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Asymmetric synthesis of *aza*-diospongin A as an iNOS inducer

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ABSTRACT

The synthesis of the *aza*-analogue of diospongin A is described. The key steps in the synthetic sequence are Mitsunobu inversion, cross olefin metathesis and intramolecular *aza*-Michael addition reactions. The biological activity of this new analogue was also evaluated in the induction of nitric oxide synthase and was found to be better when compared to its natural counterpart.

1. Introduction

Diospongins **1a** and **1b** are a new family of diaryl heptanoids, which were isolated from the rhizomes of *Dioscorea spongiosa* in 2004 by Kadota et al.[1] They are known to exhibit potent anti-osteoporotic activity in a bone organ culture. Due to the relatively simple structure and interesting biological activities of diospongins, they have attracted the attention of synthetic organic chemists; various approaches leading to diospongin A and/or B have been reported.[2] Apart from the natural derivatives, the synthesis of unnatural derivatives (analogues) is also equally important; such analogues are useful for the elucidation of structure–activity relationships (SARs) and may lead to more active derivatives demonstrated by the recent synthesis of *aza*-analogues of various natural molecules.[3]

The structure of the diospongins containing a tetrahydropyran ring with two phenyl groups and three asymmetric centres is a tunable scaffold for the synthesis of various analogues. Recently, Uenishi et al. have synthesized C-5 stereoisomers of (−)-diospongins A and B along with the natural compounds (Fig. 1).[4] Our interest in the synthesis of bio-active molecules and their analogues,[5] has directed us towards the synthesis of diospongin analogues. Herein, we report on the synthesis of a new type of diospongin analogue, *aza*-(−)-diospongin **2**, which is based on a piperidine skeleton rather than the natural tetrahydropyran scaffold. (Scheme 1). The *aza*-fragment can be synthesized via compound **4** from allyl alcohol **3** using Mitsunobu inversion and olefin cross-metathesis reaction as the key steps.

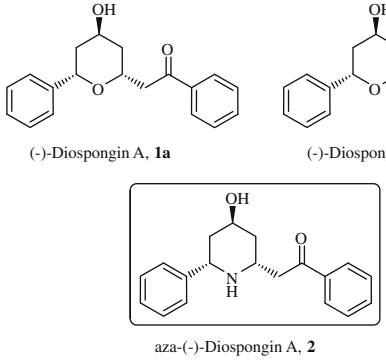
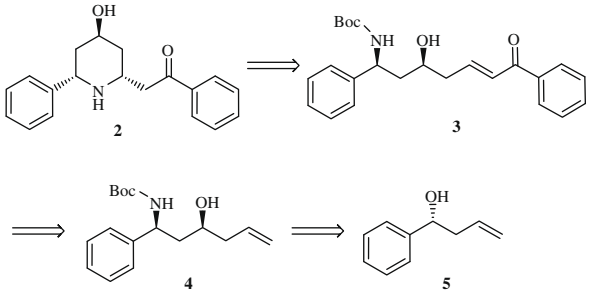


Figure 1. Structures of (−)-diospongin A, B and *aza*-(−)-diospongin A.



Scheme 1. Retrosynthetic analysis of *aza*-(−)-diospongin A.

2. Results and discussion

The synthesis began with (*R*)-phenylbutenol **5**, which can be readily obtained from benzaldehyde via a Keck allylation.[6] Alcohol **5** underwent Mitsunobu inversion with hydrazoic acid to provide azide[7] **6** in 54% yield. The reduction of the azide functionality to an amine using $LiAlH_4$ followed by protection of the primary amine with $(Boc)_2O$ afforded the protected homoallyl amine **7**. To prepare the precursor for olefin cross-metathesis, the terminal olefin of protected homoallyl amine **7** was subjected to dihydroxylation

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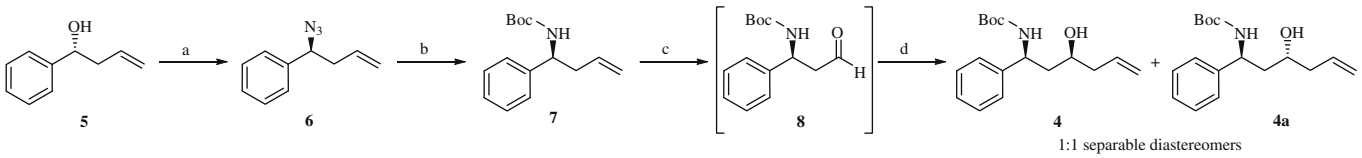
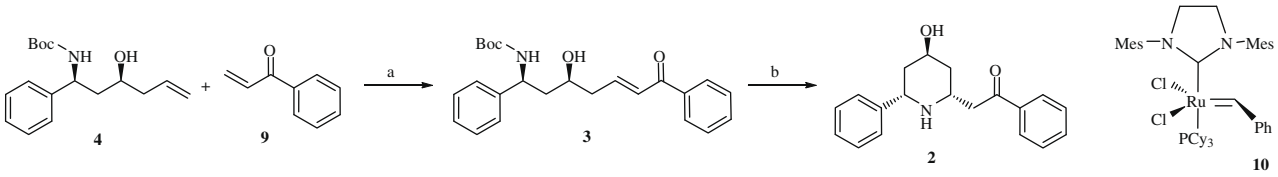


Scheme 2. Reagents and conditions: (a) HN_3, DIAD, TPP, benzene, rt, 3 h, 54%; (b) (i) $LiAlH_4$, THF, 0 °C to rt, 1 h, (ii) $(Boc)_2O$, 3 h, 90% (for two steps); (c) (i) NMO, OsO_4 (cat), acetone–water (4:1), rt, over night, (ii) NaIO4, THF, rt, 15 min, 95% (for two steps); (d) allyl bromide, Zn, DMF, rt, 30 min, 70%.



Scheme 3. Reagents and conditions: (a) **10** (20 mol %), CH_2Cl_2, rt, 1 h, 70%; (b) TFA, CH_2Cl_2, 0 °C, 5 h, 96%.

followed by periodate cleavage to give aldehyde **8**, which was immediately treated with allyl bromide in the presence of zinc.[8] The allylation of aldehyde **8** provided a mixture of diastereomers **4** and **4a** in a 1:1 ratio, which were separated by column chromatography (Scheme 2). The required isomer 1,3-amino alcohol **4** was submitted to olefin cross-metathesis with phenyl vinyl ketone[9] **9** using Grubb's 2nd generation catalyst (20 mol %) in dichloromethane. The reaction proceeded in excellent yield of the enone product **3**, which was ready for *aza*-Michael addition reaction. As expected, amino enone **3** underwent Boc-deprotection followed by *aza*-Michael reaction in one-pot to afford the target product, *aza*-diospongin-A **2** in 96% yield (with 99% enantiomeric purity)[10] with the exclusive formation of *cis*-isomer (Scheme 3). The structure of the product was confirmed by IR, Mass, ^{1}H NMR and ^{13}C NMR spectroscopic data. The *cis*-configuration of 2,6-substituents was confirmed by NOE studies.[11]

A biological profile of this analogue was also evaluated towards the induction of inducible nitric oxide synthase. Table 1 clearly illustrates that **2** showed the best induction of inducible nitric oxide synthase/nitric oxide synthase **2** (iNOS)[12] at 1 μM. The decreased biological effect observed at 10 μM might be related to the cytotoxic properties of **2**. At the same time, the compound failed to elicit any detectable increase in the immunostimulatory potential at 0.1 μM demonstrating the requirement of minimum threshold concentration required for the transcriptional activation of iNOS gene. It might be possible to argue that the immunomodulatory potential of compound **2,** which maintains improved bioactivity while showing reduced levels of cellular toxicity when compared to **1a**, is superior and can be enhanced by synthesizing the corresponding analogues.

Table 1
Nitrite production and the corresponding fold activation of compounds **1a** and **2**

Compound no.	Dose tested (μM)	Nitrite (mM) produced	Fold induction with respect to control
2	10	8.88	1.605
2	1	24.65	4.457
2	0.1	16.4	2.965
1a	10	8.81	1.593
1a	1	13.44	2.430
1a	0.1	11.79	2.132
IFN-γ		12.8	2.314
LPS+IFN-γ		60.93	11.018
Controls		11.01	1.990
Contorls DMSO		5.53	1

See the text for details. Cells were treated with 1 ng/mL IFNγ in order to prime the cells before treatment with compounds.
Abbreviations: BHK-21 (baby hamster kidney)-21; iNOS/NOS2: inducible nitric oxide synthase/nitric oxide synthase **2**; LPS: lipopolysaccharide; IFNγ: interferon gamma; ng: nanogram.

3. Conclusions

In conclusion, the *aza*-derivative of (−)-diospongin-A has been synthesized; this is the first *aza*-analogue of this class of molecules. The key steps of this synthesis are the Mitsunobu inversion, cross olefin metathesis and intramolecular *aza*-Michael addition reactions. The, *aza*-analogue of diospongin A was tested towards the induction of the inducible nitric oxide synthase enzyme by comparison with the natural diospongin-A and it was found that the *aza*-analogue showed a better activity when compared to the natural one.

4. Experimental section

4.1. General

All solvents and reagents were purified by standard techniques. Crude products were purified by column chromatography on silica gel of 60–120 mesh. IR spectra were recorded on Perkin–Elmer 683 spectrometer. Optical rotations were obtained on Perkin–Elmer digital polarimeter. ^{1}H and ^{13}C NMR spectra were recorded in $CDCl_3$ solution on a Varian Gemini 200 and Brucker Avance 300. Chemical shifts were reported in parts per million (ppm) with respect to internal TMS. Coupling constants (*J*) are quoted in Hz. Mass spectra were obtained on an Agilent Technologies LC/MSD Trap SL.

4.1.1. (*S*)-(1-Azidobut-3-enyl) benzene 6

To a solution of **5** (2.0 g, 0.01 mol) and TPP (5.3 g, 0.02 mol) in benzene (30 mL) under N_2 was added HN_3 (20.2 mL, 40.5 mmol) in benzene. A solution of DIAD (3.5 mL, 17.5 mmol) in benzene was added slowly via syringe resulting in a cloudy mixture. The reaction mixture was stirred at rt for 3.5 h, after which ethyl acetate was added (200 mL) and the solutions were washed with 1 M NaOH (3 × 100 ml) and dilute brine (100 mL). The organic layer was dried over Na_2SO_4 and concentrated in vacuo to obtain crude liquid which was purified by column chromatography eluting with hexanes/EtOAc (98:2) to give **6** as a pale yellow oil (1.25 g, 1.1 mmol, 54%). and **5** was recovered in 48%. $[\alpha]_D^{25} =$

−92.9 (*c* 1.0, $CHCl_3$), ^1H NMR (300 MHz, $CDCl_3$): δ 2.64–2.4 (m, 2H), 4.48 (t, *J* = 7.5 Hz, 1H), 5.15–5.02 (m, 2H), 5.8–5.62 (m, 1H), 7.4–7.23 (m, 5H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 40.5, 65.7, 118.1, 126.8, 128.2, 128.7, 133.6, 139.1. IR (neat): γ 2925, 2095, 1247, 700 cm^{-1}; MS-ESI: (*m*/*z*) 146.1 $[M-28+H]^+$.

4.1.2. (*S*)-*tert*-Butyl 1-phenylbut-3-enylcarbamate 7

A solution of compound **6** (2.5 g, 14.4 mmol) in THF (20 mL) was added to a suspension of $LiAlH_4$ (0.8 g, 21.6 mmol) in THF (3 mL) slowly at 0 °C. The reaction mixture was stirred at rt for 1 h and then the reaction was quenched with 15% NaOH (0.8 mL) and water (2.5 mL). A solution of $(Boc)_2O$ (4.9 mL, 21.6 mmol) in THF (5 mL) was then added and stirred for another 3 h and was filtered through Celite. The filtrate was diluted with EtOAc (50 mL) and the organic layer was washed with brine (50 mL), dried over Na_2SO_4 and concentrated in vacuo to obtain the crude compound, which was purified by column chromatography eluting with hexanes/EtOAc (85:15) to give **7** (3.1 g, 12.6 mmol, 90% over two steps) as an off-white powder: Mp: 64–66 °C. $[\alpha]_D^{25} = -40.5$ (*c* 1.0, $CHCl_3$), ^1H NMR (300 MHz, $CDCl_3$): δ 1.43 (br s, 9H), 2.54 (merged t, 2H), 4.84–4.61 (m, 2H), 5.15–5.02 (m, 2H), 5.75–5.56 (m, 1H), 7.34–7.16 (m, 5H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 28.3, 41.1, 54.0, 79.4, 118.0, 126.1, 127.0, 128.4, 133.9, 142.4, 155.1; IR (KBr): 3388, 2980, 2934, 1683, 1516, 1271, 1174, 754, 700 cm^{-1}; MSESI: (*m*/*z*) 248 $[M+H]^+$; HRMS: (*m*/*z*) calcd for $C_{15}H_{21}NO_2Na$, 270.1469; found 270.1462 $[M+Na]^+$.

4.1.3. *tert*-Butyl (1*S*,3*S*)-3-hydroxyl-1-phenylhex-5-enylcarbamate 4

To a solution of compound **7** (1.0 g, 4.0 mmol) in acetone–water (4:1, 20 mL) were added NMO (0.82 g, 6.0 mmol) and OsO_4 (50% sol in toluene, 14 mg, 0.05 mmol) and was stirred overnight. The solvent was evaporated in vacuo and the residue was dissolved in THF: water (9:1, 20 mL) and $NaIO_4$ (1.73 g, 8.0 mmol) were added and stirred for 15 min, the reaction mixture was filtered and the filtrate was washed with satd $NaHCO_3$ (25 mL), brine (25 mL), dried over Na_2SO_4 and concentrated in vacuo to obtain the crude solid as an off-white powder which was pure enough to carry out the next reaction without further purification. To a solution of **8** (0.7 g, 2.8 mmol) and allyl bromide (0.36 mL, 4.2 mmol) in DMF (5 mL) was added zinc (0.27 g, 4.2 mmol) and the reaction mixture was stirred for 0.5 h, satd NH_4Cl (10 mL) solution was poured and the compound was extracted with ether (3 × 30 mL). The organic layer was dried over Na_2SO_4 and concentrated in vacuo to obtain the crude solid which was purified by column chromatography eluting with hexanes/EtOAc (85:15) to give **4** and **4a** in 1:1 ratio (0.56 g,2.2 mmol, 70%) as an off-white powder: Mp: 95–97 °C; $[\alpha]_D^{25} = -12$ (*c* 1.0, $CHCl_3$); ^1H NMR (300 MHz, $CDCl_3$): δ 1.43 (br s, 9H), 1.55 (br s, 1H), 2.02–1.71 (m, 2H), 2.33–2.02 (m, 2H), 3.65–3.52 (m, 1H), 4.77 (br s, 1H), 5.18–5.03 (m, 3H), 5.84–5.65 (m, 1H), 7.35–7.16 (m, 5H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 28.3, 42.3, 43.7, 53.6, 68.6, 79.5, 118.3, 126.3, 127.2, 128.6, 134.2, 142.9, 155.4; IR (KBr): γ 3369, 3256, 2925, 1676, 1367, 1174, 1015, 703 cm^{-1}; MSESI: (*m*/*z*) 292 ($[M+H]^+$; HRMS: *m*/*z* calcd for $C_{17}H_{25}NO_3Na$ 314.1732; found: 314.1736 $[M+Na]^+$.

4.1.4. *tert*-Butyl (1*S*,3*S*)-3-hydroxy-7-oxo-1,7-diphenyl hept-5-enylcarbamate 3

To a solution of **4** (0.1 g, 0.3 mmol) and **9** (0.136 g, 10 mmol) in CH_2Cl_2 (1 mL) was added catalyst **10** (23 mg, 0.06 mmol) and was stirred at rt for 1 h. The solvent was evaporated in vacuo and the residue was purified by flash chromatography to give **3** (96 mg, 0.17 mmol, 70%) as an off-white powder: Mp: 143–146 °C; $[\alpha]_D^{25} = -17$ (*c* 0.25, $CHCl_3$), ^1H NMR (300 MHz, $CDCl_3$): δ 1.43 (s, 9H), 1.53 (br s, 1H), 2.15–1.8 (m, 2H), 2.57–2.41 (m, 2H), 3.76–3.85 (m, 1H), 4.77 (br s, 1H), 5.02 (br s, 1H), 7.1–6.86 (m, 2H), 7.6–7.2 (m, 8H), 7.96 (d, *J*= 7.3 Hz, 2H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 28.3, 41.1, 44.1, 68.6, 79.8, 126.4, 127.5, 128.5, 128.5, 128.5, 128.8, 132.8, 137.6, 145.0, 155.5, 190.4; IR (KBr): γ 3361, 3264, 2921, 1673, 1179, 1010, 697 cm^{-1}; MSESI: (*m*/*z*) 396 $[M+H]^+$; HRMS: (*m*/*z*) calcd for $C_{24}H_{29}NO_4Na$ 418.1994; found 418.1983 $[M+Na]^+$.

4.1.5. 2-((2*R*,4*S*,6*S*)-4-Hydroxy-6-phenylpiperidin-2-yl)-1-phenylethanone 2

To a solution of **3** (50 mg, 0.1 mmol) in CH_2Cl_2 (0.5 mL) at 0 °C was added TFA (0.04 mL, 0.5 mmol). After 5 h, the reaction was brought to pH 8–9 using satd $NaHCO_3$ and the compound was extracted with CH_2Cl_2 (3 × 3 mL). The organic layer was dried over Na_2SO_4 and concentrated in vacuo to obtain crude compound, which was purified by column chromatography eluting with hexanes/EtOAc 1:1 to give pure **2** (36 mg, 0.1 mmol, 96%) as an off-white powder; Mp: 148–150 °C; $[\alpha]_D^{25} = -23.7$ (*c* 1.25, $CHCl_3$); ^1H NMR (300 MHz, $CDCl_3$): δ 1.96–1.62 (m, 4H), 2.18 (br s, 3H), 3.18–3.11 (m, 2H), 3.9–3.8 (m, 1H), 4.35–4.23 (m, 2H), 7.37–7.21 (m, 3H), 7.52–7.38 (m, 4H), 7.58 (t, *J* = 7.5 Hz, 1H), 7.96 (d, *J* = 7.5 Hz, 2H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 38.9, 41, 45.2, 47.5, 55.5, 65.7, 127, 127.3, 128.2, 128.6, 128.8, 133.4, 137, 144.6, 199.4; IR (neat): γ 3449, 2926, 2859, 1754, 1248, 1080, 760, 695 cm^{-1}; MSESI: (*m*/*z*) 296 $[M+H]^+$; HRMS: *m*/*z* calcd for $C_{19}H_{22}NO_2$ 296.1650; found 296.1645 $[M+H]^+$.

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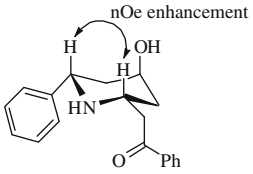
9. Compound **9** was prepared from benzaldehyde in two steps: addition of vinyl magnesium bromide followed by IBX oxidation. See ref: Wu, F.; Li, H.; Hong, R.; Deng, L. *Angew. Chem., Int. Ed.* **2006**, *45*, 947.
10. Enantiomeric excess was determined by chiral HPLC with chiral column: OD-H, 250 × 4.6 mm, 5 μm; mobile phase: 30% *i*-propanol in hexanes; flow rate: 1 mL/min.; detection: PDA; run time: 30 min.; retention time: 6.5.

11. NOE experiment has been carried out on *aza*-diospongin-A **2** and key NOE enhancements are shown below.



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