

## Article

# *N,N'*-Di-Boc-2H-Isoindole-2-carboxamide—First Guanidine-Substituted Isoindole

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**Abstract:** Synthesis of *N,N'*-Di-Boc-2H-isoindole-2-carboxamide, the first representative of isoindoles containing guanidine functionality, was carried out. The cycloaddition reactivity of this new Diels–Alder heterodiene was studied and the title compound was employed as a cycloaddition delivery reagent for guanidine functionality. Higher reactivity was found in comparison with the corresponding pyrrole derivative. Substitution with fluorine or guanidine functionality does not change the reactivities of isoindoles, and these findings are in good accord with computational results.

**Keywords:** cycloaddition; Diels–Alder reaction; heterocycles; organic synthesis; indoles; DFT calculations



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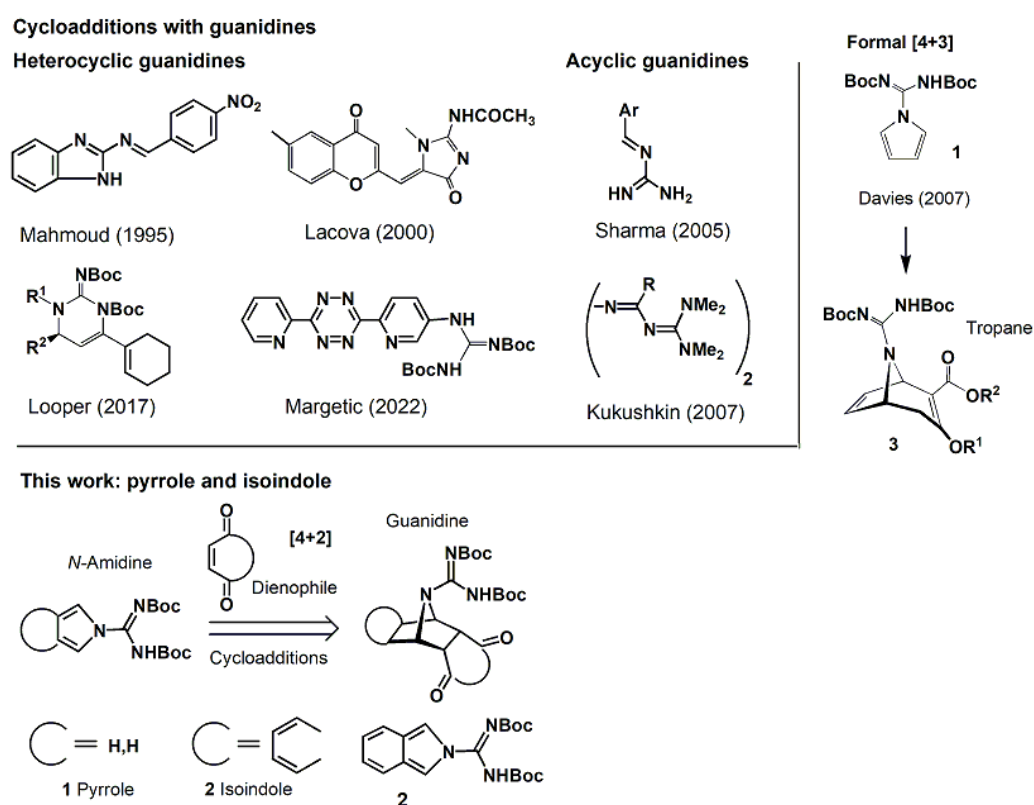
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## 1. Introduction

Guanidines are a class of nitrogen-containing molecules with very interesting physico-chemical properties, [1] especially very high basicity [2] and biological activity [3]. Hence, various aspects of guanidine chemistry were extensively studied computationally and experimentally, including their synthesis. The most viable synthetic routes towards polycyclic, complex organic molecules include the introduction of guanidine functionality at the later stages of multi-step synthesis, such as in tetrodotoxin synthesis [4,5]. One of the most efficient ways for the construction of polycyclic molecules is Diels–Alder (DA) cycloaddition; however, the cycloaddition approach to polycycles containing guanidine functionality has been rarely utilized. Diels–Alder cycloadditions, which involve diene or dienophile partners possessing guanidine functionality, were summarized in Figure 1 [6,7] which also depicts guanidine delivery cycloaddition reagents [8,9]. All of these are unsymmetrical diene molecules, and their cycloaddition reactions lead to the formation of unsymmetrical products.

For the purpose of our studies towards the synthesis of polycyclic molecules, symmetrical diene reagents were required, and, in view of earlier studies [10,11], pyrrole and isoindole *N*-carboxamide derivatives **1** and **2** were selected. These molecules possess  $C_{2v}$  symmetry [12] and upon cycloaddition form guanidine functionality which is ‘protected’ in pyrrole moiety. Whereas pyrrole-2-carboxamide **1** is a known compound, its DA cycloaddition properties were not reported. Only rhodium-catalyzed [4+3] cycloaddition was utilized to prepare tropane bicyclo [3.2.1] octane skeleton **3**, where pyrrole acted as a dipolarophile partner (Figure 1) [13]. Corresponding isoindole-2-carboxamides have not been synthesized previously.

The objective of this work is to prepare isoindole-2-carboxamide **2**, the first representative of an isoindole guanidine cycloaddition delivery reagent, and assess its cycloaddition properties experimentally and computationally. Our preliminary DFT computational study revealed that this approach is feasible and both pyrrole and isoindole dienes are predicted to have sufficient reactivity [14].



**Figure 1.** Used guanidines in Diels–Alder reactions and 2-carboxamidine dienes described in this work [6,7,13].

## 2. Results and Discussion

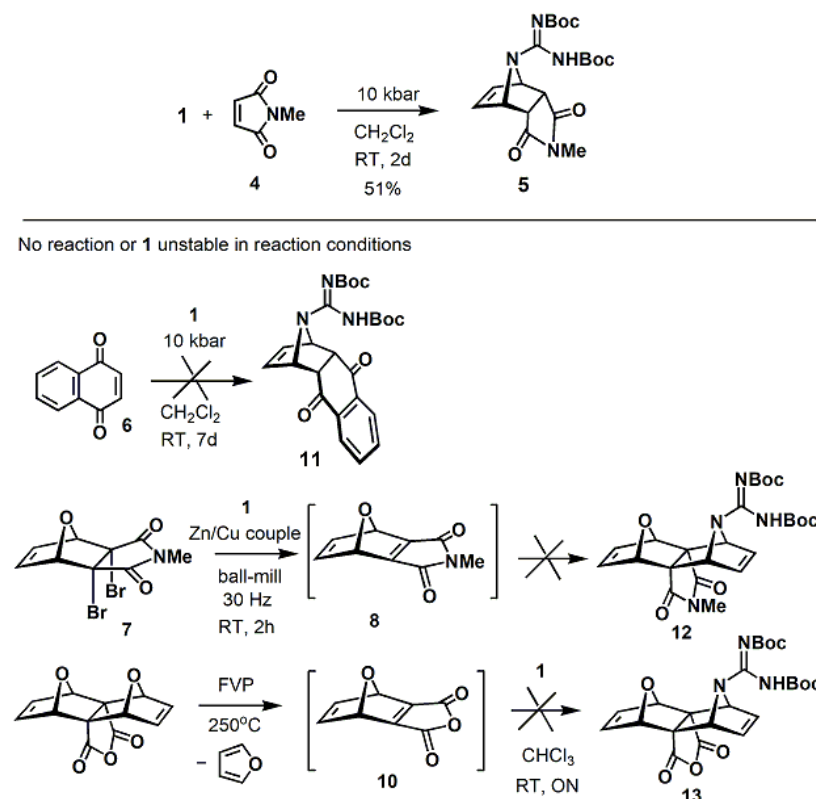
### 2.1. Cycloadditions of 1-Carboxamidine Pyrrole

The 1-(*N,N'*-Di-Boc)pyrrole carboxamidine **1** was prepared according to the literature starting from 3-pyrroline [15,16]. Pyrrole **1** showed poor reactivity in cycloaddition reactions when acting as 1,3-diene (Scheme 1). For instance, the thermal reaction of **1** with *N*-methylmaleimide did not provide the expected cycloadduct **5**. In order to increase its reactivity, an extremely high-pressure technique was employed [17]. The pressurization at 10 kbar, for 2 days, in dichloromethane resulted in the formation of **5** in 51% yield. It was found that product **5** was unstable in  $\text{CHCl}_3$  solution (also in solid state) and quickly cycloreverses back to reactants. This behavior could explain the failure of thermal conditions. An alternative way to increase dienes' reactivity is to employ more reactive dienophiles. Thermal and high-pressure reactions with naphthoquinone did not provide conclusive evidence for the formation of a cycloadduct. Equally unsuccessful were reactions of mechanochemically solid state in-situ-generated imide **8** [18] in a ball mill, due to harsh conditions for the guanidine moiety of **1**. Reaction with related anhydride **10** [19] also provided a complex reaction mixture.

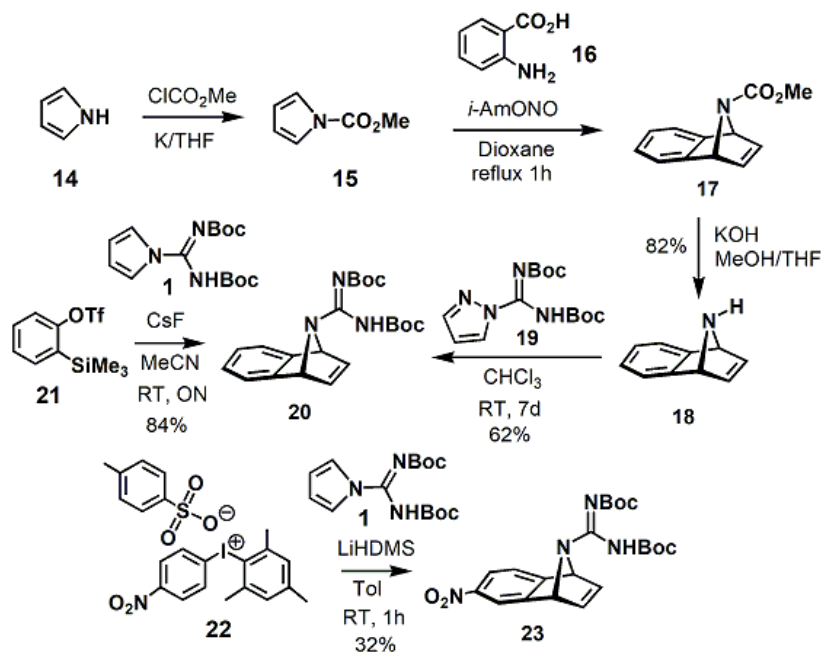
### 2.2. Synthesis of Isoindoles and Their Reactivity

Further increase in the reactivity of pyrroles could be achieved by the addition of a benzene ring, i.e., to use isoindole derivatives as dienes. Synthesis of isoindole precursor **20** in four reaction steps is shown in Scheme 2. It follows the already established synthesis of 7-azabenzonornbornadiene **18**, and subsequent guanylation with *N,N'*-Di-Boc-1H-pyrazole-1-carboxamidine provided **20** in 62% yield. Alternatively, **20** could be prepared by in-situ-generated benzyne cycloaddition with pyrrole **1** (84%). Preparation of a nitro derivative of **20** was achieved by in-situ generation of 4-nitro benzyne from iodonium salt **22** [20] and its reaction with pyrrole **1**, which provided cycloadduct **23** in 32% yield. These reactions

show that the reactivity of pyrrole-1-carboxamide **1** could be increased by the presence of a highly reactive dienophile such as arynes.



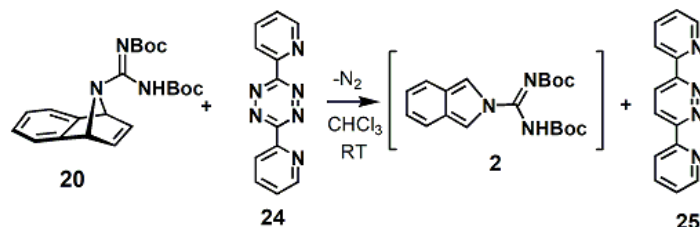
Scheme 1. Cycloaddition reactions of pyrrole **1**.



Scheme 2. Synthesis of isoindole precursors **20** and **23**.

Warrener's cycloaddition/elimination/cycloreversion method employing bis(2-pyridyl)-*sym*-1,2,4,5-tetrazine **24** [21,22] was used for the generation of isoindole **2** (Scheme 3). The formation of **2** was confirmed by  $^1\text{H}$  NMR spectroscopy, by spectrum recorded 30 min after

the addition of **24** to a solution of **20** in an NMR tube (Figure 2). The most characteristic signals which indicate the presence of **2** are a singlet of H<sub>1,3</sub> appearing at  $\delta$  7.57, whereas aromatic multiplets of H<sub>4,7</sub> and H<sub>5,6</sub> are found at  $\delta$  7.36 and 6.85. However, trapping experiments offer indirect but more solid evidence of its formation.



Scheme 3. Generation of isoindole **2** using tetrazine method.

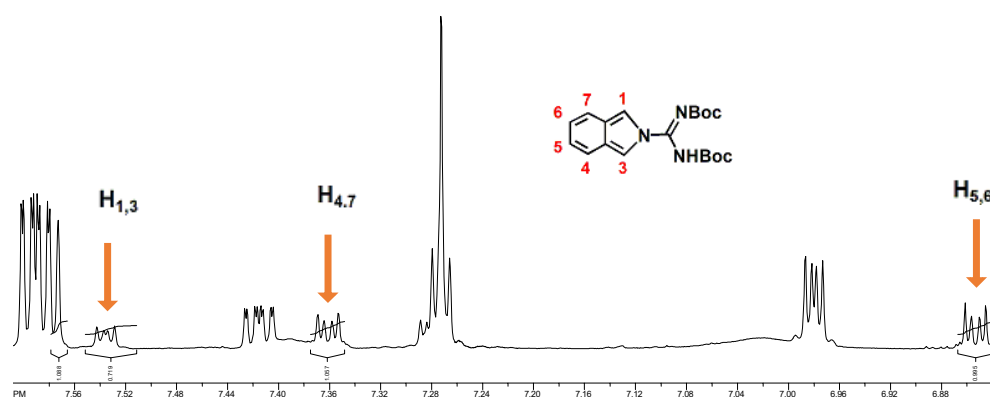


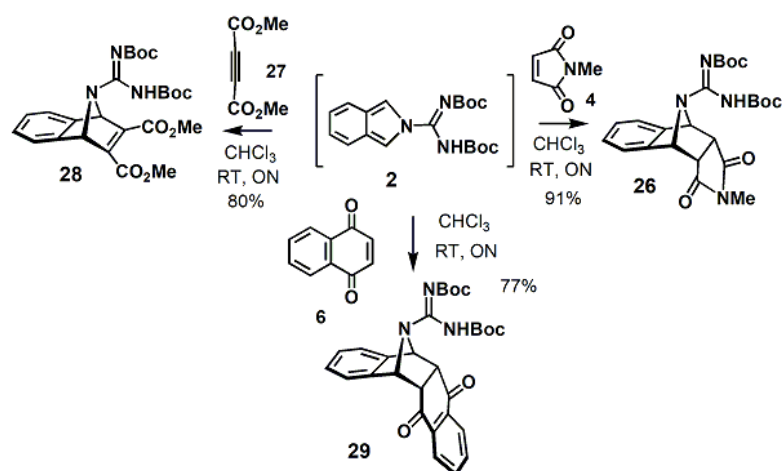
Figure 2. Section of <sup>1</sup>H NMR spectra (in CDCl<sub>3</sub>) with assigned peaks of isoindole **2**.

Scheme 4 summarizes the cycloaddition properties of isoindole-2-carboxamide **2**. When **2** was generated in the presence of dienophiles *N*-methylmaleimide, dimethylacetylenedicarboxylate (DMAD), and benzoquinone, corresponding cycloadducts **26**, **28**, and **29** were obtained (in 91, 80 and 77% yields, respectively). In variance, norbornenes **10**, **20**, **30**, and **31** did not react or afforded intractable mixtures, regardless of reaction conditions (thermal or high pressure). The *endo*-adduct **26** was solely formed, as shown by the single methyl resonance at  $\delta$  2.28 in the <sup>1</sup>H NMR spectrum (see Supplementary Materials), and the *endo*-configuration is proven by the shielding of the *N*-methyl protons by the ring current effect of the aromatic ring [22]. Furthermore, the *exo*-protons are multiplets, is characteristic of the *endo*-adducts of isoindoles [23,24]. This *endo*-stereospecificity is similar to *N*-benzyl-isoindole cycloaddition [25] and in variance with maleic anhydride reactions of isoindoles where *exo/endo* mixtures were formed, [24,26] whereas the outcome of cycloadditions of 2-substituted isoindoles with tolyl-maleimide was not specified [27].

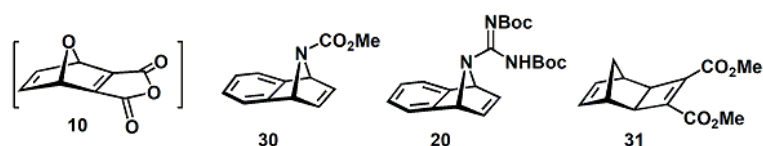
An interesting feature of the <sup>1</sup>H NMR spectra of cycloaddition products **26** and **28** is the broadness of bridgehead signals at 20 °C. Recording the spectra at 50 °C led to the sharpening of the signal, whereas cooling down to 5 °C gives two sets of bridgehead signals, which are associated with nitrogen inversion [28]. The *N*-inversion barrier in **26** is estimated to be low, 13.5 kcal mol<sup>-1</sup> in deuterated chloroform. A similar broadness of bridgehead protons was observed for pyrrole cycloadduct **5**; however, this adduct is thermally unstable and quickly cycloreverses.

In continuation, the electronics of isoindoles were altered by fluorine substituents on the aromatic ring and positioning of the guanidine functionality. Tetrafluoro isoindole precursor **37** was prepared in 35% yield by mechanochemical guanylation [29] of the known 7-azabenzonorbornadiene **36** [30,31] (Scheme 5). It was found that fluorine substitution did not have noticeable effects on the cycloaddition reactivity of isoindole. When tetrafluoroisoindole **38** was trapped in a tetrazine reaction with **37**, the *endo*-cycloadduct **39** was

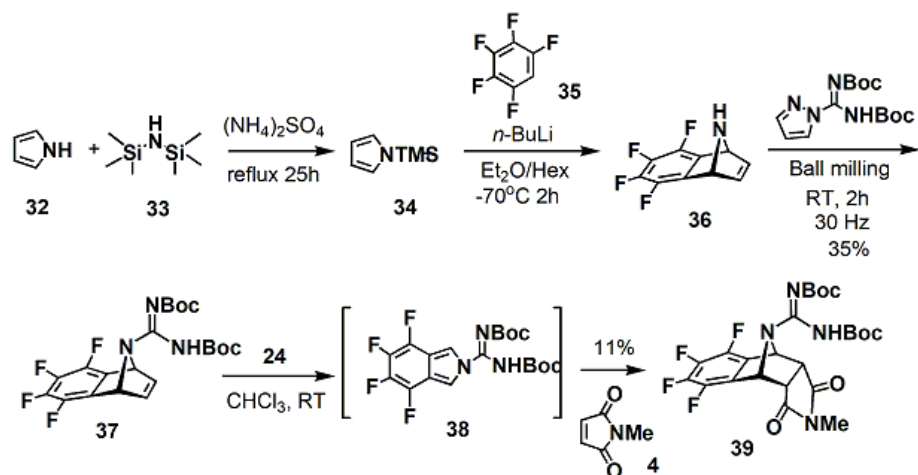
obtained in 11% yield, while, similarly to isoindole 2, tetrafluoro derivative 38 also did not react with its precursor 37.



Cycloadduct was not obtained with dienophiles



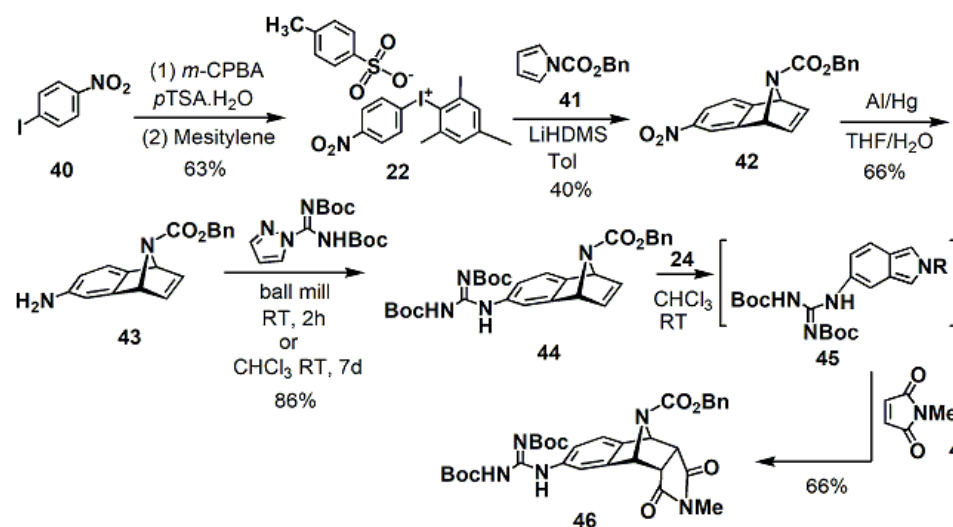
**Scheme 4.** Cycloaddition reactions of isoindole 2.



**Scheme 5.** Synthesis and reactions of tetrafluoro isoindole 38.

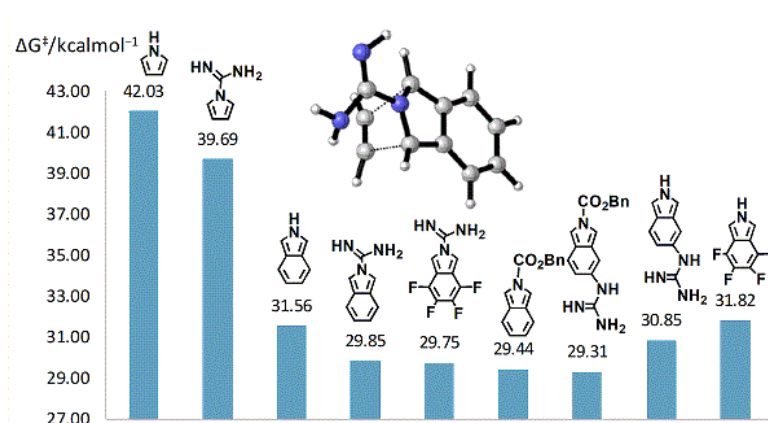
Until now, isoindoles substituted on an aromatic ring with the nitrogen atom have been known only with the nitro group, [32–35] and we prepared the first example of a guanidine aromatic-ring-substituted isoindole. The synthetic route for the introduction of guanidine functionality at position 5 of the isoindole ring in 45 is depicted in Scheme 6. In-situ-generated 4-nitro-benzyne was reacted with 1-benzyloxycarbonyl pyrrole 41 to afford the known cycloadduct 42 [23]. The nitro group was reduced by Al/Hg and the amine 43 was obtained in 66% yield. Guanylation in solution led to the formation of isoindole precursor 44 in 86% yield. This compound was treated with tetrazine 24 in chloroform and intermediate isoindole 45 was trapped as *N*-methylmaleimide cycloadduct 46 (66%). The change in the position of guanidine functionality and *N*-CBz substitution did not increase the cycloaddition reactivity of isoindole. Analogously to isoindoles 5 and 38, in the case

of **45**, a reaction with **44** as a dienophile was not observed. These results indicate the similar cycloaddition reactivity of all three investigated guanidine isoindoles.



**Scheme 6.** Synthesis and reactions of isoindole **45**.

Previous density functional theory (DFT) calculations B3LYP/6-31G(d) predict that activation energies ( $E_a$ ) for reactions of pyrrole and isoindole-2-carboxamide with DMAD are 32.37 and 23.17 kcal mol<sup>-1</sup>, respectively, indicating that the amidine substitution decreases  $E_a$  by 4–5 kcal mol<sup>-1</sup> in comparison to parent unsubstituted dienes, whereas Boc protection of amidinopyrrole causes a further drop in  $E_a$  by 2.5 kcal mol<sup>-1</sup>. Now, these theoretical predictions are supplemented with the M062X/6-311+G\*\* calculations [23] of the reaction of acetylene with pyrrole and isoindoles. All located transition states possess structures resembling the synchronous concerted mechanism of Diels–Alder reactions, such as the one illustrated in Figure 3. Computed activation-free energies ( $\Delta G^\ddagger$  values) are given in Figure 3 and reveal similar predictions to the previously obtained B3LYP calculations. Firstly, *N*-substitution with amidine lowers  $\Delta G^\ddagger$  by 1.5–2.3 kcal mol<sup>-1</sup>. The largest difference in  $\Delta G^\ddagger$  values was obtained when pyrrole was fused with a benzene ring in isoindoles, which is in qualitative accordance with published AM1 results [23]. The position of an amidine (guanidine) substituent and the addition of fluorine atoms has only a marginal effect on the  $\Delta G^\ddagger$  values, with differences in the reactivity of three experimentally studied isoindoles within 0.54 kcal mol<sup>-1</sup>. These predictions are in full accordance with almost identical experimentally observed reactivities of three isoindoles.



**Figure 3.** Activation-free energies of the Diels–Alder reaction of pyrrole and isoindoles with acetylene and optimized transition state structure of the reaction with isoindole-2-carboxamide as calculated by M062X/6-311+G\*\*.



### 3. Materials and Methods

#### 3.1. General

Solvents and chemicals were obtained from Tokyo Chemical Industry (Tokyo, Japan) and Sigma Aldrich (Burlington, VT, USA). Kemika (Zagreb, Croatia), Sigma Aldrich, and VWR Chemicals (Radnor, PA, USA) supplied the solvents, which were used without further purification, unless otherwise stated. The NMR spectra were recorded on Bruker Avance 300 MHz and Bruker Avance 600 MHz spectrometers in deuterated solvents. Chemical shifts ( $\delta$ ) are given in ppm using tetramethylsilane (TMS) as an internal standard, whereas coupling constants ( $J$ ) are expressed in Hertz (Hz). The following abbreviations were used to describe multiplicity in the  $^1\text{H}$  spectra: (s) singlet; (d) doublet; (dd) doublet of doublets; (t) triplet; (m) multiplet; (brs) broad signal. Fourier Transform Infrared Attenuated Total Reflection PerkinElmer UATR Two Spectrometer (range 400–4000  $\text{cm}^{-1}$ ) was used to record infrared spectra (FTIR-ATR). Milling reactions were carried out in Retsch MM400 vibrational mill (frequency 30 Hz), using stainless steel (SS) vials (10 mL) and one 12 mm size SS milling ball. High-pressure reactions were performed in Teflon vials ( $V = 1.5$  mL) using a high-pressure-piston cylinder apparatus (Unipress, Polish Academy of Sciences), and pentane as a pressure-transmitting liquid. Thin-layer chromatography (TLC) was performed on silica-gel plates (silica gel 60 F<sub>254</sub>, Merck), whereas silica gel (Silica gel 60, 0.063–0.200 mm, Merck, Darmstadt, Germany) was used for column chromatography. High-resolution mass spectra (HRMS) were recorded on Agilent 6550 Series Accurate-Mass-Quadrupole Time-of-Flight (Q-TOF) Agilent 1290 Infinity II instrument.

#### 3.2. Synthesis of Cycloadduct 5

Pyrrole **1** (20 mg, 0.065 mmol) and *N*-methylmaleimide (11 mg, 0.098 mmol) were dissolved in  $\text{CH}_2\text{Cl}_2$  (1 mL) and the solution was subjected to high pressure at 10 kbar for 48 h at room temperature. The reaction mixture was evaporated and purified by column chromatography, starting with petroleum ether/EtOAc mixture from 5:1 to 2.5:1. Two fractions were isolated, pyrrole **1** (11 mg) and product **5** as colorless solid (14 mg, 51%).

$^1\text{H}$  NMR ( $\text{CDCl}_3$ ),  $\delta$ /ppm: 1.49 (s, 18H, *t*-Bu), 2.84 (s, 3H,  $\text{NCH}_3$ ), 3.69 (dd, 1H,  $J = 3.3$ , 1.5 Hz, *exo*-H), 5.31 (brs, 2H, N bridge), 6.39 (brs, 2H, C=CH), 10.60 (brs, 1H, NH),

FTIR-ATR  $\nu_{\text{max}}$ / $\text{cm}^{-1}$ : 2979, 1700 (C=O), 1600 (C=O), 1275, 1121.

#### 3.3. Synthesis of 20

Benzenorbornadiene **18** (25 mg, 0.175 mmol) and *N,N'*-Di-Boc-1H-pyrazole-1-carboxamidine (49 mg, 0.158 mmol) were dissolved in  $\text{CHCl}_3$  (1 mL) and stirred at room temperature for 7 days. The reaction mixture was purified by radial chromatography using  $\text{CH}_2\text{Cl}_2$ . Product **20** was isolated as a white solid (42 mg, 62%).

m.p. 158–160 °C,

$^1\text{H}$  NMR ( $\text{CDCl}_3$ ),  $\delta$ /ppm: 1.49 (s, 9H, *t*-Bu), 1.50 (s, 9H, *t*-Bu), 5.84 (brs, 2H, N bridge), 6.98 (dd, 1H,  $J = 5.2$ , 3.2 Hz, Ar), 7.02 (d, 2H,  $J = 2.6$  Hz, C=CH), 7.27 (dd, 1H,  $J = 5.2$ , 3.2 Hz, Ar), 10.63 (brs, 1H, NH),

$^{13}\text{C}$  NMR ( $\text{CDCl}_3$ ),  $\delta$ /ppm: 28.1 (*t*-Bu), 28.2 (*t*-Bu), 65.8 (N bridge), 68.2 (N bridge), 79.8, 82.2, 120.9 (C=C), 125.3, 144.1, 147.5, 150.2 (C=O), 151.2 (C=N), 162.8 (C=O),

FTIR-ATR  $\nu_{\text{max}}$ / $\text{cm}^{-1}$ : 2977, 1756 (C=O), 1636 (C=O),

HRMS-MALDI found: 386.2087, calculated for  $\text{C}_{21}\text{H}_{28}\text{N}_3\text{O}_4$   $[\text{MH}]^+$ : 386.2080.

#### 3.4. Synthesis of Cycloadduct 23

Under argon, dry toluene (1 mL) was added to pyrrole **1** (20 mg, 0.064 mmol) and iodonium salt **22** (35 mg, 0.064 mmol). LiHDMS solution in toluene (64  $\mu\text{L}$ , 0.064 mmol, 1 M) was added dropwise and the resulting mixture was stirred for 1 h at room temperature. The reaction was quenched with saturated  $\text{NH}_4\text{Cl}$  solution (5 mL) and extracted with EtOAc ( $3 \times 10$  mL), and combined extracts were dried with  $\text{Na}_2\text{SO}_4$  and evaporated. The crude mixture was purified by radial chromatography using petroleum ether/diethyl ether

5:1 and gradually increasing polarity to 1:1. Product **23** was isolated as a yellow solid (9 mg, 32%).

m.p. 88–90 °C,

$^1\text{H NMR}$  ( $\text{CDCl}_3$ ),  $\delta/\text{ppm}$ : 1.50 (s, 18H, *t*-Bu), 5.93 (brs, 2H, N bridge), 7.05 (brs, 2H, C=CH), 7.39 (d, 1H,  $J = 7.9$  Hz, Ar), 7.99 (dd, 1H,  $J = 7.9, 1.9$  Hz, Ar), 8.08 (d, 1H,  $J = 1.9$  Hz, Ar), 10.68 (brs, 1H, NH),

$^{13}\text{C NMR}$  ( $\text{CDCl}_3$ ),  $\delta/\text{ppm}$ : 28.0 (*t*-Bu), 28.1 (*t*-Bu), 80.3 (N bridge), 82.5, 82.6, 82.7 (N bridge), 122.4 (C=C), 145.9, 149.6, 149.8, 150.0, 150.1, 151.9, 155.0 (C=N), 162.5 (C=O), 162.6 (C=O),

FTIR-ATR  $\nu_{\text{max}}/\text{cm}^{-1}$ : 2979, 1754 (C=O), 1683 (C=O),

HRMS-MALDI found: 431.1942, calculated for  $\text{C}_{21}\text{H}_{27}\text{N}_4\text{O}_6$   $[\text{MH}]^+$ : 431.1931.

### 3.5. Synthesis of Cycloadduct 26

Isoindole precursor **20** (98 mg, 0.25 mmol), bis(2-pyridyl)-*sym*-1,2,4,5-tetrazine **24** (59 mg, 0.25 mmol) and *N*-methylmaleimide (28 mg, 0.25 mmol) were dissolved in  $\text{CHCl}_3$  (2.5 mL) and stirred overnight at room temperature. The reaction mixture was purified by radial chromatography using  $\text{CH}_2\text{Cl}_2/\text{MeOH}$  mixture 99:1. Cycloadduct **26** was isolated as a white solid (107 mg, 91%).

m.p. 120–122 °C,

$^1\text{H NMR}$  ( $\text{CDCl}_3$ ),  $\delta/\text{ppm}$ : 1.50 (s, 18H, *t*-Bu), 2.28 (s, 3H,  $\text{NCH}_3$ ), 3.88 (d, 1H,  $J = 1.9$  Hz, *exo*-H), 5.76 (brs, 2H, N bridge), 7.21 (dd, 1H,  $J = 5.5, 3.1$  Hz, Ar), 7.28 (dd, 1H,  $J = 5.5, 3.1$  Hz, Ar), 10.60 (brs, 1H, NH),

$^{13}\text{C NMR}$  ( $\text{CDCl}_3$ ),  $\delta/\text{ppm}$ : 23.9 (*exo*-H), 28.1 (*t*-Bu), 47.2 ( $\text{NCH}_3$ ), 62.6 (N bridge), 80.4, 82.8, 121.6, 126.0, 139.3, 150.2 (C=O), 152.6, (C=O), 162.6 (C=N), 174.5 (C=O),

FTIR-ATR  $\nu_{\text{max}}/\text{cm}^{-1}$ : 2975, 1751 (C=O), 1704 (C=O), 1650 (C=O),

HRMS-MALDI found: 471.2255, calculated for  $\text{C}_{24}\text{H}_{31}\text{N}_4\text{O}_6$   $[\text{MH}]^+$ : 471.2244.

### 3.6. Synthesis of Cycloadduct 28

Isoindole precursor **20** (50 mg, 0.13 mmol), bis(2-pyridyl)-*sym*-1,2,4,5-tetrazine **24** (31 mg, 0.13 mmol), and DMAD (8.0  $\mu\text{L}$ , 0.065 mmol) were dissolved in  $\text{CHCl}_3$  (1 mL) and stirred overnight at room temperature. The reaction mixture was purified by radial chromatography using  $\text{CH}_2\text{Cl}_2$ . Cycloadduct **28** was isolated as an off-white solid (52 mg, 80%).

m.p. 70–72 °C,

$^1\text{H NMR}$  ( $\text{CDCl}_3$ ),  $\delta/\text{ppm}$ : 1.49 (s, 18H, *t*-Bu), 3.80 (s, 6H,  $\text{OCH}_3$ ), 6.12 (brs, 2H, N bridge), 7.07 (dd, 1H,  $J = 5.4, 3.1$  Hz, Ar), 7.43 (dd, 1H,  $J = 5.4, 3.1$  Hz, Ar), 10.61 (brs, 1H, NH),

FTIR-ATR  $\nu_{\text{max}}/\text{cm}^{-1}$ : 2979, 1755 (C=O), 1722 (C=O),

$^{13}\text{C NMR}$  ( $\text{CDCl}_3$ ),  $\delta/\text{ppm}$ : 28.1 (*t*-Bu), 52.4 (N bridge), 109.8 (C=C), 122.2, 126.2, 128.9, 139.1, 142.7 (C=O), 145.0 (C=O), 162.4 (C=O), 162.7 (C=N),

HRMS-MALDI found: 502.2197, calculated for  $\text{C}_{25}\text{H}_{32}\text{N}_3\text{O}_8$   $[\text{MH}]^+$ : 502.2189.

### 3.7. Synthesis of Cycloadduct 29

Isoindole precursor **20** (50 mg, 0.13 mmol), bis(2-pyridyl)-*sym*-1,2,4,5-tetrazine **24** (31 mg, 0.13 mmol) and naphthoquinone monohydrate (23 mg, 0.13 mmol) were dissolved in  $\text{CHCl}_3$  (1 mL) and stirred overnight at room temperature. The reaction mixture was purified by radial chromatography using  $\text{CH}_2\text{Cl}_2$ . Cycloadduct **29** was isolated as a brown solid (52 mg, 77%).

m.p. 95–97 °C,

$^1\text{H NMR}$  ( $\text{CDCl}_3$ ),  $\delta/\text{ppm}$ : 1.52 (s, 18H, *t*-Bu), 4.01 (s, 2H, *exo*-H), 5.92 (brs, 2H, N bridge), 6.87 (dd, 1H,  $J = 6.9, 3.2$  Hz, Ar), 7.10 (dd, 1H,  $J = 6.9, 3.2$  Hz, Ar), 7.52 (dd, 1H,  $J = 5.9, 3.3$  Hz, Ar), 7.77 (dd, 1H,  $J = 5.9, 3.3$  Hz, Ar), 10.58 (brs, 1H, NH),

$^{13}\text{C NMR}$  ( $\text{CDCl}_3$ ),  $\delta/\text{ppm}$ : 28.2 (*t*-Bu), 49.6 (*exo*-H), 66.8 (N bridge), 80.2, 82.8, 121.8, 126.6, 127.4, 134.1, 134.6, 140.6, 150.2 (C=O), 152.3, (C=O), 162.6 (C=N), 194.4 (C=O),



FTIR-ATR  $\nu_{\max}/\text{cm}^{-1}$ : 2980, 1732 (C=O), 1677 (C=O),  
HRMS-MALDI found: 518.2295, calculated for  $\text{C}_{29}\text{H}_{32}\text{N}_3\text{O}_6$   $[\text{MH}]^+$ : 518.2291.

### 3.8. Synthesis of 37

Tetrafluoroazabenzonorbornadiene **36** (215 mg, 1.0 mmol) and *N,N'*-Di-Boc-1H-pyrazole-1-carboxamide (310 mg, 1.0 mmol) were grinded in a ball mill for 2 h. The reaction mixture was purified by radial chromatography using  $\text{CH}_2\text{Cl}_2$ /hexane mixture 70:30. Cycloadduct **37** was isolated as a brown solid (158 mg, 35%).

m.p. 84–86 °C,

$^1\text{H}$  NMR ( $\text{CDCl}_3$ ),  $\delta/\text{ppm}$ : 1.50 (s, 18H, *t*-Bu), 6.10 (brs, 2H, N bridge), 7.06 (brs, 2H, C=CH), 10.61 (brs, 1H, NH),

$^{13}\text{C}$  NMR ( $\text{CDCl}_3$ ),  $\delta/\text{ppm}$ : 28.1 (*t*-Bu), 62.9 (N bridge), 65.4 (N bridge), 80.5, 82.8, 129.2 (d,  $^4J_{\text{CF}} = 17$  Hz, C=C), 138.0 (t,  $^2J_{\text{CF}} = 16.1$  Hz, Ar), 139.6 (t,  $^2J_{\text{CF}} = 16.1$  Hz, Ar), 141.2 (m, Ar), 144.1 (m, Ar), 149.9 (C=O), 151.6 (C=O), 162.4 (C=N),

FTIR-ATR  $\nu_{\max}/\text{cm}^{-1}$ : 2981, 1760 (C=O), 1653 (C=O),

HRMS-MALDI found: 458.1716, calculated for  $\text{C}_{21}\text{H}_{24}\text{F}_4\text{N}_3\text{O}_4$   $[\text{MH}]^+$ : 458.1703.

### 3.9. Synthesis of Cycloadduct 39

Isoindole precursor **38** (15 mg, 0.033 mmol), bis(2-pyridyl)-*sym*-1,2,4,5-tetrazine **24** (8 mg, 0.033 mmol) and *N*-methylmaleimide (4 mg, 0.036 mmol) were dissolved in  $\text{CHCl}_3$  (0.5 mL) and stirred overnight at 60 °C. The reaction mixture was purified by radial chromatography using  $\text{CH}_2\text{Cl}_2$ . Cycloadduct **39** was isolated as a white solid (2 mg, 11%).

m.p. 108–110 °C,

$^1\text{H}$  NMR ( $\text{CDCl}_3$ ),  $\delta/\text{ppm}$ : 1.49 (s, 9H, *t*-Bu), 1.52 (s, 9H, *t*-Bu), 2.56 (s, 3H,  $\text{NCH}_3$ ), 3.96 (dd, 2H,  $J = 3.8, 1.8$  Hz, *exo*-H), 5.99 (brs, 2H, N bridge), 10.59 (brs, 1H, NH),

$^{13}\text{C}$  NMR ( $\text{CDCl}_3$ ),  $\delta/\text{ppm}$ : 24.6 ( $\text{NCH}_3$ ), 28.0 (*t*-Bu), 47.1, 60.0 (N bridge), 65.2 (N bridge), 81.1, 83.5, (140.3, 146.6, 149.9), 152.5 (C=O), 162.1 (C=N), 173.2 (C=O),

FTIR-ATR  $\nu_{\max}/\text{cm}^{-1}$ : 2978, 1741 (C=O), 1709 (C=O), 1662 (C=O),

HRMS-MALDI found: 543.1874, calculated for  $\text{C}_{24}\text{H}_{27}\text{F}_4\text{N}_4\text{O}_6$   $[\text{MH}]^+$ : 543.1867.

### 3.10. Synthesis of 42

Under argon, dry toluene (11 mL) was added to Cbz-pyrrole **41** (200 mg, 1.0 mmol) and idonium salt **22** (539 mg, 1.0 mmol). LiHDMS solution in toluene (1.0 mL, 1.0 mmol, 1 M) was added dropwise and the resulting mixture was stirred for 1 h at room temperature. The reaction was quenched with saturated  $\text{NH}_4\text{Cl}$  solution (40 mL) and extracted with EtOAc (3x30 mL); combined extracts were dried with  $\text{Na}_2\text{SO}_4$  and evaporated. The crude mixture was purified by radial chromatography using  $\text{CH}_2\text{Cl}_2$  and gradually increasing polarity with MeOH. Product **42** was isolated as a viscous yellow oil (128 mg, 40%).

$^1\text{H}$  NMR ( $\text{CDCl}_3$ ),  $\delta/\text{ppm}$ : 5.06 (s, 2H,  $\text{CH}_2$ ), 5.67 (brs, 2H, N bridge), 7.02 (d, 2H,  $J = 10.1$  Hz, C=CH), 7.23–7.25 (m, 2H, Ar), 7.30–7.37 (m, 4H, Ar), 7.95 (dd, 1H,  $J = 7.9, 1.8$  Hz, Ar), 8.03 (brs, 1H, Ar),

$^{13}\text{C}$  NMR ( $\text{CDCl}_3$ ),  $\delta/\text{ppm}$ : 66.1 (N bridge), 67.0 ( $\text{OCH}_2$ ), 67.6 (N bridge), 122.2 (C=C), 127.9, 128.2, 128.3, 128.6, 128.7, 128.8, 128.9, 129.0, 135.8, 145.7, 155.0 (C=O),

FTIR-ATR  $\nu_{\max}/\text{cm}^{-1}$ : 2955, 1708 (C=O), 1517 (N-O), 1323 (N-O),

HRMS-MALDI found: 323.1038, calculated for  $\text{C}_{18}\text{H}_{15}\text{N}_2\text{O}_4$   $[\text{MH}]^+$ : 323.1032.

### 3.11. Synthesis of 43

Azabenzonorbornadiene **42** (89 mg, 0.28 mmol) was dissolved in THF/ $\text{H}_2\text{O}$  mixture (40 mL, 10%  $\text{H}_2\text{O}$ ) and heated to 60 °C. Aluminium amalgam was prepared by immersing aluminium foil (400 mg) in a solution of  $\text{HgCl}_2$  (500 mg) in water (50 mL) for 1 min, followed by washing in ethanol (50 mL) and diethyl ether (50 mL). Amalgam was added to the solution and the mixture was continuously heated for 1 h, filtered through Celite and washed with THF. The filtrate was evaporated and purified by radial chromatography

using CH<sub>2</sub>Cl<sub>2</sub> and gradually increasing polarity with MeOH. Product **43** was isolated as a viscous brown oil (53 mg, 66%).

<sup>1</sup>H NMR (CDCl<sub>3</sub>), δ/ppm: 3.56 (brs, 2H, NH<sub>2</sub>), 5.06 (s, 2H, CH<sub>2</sub>), 5.49 (d, 2H, *J* = 6.6 Hz, N bridge), 6.22 (dd, 1H, *J* = 7.6, 2.0 Hz, Ar), 6.69 (brs, 1H), 6.85–6.95 (m, 3H, C=C, Ar), 7.24–7.33 (m, 5H, Ar),

<sup>13</sup>C NMR (CDCl<sub>3</sub>), δ/ppm: 65.8 (N bridge), 67.2 (N bridge), 67.3 (OCH<sub>2</sub>), 112.7 (C=C), 121.4 (C=C), 127.7, 127.8, 128.1, 128.2, 128.5, 128.6, 130.1, 130.2, 136.1, 136.2, 155.2 (C=O),

FTIR-ATR ν<sub>max</sub>/cm<sup>-1</sup>: 3361 (N-H<sub>2</sub>), 1699 (C=O),

HRMS-MALDI found: 293.1292, calculated for C<sub>18</sub>H<sub>17</sub>N<sub>2</sub>O<sub>2</sub> [MH]<sup>+</sup>: 293.1290.

### 3.12. Synthesis of Guanidine **44**

Azabenzonorborene **43** (167 mg, 0.572 mmol) and *N,N'*-Di-Boc-1H-pyrazole-1-carboxamide (177 mg, 0.572 mmol) were dissolved in CHCl<sub>3</sub> (5 mL) and stirred at room temperature for 2 days. The crude mixture was purified by radial chromatography using petroleum ether and gradually increasing polarity with CH<sub>2</sub>Cl<sub>2</sub>. Guanidine **44** was isolated as a viscous yellow oil (262 mg, 86%).

<sup>1</sup>H NMR (CDCl<sub>3</sub>), δ/ppm: 1.50 (s, 9H, *t*-Bu), 1.53 (s, 9H, *t*-Bu), 5.06 (d, 2H, *J* = 13.8 Hz, CH<sub>2</sub>), 5.56 (d, 2H, *J* = 16.1 Hz, N bridge), 6.92 (brs, 1H, C=CH), 6.97 (brs, 1H, C=CH), 7.05–7.21 (m, 3H, Ar), 7.26–7.34 (m, 5H, Ar), 10.29 (brs, 1H; NH), 11.64 (brs, 1H, NH),

<sup>13</sup>C NMR (CDCl<sub>3</sub>), δ/ppm: 28.1 (*t*-Bu), 28.2 (*t*-Bu), 65.9 (N bridge), 66.4 (N bridge), 67.2 (OCH<sub>2</sub>), 116.5 (C=C), 118.3, 120.9 (C=C), 127.8, 128.0, 128.5, 129.0, 133.9, 136.3, 142.8, 144.3, 149.2, 153.3 (C=O), 153.6 (C=O), 155.1 (C=N), 163.5 (C=O),

FTIR-ATR ν<sub>max</sub>/cm<sup>-1</sup>: 2978, 1713 (C=O),

HRMS-MALDI found: 535.2572, calculated for C<sub>29</sub>H<sub>35</sub>N<sub>4</sub>O<sub>6</sub> [MH]<sup>+</sup>: 535.2557.

### 3.13. Synthesis of Cycloadduct **46**

Isoindole precursor **44** (15 mg, 0.028 mmol), bis(2-pyridyl)-*sym*-1,2,4,5-tetrazine **24** (7.0 mg, 0.029 mmol) and *N*-methylmaleimide (3.0 mg, 0.027 mmol) were dissolved in CHCl<sub>3</sub> (0.5 mL) and stirred overnight at room temperature. The reaction mixture was purified by radial chromatography using CH<sub>2</sub>Cl<sub>2</sub>. Cycloadduct **46** was isolated as a white solid (11 mg, 66%).

m.p. 100–102 °C,

<sup>1</sup>H NMR (CDCl<sub>3</sub>), δ/ppm: 1.49 (s, 9H, *t*-Bu), 1.53 (s, 9H, *t*-Bu), 2.36 (s, 3H, NCH<sub>3</sub>), 3.69 (brs, 2H, *exo*-H), 5.08 (brs, 2H, CH<sub>2</sub>), 5.52 (d, 1H, *J* = 4.4 Hz, N bridge), 5.56 (d, 1H, *J* = 4.4 Hz, N bridge), 7.21 (d, 1H, *J* = 8.0 Hz, Ar), 7.31–7.38 (m, 5H, Ar), 7.49 (brs, 2H, Ar), 10.29 (brs, 1H, NH), 11.58 (brs, 1H, NH),

<sup>13</sup>C NMR (CDCl<sub>3</sub>), δ/ppm: 28.1 (*t*-Bu), 28.2 (*t*-Bu), 45.6 (*exo*-H), 46.6 (*exo*-H), 62.4 (CH<sub>2</sub>), 62.8 (N bridge), 67.8 (N bridge), 79.8, 83.9, 116.2, 122.1, 128.1, 128.4, 128.5, 128.6, 135.7, 135.9, 136.8, 140.5, 153.3 (C=O), 153.6 (C=O), 154.6 (C=O), 163.4 (C=N), 174.1 (C=O), 174.2 (C=O),

FTIR-ATR ν<sub>max</sub>/cm<sup>-1</sup>: 2980, 1704 (C=O), 1634 (C=O),

HRMS-MALDI found: 620.2730, calculated for C<sub>32</sub>H<sub>38</sub>N<sub>5</sub>O<sub>8</sub> [MH]<sup>+</sup>: 620.2720.

## 4. Conclusions

Novel isoindoles possessing guanidine substituents were synthesized and their cycloaddition reactivity was explored. These show higher reactivity than the corresponding pyrrole-1-carboxamide and lead to the formation of polycyclic structures which incorporate guanidine functionality into the 7-azanobornene skeleton. New isoindoles were reactive towards dienophiles possessing electron-withdrawing groups (*N*-methylmaleimide, DMAD, naphthoquinone) and highly reactive arynes, whereas reactions with 7-azabenzonorborene did not occur. In addition, 2H-isoindole-2-carboxamide, its tetrafluoro counterpart and 4-guanidino isoindole showed similar reactivity. Experimentally observed reactivities are in good accord with theoretical predictions obtained at the M062X/6-311+G\*\* level. Only small (about 0.5 kcal mol<sup>-1</sup>) differences in energy barriers for cycloaddition reactions of isoindoles were predicted.

**Supplementary Materials:** The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/molecules27248954/s1>,  $^1\text{H}$  and  $^{13}\text{C}$  NMR and IR spectra for the synthesized compounds.

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## References

1. Margetić, D. Physico-Chemical Properties of Organosuperbases. In *Superbases for Organic Synthesis: Guanidines, Amidines, Phosphazenes and Related Organocatalysts*; Ishikawa, T., Ed.; Wiley: Chichester, UK, 2009; Chapter 2; pp. 9–48. [CrossRef]
2. Vazdar, K.; Margetić, D.; Kovačević, B.; Sundermeyer, J.; Leito, I.; Jahn, U. Design of novel uncharged organic superbases: Merging basicity and functionality. *Acc. Chem. Res.* **2021**, *54*, 3108–3123. [CrossRef] [PubMed]
3. Saczewski, F.; Balewski, L. Biological activities of guanidine compounds. *Expert Opin. Ther. Pat.* **2009**, *19*, 1417–1448. [CrossRef] [PubMed]
4. Nishikawa, T.; Urabe, D.; Isobe, M. An Efficient Total Synthesis of Optically Active Tetrodotoxin. *Angew. Chem. Int. Ed.* **2004**, *43*, 4782–4785. [CrossRef] [PubMed]
5. Konrad, D.B.; Rühmann, K.-P.; Ando, H.; Hetzler, B.E.; Strassner, N.; Houk, K.N.; Matsuura, B.S.; Trauner, D. A concise synthesis of tetrodotoxin. *Science* **2022**, *377*, 411–415. [CrossRef] [PubMed]
6. Margetić, D. Cycloadditions of guanidines. In *Cycloaddition Reactions: Advances in Research and Applications*; Margetić, D., Ed.; Nova Science Publishers: New York, NY, USA, 2019; Chapter 7; pp. 243–280.
7. Briš, A.; Murata, Y.; Hashikawa, Y.; Margetić, D. Utilization of *sym*-Tetrazines as Guanidine Cycloaddition Delivery Reagents. An Experimental and Computational Study. *J. Mol. Struct.* **2023**, *1272*, 134207. [CrossRef]
8. Margetić, D.; Russell, R.A.; Warrenner, R.N. Cycloadditions Reagents for Rigidly Attaching the 1,4-Dimethoxynaphthalene Chromophore to Scaffold Alkenes. *Org. Lett.* **2000**, *2*, 4003–4006. [CrossRef]
9. Warrenner, R.N.; Schultz, A.C.; Houghton, M.A.; Butler, D.N. Rigid molecular racks featuring the 1,10-phenanthroline ligand especially those co-functionalised with redox-active groups or other bidentate ligands. *Tetrahedron* **1997**, *53*, 3991–4012. [CrossRef]
10. Malpass, J.R.; Sun, G.; Fawcett, J.; Warrenner, R.N. Novel ‘windscreen wiper’ cavity structures formed by the cycloaddition of N-substituted isoindoles onto molrac bis-alkenes. *Tetrahedron Lett.* **1998**, *39*, 3083–3086. [CrossRef]
11. Warrenner, R.N.; Margetić, D.; Sun, G.; Russell, R.A. Position-Addressable Nano-Scaffolds. I. The Preparation of N,O-, N,C- and N,N-Bridged Sesquiorbornadiene Succinimides as Compact, Highly Functionalized Addressable Building Blocks. *Aust. J. Chem.* **2003**, *56*, 263–267. [CrossRef]
12. Donohoe, T.J. Product class 14: 1H- and 2H-isoindoles. *Sci. Synth.* **2001**, *10*, 653–692. [CrossRef]
13. Reddy, R.P.; Davies, H.M.L. Asymmetric Synthesis of Tropanes by Rhodium-Catalyzed [4+3] Cycloaddition. *J. Am. Chem. Soc.* **2007**, *129*, 10312–10313. [CrossRef]
14. Antol, I.; Barešić, L.; Glasovac, Z.; Margetić, D. Computational Study of Electronic Influence of Guanidine Substitution on Diels-Alder Reactions of Heterocyclic Dienes. *Croat. Chem. Acta* **2019**, *92*, 279–286. [CrossRef]
15. Parr, B.T.; Economou, C.; Herzon, S.B. A concise synthesis of (+)-batzelladine B from simple pyrrole-based starting materials. *Nature* **2015**, *525*, 507–510. [CrossRef]
16. Economou, C.; Romaine, J.P.; Scott, T.Z.; Parr, B.T.; Herzon, S.B. A convergent approach to batzelladine alkaloids. Total syntheses of (+)-batzelladine E, (–)-dehydrobatzelladine C, and (+)-batzelladine K. *Tetrahedron* **2018**, *74*, 3188–3197. [CrossRef]
17. Margetić, D. *High Pressure Organic Synthesis*; Verlag Walter de Gruyter: Berlin, Germany, 2019; ISBN 978-3-11-055602-5. [CrossRef]
18. Štrbac, P.; Margetić, D. Complementarity of solution and solid state mechanochemical reaction conditions demonstrated by 1,2-debromination of tricyclic imides. *Beilstein J. Org. Chem.* **2022**, *18*, 746–753. [CrossRef]
19. Butler, D.N.; Margetić, D.; O'Neill, P.J.C.; Warrenner, R.N. Parity Reversal: A New Diels-Alder Strategy for the Synthesis of Sesquiorbornadienes, Including Those with Heterobridges and Those of Unusual Stereochemistry. *Synlett* **2000**, *1*, 98–100. [CrossRef]
20. Juršić, B.S. AM1 semiempirical study of benzopyrroles as dienes for Diels-Alder reaction. *Can. J. Chem.* **1996**, *74*, 114–120. [CrossRef]

21. Warrenner, R.N.; Butler, D.N.; Margetić, D. Preparation of the First Isobenzofuran Containing Two Ring Nitrogens: A New Diels-Alder Diene for the Synthesis of Molecular Scaffolds Containing one or more End-Fused 3,6-di(2-pyridyl)pyridazine Ligands. *Aust. J. Chem.* **2003**, *56*, 811–817. [[CrossRef](#)]
22. Warrenner, R.N. Isolation of isobenzofuran, a stable but highly reactive molecule. *J. Am. Chem. Soc.* **1971**, *93*, 2346–2348. [[CrossRef](#)]
23. Priestley, G.M.; Warrenner, R.N. A new route to isoindole (benzo[c]indole) and its derivatives. *Tetrahedron Lett.* **1972**, *42*, 4295–4298. [[CrossRef](#)]
24. Kreher, R.P.; Use, G. Untersuchungen zur Chemie von Isoindolen und Isoindoleninen, XXIX. Reaktionen des 2H-Isoindols mit Maleinimiden: Ein einfaches Herstellungsverfahren für 7-Azabicyclo[2.2.1]heptene. *Eur. J. Inorg. Chem.* **1988**, *121*, 927–934. [[CrossRef](#)]
25. Ohwada, T.; Ishikawa, S.; Mine, Y.; Inami, K.; Yanagimoto, T.; Karaki, F.; Kabasawa, Y.; Otani, Y.; Mochizuki, M. 7-Azabicyclo[2.2.1]heptane as a structural motif to block mutagenicity of nitrosamines. *Bioorganic Med. Chem.* **2011**, *19*, 2726–2741. [[CrossRef](#)] [[PubMed](#)]
26. Kreher, R.P.; Seubert, J.; Kohl, N. Investigations on the Chemistry of Isoindoles and Isoindolenines. Part 26. Simple Methods for the Preparation of 2H-Isoindole. *Chem. Ztg.* **1987**, *111*, 349–356. [[CrossRef](#)]
27. Kreher, R.P.; Kohl, N. A Rational Synthetic Method for 2H-Isoindoles. *Angew. Chem. Int. Ed.* **1984**, *23*, 517–518. [[CrossRef](#)]
28. Davies, J.W.; Malpass, J.R.; Moss, R.E. Barriers to inversion at nitrogen in bicyclic amines and hydrazines. *Tetrahedron Lett.* **1985**, *26*, 4533–4536. [[CrossRef](#)]
29. Dud, M.; Glasovac, Z.; Margetić, D. The utilization of ball-milling in synthesis of aryl guanidines through guanidinylation and N-Boc-deprotection sequence. *Tetrahedron* **2019**, *75*, 109–115. [[CrossRef](#)]
30. Hewson, M.J.C.; Schmutzler, R. Phosphorus-fluorine chemistry part XLIII. Pyrrole-substituted fluorophosphoranes. *Phosphorus Sulfur Relat. Elem.* **1980**, *8*, 9–26. [[CrossRef](#)]
31. Davies, J.W.; Durrant, M.L.; Walker, M.P.; Belkacemi, D.; Malpass, J.R. Preparation and spectroscopic studies of the 1,4-dihydro-1,4-iminonaphthalene (7-azabenzonornadiene) ring system. *Tetrahedron* **1992**, *48*, 861–884. [[CrossRef](#)]
32. Wojciechowski, K. Synthesis of 4-nitro-2H-isoindole derivatives. *Liebigs Ann. Chem.* **1991**, *1991*, 831–832. [[CrossRef](#)]
33. Murashima, T.; Tamai, R.; Nishi, K.; Nomura, K.; Fujita, K.; Uno, H.; Ono, N. Synthesis and X-ray structure of stable 2H-isoindoles. *J. Chem. Soc. Perkin Trans.* **2000**, *6*, 995–998. [[CrossRef](#)]
34. Use, G.; Kreher, R. Studies of the chemistry of isoindoles and isoindolenines 18. 2-tert-butyl-5-nitro-2H-isoindole. Preparation and reactions. *Chem. Ztg.* **1982**, *106*, 143–144. [[CrossRef](#)]
35. Lin, C.; Zhen, L.; Cheng, Y.; Du, H.-J.; Zhao, H.; Wen, X.; Kong, L.-Y.; Xu, Q.-L.; Sun, H. Visible-Light Induced Isoindoles Formation To Trigger Intermolecular Diels-Alder Reactions in the Presence of Air. *Org. Lett.* **2015**, *17*, 2684–2687. [[CrossRef](#)]