromethane gave 448 mg (75%) of the desired product: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.45 (t, J = 7.6 Hz, 1H), 7.31 (d, J = 7.8 Hz, 1H), 7.16 (d, J = 7.4 Hz, 1H), 4.13 (q, J = 7.2 Hz, 2H), 3.08 (t, J = 7.4 Hz, 2H), 2.78 (t, J = 7.4 Hz, 2H), 1.24 (t, J = 7.2 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 172.4, 161.5, 141.3, 138.5, 125.5, 121.7, 60.2, 33.0, 32.3, 14.0; IR (film) 3213, 2051, 1536, 1435, 1410, 1347, 978, 863 cm^{-1}; HRMS (ESI) m/z calcd for $C_{10}H_{12}BrNO_2Na$ $[M + Na]^+$ 279.9949, found 279.9957.

Synthesis of Ester 25. A flame-dried 50 mL round-bottom flask was charged with **24** (194 mg, 0.75 mmol), $PdCl_2(PPh_3)_2$ (52 mg, 0.074 mmol), PPh_3 (39 mg, 0.15 mmol), CuI (14 mg, 0.076 mmol), distilled diisopropylamine (0.96 mL, 6.8 mmol), and distilled THF (3 mL).

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The reaction was degassed with argon for 15 min, and then **19** (186 mg, 1.65 mmol) was added dropwise. The reaction mixture was heated at 50 °C for 1.5 h under argon. Flash chromatography with 40% ethyl acetate in hexanes gave 206 mg (91%) of the desired product: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.56 (t, J = 7.8 Hz, 1H), 7.26 (d, J = 7.8 Hz, 1H), 7.16 (d, J = 7.8 Hz, 1H), 5.28 (brs, 1H), 4.24 (d, J = 5.7 Hz, 2H), 4.11 (q, J = 7.2 Hz, 2H), 3.71(s, 3H), 3.09 (t, J = 7.5 Hz, 2H), 2.78 (t, J = 7.5 Hz, 2H), 1.23 (t, J = 7.2 Hz, 3H).

A 25 mL round-bottom flask was charged with $Ni(OAc)_2{\cdot}4H_2O$ (31 mg, 0.12 mmol) and 95% ethanol (1 mL), and a hydrogen atmosphere was applied. A solution of $NaBH_4$ (0.15 mL, 1 N, 0.015 mmol) in 95% ethanol with 0.1 N NaOH was added to the flask within 10 s. After 3 min, ethylenediamine (17 μL, 0.25 mmol) was added, followed by another 3 min of stirring. The alkyne (90 mg, 0.31 mmol) was added. After 1 h, the reaction mixture was partitioned between diethyl ether and water. The organic phase was dried with sodium sulfate, filtered and concentrated. Flash chromatography with 40% ethyl acetate in hexanes gave 59 mg (65%) of the desired product: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.57 (t, J = 7.7 Hz, 1H), 7.05 (d, J = 6.9 Hz, 1H), 7.02 (d, J = 6.6 Hz, 1H), 6.46 (d, J = 11.7 Hz, 1H), 5.97 (dt, J = 6.9, 11.5 Hz, 1H), 5.97 (brs, 1H), 4.30 (t, J = 6.1 Hz, 2H), 4.15 (q, J = 7.1 Hz, 2H), 3.68 (s, 3H), 3.13 (t, J = 7.4 Hz, 2H), 2.82 (t, J = 7.3 Hz, 2H), 1.22 (t, J = 7.1 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 173.0, 159.3, 157.1, 154.9, 136.6, 133.0, 129.9, 122.1, 120.9, 60.3, 51.8, 39.2, 32.9, 32.7, 14.0; IR (film) 3422, 3201, 3144, 2047, 1600, 1553, 1370, 1324, 853 cm^{-1}; HRMS (ESI) m/z calcd for $C_{15}H_{20}N_2O_4Na$ $[M + Na]^+$ 315.1321, found 315.1311.

Synthesis of Allylic Alcohol 28. To a suspension of 60% sodium hydride (63 mg, 1.6 mmol) in THF (5 mL) was added $(EtO)_2P(O)$-CH_2CO_2Me (0.41 mL, 2.53 mmol) dropwise at 0 °C under nitrogen. One hour later, 5-bromofuran-2-carbaldehyde **27** (204 mg, 1.17 mmol) in 5 mL of THF was added dropwise, and the reaction mixture was allowed to warm rt over 0.5 h. The reaction mixture was partitioned between diethyl ether and water. The organic phase was dried, filtered, and concentrated under vacuum. Flash chromatography with 20% diethyl ether in hexanes gave 261 mg (97%) of the desired ester: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.32 (d, J = 15.7 Hz, 1H), 6.54 (d, J = 2.9 Hz, 1H), 6.40 (d, J = 3.0 Hz, 1H), 6.29 (d, J = 15.7 Hz, 1H), 3.78 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 166.9, 152.5, 129.7, 125.3, 116.6, 115.7, 114.0, 51.5.

To a flame-dried 25 mL round-bottom flask was charged the ester (310 mg, 1.34 mmol), $PdCl_2(PPh_3)_2$ (93 mg, 0.13 mmol), PPh_3 (69.8 mg, 0.266 mmol), CuI (25.8 mg, 0.136 mmol), distilled triethylamine (1.70 mL, 12.2 mmol), and distilled THF (5 mL). The reaction was degassed with argon for 15 min, and then methyl prop-2-ynylcarbamate **19** (310 mg, 2.75 mmol) was added dropwise. The reaction mixture was heated at 50 °C for 1.5 h under argon. Flash chromatography with 40% ethyl acetate in hexanes gave 289 mg (82%) of the coupled product: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.34 (d, J = 15.7 Hz, 1H), 6.59 (d, J = 3.5 Hz, 1H), 6.57 (d, J = 3.5 Hz, 1H), 6.33 (d, J = 15.7 Hz, 1H), 5.79 (brs, 1H), 4.25 (d, J = 5.6 Hz, 2H), 3.78 (s, 3H), 3.71 (s, 3H).

A 25 mL round-bottom flask was charged with $Ni(OAc)_2{\cdot}4H_2O$ (47 mg, 0.19 mmol) and 95% ethanol (1 mL), and a hydrogen atmosphere was applied. A solution of $NaBH_4$ (0.25 mL, 1 N, 0.015 mmol) in 95% ethanol with 0.1 N NaOH was added to the flask within 10 s. After 3 min, ethylenediamine (26 μL, 0.37 mmol) was added, followed by another 3 min of stirring. The coupled product (123 mg, 0.467 mmol) was added to the resulting black suspension followed by washing wth 95% EtOH. After 1.5 h, the reaction mixture was filtered through a pad of silica gel and concentrated under vacuum. Flash chromatography with 30% THF in hexanes gave 80 mg (65%) of the reduced product: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.40 (d, J = 15.7 Hz, 1H), 6.62 (d, J = 3.5 Hz, 1H), 6.35 (d, J = 3.5 Hz, 1H), 6.27 (d, J = 15.6, 1H), 6.22 (d, J = 10.4 Hz, 1H), 5.68 (dt, J = 6.4, 11.8 Hz, 1H), 5.11 (brs, 1H), 4.29 (t, J = 5.4 Hz, 2H), 3.79 (s, 3H), 3.70 (s, 3H).

The (*Z*)-alkene (60 mg, 0.23 mmol) in dichloromethane (3 mL) was cooled to −78 °C under nitrogen. DIBAL-H (1.34 mL, 1 M in hexanes) was added dropwise, followed by 1 h of stirring at −78 °C. The reaction was quenched with saturated ammonium chloride solution, extracted with ethyl acetate (3×), and concentrated under vacuum. Flash chromatography with 30% hexanes in ethyl acetate gave 51 mg (95%) of alcohol **28**: ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.44−6.12 (m, 5H), 5.51 (dd, J = 6.0, 11.7 Hz, 1H), 5.09 (br, 1H), 4.30 (br, 4H), 3.68 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 156.9, 154.9, 152.0, 129.1, 126.2, 123.6, 118.4, 116.0, 113.1, 59.8, 52.1, 42.0; IR (film) 3429, 3283, 1636, 1527, 1438, 1417, 1182, 966, 869 cm^{-1}; HRMS (ESI) m/z calcd for $C_{12}H_{15}NO_4Na$ $[M + Na]^+$ 260.0899, found 260.0908.

Synthesis of Furan-Containing Side-Chain Ester 29. Alcohol **28** (51 mg) was dissolved in CH_2Cl_2 (3.3 mL) and acetonitrile (1.2 mL) and cooled to 0 °C. Pyridine (28 μL, 0.35 mmol) was added, followed by the Dess−Martin periodinane (144 mg, 0.34 mmol). After 2 h of stirring at 0 °C, the reaction mixture was concentrated and purified with 40% ethyl acetate in hexanes to afford 35 mg (71%) of the desired aldehyde: ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.63 (d, J = 7.9 Hz, 1H), 7.20 (d, J = 15.6 Hz, 1H), 6.79 (d, J = 3.5 Hz, 1H), 6.55 (dd, J = 7.9, 15.6 Hz, 1H), 6.43 (d, J = 3.5 Hz, 1H), 6.26 (d, J = 11.8 Hz, 1H), 5.75 (dt, J = 6.3, 11.8 Hz, 1H), 5.07 (brs, 1H), 4.30 (t, J = 5.8 Hz, 2H), 3.71 (s, 3H).

A 25 mL round-bottom flask was charged with methyl bis-(trifluoroethyl)phosphonoacetate (86 μL, 0.41 mmol), 18-crown-6 (330 mg, 1.23 mmol), and THF (4 mL). The mixture was cooled to −78 °C, and KHMDS (61 mg, 0.29 mmol) in 2 mL of THF was added dropwise under nitrogen. After 30 min of stirring, the freshly prepared aldehyde (35 mg, 0.15 mmol) in 4 mL of THF was added dropwise, followed by 3 h of stirring at −78 °C. The reaction was quenched with saturated aqueous NH_4Cl solution, extracted with ethyl acetate, and concentrated under vacuum. Flash chromatography first with 30% ethyl acetate in pentane, and then 20% ethyl acetate in toluene gave 30 mg (70%) of the desired product **29**: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.93 (dd, J = 11.9, 15.5 Hz, 1H), 6.66 (dd, J = 11.6, 11.6 Hz, 1H), 6.56 (d, J = 15.5 Hz, 1H), 6.49 (d, J = 3.5 Hz, 1H), 6.32 (d, J = 3.5 Hz, 1H), 6.22 (d, J = 11.6 Hz, 1H), 5.69 (d, J = 11.2 Hz, 1H), 5.60−5.70 (m, 1H), 5.12 (brs, 1H), 4.29 (td, J = 1.5, 6.2 Hz, 2H), 3.75 (s, 3H), 3.69 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 166.9, 157.1, 153.3, 152.3, 143.8, 128.5, 127.1, 123.8, 118.1, 117.2, 113.6, 113.0, 52.1, 51.2, 40.0; IR (film) 3340, 2950, 1708, 1608, 1528, 1438, 1253, 1197, 1169, 1020, 785 cm^{-1}; HRMS (ESI) m/z calcd for $C_{15}H_{17}NO_5Na$ $[M + Na]^+$ 314.1004, found 314.1028.

Synthesis of Oxazole 32. To a suspension of 60% sodium hydride (63 mg, 1.3 mmol) in THF (5 mL) was added $(EtO)_2P(O)$-CH_2CO_2Me (0.41 mL, 2.5 mmol) dropwise at 0 °C under nitrogen. Thirty minutes later, 2-methyloxazole-4-carbaldehyde **31** (107 mg, 0.96 mmol) in 2 mL of THF was added dropwise, and the reaction mixture was kept at 0 °C for 1.5 h. The reaction mixture was partitioned between diethyl ether and water. The organic phase was dried, filtered, and concentrated under vacuum. Flash chromatography with 5% diethyl ether in hexanes gave 151 mg (93%) of the desired ester: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.69 (s, 1H), 7.47 (d, J = 15.6, 1H), 6.58 (dt, J = 2.6, 15.8 Hz, 1H), 3.78 (s, 3H), 2.49 (s, 1H).

The ester (71 mg, 0.42 mmol) was stirred under a hydrogen atmosphere in methanol (10 mL) with 10% Pd/C (71 mg) for 2 h. The resulting mixture was then filtered through Celite and purified by flash chromatography with 40% ethyl acetate in hexanes to give 59 mg (82%) of the desired product **32**: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.30 (t, J = 1.1 Hz, 1H), 3.68 (s, 3H), 2.81 (t, J = 7.5 Hz, 2H), 2.65 (td, J = 1.1, 7.1 Hz, 2H), 2.42 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 175.1, 160.3, 136.2, 128.5, 51.7, 32.4, 20.4, 14.4; IR (film) 3158, 1710, 1549, 1442, 1426, 1359, 1226, 1145, 841 cm^{-1}; HRMS (ESI) m/z calcd for $C_8H_{11}NO_3Na$ $[M + Na]^+$ 192.0637, found 192.0635.

Synthesis of Des-carbamyl Side-Chain Ester 33. A 25 mL round-bottom flask was charged with **32** (48 mg, 0.28 mmol) and dichloromethane (3 mL). The reaction was cooled to −78 °C, and a 1 M DIBAL-H solution in hexanes (0.42 mL, 0.42 mmol) was added dropwise under nitrogen. After 1.5 h of stirring at −78 °C, the reaction was quenched with saturated aqueous ammonium chloride solution. Flash chromatography with 40% ethyl acetate in hexanes gave 27 mg (69%) of the desired aldehyde: ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.82 (s, 1H), 7.29 (t, J = 5.2 Hz, 1H), 2.81 (app s, 4H), 2.42 (s, 1H).

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A 25 mL round-bottom flask was charged with methyl bis-(trifluoroethyl)phosphonoacetate (114 μL, 0.54 mmol), 18-crown-6 (430 mg, 1.63 mmol), and THF (5 mL). The mixture was cooled to −78 °C, and KHMDS (80 mg, 0.40 mmol) in 5 mL of THF was added dropwise under nitrogen. After 30 min of stirring, the aldehyde (26 mg, 0.19 mmol) in 5 mL of THF was added dropwise, followed by 5 h of stirring at −78 °C. The reaction was quenched with saturated aqueous NH_4Cl solution and extracted with ethyl acetate. Flash chromatography with 30% ethyl acetate in pentane gave 23 mg (71%) of the desired product **33**: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.29 (s, 1H), 6.26 (dt, J = 7.3, 11.5 Hz, 1H), 5.82 (dt, J = 1.7, 11.5 Hz, 1H), 3.71 (s, 3H), 2.98 (dtd, J = 1.6, 7.2, 7.2 Hz, 2H), 2.64 (dd, J = 6.9, 7.6 Hz, 2H), 2.42 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 166.7, 161.3, 149.1, 139.7, 133.9, 120.1, 51.1, 27.5, 25.6, 13.9; IR (film) 2921, 1722, 1646, 1581, 1439, 1201, 1177, 1096, 820 cm^{-1}; HRMS (ESI) m/z calcd for $C_{10}H_{13}NO_3Na$ $[M + Na]^+$ 218.0793, found 218.0786.

Representative Protocol for Side-Chain Ester Cleavage. The side- chain ester was dissolved in THF (~0.2 M) at rt. Aqueous LiOH (10 equiv) was added, and the reaction mixture was stirred at rt for 4 h. The reaction mixture was acidified with 2 N HCl at 0 °C to pH 3 and extracted with ethyl acetate (3×). The organic phase was dried with Na_2SO_4, filtered, and concentrated under vacuum. Flash chromatography with 5% methanol in dichloromethane gave the side chain acids.

Benzene-containing side-chain acid: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.26 (t, J = 7.8 Hz, 1H), 7.03−7.10 (m, 3H), 6.55 (d, J = 11.6 Hz, 1H), 6.34 (dt, J = 7.5, 11.3 Hz, 1H), 5.81 (d, J = 11.5 Hz, 1H), 5.64 (dt, J = 6.5, 11.8 Hz, 1H), 4.84 (brs, 1H), 4.09 (app s, 2H), 3.69 (s, 3H), 3.00 (dt, J = 7.5, 7.5 Hz, 2H), 2.77 (t, J = 7.6 Hz, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 169.9, 157.2, 150.5, 141.0, 136.4, 131.6, 128.8, 128.3, 128.1, 127.6, 126.6, 120.2, 52.4, 39.6, 34.9, 30.6; IR (film) 3336, 2924, 1700, 1641, 1531, 1435, 1260, 1194, 1152, 803 cm^{-1}; HRMS (ESI) m/z calcd for $C_{16}H_{19}NO_4Na$ $[M + Na]^+$ 312.1212, found 312.1215.

Furan-containing side chain acid: ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.01 (dd, J = 12.0, 15.4 Hz, 1H), 6.78 (t, J = 11.5 Hz, 1H), 6.60 (d, J = 15.5 Hz, 1H), 6.50 (d, J = 3.5 Hz, 1H), 6.33 (d, J = 3.3 Hz, 1H), 6.23 (d, J = 11.9 Hz, 1H), 5.73 (d, J = 11.2 Hz, 1H), 5.66 (dt, J = 6.4, 11.9 Hz, 1H), 5.12 (brs, 1H), 4.32 (app s, 1H), 3.70 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.7, 157.3, 153.5, 152.3, 145.1, 128.6, 127.1, 124.2, 117.9, 117.1, 113.8, 112.9, 52.2, 40.3; IR (film) 3452, 2941, 1701, 1524, 1253, 1189, 1173, 788 cm^{-1}; HRMS (ESI) m/z calcd for $C_{14}H_{15}NO_5Na$ $[M + Na]^+$ 300.0848, found 300.0859.

Des-carbamyl side-chain acid: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.31 (s, 1H), 6.25 (dt, J = 7.3, 11.1 Hz, 1H), 5.85 (d, J = 11.0 Hz, 1H), 2.97 (dt, J = 7.1, 7.1 Hz, 2H), 2.68 (t, J = 7.0 Hz, 2H), 2.45 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.0, 161.8, 148.6, 139.1, 134.1, 121.2, 27.5, 24.9, 13.7; IR (film) 3139, 2922, 2537, 1700, 1643, 1577, 1437, 1203, 1096, 1017, 935, 822 cm^{-1}; HRMS (EI) m/z calcd for $C_9H_{11}NO_3$ $[M^+]$ 181.0739, found 181.0737.

General Procedure for the Mitsunobu Coupling. A one-dram vial was charged with the macrocyclic alcohol (1 equiv), oxazolic acid (4 equiv), and PPh_3 (4 equiv), and benzene (~0.05 M alcohol concentration) was added . DIAD (4 equiv) was added to the reaction mixture. After an indicated period (see individual reactions for details), the mixture was concentrated under vacuum. The resulting residue was purified by flash column chromatography to afford the desired product.

Hydroxy Analogue 13. The general esterification procedure was followed with **10** (5.8 mg, 0.017 mmol), **12** (7.0 mg, 0.025 mmol), PPh_3 (8.8 mg, 0.034 mmol), and DIAD (6.7 μL, 0.034 mmol) in benzene. Two hours later, the reaction was quenched with 1 drop of water, concentrated under vacuum, and purified with flash chromatography (30% THF in pentane) to afford the desired product (4.5 mg, 42%): ^{1}H NMR (400 MHz, CD_3OD) δ 7.70 (s, 1H), 6.39 (dt, J = 7.7, 12.0 Hz, 1H), 6.31 (d, J = 11.9 Hz, 1H), 6.06 (dt, J = 6.1, 12.2 Hz, 1H), 5.90 (d, J = 10.6 Hz, 1H), 5.31 (aps, 1H), 4.33 (d, J = 5.6 Hz, 2H), 4.07 (dd, J = 6.8, 9.4 Hz, 1H), 3.75 (apt, J = 6.1 Hz, 1H), 3.67 (s, 3H), 3.49 (apt, J = 10.1 Hz, 1H), 3.39−3.43 (m, 1H), 3.33 (s, 3H), 3.19 (dd, J = 2.0, 8.5 Hz, 1H), 3.04 (dt, J = 7.4, 7.4 Hz, 2H), 2.74 (t, J = 7.4 Hz, 2H), 2.66 (dd, J = 4.2, 13.7 Hz, 1H), 2.30 (dd, J = 10.1, 13.6 Hz, 1H), 2.05 (apq, J = 12.7 Hz, 2H), 1.76−1.90 (m, 3H), 1.51−1.67 (m, 4H), 1.22−1.38 (m, 4H), 0.97 (d, J = 7.2 Hz, 3H), 0.94 (t, J = 7.3 Hz, 3H); ^{13}C NMR (100 MHz, CD_3OD) δ 173.1, 167.0, 162.0, 150.1, 142.4, 139.3, 136.1, 121.8, 116.1, 78.7, 77.9, 77.6, 71.1, 69.0, 68.9, 56.6, 52.7, 43.3, 41.2, 40.0, 39.7, 38.3, 36.7, 36.0, 33.8, 29.1, 26.5, 20.2, 17.0, 14.3; IR (film) 3366, 2958, 2924, 2873, 1714, 1520, 1461, 1417, 1264, 1182, 1154, 1091, 820 cm^{-1}; HRMS (ESI) m/z calcd for $C_{31}H_{46}N_2O_{10}Na$ $[M + Na]^+$ 629.3050, found 629.3055; $[\alpha]^{25}_D$ = +13.3 ($CHCl_3$, c = 0.36).

Hydroxy Analogue 14. The general esterification procedure was followed with **11** (2.6 mg, 0.0075 mmol), **12** (3.6 mg, 0.013 mmol), PPh_3 (4.4 mg, 0.017 mmol), and DIAD (3.4 μL, 0.017 mmol) in benzene (0.3 mL) After 2 h, the reaction was quenched with 1 drop of water, concentrated under vacuum, and purified with flash chromatography (30% THF in pentane) to afford the desired product (2.1 mg, 45%): ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.39 (s, 1H), 6.34 (dt, J = 7.3, 11.5 Hz, 1H), 6.29 (dt, J = 1.5, 11.6 Hz, 1H), 6.10 (dt, J = 6.3, 11.9 Hz, 1H), 4.89 (dt, J = 1.6, 11.4 Hz, 1H), 5.52 (brs, 1H), 5.20−5.28 (m, 2H), 4.32 (t, J = 5.8 Hz, 2H), 4.22 (apt, J = 14.9 Hz, 1H), 3.69 (s, 3H), 3.65 (dd, J = 1.7, 12.1 Hz, 1H), 3.79−3.42 (m, 1H), 3.32 (s, 3H), 3.21 (d, J = 9.7 Hz, 1H), 3.04 (dtd, J = 1.6, 7.5, 7.5 Hz, 2H), 2.72 (t, J = 7.1 Hz, 2H), 2.61 (dd, J = 3.8, 15.4 Hz, 1H), 2.42 (dd, J = 11.4, 15.4 Hz, 1H), 2.10−2.17 (m, 1H), 2.00 (ddd, J = 3.0, 12.1, 15.7 Hz, 1H), 1.80−1.88 (m, 2H), 1.60−1.69 (m, 2H), 1.41−1.56 (m, 4H), 1.25−1.38 (m, 4H), 1.07 (d, J = 7.1 Hz, 3H), 0.92 (t, J = 7.3 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.4, 165.3, 149.6, 136.4, 133.9, 133.1, 120.5, 116.7, 78.3, 72.9, 70.4, 67.5, 56.6, 53.4, 42.0, 39.3, 37.3, 35.7, 34.9, 33.7, 33.2, 31.9, 27.7, 25.6, 23.9, 22.7, 18.8, 13.9; IR (film) 3406, 2956, 2923, 2853, 1713, 1643, 1533, 1377, 1261, 1151, 1117, 798 cm^{-1}; HRMS (ESI) m/z calcd for $C_{31}H_{46}N_2O_{10}Na$ $[M + Na]^+$ 629.3050, found 629.3079; $[\alpha]^{25}_D$ = +6.7 ($CHCl_3$, c = 0.21).

Benzene-Containing Side-Chain Analogue 22. The general esterification procedure was followed by **15** (2.2 mg, 0.0067 mmol), the phenyl-containing side chain acid **74** (7.1 mg, 0.024 mmol), PPh_3 (7.0 mg, 0.027 mmol), and DIAD (5.4 μL, 0.027 mmol) in benzene (0.5 mL). The mixture was stirred for 10 min and then was purified by flash column chromatography (30% THF in hexanes) to yield 3.5 mg (87%) of the desired product: ^{1}H NMR (400 MHz, CD_3OD) δ 7.27 (t, J = 7.8 Hz, 1H), 7.13 (d, J = 5.8 Hz, 1H), 7.13 (s, 1H), 7.09 (d, J = 7.8 Hz, 1H), 6.52 (d, J = 11.6 Hz, 1H), 6.36 (dt, J = 7.6, 11.6 Hz, 1H), 5.85 (dt, J = 1.5, 11.6 Hz, 1H), 5.65 (dt, J = 6.5, 11.6 Hz, 1H), 5.14−5.20 (m, 2H), 4.02−4.07 (m, 1H), 3.99 (apd, J = 5.3, 2H), 3.66 (apt, J = 9.7 Hz, 1H), 3.64 (s, 3H), 3.54 (apt, J = 9.5 Hz, 1H), 3.27 (s, 3H), 3.00 (dt, J = 7.8, 7.8 Hz, 2H), 2.79 (t, J = 7.4 Hz, 2H), 2.67 (dd, J = 4.3, 14.8 Hz, 1H), 2.28 (dd, J = 11, 14.8 Hz, 1H), 1.75−1.89 (m, 3H), 1.61−1.73 (m, 2H), 1.44−1.60 (m, 4H), 1.25−1.42 (m, 5H), 1.11 (ddd, J = 2.0, 11.0, 13.0 Hz, 1H), 0.96 (d, J = 6.7 Hz, 3H), 0.93 (t, J = 7.3 Hz, 3H); ^{13}C NMR (100 MHz, CD_3OD) δ 173.1, 166.9, 150.6, 142.6, 138.0, 131.8, 130.0, 129.4, 128.4, 127.6, 121.4, 77.1, 77.0, 73.9, 71.3, 69.1, 56.4, 52.4, 45.3, 43.5, 43.2, 41.1, 40.4, 38.0, 37.4, 36.2, 36.0, 32.6, 31.9, 26.0, 22.3, 20.0, 14.1; IR (film) 3338, 2956, 2923, 2854, 1717, 1643, 1527, 1460, 1375, 1250, 1180, 1156, 1111, 1084, 1033, 800 cm^{-1}; HRMS (ESI) m/z calcd for $C_{34}H_{49}NO_8Na$ $[M + Na]^+$ 622.3356, found 622.3322; $[\alpha]^{25}_D$ = +14.2 (MeOH, c = 0.33).

Pyridyl-Containing Side-Chain Analogue 26. A 25 mL round-bottom flask was charged with **25** (80 mg, 0.27 mmol) and dichloromethane (3.5 mL). The mixture was cooled to −78 °C, and a 1 M DIBAL-H solution in hexanes (0.33 mL, 0.33 mmol) was added dropwise under nitrogen. After 2 h of stirring at −78 °C, the reaction was quenched with saturated aqueous ammonium chloride solution. Flash chromatography with 40% ethyl acetate in hexanes gave 28 mg (42%) of the desired product, 14 mg (18%) of recovered starting material, and 25 mg (37%) of overreduced alcohol: ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.88 (t, J = 1.2 Hz, 1H), 7.57 (t, J = 7.7 Hz, 1H), 7.05(d, J = 7.5 Hz, 1H), 7.03 (d, J = 7.7 Hz, 1H), 6.45 (d, J = 11.7 Hz, 1H), 5.95 (dt, J = 6.6, 11.7 Hz, 1H), 5.76 (brs, 1H), 4.31 (td, J = 1.6, 6.5 Hz, 2H), 3.68 (s, 3H), 3.15 (t, J = 6.8 Hz, 2H), 2.98 (t, J = 7.2 Hz, 2H).

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A 25 mL round-bottom flask was charged methyl bis(trifluoroethyl)-phosphonoacetate (44 μL, 0.21 mmol), 18-crown-6 (165 mg, 0.624 mmol), and THF (2 mL). The mixture was cooled to −78 °C, and KHMDS (30 mg, 0.15 mmol) in 3 mL of THF was added dropwise under nitrogen. After 30 min of stirring, the aldehyde (27 mg, 0.11 mmol) in 3 mL of THF was added dropwise, followed by 4 h of stirring at −78 °C. The reaction was quenched with saturated aqueous NH_4Cl solution and extracted with ethyl acetate. Flash chromatography with 10% THF in dichloromethane gave 24 mg (71%) of the desired ester: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.57 (t, J = 7.7 Hz, 1H), 7.04 (d, J = 7.1 Hz, 1H), 7.02 (d, J = 6.7 Hz, 1H), 6.48 (d, J = 11.7 Hz, 1H), 6.30 (dt, J = 7.5, 11.5 Hz, 1H), 6.06 (brs, 1H), 6.01 (dt, J = 6.1, 11.4 Hz, 1H), 5.81 (dt, J = 1.6, 11.5 Hz, 1H), 4.29 (t, J = 6.3 Hz, 2H), 3.70 (s, 3H), 3.67 (s, 3H), 3.12 (dtd, J = 1.0, 7.4, 7.4 Hz, 2H), 2.95 (t, J = 7.8 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 166.7, 160.2, 157.3, 155.0, 149.2, 136.8, 132.9, 130.4, 122.1, 120.9, 120.0, 52.0, 51.1, 39.1, 37.4, 28.7; IR (film) 3399, 1719, 1643, 1570, 1530, 1453, 1521, 1147, 1023, 819 cm^{-1}; HRMS (ESI) m/z calcd for $C_{16}H_{20}N_2O_4Na$ [M + Na]$^+$ 327.1321, found 327.1302.

The methyl ester (18 mg, 0.060 mmol) was dissolved in THF (0.35 mL) at rt. After 1 N LiOH aqueous solution (0.90 mL, 0.90 mmol) was added, the reaction mixture was stirred at room temperature for 5 h. The reaction mixture was adjusted to pH 8 at 0 °C, and was extracted with 2 mL of ethyl acetate. The aqueous layer was acidified to pH 2, with ethyl acetate extraction at every 2 pH intervals. The combined organic phase was dried with $MgSO_4$, concentrated, redissolved in 10% methanol in dichlromethane, filtered through a thin plug of silica gel and concentrated under vacuum. The salt was obtained (15 mg, 85%) and used directly in the next Mitsunobu reaction without further purification.

The general esterification procedure was followed with **15** (2.6 mg, 0.008 mmol), the pyridine-containg side chain acid (9.8 mg, 0.034 mmol), PPh_3 (16.8 mg, 0.064 mmol), and DIAD (12.5 μL, 0.064 mmol) in benzene (0.5 mL). The mixture was stirred for 10 min and then was purified by flash column chromatography (30% THF in pentane, then 50% EtOAc in pentane) to yield 3.2 mg (67%) of the desired analogue: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.59 (t, J = 7.7 Hz, 1H), 7.00−7.09 (m, 3H), 6.49 (d, J = 11.7 Hz, 1H), 5.94−6.04 (m, 1H), 5.94 (dt, J = 1.4, 15.6 Hz, 1H), 5.10−5.21 (m, 2H), 5.04 (brs, 1H), 4.31 (t, J = 6.5 Hz, 2H), 4.06 (tdd, J = 2.2, 4.2, 11.3 Hz, 1H), 3.69 (s, 3H), 3.51−3.60 (m, 2H), 3.32 (s, 3H), 2.99 (t, J = 8.0 Hz, 2H), 2.72 (dd, J = 6.8, 14.9 Hz, 2H), 2.58 (dd, J = 4.4, 9.3 Hz, 1H), 2.36 (dd, J = 7.0, 10.8 Hz, 1H), 1.85−1.96 (m, 1H), 1.62−1.79 (m, 3H), 1.45−1.58 (m, 4H), 1.11−1.43 (m, 10H), 0.98 (d, J = 6.4 Hz, 3H), 0.92 (t, J = 7.3 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.9, 165.8, 159.7, 157.2, 155.2, 148.5, 136.9, 133.1, 130.3, 122.3, 121.9, 120.9, 75.6, 75.4, 73.4, 69.8, 67.8, 56.2, 52.0, 44.0, 42.4, 42.3, 40.0, 36.9, 36.5, 36.2, 35.3, 31.7, 31.0, 25.6, 19.0, 13.9; IR (film) 2955, 2923, 1722, 1651, 1570, 1455, 1375, 1264, 1182, 1085, 1031, 966, 820 cm^{-1}; HRMS (ESI) m/z calcd for $C_{33}H_{48}N_2O_8Na$ [M + Na]$^+$ 623.3308, found 623.3356; $[\alpha]^{25}_D$ = +22.4 ($CHCl_3$, c = 0.32).

Furan-Containing Side-Chain Analogue 30. The general esterification procedure was followed with **15** (2.2 mg, 0.0067 mmol), the furan-containing side-chain acid (5.6 mg, 0.013 mmol), PPh_3 (3.9 mg, 0.015 mmol), and DIAD (3.0 μL, 0.015 mmol) in benzene (0.5 mL). The mixture was stirred for 10 min and then was purified with flash column chromatography (50% EtOAc in pentane) to afford 2.0 mg (50%) of the desired analogue: ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.01 (dd, J = 12.0, 16.0 Hz, 1H), 6.74 (t, J = 11.7 Hz, 1H), 6.60 (d, J = 15.6 Hz, 1H), 6.51 (d, J = 3.4 Hz, 1H), 6.33 (d, J = 3.5 Hz, 1H), 6.24−6.28 (m, 1H), 5.76 (d, J = 11.1 Hz, 1H), 5.60−5.69 (m, 1H), 5.23−5.26 (m, 1H), 5.12−5.19 (m, 1H), 5.07 (brs, 1H), 4.28 (t, J = 6.6 Hz, 2H), 4.10 (apt, J = 11.4 Hz, 1H), 3.70 (s, 3H), 3.57−3.62 (m, 2H), 3.32 (s, 3H), 2.60 (dd, J = 4.4, 14.5 Hz, 1H), 2.36 (dd, J = 10.8, 14.1 Hz, 1H), 1.82−1.94 (m, 2H), 1.70−1.75 (m, 2H), 1.45−1.62 (m, 4H), 1.23−1.39 (m, 6H), 1.12−1.19 (m, 1H), 0.98 (d, J = 6.5 Hz, 3H), 0.92 (t, J = 7.3 Hz, 3H); ^{13}C NMR (100 MHz, CD_3OD) δ 173.1, 167.1, 154.8, 145.2, 130.1, 128.7, 125.1, 118.7, 114.9, 114.3, 77.1, 77.0, 73.9, 71.3, 69.1, 56.4, 52.5, 45.2, 43.5, 41.1, 40.4, 37.9, 37.4, 36.0, 32.6, 26.0, 20.0, 14.1; IR (film) 2947, 2854, 1713, 1528, 1376, 1249, 1190, 1064, 994 cm^{-1}; HRMS (ESI) m/z calcd for $C_{32}H_{45}NO_9Na$ [M + Na]$^+$ 610.2992, found 610.3017; $[\alpha]^{25}_D$ = +5.1 (MeOH, c = 0.20).

Des-carbamyl Analogue 34. The general esterification procedure was followed with **15** (2.2 mg, 0.0067 mmol), the des-carbamyl side-chain acid (4.9 mg, 0.027 mmol), PPh_3 (9.4 mg, 0.036 mmol), and DIAD (7.1 μL, 0.036 mmol) in benzene (0.5 mL). The mixture was stirred for 10 min and then was purified by flash column chromatography (20% EtOAc in DCM, then 40% EtOAc in hexanes) to yield 2.5 mg (76%) of the desired analogue: ^{1}H NMR (400 MHz, CD_3OD) δ 7.54 (t, J = 1 Hz, 1H), 6.34 (dt, J = 7.4, 11.5 Hz, 1H), 5.88 (dt, J = 1.7, 11.5 Hz, 1H), 5.14−5.20 (m, 2H), 4.07 (dddd, J = 2.2, 2.2, 11.5, 11.5 Hz, 1H), 3.67 (app t, J = 9.7, 1H), 3.57 (app t, J = 10.8 Hz, 1H), 3.28 (s, 3H), 2.97 (dtd, J = 1.6, 7.1, 7.1 Hz, 2H), 2.70 (dd, J = 4.3, 14.8 Hz, 1H), 2.64 (t, J = 7.3, 2H), 2.30 (dd, J = 11.0, 14.8 Hz, 1H), 1.87 (dd, J = 10.9, 14.0 Hz, 1H), 1.80−1.86 (m, 1H), 1.66−1.76 (m, 2H), 1.46−1.62 (m, 4H), 1.26−1.44 (m, 6H), 1.29 (s, 3H), 1.12 (ddd, J = 1.8, 11.0, 12.8 Hz, 1H), 0.97 (t, J = 5.8 Hz, 3H), 0.94 (t, J = 7.3 Hz, 3H); ^{13}C NMR (100 MHz, CD_3OD) δ 173.0, 167.7, 164.4, 150.8, 141.6, 136.9, 122.5, 78.0, 77.9, 74.8, 72.2, 70.0, 57.3, 46.1, 44.3, 44.1, 41.9, 38.8, 38.3, 37.1, 33.4, 29.8, 27.1, 26.8, 20.9, 15.0, 14.4; IR (film) 2956, 2922, 2836, 1718, 1642, 1580, 1458, 1382, 1334, 1261, 1175, 1087, 1030, 797 cm^{-1}; HRMS (ESI) m/z calcd for $C_{27}H_{41}NO_7Na$ [M + Na]$^+$ 514.2781, found 514.2766; $[\alpha]^{25}_D$ = +27.8 (MeOH, c = 0.21).

Growth Inhibition Assays. Cell proliferation was evaluated uing the MTS (3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2*H*-tetrazolium) dye reduction assay with PMS (phenazine methosulfate) as electron acceptor performed in triplicate or quintuplicate. Briefly, cells were seeded in 96-well plates at 1000 or 2000 cells per well and incubated for 24 h at 37 °C. Cells were then exposed to various concentrations (serial 3-fold dilutions) of the indicated compounds for 72 h at 37 °C. An MTS solution was added, and the cells were incubated at 37 °C for 2 h. The absorption signals were measured at 490 and 650 nm. Growth inhibition was calculated as defined by the National Cancer Institute [GI_{50} = 100 × (T - T_0)/(C − T_0); T_0 = cell density at time zero; T = cell density of the tested well after period of exposure to tested compound; C = cell density of the vehicle treated].

■ ASSOCIATED CONTENT

Supporting Information

^{1}H and ^{13}C NMR spectra for all compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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