# Synthesis of the diketopiperazine dipodazine

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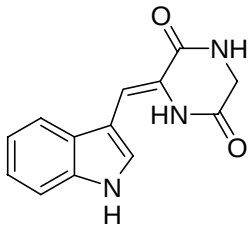
**Abstract**
The diketopiperazine derivative dipodazine (1), isolated from *Penicillium dipodomyis*, has been synthesized via a stereoselective aldol condensation from *N*-protected indole-3-carboxaldehyde and 1,4-diacetyl-2,5-piperazinedione (3) in the presence of cesium carbonate.

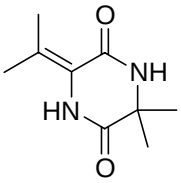
**Keywords:** Dipodazine, aldol condensation, 2-benzenesulfonylethyl protection

## Introduction

The mold Penicillium dipodomyis, isolated from seed caches and cheek pouches of kangaroo rats, and *Penicillium nalgiovese*, from mould-fermented sausages, both produce blue-fluorescent metabolites, such as 3-[1-(1*H*-indol-3-yl)-meth-(*Z*)-ylidene]-piperazine-2,5-dione (**1**), or dipodazine (Figure 1). Dipodazine (**1**) has been isolated and characterized as a major metabolite from *P. dipodomyis*, and is also present in the meat-associated *P. nalgiovese*.[1] Dipodazine (**1**) is a cyclic dipeptide composed of dehydrotryptophan and glycine. Structures related to **1** are known in the literature, and this particular molecule **1** has been mentioned as an intermediate in synthetic studies of euchinulines.[2] However, at that time no spectral or physical data for dipodazine (**1**) were provided.




**1**




**2**

**Figure 1**

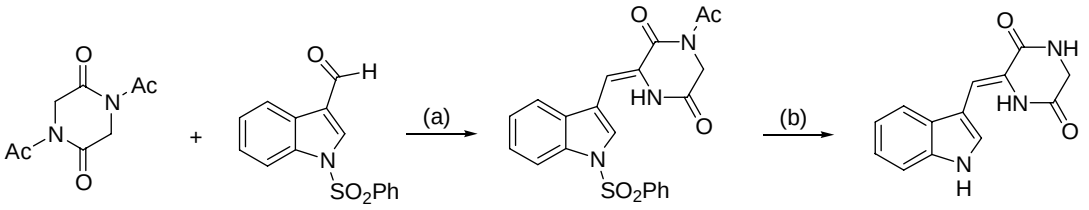
 

Due to steric effects, 3-ylidenepiperazine-2,5-diones, like **2** (Figure 1), are usually more stable in the *Z*-configuration[3] and dipodazine (**1**) is not an exception, as the stereochemistry of the molecule was deduced as *Z*-, based upon spectral data.[1]

## Results and Discussion

Herein, we report a concise synthesis of dipodazine (**1**), which also confirms the structural assignment of the natural product. 3-Ylidenepiperazine-2,5-diones, *e.g.,* (**2**), can either be constructed from an open-chain dehydroamino acid precursor, or from a preformed diketopiperazine with subsequent introduction of the unsaturated side chain.[3] Since dehydrotryptophan is not readily available, the former method is of limited applicability in this particular case. Commonly used methods to introduce ylidene groups to diketopiperazines encompass Wittig type reactions,[4] or aldol condensations, usually performed in basic media[5] or in NaOAc–$Ac_2O$.[6] The most straightforward synthetic route towards 1 would include an aldol condensation between an *N*-protected indole-3-carbaldehyde and the readily available partner 1,4-diacetyl-2,5-piperazinedione (**3**)[7].

Condensation of **3** with (1-benzenesulfonyl)indole-3-carbaldehyde[8] (**4**) using cesium carbonate[9] as the base exclusively afforded the *Z*- form of **5** in 77% isolated yield. The *Z*-configuration was deduced from a ROESY-experiment, which displayed a cross-peak indicating a NOE between the 2-hydrogen of the indole unit and the 4-hydrogen of the diketopiperazine. The anticipated loss of one acetyl group was also observed.[5] This concise synthesis was completed by removal of both protecting groups using ethanolic aqueous NaOH to give dipodazine (**1**). The spectral and physical data of synthetic dipodazine (**1**) were identical in all aspects to those reported for the natural product.[1]

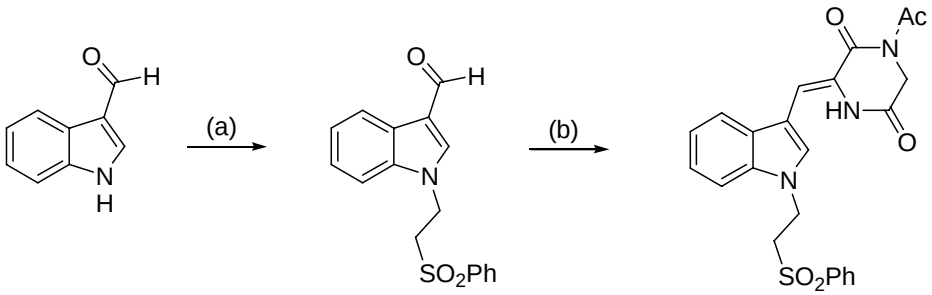



**3** **4** **5** (77%) **1** (53%)

**Scheme 1.** (a) $Cs_2CO_3$, 4Å ms, DMF, rt, 18h. (b) NaOH, $H_2O$/EtOH, reflux, 3h.

In an extension of this study, the potential use of the 2-benzenesulfonylethyl group for *N*-protection was also evaluated, as it has been only rarely used for protection of indoles and only a few examples are reported in the literature.[10,11] To explore the features and applicability of this particular protecting group further, 1-(2-benzensulfonylethyl)-1*H*-indole-3-carbaldehyde[12] (**6**)

was subjected to the above- mentioned aldol condensation with the diketopiperazine **3**, which gave the expected product **7**, although the yield was significantly lower than for the benzenesulfonyl analogue. Also in this case, only the *Z*-isomer was produced (Scheme 2). Deprotection of **7** proved to be difficult, as both methods reported in the literature, *i.e*., NaH- or t-BuOK in DMF, failed to produce the desired natural product **1**. The latter method did, in fact, prove to be unsuitable for systems like **7**, since t-BuOK cleaves the double bond at the same time as the 2-benzenesulfonylethyl group is removed, giving indole-3-carbaldehyde as the major product.




**6** (97%) **7** (12%)

**Scheme 2.** (a) 2-Chloro-1-phenylsulfonylethane, $Bu_4NHSO_4$, KOH, $CH_2Cl_2$, rt, 3h. (b) $Cs_2CO_3$, 4Å ms, DMF, 24 h, rt.

## Experimental Section

**General Procedures.** NMR spectra were recorded at 300 MHz for $^1H$, and 75 MHz for $^{13}C$, respectively. Coupling constants are given in Hz. The IR spectra were acquired using a FT-IR instrument. Elemental analyses were performed by H. Kolbe Mikroanalytisches Laboratorium, Mülheim an der Ruhr, Germany. Melting points were determined on a capillary melting point apparatus or a Kofler hot stage and are uncorrected. All reagents were purchased from Aldrich or Lancaster and were used as received. All solvents were purified by distillation or were of analytical grade.

**1-Acetyl-3-[1-(1-benzenesulfonyl-1*H*-indol-3-yl)meth-(*Z*)-ylidene]-piperazine-2,5-dione(5).** Benzenesulfonyl-1*H*-indol-3-yl-carbaldehyde [8] (**4**) (570 mg, 2.0 mmol) and 1,4-piperazine-2,5-dione (**3**) (792 mg, 4.0 mmol) were dissolved in dry DMF (5 mL) containing 4Å molecular sieves. $Cs_2CO_3$ (717 mg, 2.2 mmol) was added to the solution and the reaction mixture was stirred at room temperature for 18 h. The molecular sieves were removed by filtration, and water (20 mL) was added to the filtrate. The resulting precipitate was collected and washed with water to afford 5 as a yellow solid (650 mg, 77%). Mp: 252.5–254.0°C. IR (KBr): 3488, 1715, 1682,

1634, 1448, 1376, 1228, 1173, 1137, 1096, 980, 750, 726, 568 $cm^{-1}$. $^1$H-NMR (DMSO-$d_6$) δ 10.56 (s, 1H), 8.45 (s, 1H), 8.12–8.09 (m, 2H), 7.95 (d, *J* = 8.2, 1H), 7.78–7.70 (m, 2H), 7.64–7.59 (m, 2H), 7.44–7.32 (m, 2H), 7.10 (s, 1H), 4.41 (s, 2H), 2.52 (s, 3H). $^{13}$C-NMR (DMSO-$d_6$) δ 171.8 (s), 164.4 (s), 161.3 (s), 136.7 (s), 134.9 (d), 133.5 (s), 129.9 (d), 129.6 (s), 127.2 (s), 127.0 (d), 126.7 (d), 125.5 (d), 124.0 (d), 119.7 (d), 114.3 (s), 113.1 (d), 108.2 (d), 45.7 (t), 26.7 (q). MS (ESI) m/z 424 $(M+H)^+$. Anal. Calcd. for $C_{21}H_{17}N_3O_5S$: C, 59.57; H, 4.05; N 9.92. Found: C, 59.46; H, 4.04; N 10.03%.

**3-[1-(1*H*-indol-3-yl)-meth-(*Z*)-ylidene]-piperazine-2,5-dione, dipodazine (1).** Compound **5** (150 mg, 0.35 mmol) was dissolved in a mixture of $H_2O$ and EtOH (10 mL/10 mL) containing NaOH (238 mg, 5.95 mmol). The resulting solution was heated at reflux for 3 h, cooled on ice and thereafter acidified to pH~4 with AcOH. After evaporation of the EtOH, 5 mL of $H_2O$ was added. The precipitated yellow solid was collected to afford the title compound **1** (45 mg, 53%). Mp: ~285°C (dec.). IR (KBr): 3391, 3189, 3052, 1693, 1654, 1622, 1458, 1233, 741 $cm^{-1}$. $^1$H-NMR (DMSO-$d_6$) δ 11.63 (s, 1H), 9.47 (s, 1H), 8.11 (s, 1H), 7.94 (d, J = 2.5, 1H), 7.65 (d, J = 7.6, 1H), 7.44 (d, J = 7.8, 1H), 7.20 (m, 2H), 7.01 (s, 1H), 4.01 (d, J = 1.7, 2H).

**1-(2-Benzenesulfonyl-ethyl)-1*H*-indole-3-carbaldehyde (6).** A mixture of 1H-indole-3-carbaldehyde (2.90 g, 20 mmol), $Bu_4NHSO_4$ (0.68 g, 2.0 mmol), 2-chloro-1-benzenesulfonylethane (4.92 g, 24 mmol) and aqueous KOH (50%, 30 mL) in $CH_2Cl_2$ (100 mL) was vigorously stirred for 4.5 h at room temperature. The organic phase was separated from the mixture, dried over $MgSO_4$ and evaporated to afford a dark yellow oil. The crude product was subjected to gradient column chromatography starting with n-hexane/ethyl acetate (60:40) with increasing amounts of ethyl acetate to yield the title compound **6** (6.08 g, 97%) as a beige solid. Mp: 128–129°C. IR (KBr): 1655, 1536, 1466, 1402, 1304, 1136, 756, 685, 536 $cm^{-1}$. $^1$H-NMR (DMSO-$d_6$) δ 9.82 (s, 1H), 8.19 (s, 1H), 8.04–8.02 (m, 1H), 7.80–7.77 (m, 2H), 7.68–7.63 (m, 1H), 7.54–7.54 (m, 3H), 7.31–7.23 (m, 2H), 4.66 (t, J=6.5, 2H), 4.05 (t, J = 6.5, 2H). $^{13}$C-NMR (DMSO-$d_6$) δ 184.5 (d), 140.3 (d), 138.7 (s), 136.6 (s), 133.8 (d), 129.2 (d), 127.3 (d), 124.5 (s), 123.6 (d), 122.5 (d), 121.0 (d), 117.4 (s), 110.8 (d), 53.5 (t), 40.3 (t). MS (ESI) *m/z* 314 $(M+H)^+$. Anal. Calcd. for $C_{17}H_{15}NO_3S$: C, 65.16; H, 4.82; N 4.47. Found: C, 64.97; H, 4.75; N 4.36%.

**1-Acetyl-3-[1-[1-(2-benzenesulfonyl-ethyl)-1*H*-indol-3-yl]-meth-(*Z*)-ylidene]-piperazine-2,5-dione (7).** 1-(2-Benzenesulfonyl-ethyl)-1H-indole-3-carbaldehyde (**6**) (1.06 g, 3.39 mmol) and 1,4-piperazine-2,5-dione (**3**) (1.34 mg, 6.78 mmol) were dissolved in 5 mL of dry DMF together with 4Å molecular sieves. $Cs_2CO_3$ (1.21 g, 3.73 mmol) was added to the solution and the reaction mixture was stirred at room temperature for 18 h. The molecular sieves were filtered off and $H_2O$ (15 mL) was added to the residue. The mixture was extracted with CH2Cl2 (3×30 mL) and the combined organic phases were washed with $H_2O$ (20 mL), brine (20 mL), dried over $MgSO_4$ and finally evaporated. The resulting brown, oily residue was treated with MeOH and **7** was collected by filtration as a yellow solid. Yield: 185 mg (12%). Mp: 230.0–232.0°C. IR (KBr): 1684, 1625, 1439, 1364, 1255, 1212, 1139, 736 $cm^{-1}$. $^1$H-NMR (DMSO-$d_6$) δ 9.71 (s, 1H), 8.01 (s, 1H), 7.83–7.81 (m, 2H), 7.70–7.64 (m, 2H), 7.54–7.49 (m, 2H), 7.40 (d, J = 8.1, 1H), 7.26–7.13 (m, 3H), 4.60 (t, J = 6.7, 2H), 4.39 (s, 2H), 4.04 (t, J = 6.7, 2H), 2.51 (s, 3H). $^{13}$C-

NMR (DMSO-$d_6$) δ 171.8 (s), 164.0 (s), 161.7 (s), 138.6 (s), 135.2 (s), 133.8 (d), 130.4 (d), 129.2 (d), 127.5 (s), 127.3 (d), 122.6 (d), 122.3 (s), 120.8 (d), 118.2 (d), 111.9 (d), 110.2 (d), 108.0 (s), 53.9 (t), 45.7 (t), 39.9 (t), 26.6 (q). MS (ESI) m/z 450 $(M–H)^-$.

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