CHAPTER 2

Synthesis of Unit-B of Cryptophycin-24 via Sharpless Asymmetric Dihydroxylation
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2.1. Introduction

The cryptophycins\textsuperscript{1} are potent antitumour selective cytotoxins, isolated from blue green algae NOSTOC Sp. ATCC 53789\textsuperscript{2} and NOSTOC Sp. GSV 224.\textsuperscript{3} Schwartz at Merck\textsuperscript{2} for the first time isolated the first Cryptophycin, Cryptophycin A. Subsequently in 1994, Moore and his colleagues\textsuperscript{3a} discovered the potent and selective antitumor properties of the cryptophycin class of compounds. In addition to cryptophycin A, several other cytotoxin analogues were isolated from blue green algae belonging to Nostocaceae.\textsuperscript{2,4}

Subsequently studies were conducted to determine structure, chemical stability and antitumour activity of the new analogues of cryptophycin and provided a structure–activity relationship (SAR).\textsuperscript{4} The Hawaii group was the first to publish the relative and absolute stereochemistry for the cryptophycin in 1994.\textsuperscript{5} In 1995, they synthesized and corrected the structure of Cryptophycin-1 and-3. For the purpose of retrosynthetic analysis, the cryptophycin-1 can be disconnected into four units (Figure 2.1). Unit A is a structurally unique δ-hydroxy acid with four stereogenic centres. Unit B is a D-tyrosine derived α-amino acid, unit C is a β-alanine derivative and unit D is L-leucine acid. The individual building blocks are synthesized as protected precursors which are subsequently coupled to give an acyclic depsipeptide and then cyclized. Ring closure can be affected by either macrolactamization or ring closing metathesis (RCM) as pioneered by Georg & Tripathy in 2004.\textsuperscript{6}
Several elegant synthetic approaches, as well as extended SAR studies, have been performed for all four units of the cryptophycin backbone (Figure 2.1). As cryptophycin represents a new class of extraordinarily potent, solid tumor-selective cytotoxins, it led to the development of a number of total syntheses, as well as syntheses of cryptophycin analogues and fragments.

Structure-activity relationship (SAR) studies of the C10 side chain of Cryptophycin-1 shows that absence of C3'-Cl decrease the activity of the cryptophycin by a factor of 10. A factor of 30 decrease inactivity was observed due the absence of C4'-OMe group. The presence of a second Cl- substituent at the C5' position of the C10 side chain decreases the activity by a factor of 120. These data indicate that the presence of C3'-Cl and C4'-OMe are necessary of optimal activity of the cryptophycin. Analogues lacking the epoxide ring in unit A (Figure 2.1) and the intact macrolide ring also showed diminished cytotoxicity which was consistent with marginal to -ve activities in vivo. More recently it was revealed that the cytotoxicity of cryptophycin can be more dramatically affected by altering the C10 side chain. In a study carried out by Patel and co-workers on human leukemia CCRF-CEM tumor cell lines by using cryptophycin-52 analogues, they established that 3'-Cl substituent provides an in vitro activity. The SAR determined from isolated analogues of Cryptophycin-1 and synthesized cryptophycin-52 analogues suggested that any synthesized cryptophycin analogue should retain a benzyl moiety on C10 side chain with R stereochemistry for optimal activity.

Structurally simpler cryptophycin, cryptophycin-24 was isolated from an Okinawan marine sponge, Dysidea arnaria. Though cryptophycin-24 lacks chlorine substituent on the aryl ring of the C-10 side chain and the methyl group at C6 (Fig 2.1), it was extremely potent against KB cells (ED50 = 5 pm). In vivo,
however, cryptophycin-24 showed nil to marginal antitumor activity which was not surprising based on the reported SAR for cryptophycin 1. But cryptophycin-24 has a half-life of approximately 10 min in mouse serum due to ester hydrolysis. The present work is based on synthesis of B fragment for cryptophycin-24 (figure 2.2)

![Figure 2.2](image)

### 2.2. Review of Literature

Commercial availability of the α-amino acids provides an easy access to B fragment. Tius achieved the synthesis of the B-fragment from D-tyrosine. Initial step for the synthesis was Boc-protection of the amino group of the precursor α-amino acid. A suspension of D-tyrosine in 50% aqueous dioxane was treated with 1.2 equivalent of di-tert butyl dicarbonate in presence of 1.2 equivalents of triethylamine. The resulting product was dimethylated with dimethyl sulfate in presence of K₂CO₃ in refluxing acetone in 85% yield. The methyl ester was then cleaved by careful saponification with NaOH in aqueous dioxane to yield Boc-O-methyl-D-tyrosine (Scheme 2.1). For the synthesis of Boc protected Chloro-O-methyl-D-tyrosine, D-tyrosine was chlorinated with sulfuryl chloride in glacial acetic acid and the product of this reaction was converted to Boc protected chloro-O-methyl-D-tyrosine using the procedure for synthesis of Boc-O-methyl-D-tyrosine as explained above.

![Scheme 2.1](image)

**Scheme 2.1.** Reagents and conditions: a) Boc₂O, Et₃N, dioxane/H₂O (1:1), rt (quant.) b) Me₂SO₄, K₂CO₃, acetone, reflux, 4 h (85%); c) NaOH, H₂O/dioxane rt, (93%).

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A new synthesis based on the highly enantioselective alkylation of glycine derivatives\(^\text{15}\) was developed by Maier.\(^\text{16}\) The synthetic route is shown in the Scheme 2.2. The OH group of phenol 4 was converted to methyl ether 5 with dimethyl sulfate for making of the tyrosine core. The methyl group of 5 was then brominated with NBS to form benzyl bromide 6. The enantioselective alkylation of glycine imine with benzylic bromide 6 was carried out with the use of chiral cinchona-derived phase-transfer catalyst (7) to produce benzylated imine 8 in good yield. The \(ee\) of compound 8 was found to be 96%. Hydrolysis of imine 8 was done by 15% citric acid to provide free amine 9 which was then protected with (fluorenylethoxycarbonyl)chloride (FmocCl) to form protected amino acid 10. Treatment of the amino acid 10 with trifluoroacetic acid (TFA) provided the desired amino acid 11.

Sewald et al.\(^\text{14}\) had carried out a novel and highly enantioselective two step process for the synthesis of unit B precursor of cryptophycin. The key step of the synthetic route is an asymmetric hydrogenation using commercially available \([(\text{COD})\text{Rh}-\text{(R,R)-Et-DuPhos}]\text{BF}_4\) catalyst. In the first step of the synthesis, \(\text{rac-Boc-}\)
α-phosphonoglycine trimethyl ester 12 (Scheme 2.3) was reacted with 3-chloro-4-methoxy benzaldehyde to furnish the olefin 13 in a completely Z-selective Horner-Wadsworth-Emmons (HWE) reaction. Asymmetric hydrogenation using commercially available [(COD)Rh-(R,R)-Et-DuPhos]BF<sub>4</sub> catalyst gave the methyl ester 14 in 97% yield with an ee exceeding 98%.

Scheme 2.3. Reagents and conditions: (a) 3-Chloro-4-methoxybenzaldehyde, 1,1,3,3-tetramethylguanidine, CH<sub>2</sub>Cl<sub>2</sub>, 0°C to rt, 16h (84%); (b) [(COD)Rh-(R,R)-Et-DuPhos]BF<sub>4</sub> (1.9 mol%), H<sub>2</sub>, dry and degassed MeOH, 3-6 bar, 21.5h (97%; 98% ee)

Although a number of methods are available for synthesis of enantiomerically pure α-amino acids, these methods are not conventionally used for the synthesis of unit B of cryptophycin. Most of the processes developed for synthesis of enantiomerically pure phenyl alanine analogues are based on either asymmetric hydrogenation of dehydroaryl alanine derivatives or the reaction of nucleophilic glycine derivatives with benzylic halide. Negishi cross coupling reaction of the serine derived organozinc reagent with aryl halide under Pd catalyst has proven to be a convenient method for the direct preparation of protected phenylalanine analogues.
2.3. Present Work

Objective

Although, unit-B of cryptophycin could be derived from commercially available α-amino acid, alternative pathways were also developed to synthesize the target amino-acid fragment. In this chapter, we intend to develop a new synthetic strategy for unit-B of cryptophycin-24 using Sharpless asymmetric dihydroxylation as the key step. The retrosynthetic sequence is represented in Scheme 2.4.

Scheme 2.4. Retrosynthetic sequence for unit-B of Cryptophycin 24.
2.4. Results and Discussion

To realize the synthesis of unit B of Cryptophycin-24, we have initiated the synthesis, using 4-methoxy cinnamic acid as the starting material (scheme 2.5). The acid was converted to methyl ester (16) in 87% yield using methanol and thionyl chloride (SOCl₂).

![Scheme 2.5](image)

The compound was characterized using IR and NMR spectroscopy. In the $^1$H spectra of the compound (Figure 2.3), the two olefinic proton appears at 7.58 $\delta$ and 6.24 $\delta$ as doublet with $J = 16$ Hz. The O-CH$_3$ protons appear at 3.76 $\delta$ and 3.72 $\delta$. The O-CH$_3$ proton of the ester group comes at downfield position with respect to that of the ring O-CH$_3$ proton. In the $^{13}$C spectrum (Figure 2.4) the olefinic carbons appear at 144.5 $\delta$ and 115.2 $\delta$ while the O-CH$_3$ carbon comes at 55.3 and 51.5 $\delta$.

![Figure 2.3. $^1$H NMR of compound 16](image)
Thereafter, we planned to follow a pathway as shown in scheme 2.6. The ester 16 was subjected to asymmetric dihydroxylation reaction using Sharpless Asymmetric Dihydroxylation procedure using (DHQ)$_2$PHAL as the chiral ligand.$^{21}$ The corresponding diol 17 was obtained in 87% yield.

Scheme 2.6. Reagents and condition: (a) K$_2$O$_2$SO$_4$(OH)$_2$, (DHQ)$_2$PHAL, MeSO$_2$NH$_2$ BuOH: H$_2$O (1:1), 0°C (b) Pd/C, H$_2$, MeOH (c) CBr$_4$, TPP, dry DCM, rt (d) NaN$_3$, DMF, rt (e) H$_2$, EtOAc, Boc$_2$O (f) K$_2$CO$_3$, H$_2$O, MeOH, rt
In the IR spectra, the absorption frequency at 3391 cm\(^{-1}\) indicates the presence of OH group. The CH protons of C2 and C3 appear at 4.90 \(\delta\) and 4.28 \(\delta\) in \(^1\)H spectrum (Figure 2.5). The OH protons appear as broad signal around 3.01 \(\delta\) and 2.56 \(\delta\). In the \(^{12}\)C spectrum, C2 and C3 carbons appear at 74.7 and 74.3 (Figure 2.6).

The optical purity of the compound was determined by comparing of data reported in literature.\(^{22}\) The reported value for the same compound is +5.5\(^{\circ}\) (c 1, ethanol). The optical rotation of the compound was found to be +5.1\(^{\circ}\) (c 1, EtOH). Spectroscopic data are consistent with that previously reported.

**Figure 2.5.** \(^1\)H NMR of compound 17

**Figure 2.6.** \(^{12}\)C NMR of compound 17

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Hydrogenolysis of diol 17 using Pd/C and hydrogen gas in methanol produced the corresponding alcohol 18 in 65% yields. The signal appearing as doublet of doublet (dd) at 3.06 δ and 2.90 δ in 1H spectrum (figure 2.7) shows the reduction of the benzylic carbon while the proton of C2 atom appears at 4.43-4.39 δ as a multiplet. The benzylic carbon appears at 39.5 δ in 13C spectrum (figure 2.8). The measured optical rotation was found to be +11.3° (c 1, CHCl3).

Figure 2.7. 1H NMR of compound 18

Figure 2.8. 13C NMR of compound 18
The next step our strategy was to convert the alcohol functionality to the azide. The alcohol 18 was transformed to corresponding bromide 19 and then to the azido compound 20. Bromination was carried out using CBr₄ in the presence of PPh₃. The proton on C2 possesing the Br atom now comes at slightly downfield position (4.38-4.32 δ) in comparison to the same proton in the compound 18 (Figure 2.9) in ¹H NMR spectra while in ¹³C spectrum (Figure 2.10) the C2 carbon shift to 45.3 δ from 71.3 δ of the same carbon in compound 18.

![Figure 2.9. ¹H NMR of compound 19](image1)

![Figure 2.10. ¹³C NMR of compound 19](image2)

The measured optical rotation of the bromo compound was found to be -3.6° (c 1, CHCl₃). The bromo ester 19 was then converted to azide 20 using NaN₃ in dry DMP. The IR absorption frequency around 2109 cm⁻¹ indicates the formation of the azido compound. The C3 proton of the compound 20 appears as doublet of
doublet (dd) at 3.12 δ and 2.96 δ in 1H spectrum (Figure 2.11). The C2 carbon appears at 63.3 δ in 13C spectrum (Figure 2.12). The measured optical rotation of the azide 20 was found to be +14.9° (c 1, CHCl3).

![Figure 2.11. 1H NMR of compound 20](image)

In the next step we converted the azide 20 to Boc protected amino compound 21 through one pot synthesis.26 In the 1H NMR spectrum (Figure 2.13) of the compound the protons appearing at 1.40 δ indicates the Boc protection while the broad signal appearing at 4.95 δ is due to the NH group. The carbon signals of the Boc group appear at 28.2 δ in 13C spectrum (Figure 2.14).

![Figure 2.12. 13C NMR of compound 20](image)

In the next step we converted the azide 20 to Boc protected amino compound 21 through one pot synthesis.26 In the 1H NMR spectrum (Figure 2.13) of the compound the protons appearing at 1.40 δ indicates the Boc protection while the broad signal appearing at 4.95 δ is due to the NH group. The carbon signals of the Boc group appear at 28.2 δ in 13C spectrum (Figure 2.14).
The optical rotation of the compound was found to be $-16.1^\circ$ (c 1, CHCl$_3$). The optical purity of compound 21 was found to be very low as compared to that previously reported (Lit. $-58.4^\circ$). This may be due to partial racemisation of the product during bromination of hydroxyl functionality of compound 18, located at $\alpha$-position to the ester functionality.

Hence, we designed a new strategy to synthesize the target molecule. The new synthetic route is shown in the **Scheme 2.7**.
Accordingly, we planned to synthesize the azido compound directly from monohydroxy compound in one step. We have carried out the AD reaction of the compound 16, using (DHQD)$_2$PHAL as the chiral ligand. The product was obtained with 89% yield with high optical purity. The measured optical rotation for the diol was found to be -5.4° (c 1, CH$_2$Cl$_2$). The optical rotation value of compound 23, reported in lit. 28 is -5.8° (c 1, CH$_2$Cl$_2$). Thereafter the compound 23 was subjected to hydrogenolysis following the same method used previously to produce the compound 24 with 67% yield. The optical rotation of the compound recorded to be -11.8° (c, 1, CHCl$_3$). The compound 23 and 24 was characterized by using IR and NMR spectroscopy and data are found to be similar to those reported in the previous case.

In the next step we synthesized the azide 25 directly from 24 by using Diphenylphosphoryl azide (DPPA). 29 The reaction was carried out by treating the compound 24 with DPPA in presence of DBU in THF under ice cool condition and continuing the reaction at room temperature for 24 h. Corresponding azide 25 was obtained in 75% yield and with high optical activity. The measured optical rotation of the azide 25 was found to be +48.1° which is far better in comparison to the previous synthesis (Scheme 2.6). Then we continued the synthesis to produce the Boc-protected α-amino acid ester 26 under hydrogenation condition. The present method resulted in significant improvement of the optical purity of compound 26 and the optical rotation was found to be -58.1° (c 1, CHCl$_3$). The enantiomeric

Scheme 2.7. Reagents and condition: (a) K$_2$O$_2$(OH)$_2$ (DHQD)$_2$PHAL, MeSO$_2$NH$_2$'BuOH: H$_2$O (1:1), 0°C (b) Pd/C, H$_2$, MeOH (c) DPPA, DBU, dry THF, 0°C (d) H$_2$, EtOAc, Boc$_2$O (e) K$_2$CO$_3$, H$_2$O, MeOH, rt
excess of the product was analysed by using HPLC and found to be 99% (Figure 2.15).

Figure 2.15
The amino ester was finally converted to corresponding amino acid 22 using K$_2$CO$_3$ in methanol$^{30}$ to yield the product as a white solid. The C3 proton of the compound 22 appears as doublet of doublet at 3.00 $\delta$ and 2.81$\delta$ (Figure 2.16). The C2 proton appears as a multiplet at 3.91-3.87 $\delta$. In Mass spectral data of this compound, the molecular ion peak is found to be m/z = 318.2 [C$_{15}$H$_{21}$NO$_5$ (M$^+$ + Na) requires 318.321]. It confirms the formation of the desired product. The melting point of the compound is found to be 88-90 °C (Lit.$^{31}$ 89-90°C). The optical rotation of the compound was found to be $-26.9$ (c 1, EtOH). The overall yield of the synthetic route (Scheme 2.7) was found to be 29%.

Figure 2.16. $^1$H NMR of compound 22
After getting success in the synthesis of unit B of cryptophycin 24, we made an attempt to synthesize unit B of other cryptophycin bearing a chloro substituent on the aromatic ring. We have started the synthesis using methyl ester of 3-chloro-4-methoxy cinnamic acid (27). The asymmetric dihydroxylation proceeds of the ester proceeds with high optical purity of the compound (28) with ee of 99% (HPLC). However in the proceeding step of hydrogenolysis of the diol results cleavage of the chlorine atom from the aromatic ring along with the cleavage of benzylic –OH bond (29) (Scheme 2.8).

\[ \text{Scheme 2.8. Reagents and condition: (a) SOCl}_2, \text{MeOH (b) K}_2\text{OsO}_4(\text{OH})_4, (\text{DHQD})_2\text{PHAL, MeSO}_2\text{NH}_2^\text{BuOH: H}_2\text{O (1:1), 0°C (c) Pd/C, H}_2, \text{MeOH} } \]

Since the hydrogenolysis step was unsuccessful, we did not proceed further.
2.5. Conclusion

In conclusion we have developed a new asymmetric synthetic pathway for the synthesis of unit-B of cryptophycin-24 using Sharpless asymmetric dihydroxylation as the key step. Initial attempt to convert α-hydroxy acid ester to corresponding α-amino acid ester via bromocompound was unsuccessful due to partial racemization of the product during bromination. However, direct azidation route was successful. This study shows that direct azidation using DPPA is beneficial for asymmetric synthesis of α-amino acid from α-hydroxy acid without the loss of chirality during the transformation.

2.6. Experimental Section

2.6.1. General Remark

All reagents were purchased from Sigma-Aldrich. IR spectra were recorded on PERKIN ELMER Spectrum RX I FT-IR System. NMR spectra were recorded on Bruker 300 MHz and 400 MHz instruments. Optical rotations were measured with Perkin Elmer Model 343 Polarimeter. Enantiomeric excess of compound 26 was determined using chiralcel R column.

**Synthesis of Methyl3-(4-methoxyphenyl)acrylate (16)**

\[
\begin{array}{c}
\text{MeO} \\
\text{C} \text{= C} \text{= C} \text{O} \\
\text{Me}
\end{array}
\]

To an ice-cold solution of 4-Methoxy cinnamic acid (2 g, 11.2 mmol) in methanol (10 mL), SOCl₂ (1mL) was added drop wise and the reaction mixture was stirred at room temperature for 1 h. After completion of the reaction, methanol was removed under vacuum and water was added to the reaction mixture. The aqueous layer was extracted with ethyl acetate twice and combined organic layer was dried over anhydrous Na₂SO₄. The solvent was then evaporated under reduced pressure and the crude product was purified with column chromatography (petroleum ether /ethylacetate) (4:1) to afford pure product as a white solid.
Yield : 87% (1.87 g)
IR (KBr, cm⁻¹) : 3028, 2950, 1714, 1636, 1597, 1508, 1287, 1253, 1170, 1018
\(^1\)H NMR (400MHz, CDCl₃) : δ 7.58 (d, \(J = 16\)Hz, 1H), 7.40(d, \(J = 8\)Hz, 2H), 6.81(d, \(J = 8\) Hz, 2H), 6.24(d, \(J = 16\)Hz, 1H), 3.76 (s, 3H), 3.72 (s, 3H)
\(^13\)C NMR (100 MHz, CDCl₃): δ 167.7, 161.3, 144.5, 129.7, 127.1, 115.2, 114.3, 55.3, 51.5

Synthesis of Methyl 2,3-dihydroxy3-(4-methoxyphenyl)propanoate (17)

A 100 ml RB flask was charged with 20 ml of tert-butanol and water (1:1). K₃Fe(CN)₆ (4.269 g, 12.968 mmol) and K₂CO₃ (1.792 g, 12.968 mmol) was added to the solvent and stirred for 5-10 minutes. To this mixture, K₂OsO₂(OH)₄ (0.006 g, 0.017 mmol), (DHQ\(^\circ\)PHAL (0.033 g, 0.043 mmol) and methanesulfonamide (0.411 g, 4.322 mmol) were added successively. The reaction mixture was cooled to 0 °C and then the olefin 2 (0.830g, 4.322 mmol) was added. The reaction was stirred for 24 hrs at 0 °C. The progress of the reaction was monitored by TLC. After completion of the reaction the reaction was quenched with sodium sulfite and allowed to run for another 30 minutes at RT. The reaction mixture was then washed with brine solution and extracted with EtOAc twice. The separated organic layer was dried over anhydrous Na₂SO₄. The solvent was then evaporated under reduced pressure and the crude product was purified with column chromatography (petroleum ether/ethylacetate) (1:1)

Yield : 87% (0.850 g)
mp : 102-104 ° (Lit.\(^{22}\) value 106-107°)
\([\alpha]_D^{28}\) : \([\alpha]_D^{26} = +5.2 (c 1, EtOH) [\text{Lit}^{22} +5.5 (c 1, EtOH)]
IR (KBr, cm⁻¹) : 3489, 3391, 3018, 2960,1709, 1606, 1513, 1444, 1390, 1317, 1253, 1170, 1018
\(^1\)H NMR (400MHz, CDCl₃) : δ7.27 (d, \(J = 8\)Hz, 2H), 6.84 (d, \(J = 8\)Hz, 2H), 4.90(
The compound 23 (0.750 g, 3.318 mmol) was charged with Pd/C (0.075 g) and MeOH (50 ml). Hydrogen gas was allowed to pass through the reaction mixture with constant stirring for 15 hrs. The progress of the reaction was monitored by TLC. The Pd/C was then filtered off and the solvent was evaporated under reduced pressure. The crude was purified by column chromatography (ethyl acetate/petroleum ether) (1:4) to afford pure product as a white solid.

Yield : 65 % (0.452 g)

\[ \delta_{D26} : +11.3 (c 1, CHCl_3) \]

IR (KBr, cm\(^{-1}\)) : 3474, 2999, 2954, 1742, 1612, 1514, 1443, 1297, 1248, 1179, 1033

\(^1\)H NMR (300MHz, CDCl\(_3\)) : \(\delta\) 7.12 (d, \(J = 9\)Hz, 2H), 6.83(d, \(J = 9\)Hz, 2H), 4.43-4.39 (m, 1H), 3.78(s, 3H), 3.76 (s, 3H), 3.06(dd, \(J = 15\)Hz, 6 Hz , 1H) 2.90(dd, \(J = 15\) Hz, 6 Hz, 1H), 2.64 (br, 1H)

\(^{13}\)C NMR (75 MHz, CDCl\(_3\)) : \(\delta\) 174.5, 158.4, 130.4, 128.1, 113.7, 71.3, 55.1, 52.3, 39.5

**Synthesis of Methyl 2-bromo-3-(4-methoxyphenyl)propanoate (19)**

Triphenyl phosphine (0.435 g, 1.66 mmol) in 4 ml of dry DCM was added drop wise through a capillary during a period of minutes to a well stirred solution of alcohol 18 (0.350 g, 1.66 mmol) and CBr\(_4\) (0.825 g, 2.49 mmol) in 4 ml of dry DCM.
at room temperature. After an additional one hour of stirring, the reaction mixture was treated with n-pentane and the resulting precipitate of triphenyl phosphine oxide was removed by filtration and washed several times with n-pentane. The combined n-pentane solution was then washed with 5% Na\textsubscript{2}CO\textsubscript{3}, water and NaCl solution and then dried over anhydrous Na\textsubscript{2}SO\textsubscript{4}. The solvent was evaporated under reduced pressure. The purification of the crude product with flash column chromatography (ethyl acetate/ petroleum ether) (1.5:8.5) yield the pure product as a colorless liquid.

Yield : 65% (0.295 g)

$\left[\alpha\right]_D^{26} : -3.6$ (c 1, CHCl\textsubscript{3})

IR (Neat, cm\textsuperscript{-1}) : 2930, 1739, 1606, 1508, 1440, 1356, 1292, 1248, 1175, 1027

$^1$H NMR (300MHz, CDCl\textsubscript{3}) : δ 7.12 (d, $J = 9$Hz, 2H), 6.83 (d, $J = 9$Hz, 2H), 4.38-4.32 (m, 1H), 3.79 (s, 3H), 3.72 (s, 3H), 3.40 (dd, $J = 15$ Hz, 9Hz, 1H), 3.18 (dd, $J = 15$ Hz, 9 Hz, 1H)

$^{13}$C NMR (75 MHz, CDCl\textsubscript{3}) : δ 169.9, 158.7, 130.2, 128.7, 114.0, 55.2, 52.8, 45.3, 40.2

**Synthesis of Methyl 2-azido-3-(4-methoxyphenyl)propanoate (20)**

To a solution of the bromo ester 19 (0.180 g, 0.766 mmol) in dry DMF was added NaN\textsubscript{3} (0.248 g, 3.83 mmol). The reaction mixture was allowed to stir for 3 hrs at room temperature. The progress of the reaction was monitored by TLC. After completion of the reaction water was added to the reaction mixture. It was then extracted with diethyl ether. The organic phase was separated and then dried over anhydrous Na\textsubscript{2}SO\textsubscript{4}. The solvent was then evaporated under reduced pressure. The crude product was purified by column chromatography (ethyl acetate/ petroleum ether) (1:9) to afford the pure product as a light yellowish liquid.

Yield : 65% (0.100 g)

$\left[\alpha\right]_D^{26} : +14.9$ (c 1, CHCl\textsubscript{3})
IR (Neat, cm⁻¹) : 2930, 2852, 2106, 1744, 1606, 1508, 1440, 1351, 1248, 1204, 1175, 1027.

¹H NMR (300MHz, CDCl₃) : δ 7.15 (d, J = 9Hz, 2H), 6.86(d, J = 9Hz, 2H), 4.06-4.02 (m, 1H), 3.80(s, 3H); 3.78 (s, 3H), 3.12(dd, J = 15 Hz, 6 Hz, 1H), 2.96 (dd, J = 15Hz, 6 Hz, 1H);

¹³C NMR (75 MHz, CDCl₃) : δ 170.3, 158.7, 130.1, 127.7, 114.0, 63.3, 55.1, 52.5, 36.7

Synthesis of methyl 2-[(tert-butoxycarbonyl)amino]-3-(4-methoxyphenyl)propanoate (21)

A suspension of Pd/C (0.010 g) in 6 mL EtOAc was vigorously stirred under hydrogen atmosphere until the uptake of hydrogen ceased. A mixture of compound 25 (0.080 g, 0.340 mmol) and Boc₂O (0.089 g, 0.408 mmol) in ethyl acetate (3 mL) was added to the suspension. The resulting solution was stirred under hydrogen atmosphere until disappearance of azide results. After completion of the reaction, Pd/C was filtered off and the solvent was removed under reduced pressure. The purification of the crude product through flash column chromatography using ethyl acetate and petroleum ether (2:8) results white solid as the pure product.

Yield : 85% (0.090 g)
mp : 48-50 °C (Lit. value 49-50 °C)
[α]D²⁶ : -16.1° (c 1, CHCl₃), Lit.²⁷ value [α]D²⁵ = -58.4 (c1, CHCl₃)
IR(KBr,cm⁻¹) : 3352, 3038, 1969, 1739, 1690, 1611, 1523, 1440, 1366, 1283, 1248, 1170, 1032

¹H NMR (400MHz, CDCl₃) : δ 7.04 (d, J = 8Hz, 2H), 6.83 (d, J = 8 Hz, 2H), 4.95 (br, 1H), 4.53 (m, 1H), 3.79 (s, 3H), 3.72 (s, 3H), 3.03 (m, 2H), 1.40 (s, 9H);

¹³C NMR(100MHz, CDCl₃) : δ 172.4, 158.6, 156.2, 130.2, 127.8, 113.9, 79.8, 55.1, 54.4, 52.1, 37.4, 28.2
Synthesis of Methyl 2,3-dihydroxy3-(4-methoxyphenyl)propanoate (23)

A 100 ml RB flask was charged with 20 ml of tert-butanol and water (1:1). K$_3$Fe(CN)$_6$ (4.346 g, 13.2 mmol) and K$_2$CO$_3$ (1.833 g, 13.2 mmol) was added to the solvent and stirred for 5-10 minutes. To this mixture, K$_2$OsO$_2$(OH)$_4$ (0.006 g, 0.017 mmol), (DHQD)$_2$PHAL (0.034 g, 0.044 mmol) and methanesulfonamide (0.420 g, 4.421 mmol) were added successively. The reaction mixture was cooled to 0°C and then the olefin 16 (0.850 g, 4.421 mmol) was added. The reaction was stirred for 24 hrs at 0°C. The progress of the reaction was monitored by TLC. After completion of the reaction the reaction was quenched with sodium sulfite and allowed to run for another 30 minutes at RT. The reaction mixture was then washed with brine solution and extracted with EtOAc twice. The separated organic layer was dried over anhydrous Na$_2$SO$_4$. The solvent was then evaporated under reduced pressure and the crude product was purified with column chromatography (petroleum ether/ethylacetate) (1:1).

Yield : 89 % (0.895 g)

mp : 103-105 ° (Lit. 22 value 106-107°)

[α]$_D^{28}$ : - 5.6°(c 1, CH$_2$Cl$_2$); Lit. 28 [α]$_D^{27}$ = -5.8 (c 1, CH$_2$Cl$_2$)

IR (KBr, cm$^{-1}$) : 3487, 3390, 3015, 2963, 1707, 1606, 1513, 1444, 1390, 1317, 1253, 1170, 1018

$^1$H NMR (400MHz, CDCl$_3$) : δ 7.27 (d, $J$ = 8Hz, 2H), 6.84(d, $J$ = 8Hz, 2H), 4.90(s, 1H), 4.28 (s, 1H), 3.75 (s, 3H), 3.74(s, 3H), 3.03 (br, 1H), 2.55 (br 1H)

$^{13}$C NMR (100 MHz, CDCl$_3$) : δ 173.2, 159.2, 131.9, 127.4, 113.6, 74.7, 74.3, 55.2, 52.8

Synthesis of Methyl 2-dihydroxy3-(4-methoxyphenyl)propanoate (24)
The compound 9 (0.800 g, 3.539 mmol) was charged with Pd/C (0.080 g) and MeOH (50 ml). Hydrogen gas was allowed to pass through the reaction mixture with constant stirring for 15 hrs. The progress of the reaction was monitored by TLC. The Pd/C was then filtered off and the solvent was evaporated under reduced pressure. The crude was purified by column chromatography (ethyl acetate/petroleum ether) (1:4) to afford pure product as a white solid.

Yield : 67 % (0.498 g)

$[\alpha]_D^{26}$ : -11.8 (c 1, CHCl$_3$)

IR (KBr, cm$^{-1}$) : 3476, 2995, 2956, 1742, 1612, 1514, 1443, 1295, 1245, 1179, 1033

$^1$H NMR ( 300MHz, CDCl$_3$) : δ 7.12 (d, $J = 9$Hz, 2H), 6.83(d, $J = 9$Hz, 2H), 4.42-4.38 (m, 1H), 3.78 (s, 3H), 3.76 (s, 3H), 3.06(dd, $J = 15$ Hz, 6 Hz , 1H) 2.90(dd, $J = 15$Hz, 6Hz, 1H), 2.62 (br,1H)

$^{13}$C NMR (75 MHz, CDCl$_3$) : δ 174.4, 158.4, 130.2, 128.1, 113.7, 71.3, 55.1, 52.3, 39.3

Synthesis of Methyl 2-azido-3-(4-methoxyphenyl)propanoate (25)

A 100 ml three necked flask was dried and equipped with nitrogen gas. It was then charged with compound 10 (0.350 g, 1.66 mmol), dry THF (2 ml) and diphenylphosphoryl azide (0.502 g, 1.826 mmol). The reaction mixture was then cooled under ice water bath and then added 1,8-diazabicyclo[5,4,0]undec-7-ene (DBU) (0.212 g, 1.394 mmol) drop wise via a syringe. The reaction was run at around 0°C for 1 hr and then it was warmed to room temperature and finally stirred for another 24 hrs at rt. The resulting homogeneous mixture was diluted with diethyl ether and water. The water layer was removed and the organic layer was washed with water and 0.5 M citric acid monohydrate. The organic phase was dried over anhydrous Na$_2$SO$_4$ and then the solvent was evaporated under reduced pressure. The purification of the crude product with column chromatography (ethyl acetate/petroleum ether) (1.5:8.5) yield the pure product as a colorless liquid.
Yield : 75% (0.293 g)
$\alpha_d^{[26]}$ : $+48.1$ (c 1, CHCl$_3$)
IR (Neat, cm$^{-1}$) : 2996, 2926, 2109, 1748, 1612, 1514, 1445, 1357, 1249, 1209, 1172, 1031
$^1$H NMR (300MHz, CDCl$_3$) : $\delta$ 7.14 (d, $J$ = 8.7 Hz, 2H), 6.86(d, $J$ = 8.7Hz, 2H), 4.04(m, 1H), 3.79 (s, 3H), 3.77 (s, 3H), 3.12 (dd, $J$ = 13.8 Hz, 5.4 Hz, 1H), 2.95 (dd, $J$ = 13.8Hz, 8.4Hz, 1H)
$^{13}$C NMR (75 MHz, CDCl$_3$) : $\delta$ 170.3, 158.5, 130.0, 127.5, 113.9, 63.2, 55.0, 52.1, 36.7

**Synthesis of methyl 2-[(tert-butoxycarbonyl)amino]-3-(4-methoxyphenyl)propanoate (26)**

A suspension of Pd/C (0.018 g) in 10 ml EtOAc was vigorously stirred under hydrogen atmosphere until the uptake of hydrogen ceased. A mixture of compound 11 (0.180 g, 0.765 mmol) and Boc$_2$O (0.200 g, 0.918 mmol) in ethyl acetate (4 ml) was added to the suspension. The resulting solution was stirred under hydrogen atmosphere until disappearance of azide results. After completion of the reaction, Pd/C was filtered off and the solvent was removed under reduced pressure. The purification of the crude product through flash column chromatography using ethyl acetate and petroleum ether (2:8) results white solid as the pure product.

Yield : 85% (0.200 g)
mp : 47-49 °C (Lit.$^{31}$ value 49-50°C)
$\alpha_d^{[26]}$ : -58.1° (c 1, CHCl$_3$), Lit.$^{3}$ $\alpha_d^{[26]}$ = -58.4 (c 1, CHCl$_3$)
IR(KBr,cm$^{-1}$) : 3351, 3035, 1967, 1739, 1692, 1611, 1523, 1440, 1366, 1280, 1248, 1170, 1032
$^1$H NMR (400MHz, CDCl$_3$) : $\delta$ 7.04 (d, $J$ = 8Hz, 2H), 6.83(d, $J$ = 8Hz, 2H), 4.93 (br, 1H), 4.53 (m, 1H), 3.79 (s, 3H), 3.72 (s, 3H), 3.02 (m, 2H), 1.40 (s, 9H)
$^{13}$C NMR(100MHz, CDCl$_3$) : $\delta$ 172.2, 158.5, 156.2, 130.2, 127.8, 113.9, 79.6, 55.1, 54.3, 52.1, 37.4, 28.1.
Synthesis of 2-[( tert-butoxycarbonyl)amino]-3-(4-methoxyphenyl)propanoic acid, (22)

K$_2$CO$_3$ (0.089 g, 0.608 mmol) was added to a solution of ester 26 (0.100 g, 0.323 mmol) in MeOH (4 mL) in presence of water (1 ml). The reaction mixture was stirred for 12 hrs. After completion of the reaction the MeOH was evaporated and the aqueous layer was extracted with EtOAc. The organic layer was dried over anhydrous Na$_2$SO$_4$ and then the solvent was evaporated under reduced pressure. The residue was subjected to column chromatography using ethyl acetate and petroleum ether (1:1) as eluent to yield the pure product.

Yield : 88% (0.084 g)

[α]$_D^{29}$ : -26.9 (c 1, EtOH)

mp : 89-90 °C, (Lit.\textsuperscript{31} value 89-90°C)

MS : m/z = 318.2. [C$_{15}$H$_{21}$NO$_5$ (M$^+$ + Na) requires 318.321]

IR (KBr, cm$^{-1}$) : 3432, 2978, 2935, 1694, 1612, 1587, 1514, 1440, 1367, 1249, 1176, 1037;

$^1$H NMR (400 MHz, CDCl$_3$) : δ 7.08 (d, J = 8Hz, 2H), 6.77 (d, J = 8Hz, 2H), 6.18 (br, 1H), 3.91-3.87 (m, 1H), 3.69 (s, 3H), 3.00 (dd, J = 12Hz, 8 Hz, 1H), 2.81 (dd, J = 12Hz, 8 Hz, 1H), 1.32 (s, 9H)

$^{13}$C NMR (100 MHz, CDCl$_3$) : δ 168.20, 157.6, 154.9, 130.8, 130.4, 113.3, 77.5, 56.2, 54.9, 36.4, 28.3
2.7. REFERENCES


