MICROWAVE-ASSISTED CLAISEN REARRANGMENT: SYNTHESIS OF NATURALLY OCCURRING TRAIL-RESISTANCE-OVERCOMING TYROSINE DERIVATIVE

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GRAPHICAL ABSTRACT

Abstract Tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) is a tumor necrosis factor (TNF) family ligand that binds on the death receptors, DR4 and DR5, activating apoptotic pathways selectively in cancer cells and thus has become a promising cancer therapeutic agent. Compound 1, isolated from Streptomyces sp. IFM 10937, has shown activity in overcoming TRAIL resistance in AGS cells. Synthesis of 1 has been accomplished from L-tyrosine in an overall high-yielding reaction sequence.

Keywords Cancer; olefin cross-metathesis reaction; total synthesis; TRAIL; tyrosine derivative

INTRODUCTION

Cancers are genetic diseases that result from the deregulation of cell growth and cell death pathways due to genomic alterations. Apoptosis, the process of programmed cell death, is a genetically programmed biochemical process that removes unwanted cells and maintains tissue homeostasis. Tumor necrosis factor-related apoptosis-inducing ligand (TRAIL), a tumor necrosis factor (TNF) family member,[1,2] activates apoptotic pathways selectively in cancer cells[3] through binding on the death receptors, DR4[4,5] and DR5.[6,7] After initiation by the
death-receptor pathway, TRAIL-induced apoptosis results in activation of effector caspase-3, death-inducing signaling complex (DISC) formation, and proteolytic activation of caspase-8.\(^8\) TRAIL has emerged as an attractive antineoplastic agent because of its remarkable ability to selectively kill tumoral cells while leaving normal cells unscathed.\(^9\) Unlike the other members of the TNF superfamily, in vivo administration of TRAIL has been proved to be safe.\(^10\) However, in the case of highly malignant tumors, a reasonable numbers of cancer cells have intrinsic or acquired resistance to TRAIL-induced apoptosis.\(^11\) Therefore, discovery of compounds that can abrogate TRAIL resistance has attracted a great deal of attention in anticancer drug discovery.

In a recent study, bioassay-guided fractionation of \textit{Streptomyces} sp. IFM 10937 has led to the isolation of a new tyrosine derivative 1 (Fig. 1).\(^12\) Compound 1 was evaluated for its activity in overcoming TRAIL resistance in AGS (human gastric adenocarcinoma) cells. Combined treatment of 75 or 150\(\mu\)M 1 and 100 ng/mL TRAIL with AGS cell lines reduced cell viability to 77\% and 67\% of control levels \((P < 0.01)\), respectively, which suggested a possible synergism between the two agents.

In our ongoing efforts toward the total synthesis bioactive natural products,\(^13\)–\(^16\) we now disclose the synthesis of 1.

**DISCUSSION**

Synthesis of the desired compound 1 was envisaged from the olefin cross-metathesis reaction of intermediate 4, which in turn was to be synthesized from Claisen rearrangement of intermediate 3. Thus, L-tyrosine 2 was converted to intermediate 3 by the operations of esterification, N-boc protection, and O-allylation based on literature known procedures\(^17\) (Scheme 1).

However, Claisen rearrangement of intermediate 3 either under thermal or microwave irradiation conditions at different temperatures, using N,N-dimethylaniline or DMF as solvents, were unsuccessful. For instance, under thermal conditions, reaction in N,N-dimethylaniline or dimethylformamide (DMF) at reflux left the starting material intact, whereas heating neat 3 at elevated temperatures led to the decomposition of 3, with no desired product formation. Likewise, under microwave conditions, reaction at lower temperature (200°C, 250 W, 1 h) in DMF left 3
unchanged, whereas heating the reaction at higher temperature (250 °C, 250 W, 45 min) in N,N-dimethylaniline led to the deprotection of N-Boc group. Deprotection of N-Boc group under microwave conditions using mild base or under thermolytic conditions is well known.\cite{18} To address the N-deprotection problem, we moved on to make the N-acetyl derivative 5 which in turn was synthesized from intermediate 2 according to a literature known procedure\cite{19} (Scheme 1). Claisen rearrangement of intermediate 5 in N,N-dimethylaniline under microwave irradiation at 250 °C gave the desired rearranged phenol 6 in excellent yield (75%). Acetylation of intermediate 6 under standard conditions rendered 7 in a very good yield. Olefin cross-metathesis reaction between intermediate 7 and 2-methyl-2-butene, using second-generation Grubbs catalyst, yielded the desired 8 in excellent yield (84%). It is noteworthy that O-prenylation of O-deallylated 5 followed by Claisen rearrangement would possibly have given access to the O-deacetylated 8. However, such O-prenylation would have required a palladium-catalyzed reaction between

Scheme 1. Synthesis of tyrosine derivative 1.
deallylated 5 with commercially not available isobutyl-2-methyl-3-butene-2-yIcarbonate.\textsuperscript{[20]} O-Prenylation of tyrosines in proteins has recently been accomplished with prenyltransferase LynF, an enzyme from TruF family.\textsuperscript{[21,22]} Finally, exposure of intermediate 8 under basic condition at room temperature ultimately produced the desired 1 in 43\% overall yield from 5 (Scheme 1). All the spectral data of 1 matched with those of the previously isolated material.\textsuperscript{[12]}

**EXPERIMENTAL**

Elemental analysis was carried out on a Perkin-Elmer Elemental Analyzer Series 11 model 2400 (Perkin-Elmer Inc. USA). IR spectra were recorded on a Thermo Scientific Nicolet 6700 FT-IR Perkin Elmer 16 F PC FTIR spectrophotometer (Thermo Scientific USA). \(^1\)H and \(^13\)C NMR spectra were measured in CDCl\(_3\) and CD\(_3\)OD using tetramethylsilane (TMS) as internal standard on a Jeol JNM-LA 500-MHz spectrometer (Jeol USA Inc.). Analytical thin-layer chromatography (TLC) was carried out on silica-gel 60 F\(_{254}\) plates (E. Merck); column chromatography was carried out on silica gel (200–400 mesh, E. Merck).

**\((S)\)-Methyl 2-Acetamido-3-(3-allyl-4-hydroxyphenyl)propanoate**  
(6, C\(_{15}\)H\(_{19}\)NO\(_4\))

A microwave reaction vessel containing a solution of aryl ether 5 (0.75 g, 2.70 mmol) in N,N-dimethylaniline (4 ml) had gently bubbling nitrogen for 1 min, and then the vessel was placed inside a CEM Discover S-Class microwave synthesizer, where it was exposed to microwaves at 250°C (260 W) for 2 h. After completion of the reaction, the mixture was diluted with ethyl acetate (50 mL) and extracted with 3 M hydrochloric acid (3 × 10 mL). The organic layer was washed successively with saturated sodium hydrogen carbonate (15 mL) and brine (10 mL), dried over anhydrous sodium sulfate, filtered, and concentrated under vacuum. Column chromatography purifications of the yellow oily material, eluting with ethyl acetate/hexane (1:1), yielded compound 6 as a pale yellow solid. Yield: 0.56 g, 75\%; mp 91–92°C; [\(\alpha\)]\(_D\)\(^{25}\) +25.95 (c. 1.15, CHCl\(_3\)); IR (neat): 3418, 3300, 3081, 3006, 2956, 1717, 1662, 1510, 1432, 1209, 1121 cm\(^{-1}\); \(^1\)H NMR (CDCl\(_3\), 500 MHz): \(\delta\) 1.92 (s, 3H, NCOCH\(_3\)), 2.96 (dd, 2H), 3.27 (m, 2H), 3.66 (s, 3H, OCH\(_3\)), 4.76 (m, 1H), 4.99–5.03 (m, 2H), 5.90 (m, 1H), 5.99 (d, 1H, J = 7.9 Hz, NH), 6.62 (m, 1H, aromatics), 7.2 (m, 2H, aromatics); \(^13\)C NMR (CDCl\(_3\), 500 MHz): \(\delta\) 23.05, 34.46, 37.05, 52.35, 53.31, 115.64, 116.07, 125.92, 127.26, 128.13, 131.04, 136.46, 153.39, 170.05, 172.29. Anal. calcd. for C\(_{15}\)H\(_{19}\)NO\(_4\): C, 64.97; H, 6.91; N, 5.05. Found: C, 64.93; H, 6.94; N, 5.01.

**\((S)\)-Methyl 2-Acetamido-3-(4-acetoxy-3-allylphenyl)propanoate**  
(7, C\(_{17}\)H\(_{21}\)NO\(_5\))

Triethylamine (0.75 ml, 5.41 mmol) was added to a solution of compound 6 (0.5 g, 1.80 mmol) in anhydrous dichloromethane (15 mL) at 0°C. After being stirred for 10 min, acetic anhydride (0.35 ml, 3.60 mmol) was added dropwise and the reaction was stirred for 2 h at room temperature. To the mixture was added ethyl acetate (30 mL), and it was washed successively with saturated sodium hydrogen carbonate (15 mL) and brine (10 mL). The organic layer was dried over anhydrous
sodium sulfate and evaporated under vacuum to obtain compound 7 as an off-white solid. Yield: 0.57 g, 99%; mp 104 °C; [α]D25 +56.6 (c. 1.0, CHCl3); IR (neat): 3311, 3086, 2948, 1740, 1649, 1639, 1543, 1497, 1433, 1371, 1202, 1185, 1166 cm–1; 1H NMR (CDCl3, 500 MHz): δ 1.99 (s, 3H, NCOCH3), 2.29 (s, 3H, COCH3), 3.10 (t, 2H, J = 5.5 Hz), 3.25 (d, 2H, J = 6.7 Hz), 3.72 (s, 3H, OCH3), 4.86 (m, 1H), 5.03–5.09 (m, 2H), 5.85 (m, 1H), 5.89 (d, 1H, J = 7.9 Hz, NH), 6.96 (m, 3H, aromatics); 13C NMR (CDCl3, 500 MHz): δ 23.21, 29.71, 34.45, 37.16, 52.38, 53.06, 116.47, 122.47, 128.18, 131.23, 132.03, 133.70, 135.70, 148.01, 169.35, 169.61, 171.94. Anal. calcd. for C17H21NO5: C, 63.94; H, 6.63; N, 4.39. Found: C, 63.90; H, 6.68; N, 4.32.

(S)-Methyl 2-Acetamido-3-(4-acetoxy-3-(3-methylbut-2-enyl)phenyl) propanoate (8, C19H25NO5)

To a solution of compound 7 (0.23 g, 0.72 mmol) in anhydrous dichloromethane (36 ml) was added successively 2-methyl-2-butene (4 ml) and Grubbs’ second-generation catalyst (0.018 g, 0.021 mmol) under a nitrogen atmosphere. The solution was stirred for 24 h at room temperature and concentrated under vacuum. Column chromatography of the dark brown oily material, eluting with ethyl acetate = hexane (2:3), gave compound 8 as a light yellow solid (0.21 g, 84%). Yield: 0.21 g, 84%; mp 110–112 °C; [α]D25 –59.7 (c. 0.22, CHCl3). IR (neat): 3288, 3061, 2951, 1735, 1649, 1539, 1492, 1370, 1185, 1164 cm–1; 1H NMR (CDCl3, 500 MHz): δ 1.69 (s, 3H), 1.74 (s, 3H), 1.99 (s, 3H, NCOCH3), 2.30 (s, 3H, COCH3), 3.10 (m, 2H), 3.25 (d, 2H, J = 7.3 Hz), 3.72 (s, 3H, OCH3), 4.87 (m, 1H), 5.29 (m, 1H), 5.92 (d, 1H, J = 9.7 Hz, NH), 6.93 (m, 3H, aromatics); 13C NMR (CDCl3, 500 MHz): δ 17.82, 23.14, 25.77, 28.59, 29.71, 37.15, 52.31, 53.03, 121.38, 122.31, 127.76, 130.83, 133.41, 133.61, 147.96, 169.41, 169.59, 171.96. Anal. calcd. for C19H25NO5: C, 65.69; H, 7.25; N, 4.03. Found: C, 65.63; H, 7.30; N, 3.97.

(S)-2-Acetamido-3-(4-hydroxy-3-(3-methylbut-2-enyl)phenyl) propanoic Acid (1, C16H21NO4)

Lithium hydroxide monohydrate (0.096 g, 2.3 mmol) was added to a solution of compound 8 (0.16 g, 0.46 mmol) in a mixture of tetrahydrofuran, methanol, and water (10 ml, in 3:1:1 ratio), and the mixture was stirred for 3 h at room temperature. The solvent was evaporated and residue was diluted with chloroform (20 ml) and washed with 1 M hydrochloric acid (3 ml). The organic layer was dried over anhydrous sodium sulfate, concentrated under vacuum, and passed over a plug of silica, eluting with methanol/dichloromethane (0.5:9.5) to afford compound 1 as a colorless solid (0.092 g, 69%). The spectral data of 1 coincided with literature values.[12]

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SUPPORTING INFORMATION

Supplemental data for this article can be accessed on the publisher’s website.

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