Efficient protocol to quinazolino[3,2-\(d\)][1,4]benzodiazepine-6,9-dione via Staudinger-aza-Wittig cyclization: application to synthesis of Asperlicin D

Deeb Taher, Zakariyya N. Ishtaiwi, and Naim H. Al-Said

Department of Chemistry, Tafila Technical University, Tafila, Jordan
Department of Applied Chemical Sciences, Jordan University of Science and Technology, Irbid 22110, Jordan
Department of Chemistry, King Khalid University, Abha, Saudi Arabia
E-mail: naim@just.edu.jo

Abstract
Tandem Staudinger and intramolecular aza-Wittig reactions followed by cyclodehydration of the linear \(N'-(N-(2-azidobenzoyl)-2-a minobenzoyl)glycine \) ethyl ester furnished the tetracyclic quinazolino[3,2-\(d\)][1,4]benzodiazepine-6,9-dione ring system found in some biologically active natural alkaloids. This method was successfully implemented to synthesize asperlicin D from a linear peptide containing ester and azido terminal groups.

Keywords: Quinazolinobenzodiazepine, aza-Wittig, cyclodehydration, asperlicin D

Introduction
Naturally occurring alkaloids such as benzomalvins,\(^1\) circumdatin F,\(^2\) asperlicins,\(^3,4\) and sclerotigenin\(^5\) incorporating two anthranilic acid units and one or two amino acids combined together in a quinazolino[3,2][1,4]benzodiazepine system, have been isolated from different sources. These alkaloids display various biological activities. For instance, benzomalvin A shows inhibitory activity against substance P at the guinea pig, rat and human neurokinin NK1 receptors.\(^1\) Sclerotigenin has insecticidal properties.\(^5\) The asperlicin family displays strong affinity for pancreatic and gall bladder cholecystokinin (CCK) receptors.\(^3,4\) Asperlicins A (1), B (2), C (3) and E (5) incorporate a quinazolino[3,2-\(a\)][1,4]benzodiazepine-5,13-dione, whereas asperlicin D (4) has a quinazolino[3,2-\(d\)][1,4]benzodiazepine-6,9-dione system.
Several tactical variants of the Eguchi aza-Wittig reaction have been of major value in the synthesis of this family of natural products. Globally, these tactics employed the overall sequence of selective acylation of a [1,4]benzodiazipine system with 2-azidobenzoyl chloride followed by aza-Wittig cyclization to attain the quinazolino[1,4]benzodiazipine structure after a multi-step sequence.6-11

Following on from our previous studies on the construction of the quinazolino[1,4]benzodiazipine ring system,12 here we report a new and effective strategy for the synthesis of the quinazolino[3,2-d][1,4]benzodiazipine-6,9-dione system utilizing one-pot tandem Staudinger, intramolecular aza-Wittig and cyclodehydration reactions. This paper describes in detail the data of the preliminary communication that described the first total synthesis of asperlicin D.13,14

Results and Discussion

Retrosynthetically, we envisioned that these alkaloids could be derived from two consecutive intramolecular reactions from linear peptide 6 containing two termini capable of reacting with each other (Scheme 1). Therefore, azido and ester groups were selected to study the feasibility of intramolecular aza-Wittig cyclization15-18 on a substrate incorporating amodic protons to form the proposed cyclic intermediate 7. This intermediate could furnish, after cyclization, either quinazolino[3,2-d][1,4]benzodiazipine 8 found in asperlicins A (1), B (2), C (3), and E (5) via path a or quinazolino[3,2-d][1,4]benzodiazipine 9 found in asperlicins D (4) through path b.
Scheme 1. Cyclization modes of aza-Wittig intermediate 7 generated from dipeptide 6.

As shown in Scheme 2, our study commenced with the coupling of isatoic anhydride (10) with ethyl glycinate in dry acetonitrile followed by acylation with freshly prepared 2-azidobenzoyl chloride. This reaction furnished N-[N-(2-azidobenzoyl)-2-aminobenzoy]glycine ethyl ester 11, as a model substrate, in good yield (80%).

Staudinger iminophosphorane intermediates 12, 13, 14 and 15 were generated in situ by stirring 11 in a dry solvent (benzene, toluene, mesitylene) with phosphorus reagents Ph₃P, Bu₃P, (EtO)₃P, and (PhO)₃P, respectively, at ambient temperature for an appropriate time to complete the reaction.¹⁵-¹⁹ The formation of these intermediates was confirmed by their acidic hydrolysis (PhSO₃H, H₂O, THF) at ambient temperature. Iminophosphoranes 12 and 13 gave the known amine 16,¹³,¹⁴ whereas 14 and 15 gave the corresponding amidophosphates 17 and 18, respectively. Similar results were obtained when iminophosphorane intermediates 12, 13, 14 and 15 were passed through a short column of silica gel. The amidophosphates (17 and 18) were isolated in pure form by column chromatography and their structures were confirmed by spectral data and elemental analysis.
Next, we turned to explore the viability of tandem cyclization reactions of Staudinger intermediates 12-15 shown in Scheme 2. Suitable conditions for the intramolecular aza-Wittig reaction were optimized employing 12. Initial attempts to promote cyclization of this intermediate at reflux temperature in toluene or xylene were unsuccessful even after an extended reaction time (48 h). However, when cyclization was conducted in boiling mesitylene for 40 h it furnished a significant amount of the expected imino ether, 7-ethoxy-quinazolino[3,2-d][1,4]benzodiazepine (19) along with its hydrolyzed product quinazolino[3,2-d][1,4]benzodiazepine (20) in 20% and 15% yield, respectively, after separation by column chromatography. The two products were isolated in variable proportions depending on the reaction time and temperature. The methylene protons of the seven-membered ring in 19 were observed at $\delta$ 5.87 (d, 13.2 Hz) and 3.83 (d, 13.2 Hz), indicating that they are non-equivalent on the NMR time scale due to the significant barrier to flipping of the ring. Furthermore the methylene protons of the ethoxy group were displayed at $\delta$ 4.47 and 4.24 as two broad peaks. Since the reaction conditions were anhydrous, it seemed likely that the hydrolysis of 19 to 20 occurred on the silica gel during purification. Moreover, the imino ether 19 was cleanly hydrolyzed to 20 in wet THF containing a catalytic amount of PhSO$_3$H. To simplify the separation of the desired product 20 from the reaction mixture after conducting the reaction in boiling mesitylene, the crude product was hydrolyzed after concentration to give 20 in 30-40%
yield. The structure of the final product 20 was assigned based on the reported NMR spectral data and also by elemental analysis. The spectral data of 20 were identical to those reported previously.20

The yield of the final product 20 was significantly improved by switching to the more reactive iminophosphorane 13. Stirring 11 and (Bu)3P in mesitylene at room temperature for 2 h then at reflux for 40 h, followed by hydrolysis, gave 20 in 55% yield. Lower yields of 20 (<30%) were obtained by performing the reaction in boiling xylene. The results of these experiments are summarized in Table 1. The cyclization of 14 failed in boiling xylene, affording only the hydrolysis product, amidophosphate 17. However, 20 was obtained in 20% yield along with the amidophosphate 17 by conducting the reaction in mesitylene at reflux temperature. Similarly, 15 furnished 20 along with the corresponding amidophosphate 17.

Table 1. Synthesis of Quinazolino[3,2-d][1,4]benzodiazepine-6,9-dione Ring System 20

<table>
<thead>
<tr>
<th>Phosphorous(III) reagent</th>
<th>% Yield of Method Aa</th>
<th>% Yield Method Bb</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Ph)3P</td>
<td>37</td>
<td>52</td>
</tr>
<tr>
<td>(Bu)3P</td>
<td>55</td>
<td>65</td>
</tr>
<tr>
<td>(EtO)3P</td>
<td>20</td>
<td>30</td>
</tr>
<tr>
<td>(PhO)3P</td>
<td>15</td>
<td>20</td>
</tr>
</tbody>
</table>

a In method A: the reactions were conducted in mesitylene at reflux temperature for 40 h.
b In method B: the reactions were conducted in sealed tube at 200 °C for 5 h.

An alternative procedure for the cyclization of the intermediates 12-15 was also explored. Thus, the cyclization process was performed in sealed tube. Table 1 details the efficiency of the cyclization of intermediates 12-15 at 200 °C in sealed tube followed by hydrolysis. In general, the yield of the desired product was enhanced.

Furthermore, implementation of Wipf methodology (Ph3P/I2/Et3N)21 with 16 afforded an inseparable mixture of products. Fortunately, we observed that the starting material 16 was completely consumed and a new product 21 was isolated (10% yield) in impure form when the reaction was conducted with excess reagent (Ph3P/I2/Et3N). We believe that the product 21 is formed from 20 after it has been formed in the reaction mixture. Thus, to confirm this proposal, compound 20 was allowed to react with excess Wipf reagent (Ph3P/I2/Et3N). This reaction furnished 21 in moderate yield (50%). Moreover, derivative 22 was isolated when 20 stirred with a mixture of Ph3P/I2/Bu3N. Utilizing the experimental conditions established for the unsubstituted quinazolino[3,2-d][1,4]benzodiazepine (20), we next explored the application of these conditions to prepare asperlicin D (4) starting from tryptophan azido derivative 23. This starting material was prepared in a one-pot reaction in good yield (>70%) by condensation of isatoic anhydride (10) with L-tryptophan methyl ester followed by acylation with freshly prepared 2-azidobenzoyl chloride (Scheme 3).
Staudinger iminophosphorane intermediate 24 was generated in situ by stirring 23 with (Ph)3P at room temperature until the evolution of nitrogen gas ceased (2 h). Initial attempts to promote cyclization of 24 at reflux in benzene or xylene were unsuccessful even after an extended reaction time. However, the TLC indicated the consumption of 24 when the reaction was conducted in boiling mesitylene for 40 h. This reaction afforded two products (by TLC). Fortunately, after hydrolysis (H2O, THF, PhSO3H) the crude reaction mixture furnished the natural product 4 in 30-40% yield together with amine 25. The two products were formed in variable proportions depending on the reaction time and temperature. The yield of asperlicin D was improved using (Bu)3P in mesitylene at reflux temperature. The isolated asperlicin D showed spectral properties identical to those previously described for the natural product.

**Table 2. Synthesis of asperlicin (4) using Ph3P in mesitylene**

<table>
<thead>
<tr>
<th>Temperature (°C)</th>
<th>% Yield of 4</th>
<th>% Yield 25</th>
</tr>
</thead>
<tbody>
<tr>
<td>100</td>
<td>22</td>
<td>40</td>
</tr>
<tr>
<td>125</td>
<td>28</td>
<td>33</td>
</tr>
<tr>
<td>150</td>
<td>35</td>
<td>22</td>
</tr>
</tbody>
</table>

**Conclusions**

The present work demonstrates the viability of aminophosphorane intermediates having a secondary amide proton to provide a one-step entry to quinazolino[1,4-d]benzodiazepine ring system via tandem intramolecular aza-Wittig reaction followed by cyclodehydration performed on a linear peptide. We have devised a general procedure with simple reagents for accomplishing successive cyclization reactions via Staudinger intermediates. The power of this approach has resulted in the synthesis of asperlicin D.
Experimental Section

General Procedures. Melting points (mp) were determined on an electrothermal digital melting point apparatus and are uncorrected. Infrared (IR) spectra were recorded using a Nicolet-Impact 410 FT-IR spectrophotometer. Proton nuclear magnetic resonance (1H NMR) spectra were recorded on Bruker, Avance DPX-300 (300 MHz), Bruker 250 spectrometers. Tetramethylsilane (TMS) was used as an internal reference. The spectral data are reported in delta (δ) units relative to TMS reference line. Carbon-13 nuclear magnetic resonance (13C NMR) spectra were taken using a Bruker Avance DPX-300 (75.5 MHz) spectrometer and signals are reported in delta (δ) units relative to TMS reference using the solvent peaks (CDCl3) as internal standard. Mass spectra were recorded with a Mariner Biospectrometry Workstation 4.0 by Applied Biosystems.

Ethyl N-2-[2-azidobenzoyl]glycinate (11)
Isatoic anhydride (0.327 g, 2 mmol) was added to ethyl glycinate hydrochloride (0.279 g, 2 mmol) dissolved by heating in acetonitrile (7 mL) containing triethylamine (0.212 g, 2.1 mmol). The mixture was heated and allowed to reflux for 3 h. The reaction mixture was cooled to 0 °C and then triethylamine (0.404 g, 4 mmol) was added. Then a solution of freshly prepared 2-azidobenzoyl chloride (0.417 g, 2.3 mmol) in acetonitrile (2 mL) was added to the mixture. The reaction mixture was stirred at 0 °C for 0.5 h and at room temperature for 24 h. The mixture was concentrated and extracted with ethyl acetate (2 x 40 mL) and water (20 mL). The combined organic layers were washed with brine, dried with MgSO4 and concentrated. The residue was purified by column chromatography on silica gel (30% ethyl acetate in hexane) to afford 11 (0.559 g, 77%). mp 106-107 °C; IR (KBr disk, cm⁻¹) 3312 and 3219 (NH), 2988, 2139 (N3), 1743, 1671 and 1643 (C=O); 1H NMR (400 MHz, CDCl3) δ 11.58 (br, 1H, NH), 8.73 (d, J 8.3 Hz, 1H, Ar-H), 7.91 (dd, J 1.7, 8.3 Hz, 1H, Ar-H), 7.58 (dd, J 1.3, 7.8 Hz, 1H, Ar-H), 7.52 (dq, J 1.5, 7.8 Hz, 2H, Ar-H), 7.24 (dq, J 1, 5.0 Hz, 2H, Ar-H), 7.09 (dq, J 1.3, 7.7 Hz, 1H, Ar-H), 6.89 (t, J 5.3 Hz, 1H, N-H), 4.22 (q, J 7.1 Hz, 2H, -CH2-), 4.17 (d, J 5.2 Hz, 2H, -CH2-), 1.3 (t, J 7.1 Hz, 3H, -CH3). 13C-NMR (100 MHz, CDCl3) δ 169.8, 169.0, 164.4, 139.1, 137.7, 132.8, 132.5, 131.3, 127.4, 127.2, 125.2, 123.5, 122.4, 121.7, 119.3, 61.9, 41.9, 14.3. EIMS (m/z, relative intensity) cal for C18H17N5O4: 367.1; found 367.2 (14%). Anal. Calcd for C18H17N5O4: C, 58.85; H, 4.66; N, 19.06% Found: C, 58.72; H, 4.54; N, 18.78%

Ethyl N-2-[2-aminobenzoyl]glycinate (16)
A mixture of azide 11 (0.367 g, 1 mmol) and Ph3P or Bu3P (1 mmol) in mesitylene (10 mL) was stirred at room temperature for 2 h. After concentration, the residue was stirred in wet-THF, containing a catalytic amount of PhSO3H, for 3 h. The reaction mixture was concentrated. The residue was purified by chromatography on silica gel (25% ethyl acetate in hexane) to afford 16 (0.324 g, 95%). mp 131-133 °C [lit. mp 131-132 °C13,14].

Ethyl N-2-[2-(diethylphosphate)amidobenzoyl]glycinate (17)
A solution of azide 11 (0.367 g, 1 mmol) and triethyl phosphite (0.183 g, 1.1 mmol) in mesitylene (5 mL) was stirred at room temperature for 2 h. After concentration, the residue was passed through a
short column of silica gel (25% ethyl acetate in hexane) to afford 17 (0.439 g, 92%). The same product was obtained by stirring the residue in wet-THF containing catalytic amount of PhSO$_3$H for 3 h. mp 127-128 °C; IR (KBr disk, cm$^{-1}$) 3271 (N-H), 3080, 2993, 1751, 1650, 1605, 1510; $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ J 12.13 (br s, 1H, N-H), 9.68 (d, J 11.6 Hz, N-H) 8.72 (d, J 8.5 Hz, 1H, N-H), 7.81 (d, J 8.3 Hz, 1H, Ar-H), 7.68 (d, J 7.8 Hz, 1H, Ar-H) 7.54 (t, J 7.8 Hz, 1H, Ar-H), 7.47(d, J 7.8, 1H, Ar-H), 7.42 (d, J 1 Hz , Ar-H), 7.29 (m, 1H, Ar-H), 7.14 (t, J 7.7 Hz, 1 H, Ar-H), 7.04 (t, J 7.8 Hz, 1H, Ar-H), 4.25 (q, J 7.3 Hz, 2H, OCH$_2$), 4.21 (dd, J 2.7, 8.7 Hz, 2H, NCH$_2$), 4.14 (m, 4H, 2 x POCH$_2$), 1.32 (t, J 7.0 Hz, 9H, 3 x CH$_3$) 1.27 (t, J 7.3 Hz, 3H, CH$_3$). EIMS (m/z, relative intensity) cal for C$_{22}$H$_{28}$O$_7$N$_3$P: 477.2; found 477.4 (6%). Anal. Calcd. for C$_{22}$H$_{28}$O$_7$N$_3$P: C, 55.34; H, 5.91; N, 8.80% Found: C, 55.74; H, 5.87; N, 8.90%

Ethyl N-{$\{\text{2-[(2-(diphenylphosphate)amidobenzoyl)amino\text{-}benzoyl}\text{glycinate}}$ (18). A solution of azide 11 (0.367 g, 1 mmol) and triphenyl phosphite ( 0.62 g, 1.1 mmol) in mesitylene (5 mL) was stirred at 60 °C for 5 h After concentration, the residue was stirred in wet-THF containing catalytic amount of PhSO$_3$H for 3 h. The reaction mixture was concentrated. The residue was purified by chromatography on silica gel (50% ethyl acetate in hexane) to afford 18 (0.35 g, 61%). mp 144-145 °C; IR (KBr disk, cm$^{-1}$) 3357 (N-H), 3061, 2995, 1742, 1656, 1644, 1597, 1529; $^1$H NMR (300 MHz, CDCl$_3$) $\delta$ 12.10 (br s, 1H,N-H), 10.28 (d, J 12 Hz, N-H), 8.65 (d, J 7.8 Hz, 1H, N-H), 7.82 (d, J 8.1 Hz, 1H, Ar-H), 7.75 (d, J 7.8 Hz, 1H, Ar-H), 7.63 (d, J 7.7 Hz, 1H, Ar-H), 7.53 (t, J 7.7 Hz, 1H, Ar-H), 7.49 (t, J 7.71 Hz, 1H, Ar-H), 7.33-7.10 (m, 13H), 4.26-4.21 (m, 4H, CH$_2$), 1.31 (t, J 7.2 Hz, 3H, CH$_3$). Anal. Calcd. for C$_{30}$H$_{28}$O$_7$N$_3$P: C, 62.82; H, 4.92; N, 7.33% Found: C, 62.34; H, 5.18; N, 7.42%

7-Ethoxyquinazolino[3,2-{$d$}]1,4-benzodiazepin-9(7H)-one (19) and quinazolino[3,2-{$d$}]1,4-benzodiazepine-7,9(5H,7H)-dione (20). A mixture of azide 11 (0.367 g, 1 mmol) and phosphorus(III) reagent (1.1 mmol) in mesitylene (10 mL) was stirred at room temperature for 2 h then heated to reflux for 40-60 h. After concentration, the residue was purified on silica gel (20% ethyl acetate in hexane) to give 19 (15-25%). mp 165-167 °C, IR (KBr, cm$^{-1}$) 1670 (C=O), 1655 (C=N) 1602 (C=N), 1581, 1556, 1474, 1264; $^1$H NMR (300 MHz, CDCl$_3$) $\delta$ 8.35 (d, 10.0 Hz, 1H, Ar-H), 8.16 (dd, J 1.3, 7.9 Hz, 1H, Ar-H) 7.83-7.77 (m, 2H, Ar-H), 7.59-7.48 (m, 2H, Ar-H), 7.32 (dt, J 1, 7.6 Hz, 1H, Ar-H), 7.25 (d, J 8.7, 1H, Ar-H), 5.87 (d, J 13.2, 1H, NCH$_2$), 4.47 (br s, 1H, OCH$_2$), 4.24 (br s, 1H, OCH$_2$), 3.83 (d, J 13.2 Hz, 1H, NCH$_2$), 1.38 (t, J 7.10 Hz, 3H, -CH$_3$); $^{13}$C-NMR (75 MHz, CDCl$_3$) $\delta$ 172.4, 171.0, 153.6, 148.4, 147.1, 135.0, 132.3, 131.8, 128.2, 127.5, 127.2, 127.0, 125.1, 120.2, 110.0, 73.9, 41.1, 14.4. Further eluting with (35% ethyl acetate in hexane) furnished 20 (20-25%). The spectroscopic data for this compound were identical to those reported.$^{14,20}$

**General procedure for synthesis of 20**

A mixture of azide 11 (0.367 g, 1 mmol) and phosphorus(III) reagent (1.1 mmol) in mesitylene (5 mL) was stirred at room temperature for 2 h then heated to reflux for 40-60 h. After concentration, the residue was stirred in wet-THF containing catalytic amount of PhSO$_3$H for
3 h. The reaction mixture was concentrated and then the residue was purified on silica gel (35% ethyl acetate in hexane) to give 20 (15-40%).

**General procedure for synthesis of 20 using sealed tube reactions**

A tube containing a pulverized mixture of azide 11 (0.367 g, 1 mmol) and phosphorus(III) reagent (1.1 mmol) and mesitylene (5 mL) was sealed under reduced pressure and kept in oven at 190-200 °C for 5 h. The tube was cooled, wrapped with towel and crushed. The crude reaction mixture was stirred in wet-THF containing catalytic amount of PhSO_3H for 3 h. Purification by chromatography on silica gel (20 % ethyl acetate in hexane) afforded 7. Further eluting with (35% ethyl acetate in hexane) furnished 20 (20-52%).

**6-(Diethylamino)-quinazolino[3,2-d]-1,4-benzodiazepin-9(7H)-one (21).** Triethyl- amine (0.808 g, 8 mmol), triphenylphosphine (0.786 g, 3 mmol) and iodine (0.761 g, 3 mmol) were added to benzodiazepine 3 (0.295 g, 1.06 mmol) in dry CH_2Cl_2 (20 mL). The reaction mixture was stirred for 24 h at room temperature. The mixture was washed with water (20 mL). The organic layer was dried over MgSO_4 and concentrated. The residue was purified on silica gel column (% ethyl acetate in hexane: 20%) to give 21 (0.173 g, 49%). mp 144-145 °C; IR (KBr disk, cm⁻¹) 1674 (C=O), 1608 (C=N), 1578 (C=N), 1557, 770; ¹H NMR (300 MHz, CDCl₃) δ 8.31 (d, J 7.8 Hz, 1H, Ar-H) 8.12 (dd, J 1.41, 7.82 Hz, 1H, Ar-H), 7.82-7.74 (m, 2H, Ar-H), 7.50-7.45 (m, 2H, Ar-H), 7.17-7.11 (m, 2H, Ar-H), 5.97 (d, J 13.9, 1H, -CHH-), 4.32 (br.s, 1H, -CHH-), 3.81 (br.s, 1H, -CHH-), 3.81 (d, J 13.9 Hz, -CHH-), 3.27 (br.s, 2H, -CH₂-), 1.26 (br s, 6H, 2xCH₃); ¹³C-NMR (75 MHz, CDCl₃) δ 160.83, 155.91, 154.58, 149.37, 148.28, 134.51, 131.86, 131.03, 127.83, 127.11, 126.78, 126.34, 125.81, 122.12, 119.59, 43.47, 42.81, 37.76, 15.68, 12.81. MS(EI) m/z (relative intensity %): 332.4 (M⁺ (C₂₀H₂₀ON₄)), 75), 303.3 (100), 289.2 (7) 260.3 (54), 234.3 (44).

**6-(Dibutylamino)-quinazolino[3,2-d]-1,4-benzodiazepin-9(7H)-one (22).** Tributylamine (1.48 g, 8 mmol), triphenylphosphine (0.786 g, 3 mmol) and iodine (0.761 g, 3 mmol) were added to a solution of 20 (0.295 g, 1 mmol) in dry CH_2Cl_2 (20 mL). The resulting mixture was stirred for 24 h. The reaction mixture was washed with water (20 mL). The organic layer was dried over MgSO_4 and concentrated. The residue was purified on silica gel column (% ethyl acetate in hexane: 20%) to give 22 (0.106 g, 26%). ¹H NMR (300 MHz, CDCl₃) δ 8.32 (d, J 7.8 Hz, 1H, Ar-H) 8.12 (d, J 7.7 Hz, 1H, Ar-H), 7.83-7.75 (m, 2H, Ar-H), 7.47 (dt, J 1.6, 7.1 Hz, 2H, Ar-H), 7.17-7.11 (m, 2H, Ar-H), 5.98 (d, J 13.9 Hz, 1H, -CHH-), 4.33 (br., 1H, -CHH-), 3.82 (br., 1H, -CHH-), 3.80 (d, J 13.9 Hz, -CHH-), 3.40 (br.s, 2H, -CH₂-), 1.76-0.88 (br., 6H, 2xCH₃); ¹³C-NMR (75 MHz, CDCl₃) δ 160.86, 156.24, 154.63, 149.43, 148.27, 134.51, 131.83, 130.98, 127.82, 127.11, 126.78, 126.32, 125.72, 122.00, 119.55, 49.54, 48.20, 37.85, 32.86, 29.97, 29.55, 29.51, 20.19, 13.95.

**Methyl N-[2-[(2-azidobenzoyl)amino]benzoyl]tryptophanate (23).** Tryptophan methyl ester was dissolved in acetonitrile, then triethylamine (5.5 g, 37.5 mmol) was added dropwise with stirring to the reaction mixture at 0 °C, followed by addition of isatoic anhydride 7 (4.0 g, 25.0 mmol) with stirring. The reaction mixture was refluxed for 3 h. The reaction mixture was
cooled to 0 °C then triethylamine (3.5 mL, 25.0 mmol) was added dropwise. Freshly prepared 2-
azidobenzoyl chloride (25 mmol) dissolved in acetonitrile was added dropwise to the above
solution at 0 °C with stirring over 10 min. The mixture was stirred at room temperature for 36 h.
The solvent was evaporated under reduced pressure. The crude product 23 was dissolved in ethyl
acetate (200 mL) and water (50 mL). The organic layer was separated, dried over MgSO₄,
filtered and concentrated. Purification of the residue by column chromatography on silica gel
(30% ethyl acetate in hexane) afforded pure 23 (7.72 g, 80%). IR (KBr disk,) 3290 (N-H), 3051,
2949, 2160 (N₃), 1738 (C=O), 1668 (C=O) and 1652 cm⁻¹ (C=O); ¹H NMR (400 MHz, CDCl₃) δ
11.56 (s, 1H, NH), 8.67 (d, J 8 Hz, 1H, NH), 8.17 (s, 1H, NH), 7.90 (dd, J 8, 2 Hz, 1H, Ar-H),
7.47 (m, 3H, Ar-H) 7.22 (m, 4H, Ar-H), 7.13 (t, J 8 Hz, 1H Ar-H), 7.00 (t, J 8 Hz, 1H, Ar-H),
6.96 (t, J 8 Hz, 1H, Ar-H), 6.90 (d, J 2 Hz, 1H, Ar-H), 6.67 (d, J 8 Hz, 1H, Ar-H), 5.04 (dd, J 8,
5 Hz, 1H, NCH), 3.69 (s, 3H, OCH₃), 3.42 (dd, J 15, 5 Hz, 1H, CHH), 3.36 (dd, J 15, 5 Hz, 1H,
CHH); ¹³C-NMR (75 MHz, CDCl₃) δ 172.0, 168.2, 164.2, 138.9, 137.6, 136.1, 132.6, 132.3,
131.2, 127.5, 127.2, 126.9, 125.0, 123.3, 122.8, 122.4, 122.3, 121.7, 119.9, 119.2, 118.5, 111.3,
109.8, 53.4, 52.6, 27.6.

Asperlicin D (4)
A mixture of 23 (0.468 g, 1 mmol) and Ph₃P or Bu₃P (1 mmol) in mesitylene (10 mL) was
heated at 150 °C for 18 h, then the solvent was evaporated. The residue was stirred in H₂O-THF-
PhSO₃H(cat) for 3 h. The reaction mixture was concentrated and residue was purified by column
chromatography on silica gel (60% ethyl acetate in hexane) furnishing 4¹³,¹⁴ (30-52%).

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