Transfer of Stereochemical Information in a Minimal Self-Replicating System

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SUPPORTING INFORMATION

Selected spectroscopic data obtained for the characterization of compounds:

***N*-[3-(*N'*-6-Methyl-2-pyridyl)-amidobenzylidine]-phenylamine *N*-oxide 1**: M.p. 170-172°C; Found 332.1397 [M+H$^+$], $C_{20}H_{18}N_3O_2$ requires 332.1399; ν_{max}(KBr)/cm^{-1} 3184, 3066, 1675, 1600, 1069, 785, 731; δ_H (300 MHz, $CDCl_3$) 8.89 (1H, s, NH), 8.84 (1H, s, CH=N), 8.71 (1H, d, $^3J_{H,H}$ 8, Ar CH), 8.17 (1H, d, $^3J_{H,H}$ 8, Ar CH), 8.04-7.99 (2H, m, Ar CH), 7.79-7.75 (2H, m, Ar CH), 7.66-7.57 (2H, m, Ar CH), 7.51-7.46 (3H, m, Ar CH), 6.91 (1H, d, $^3J_{H,H}$ 7, Ar CH), 2.43 (3H, s, CH_3); δ_C (75 MHz, $CDCl_3$) 164.9 (quat. C), 156.8 (quat. C), 150.5 (quat. C), 148.7 (quat. C), 138.6 (Ar CH), 134.6 (quat. C), 133.3 (Ar CH), 131.8 (Ar CH), 131.0 (quat. C), 130.1 (Ar CH), 129.4 (Ar CH), 129.1 (Ar CH), 127.4 (Ar CH), 121.5 (Ar CH), 119.4 (Ar CH), 110.9 (Ar CH), 23.8 (CH_3); *m/z* (FAB+) 332 (M+H$^+$, 100%), 316 (9).

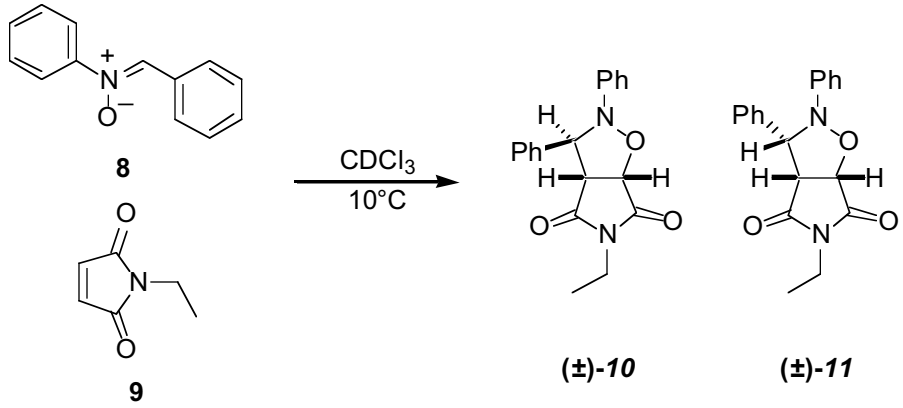
2-{3_-[*N*-(6-Methyl-2-pyridyl)-3-benzamidyl]-4,6-dioxo-2-phenyl-(3a*r*,6a*c*)-hexahydropyrrolo[3,4-*d*]isoxazol-5-yl}-ethanoic acid, methyl ester 3 and 4 (7:2 ratio of diastereoisomers): M.p. >125°C (decomp); Found: C, 64.9; H, 4.9; N, 11.1. Calc. for $C_{27}H_{24}N_4O_6$: C, 64.9; H, 4.8; N, 11.2%; Found 501.1768 [M+H$^+$], $C_{27}H_{25}N_4O_6$ requires 501.1774; ν_{max}(KBr)/cm^{-1} 3434, 1752, 1721, 1685, 1327, 1272, 792, 746, 696; δ_H (300 MHz, $CDCl_3$) 9.05 (0.7H, s, NH), 8.91 (0.3H. br s, NH), 8.22 (0.3H, d, $^3J_{H,H}$ 8, Ar CH), 8.15 (0.7H, d, $^3J_{H,H}$ 8, Ar CH), 8.05 (0.3H, s, Ar CH), 7.98 (0.7H, s, Ar CH), 7.93 (0.7H, d, $^3J_{H,H}$ 8, Ar CH), 7.87 (0.3H, d, $^3J_{H,H}$ 8, Ar CH), 7.72-7.57 (2H, m, Ar CH), 7.50-7.45 (1H, m, Ar CH), 7.22-7.17 (2H, m, Ar CH), 7.09-7.05 (2H, m, Ar CH), 7.01-6.98 (0.7H, m, Ar CH), 6.95-6.90 (1.3H, m, Ar CH), 5.52 (0.3H, s, CH), 5.21-5.16 (1H, m, CH), 4.78 (0.7H, d, $^3J_{H,H}$ 9, CH), 4.30-4.20 (1.7H, m, CH and CH_2), 4.05 (0.7H, dd, $^3J_{H,H}$ 8, $^3J_{H,H}$ 9, CH), 3.95 (0.6H, s, CH_2), 3.75 (2.1H, s, OCH_3), 3.70 (0.9H, s, OCH_3), 2.50 (0.9H, s, CH_3), 2.45 (2.1H, s, CH_3); δ_C (125 MHz, $CDCl_3$, 31°C) 173.8 (quat. C), 173.7 (quat. C), 172.5 (quat. C), 171.7 (quat. C), 166.8 (quat. C), 166.4 (quat. C), 165.4 (quat. C), 165.1 (quat. C), 156.6 (quat. C), 156.5 (quat. C), 150.9 (quat. C), 150.5 (quat. C), 147.4 (quat. C), 146.3 (quat. C), 139.3 (Ar CH), 138.8 (Ar CH), 135.1 (quat. C), 134.9 (quat. C), 134.5 (quat. C), 131.6 (Ar CH), 130.8 (Ar CH), 129.4 (Ar CH), 129.3 (Ar CH), 128.9 (Ar CH), 128.7 (Ar CH), 128.3 (Ar CH), 127.1 (Ar CH), 126.6 (Ar CH), 126.0 (Ar CH), 125.7 (Ar CH), 123.3 (Ar CH), 120.6 (Ar CH), 119.6 (Ar CH), 119.4 (Ar CH), 115.3 (Ar CH), 111.4 (Ar CH), 111.3 (Ar CH), 76.7 (CH), 76.3 (CH), 71.2 (CH), 69.6 (CH), 57.1 (CH), 54.7 (CH), 53.1 (CH_3), 52.8 (CH_3), 39.6 (CH_2), 39.5 (CH_2), 23.8 (CH_3), 23.7 (CH_3); *m/z* (LCTOF) 523 (M+Na$^+$, 97%), 501 (M+H$^+$, 8), 354 (100), 332 (25).

2-{3*c*-[*N*-(6-Methyl-2-pyridyl)-3-benzamidyl]-4,6-dioxo-2-phenyl-(3a*r*,6a*c*)-hexahydropyrrolo[3,4-*d*]isoxazol-5-yl}-ethanoic acid 6: M.p. >165°C (decomp); Found: C, 64.2; H, 4.6; N, 11.75. Calc. for $C_{26}H_{22}N_4O_6$: C, 64.2; H, 4.6; N, 11.5%; Found 487.1625 [M+H$^+$], $C_{26}H_{23}N_4O_6$ requires 487.1617; ν_{max}(KBr)/cm^{-1} 3396, 1791, 1720, 1328, 1274, 794, 753, 733, 695; δ_H (300 MHz, $CDCl_3$) 11.57 (1H, br s, CO_2H), 10.93 (1H, s, NH), 8.13 (1H, d, $^3J_{H,H}$ 8, Ar CH), 7.98 (1H, s, Ar CH), 7.88 (1H, d, $^3J_{H,H}$ 8, Ar CH), 7.82-7.76 (1H, m, Ar CH), 7.49-7.46 (1H, m, Ar CH), 7.41-7.35 (1H, m, Ar CH), 7.17-7.11 (2H, m, Ar CH), 6.99 (1H, d, $^3J_{H,H}$ 7, Ar CH), 6.93 (2H, d, $^3J_{H,H}$ 8, Ar CH), 6.89-6.84 (1H, m, Ar CH), 5.37 (1H, s, CH), 5.14 (1H, d, $^3J_{H,H}$ 7, CH), 4.05 (1H, d, $^2J_{H,H}$ 12, CH_2), 3.88 (1H, d, $^3J_{H,H}$ 7, CH), 3.83 (1H, d, $^2J_{H,H}$ 12, CH_2), 2.49 (3H, s, CH_3); δ_C (100 MHz, $CDCl_3$, 31°C) 174.9 (quat. C), 173.1 (quat. C), 171.3 (quat. C), 166.6 (quat. C), 154.8 (quat. C), 150.7 (quat. C), 147.4 (quat. C), 141.5 (Ar CH), 138.2 (quat. C), 134.4 (quat. C), 131.1 (Ar CH), 129.2 (Ar CH), 128.9 (Ar CH), 128.7 (Ar CH), 126.2 (Ar CH), 122.9 (Ar CH), 120.0 (Ar CH), 115.3 (Ar CH), 113.5 (Ar CH), 76.7 (CH), 69.4 (CH), 57.0 (CH), 40.8 (CH_2), 21.0 (CH_3); *m/z* (FAB) 487 (M+H$^+$, 81%), 457 (15), 366 (39), 332 (100).

Assignment of stereochemistry for compounds 3,4,6 and 7.

In order to assign the stereochemistry of compound **3**,**4**,**6** and **7**, a detailed investigation into the reaction between diphenyl nitrone **8** and *N*-ethyl maleimide **9** was undertaken (**Scheme 1**). ^{1}H NMR spectroscopic data obtained from the two isolated diastereoisomers **10** and **11** was then compared to spectroscopic data obtained by both Al-timari *et al*[1] and Iwakura *et al*[2].

Scheme 1.



Further structure elucidation of compounds **10** and **11** was undertaken by employing nOe techniques, the results of which are shown in **Figure 1**. Upon irradiation of the H^{3a} resonance signal a significant nOe interaction was observed to the H^{6a} resonance signal in the case of the *trans* isoxazolidine **10**. No significant nOe was observed to H^{3} as this proton is located on the opposite face of the heterocycle. Conversely, in the case of the *cis* isoxazolidine **11** strong nOes were observed to protons H^{3} and H^{6a} upon irradiation of the H^{3a} resonance signal. In the case of **11**, these protons are located on the same face of the heterocyclic ring.

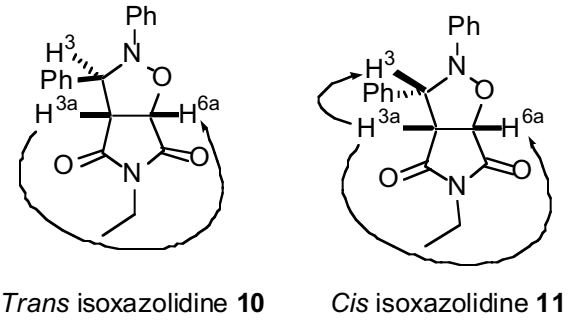
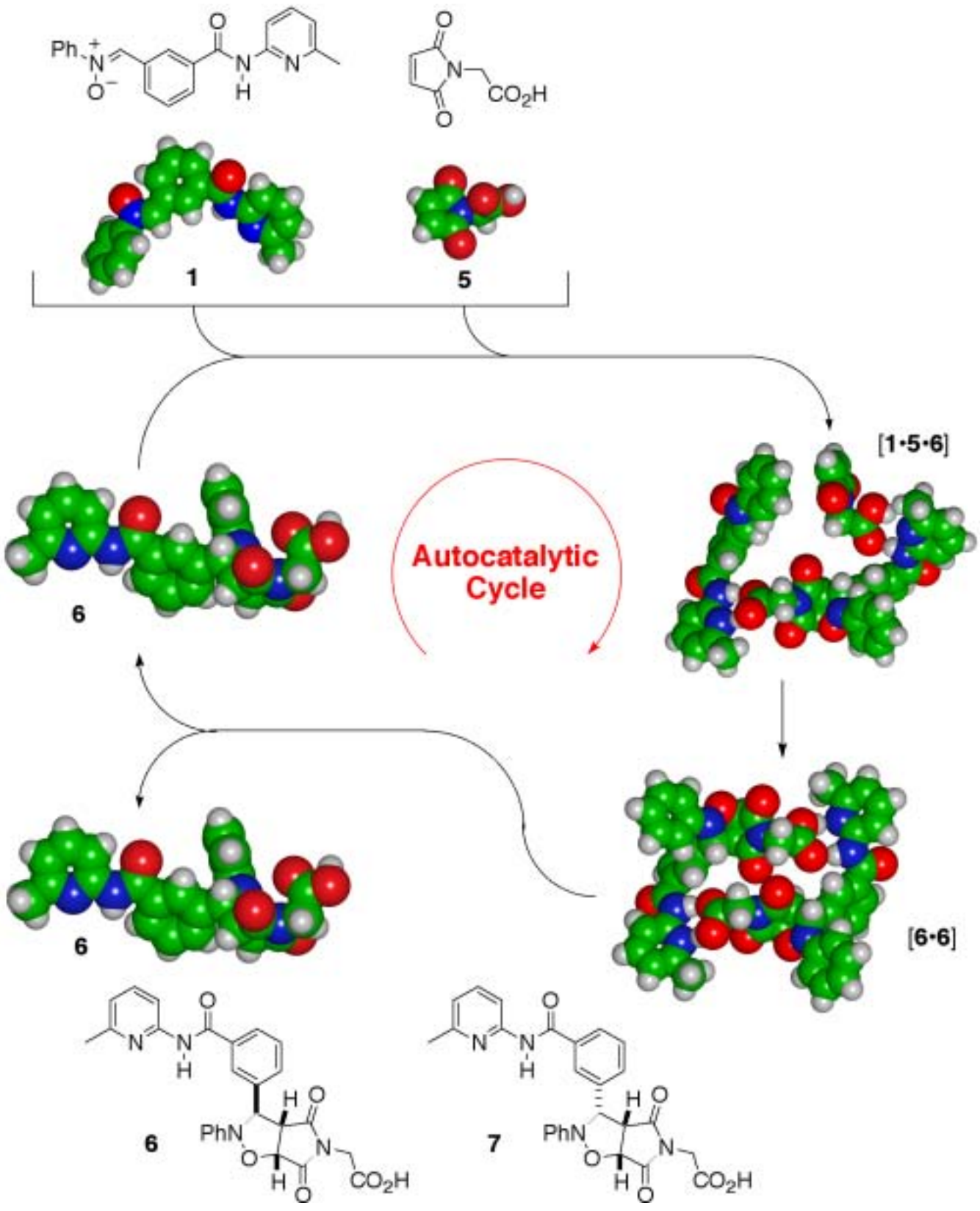


Figure 1. Positive nOes observed for the *trans* and *cis* isoxazolidines **10** and **11** arising from the reaction between diphenyl nitrone **8** and *N*-ethyl maleimide **9**.

The ^{1}H NMR spectra obtained for compounds **3** and **6**, and **4** and **7** were comparable to those obtained for cycloadducts **10** and **11** respectively when the resonance peaks due to protons attached directly to the bi-cycle were considered (H^{3}, H^{3a} and H^{6a}, **Figure 1)**. It was therefore assumed for the purposes of our investigation that the disatereoisomers displaying a similar pattern of resonance peaks to compounds **10** and **11** were the *trans* and *cis* diastereoisomers respectively.

[1] Al-timari, U. A. R.; Fisera, L.; Ertl, P.; Goljer, I.; Pronoyova, N. *Monatsch. Chem.* **1992**, *123*, 999.
[2] Iwakura, Y.; Uno, K.; Hong, S.-J.; Hongu, T. *Bull. Chem. Soc. Jpn.* **1972**, *45*, 192.

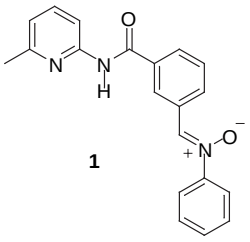
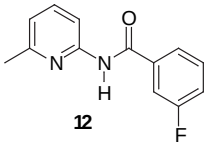
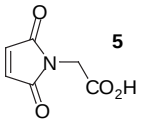
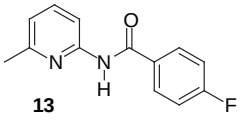
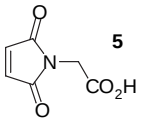
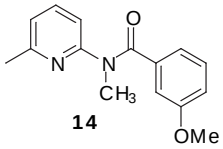
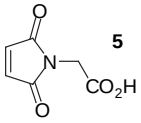
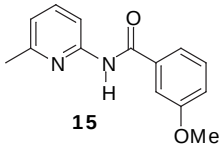
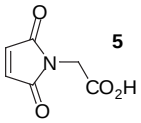
Molecular modeling representations of the autocatalytic cycle involving 1, 5 and 6



Assessment of the Association Constants for Complexes involved in the Autocatalytic Cycle

The association constant for the [**1**•**5**] complex cannot be measured directly since the reaction between **1** and **5** to afford **6** and **7** will occur within the time scale of the titration experiment. The use of the succinimide arising from hydrogenation of the double bond in **5** is also not possible as this compound is completely insoluble in the reaction solvent $CDCl_3$. We therefore adopted a strategy in which the nitrone in **1** was replaced by a group with a similar Hammett σ parameter – in this case, we chose the fluorine substituent (compounds **12** and **13**, **Table 1**). Associations involving the nitrone **1** were modeled using the complex formed between **1** and acetic acid, which has a similar pK_a to **5**. All association constants were measured by 400 MHz ^{1}H NMR titration experiments in $CDCl_3$ at 10°C and the values obtained for the association constants are given in the table below.

We have assessed the strength of the single point binding between a carboxylic acid and the amidopyridine nitrogen in other work performed in our laboratories. The association constant for the [**5**•**14**] complex was determined by a 400 MHz ^{1}H NMR titration experiments in $CDCl_3$ together with the appropriate two point association constant for the [**5**•**15**] complex for comparison (see Table).

Compound A	Compound B	K_a / M^{-1}
 **1**	CH_3COOH	130 ± 6
 **12**	 **5**	70 ± 3
 **13**	 **5**	57 ± 3
 **14**	 **5**	15 ± 1
 **15**	 **5**	115 ± 6