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Synthesis of a paramagnetic boronic acid as a useful synthetic building block and carbohydrate affinity spin probe

Tamás Kálai,[a] József Jekő[b] and Kálmán Hideg[a,*]

[a]*Institute of Organic and Medicinal Chemistry, University of Pécs, H-7602 Pécs, PO Box 99, Hungary*
[b]*ICN Hungary Ltd, H-4440 Tiszavasvári, PO Box 1, Hungary*

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Abstract—A paramagnetic boronic acid was synthesized by lithiation of 1-acetoxy-3-bromo-2,2,5,5-tetramethyl-2,5-dihydro-1*H*-pyrrole followed by treatment with trimethyl borate. This paramagnetic boronic acid proved to be a useful starting compound in Suzuki cross-coupling reactions and it exhibited affinity toward fructose and inulin.

Boronic acids are an important class of compounds in organic chemistry as starting materials in palladium-catalyzed Suzuki cross-coupling reactions with aryl and vinyl triflates and halides towards unsymmetrical biphenyls,[1] heterocycles,[2,3] sulfones,[4] and ketones.[5] The boronic acids are valuable building blocks in natural product synthesis[6] and they are used in Mannich reactions to yield amino acids.[7] The main advantages of boronic acids are their tolerance to a wide variety of functional groups, their air stability and relatively low toxicity. Regarding bioanalytical applications, very recently boronic acid-containing fluorophores have emerged as a potential alternative to current invasive glucose-monitoring techniques[8] and the application of boronic acids in therapy is also a promising area.[9]

In our laboratory, we have already synthesized paramagnetically labeled biomolecules and heterocycles in a multistep synthesis.[10] The Suzuki reaction between paramagnetic vinyl bromides and boronic acids has simplified approaches towards paramagnetic molecules, however this procedure is limited to commercially available boronic acids or those ones, which can be prepared without tedious procedures.[11] Herein we report the first (as far as we know) synthesis of paramagnetic boronic acid, which proved to be a difficult challenge. For this reaction, lithiation[12] of 1-oxyl-3-bromo-2,2,5,5-tetramethyl-2,5-dihydro-1*H*-pyrrole **1**, which is a byproduct of the NaOBr mediated Favorskii reaction of 1-oxyl-2,2,6,6-tetramethyl-4-piperidone (4-oxo-TEMPO),[13] would be feasible. However, it is well known that in these procedures the nitroxide moiety is also *O*-alkylated by the alkyllithium.[14] Therefore we decided to protect the nitroxide as diamagnetic *O*-acetate **2** by reduction of **1** with ascorbic acid to the corresponding hydroxylamine followed by treatment with acetyl chloride in the presence of Et_3N.[15] Lithiation of compound **2** in THF with 2 equiv of *n*-butyllithium at −78 °C resulted in bromine lithium exchange and *O*-acetate transformation to the corresponding hydroxylamine lithium salt, which presumably prevents *O*-alkylation. Treatment of the dilithium intermediate with trimethyl borate at −78 °C followed by ester hydrolysis and oxidation of hydroxylamine to nitroxide with activated MnO_2/O_2 afforded paramagnetic boronic acid **3**,[16] which could be further converted to boronate **4** with pinacol in MeOH.[17] Compound **4** is directly available by reaction of **1** and bis(pinacolato)diboron in the presence of PhOK as base and $PdCl_2(PPh_3)_2$ as catalyst, in toluene, at 100 °C[18] (Scheme 1).

Firstly, we investigated the affinity of compound **3** towards a monosaccharide, namely fructose (40 mM) and a polysaccharide, namely inulin from Dahlia tubers (~10 mM) in a phosphate buffer solution (PBS) at pH = 6.9 and at ambient temperature. The change in the EPR spectra was not significant, but still detectable: in the case of fructose we observed an approximately 0.4 G increase in the hyperfine splitting constant of nitrogen while in the case of the high molecular weight

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* Corresponding author. Tel.: +36 72 536220; fax: +36 72 536219; e-mail: kalman.hideg@aok.pte.hu

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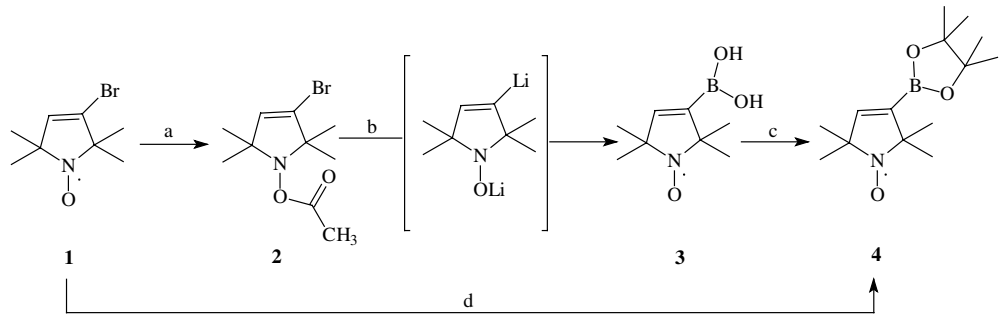


Scheme 1. Reagents and conditions: (a) ascorbic acid (5.0 equiv), dioxane/water 40 °C, 15 min under N_2, then extraction with $CHCl_3$, then Et_3N (1.1 equiv), AcCl (1.1 equiv), 0 °C → rt, 1 h, 53%; (b) *n*-BuLi (2.0 equiv), THF, −78 °C, 30 min, then $B(OCH_3)_3$ (1.2 equiv), −78 °C, 2 h, N_2, then rt, MeOH, water, 12 h, air, then extraction with Et_2O, then activated MnO_2 (1.0 equiv), O_2, 15 min, 47%; (c) pinacol (1.0 equiv), MeOH, $MgSO_4$, rt, 12 h, 68%; (d) bis(pinacolato)diboron (1.1 equiv), $PdCl_2(PPh_3)_2$ (0.03 equiv), PPh_3 (0.06 equiv), PhOK (1.5 equiv), toluene, 100 °C, 6 h, N_2, 45%.

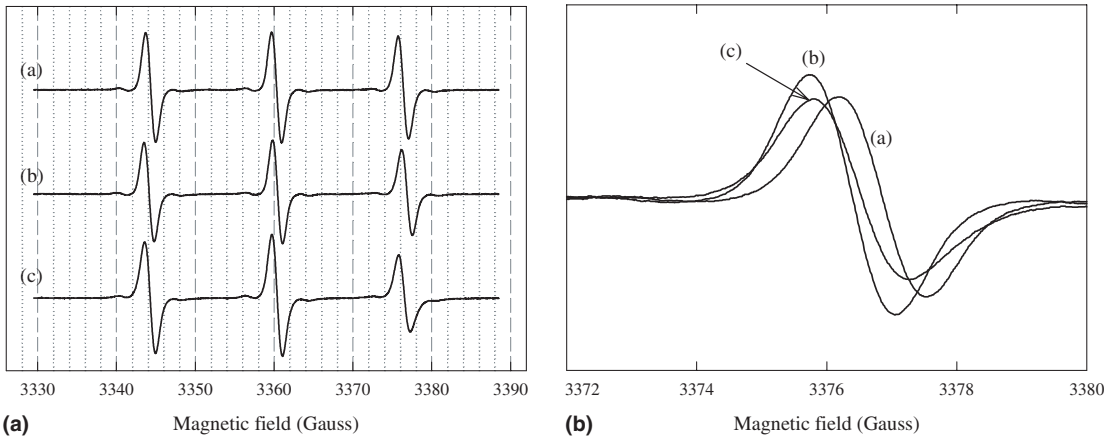
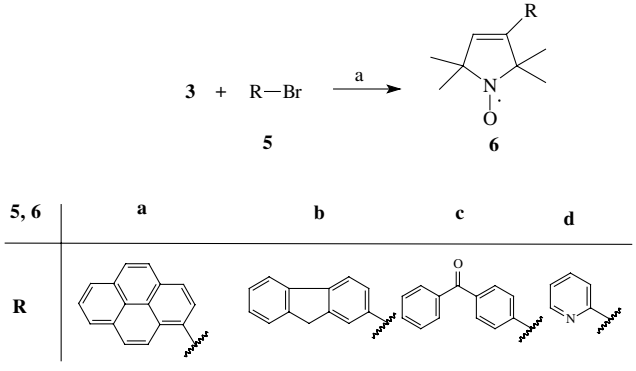


Figure 1. EPR spectra of (a) compound **3** (9.3×10^{-5} M) in PBS (pH = 6.9); (b) compound **3** (9.3×10^{-5} M) + 40 mM fructose in PBS; (c) compound **3** (9.3×10^{-5} M) + 10 mM inulin in PBS and its 3372-3380 G region, a 166% zoom.

(5–7 kDa) inulin, both the EPR line width increased and the splitting constant increased by 0.1 G (Fig. 1). The perturbation of the EPR spectra was less than in the case of paramagnetically modified mono- and polysaccharide derivatives with covalent carbohydrate-spin label bond formation,[19] however we hope that both the detection conditions and probes can be improved further.

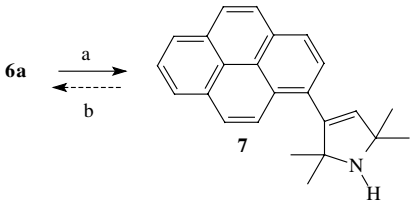
Compound **3** was tested in Suzuki reactions with polyaromatic and heterocyclic bromides as substrates. Reaction of 1-bromopyrene **5a** with pyrroline nitroxides gave a double (fluorescent and spin) sensor compound[20] **6a**, which possesses high quantum yield and long half-life fluorophore and has the ability to form excimers.[21] The reaction conditions were not optimized,[22] we used 1,2-dimethoxyethane (DME) and aq 10% Na_2CO_3 or saturated $NaHCO_3$ solution and $Pd(PPh_3)_4$ as catalyst[1,23] to give compounds **6a** and **6b** in moderate yields (42% and 35%). Suzuki cross-coupling with 4-bromobenzophenone (**5c**), as a functionalized aromatic compound, afforded **6c** in 32% yield containing a photoactivable benzophenone group.[24] Reaction of 2-bromopyridine **5d**, as a heteroaromatic compound with **3** gave compound **6d**, but only in a low, 17% yield (Scheme 2).

The fluorescence of pyrene in compound **6a** is quenched by nitroxide,[18] its reduction with Fe powder in AcOH[25]



Scheme 2. Reagents and conditions: (a) aryl bromide (1.0 equiv), $Pd(PPh_3)_4$ (0.05 equiv), DME, 10 min, under N_2, then **3** (1.1 equiv), 10% aq Na_2CO_3 or satd aq $NaHCO_3$, reflux 10 h, 42–17%.

yielded the more (about 6-fold in intensity) fluorescent diamagnetic sterically hindered amine **7**, capable of Reactive Oxygen Species (ROS) trapping yielding the low fluorescent **6a**[26] (Scheme 3). It is interesting to note, however, that the NH group of the pyrroline ring also quenches the fluorescence of pyrene because of photo-induced electron transfer (PET),[27] which could be eliminated by protonating the amine with excess trifluoroacetic acid (TFA) resulting in about a 6-fold



Scheme 3. Reagents and conditions: (a) Fe (5.0 equiv), AcOH, 70 °C, 1 h, then H_2O, K_2CO_3, extraction with $CHCl_3$, 36%; (b) ROS.

increase in the fluorescence. This shows that compound **7** can be utilized as a fluorescence sensor both for ROS and H^+ ions, but causes the opposite effect in fluorescence intensity (Fig. 2), moreover ROS scavenging can be followed by EPR also.

We also tested the synthetic usefulness of paramagnetic pinacol boronate **4**. The Suzuki reaction of compound **4** with 5-bromo-2,4-di-*tert*-butoxypyrimidine[28] in the presence of $Pd(PPh_3)_4$ in 10% aq Na_2CO_3/dioxane gave compound **8** of which treatment with acid yielded the paramagnetic uracyl **9** (Scheme 4). Utilization of boronic acid or its pinacolate offers a new approach among the well documented spin-labeled nucleic base syntheses.[29,30]

In conclusion, a paramagnetic boronic acid has been synthesized proving that the nitroxide free-radical moiety is compatible with a boronic acid group. We hope this compound may find wide application as a carbohydrate affinity spin probe and as a boronic acid reagent in Suzuki cross-coupling reactions,[31] offering an easier synthesis of challenging paramagnetic compounds or their secondary amine precursors.[32]

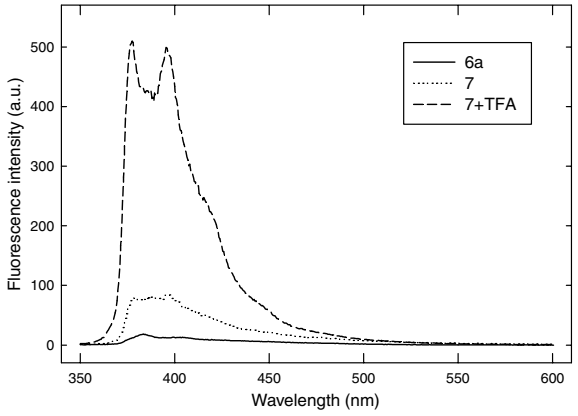
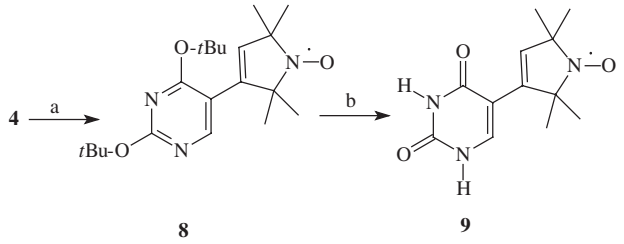


Figure 2. Emission spectra of **6a** (9.8 μM), **7** (9.8 μM) and **7** (9.7 μM) + TFA (150 mM) in acetonitrile at 24 °C, λ_{ex}: 341 nm.



8 **9**

Scheme 4. Reagents and conditions: (a) 5-bromo-2,4-di-*tert*-butoxypyrimidine (1.0 equiv), $Pd(PPh_3)_4$ (0.05 equiv), dioxane, 10 min, under N_2, then **4** (1.1 equiv), 10% aq Na_2CO_3, reflux 5 h, 55%; (b) dioxane, 30% aq (5.0 equiv), H_2SO_4, rt, 30 min, 68%.

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References and notes

1. Miyaura, N. *Cross-Coupling Reactions*; Springer: Berlin, 2002.
2. Li, J. J.; Gribble, G. W. *Palladium in Heterocyclic Chemistry*; Pergamon: Oxford, 2000.
3. (a) Mátyus, P.; Maes, U. W. B.; Riedl, Z.; Hajós, G.; Lemiére, G. L. F.; Tapolcsányi, P.; Monsieurs, K.; Éliás, O.; Dommisse, R. A.; Krajsovszky, G. *Synlett* **2004**, 1123–1139; (b) Tyrrell, E.; Brookes, P. *Synthesis* **2003**, 469–483.
4. Beaulieu, C.; Guay, D.; Wang, Z.; Evans, D. A. *Tetrahedron Lett.* **2004**, *45*, 3233–3236.
5. Eddarir, S.; Cotelle, N.; Bakkour, Y.; Rolando, C. *Tetrahedron Lett.* **2003**, *44*, 5359–5363.
6. Kawasaki, I.; Yamashita, M.; Ohta, S. *Chem. Pharm. Bull.* **1996**, *44*, 1831–1839.
7. Currie, G. S.; Drew, M. G. B.; Harwood, L. M.; Hughes, D. J.; Luke, R. W. A.; Vickers, R. J. *J. Chem. Soc., Perkin Trans. 1* **2000**, 2982–2990.
8. (a) DiCesare, N.; Lakowicz, J. R. *Tetrahedron Lett.* **2001**, *42*, 9105–9108; (b) Badugu, R.; Lakowicz, J. R.; Geddes, C. D. *Anal. Chem.* **2004**, *76*, 610–618.
9. Bacha, U.; Barilla, J.; Velazquez-Campoy, A.; Leavitt, A. S.; Freire, E. *Biochemistry* **2004**, *43*, 4906–4912.
10. (a) Sár, C. P.; Jekő, J.; Hideg, K. *Synthesis* **1998**, 1497–1500; (b) Kálai, T.; Jekő, J.; Hideg, K. *Synthesis* **2000**, 831–837.
11. Kálai, T.; Balog, M.; Jekő, J.; Hubbell, W. L.; Hideg, K. *Synthesis* **2002**, 2365–2372.
12. Clayden, J. *Organolithiums: Selectivity for Synthesis*; Elsevier: Oxford, 2002.
13. (a) Zhdanov, R. I. *Bioactive Spin Labels*; Springer: Berlin, 1992; (b) Kálai, T.; Balog, M.; Jekő, J.; Hideg, K. *Synthesis* **1998**, 1476–1482.
14. Keana, J. F. W.; Hideg, K.; Birrell, G. B.; Hankovszky, H. O.; Ferguson, G.; Parvez, M. *Can. J. Chem.* **1982**, *60*, 1439–1447.
15. Hideg, K.; Sár, P. C.; Hankovszky, H. O.; Tamás, T.; Jerkovich, G. *Synthesis* **1993**, 390–394.
16. To a stirred solution of compound **2** (2.62 g, 10.0 mmol) in anhydr. THF (20 mL), *n*-BuLi (8 mL, 20.0 mmol in 2.5 M hexane solution) was added dropwise under N_2 at −78 °C. The reaction mixture was stirred at this temperature for 30 min, then trimethyl borate (1.2 g, 12.0 mmol) in THF (5 mL) was added dropwise over 15 min and the mixture was stirred for a further 2 h at −78 °C. After warming the solution to 0 °C, methanol (1 mL) followed by water (10 mL) were added and the mixture was stirred overnight

at rt. The mixture was diluted with Et_2O (20 mL), the organic phase was separated and the aqueous phase acidified with AcOH and extracted again with Et_2O (20 mL). The combined organic phases were dried ($MgSO_4$), activated MnO_2 (10.0 mmol, 860 mg) was added and O_2 was bubbled through for 15 min. The mixture was filtered, evaporated and the residue was suspended in toluene (50 mL) and refluxed for 1 h under Dean–Stark conditions. The toluene was evaporated and the residue was crystallized from hexane/Et_2O to give a pale yellow solid 865 mg (47%) as a crude product. The solid was further purified by flash column chromatography with hexane/Et_2O (1/4). Anal. Calcd for $C_8H_{15}BNO_3$: C, 52.22; H, 8.22; N, 7.61. Found: C, 52.37; H, 8.30; N, 7.65; mp: 208–210 °C (dec.), MS (EI) *m/z*: 184 (M^+, 19), 170 (7), 154 (59), 95 (100).

17. Chaumeil, H.; Le Drian, C.; Defoin, A. *Synthesis* **2002**, 757–760.
18. Takahashi, K.; Takagi, J.; Ishiyama, T.; Miyaura, N. *Chem. Lett.* **2000**, 126–127.
19. Gnewuch, T.; Sosnovsky, G. *Chem. Rev.* **1986**, *86*, 203–238.
20. Green, S. A.; Simpson, D. J.; Zhou, G.; Ho, P. S.; Blough, N. V. *J. Am. Chem. Soc.* **1990**, *112*, 7337–7346.
21. (a) Alves, I.; Cowell, S.; Lee, S. Y.; Tang, X.; Davis, P.; Porreca, F.; Hruby, V. J. *Biochem. Biophys. Res. Commun.* **2004**, *318*, 335–340; (b) Medvedeva, N.; Martin, V. V.; Weis, A. L.; Likhtenshtein, G. I. *J. Photochem. Photobiol. A* **2004**, *163*, 45–51; (c) Wang, H. M.; Zhang, D. Q.; Guo, X. F.; Zhu, L. Y.; Shuai, Z. G.; Zhu, D. B. *Chem. Commun.* **2004**, 670–671.
22. (a) Khanapure, S. P.; Garvey, D. S. *Tetrahedron Lett.* **2004**, *45*, 5283–5286; (b) Wallace, D. J.; Chen, C. *Tetrahedron Lett.* **2002**, *43*, 6987–6990.
23. Wellmar, U.; Hörnfeldt, A.-B.; Gronowitz, S. *J. Heterocycl. Chem.* **1995**, *32*, 1159–1163.
24. Breslow, R. *Acc. Chem. Res.* **1980**, *13*, 170–177.
25. Sár, P. C.; Kálai, T.; Bárácz, M. N.; Jerkovich, G.; Hideg, K. *Synth. Commun.* **1995**, *25*, 2929–2940.
26. Barta, C.; Kálai, T.; Hideg, K.; Vass, I.; Hideg, É. *Funct. Plant. Biol.* **2004**, *31*, 23–28.
27. de Silva, A. P.; Fox, D. B.; Moody, T. S.; Weir, S. M. *Pure Appl. Chem.* **2001**, *73*, 503–511.
28. Brown, D. M.; Burdon, M. G.; Slatcher, R. P. *J. Chem. Soc. (C)* **1968**, 1051–1053.
29. (a) Gannett, P. M.; Darian, E.; Powell, J.; Johnson, E. M.; Mundoma, C.; Greenbaum, N. L.; Ramsey, C. M.; Dalal, N. S.; Budil, D. E. *Nucleic Acids Res.* **2002**, *30*, 5328–5337; (b) Okamoto, A.; Inaski, T.; Saito, T. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 3415–3418.
30. Keyes, R. S.; Bobst, A. M. In *Biological Magnetic Resonance*; Berliner, L. J., Ed.; Plenum: New York, 1998; Vol. 14, pp 283–338.
31. General procedure for the Suzuki reaction of **3**: A 50 mL two necked round-bottomed flask equipped with a condenser, magnetic stirrer, and nitrogen inlet was charged with 1.0 mmol of aryl or hetaryl bromide **5a**–**d**, $Pd(PPh_3)_4$ (58 mg, 0.05 mol) and DME (8 mL). After stirring for 10 min at rt compound **3** (202 mg, 1.1 mmol) was added, immediately followed by 10% aq Na_2CO_3 (6 mL) in the cases of **5a**–**c** or satd $NaHCO_3$ (6 mL) in the case of **5d**. The solution was refluxed for 10 h with vigorous stirring under nitrogen. After cooling to rt, the organic phase was separated and the aqueous phase was extracted with EtOAc (10 mL). The combined organic phases were washed with water (10 mL), dried ($MgSO_4$) filtered and the solvent evaporated in vacuo. The residue was purified by flash column chromatography (hexane/Et_2O or hexane/EtOAc) to give products **6a**–**d** in 42–17% yields.
32. Spectroscopic and physical data of selected compounds **2**: yellow oil. Anal. Calcd for $C_{10}H_{16}BrNO_2$: C, 45.82; H, 6.15; N, 5.34. Found: C, 45.71; H, 6.01; N, 5.22. ^{1}H NMR: ($CDCl_3$, 400 MHz) 5.74 (s, 1H), 2.11 (s, 3H), 1.26 (s, 6H), 1.24 (s, 6H). **4**: yellow solid, mp 142–144 °C. Anal. Calcd for $C_{14}H_{25}BNO_3$: C, 63.18; H, 9.47; N, 5.26. Found: C, 63.33; H, 9.59; N, 5.32. MS (EI) *m/z*: 266 (M^+, 25), 252 (24), 236 (52), 136 (100). **6b**: yellow solid, mp 122–124 °C. Anal. Calcd for $C_{21}H_{22}NO$: C, 82.86; H, 7.28; N, 4.60. Found: C, 82.66; H, 7.31; N, 4.42. MS (EI) *m/z*: 304 (M^+, 23), 290 (43), 274 (100), 231 (91). **6c**: yellow solid, mp 119–121 °C. Anal. Calcd for $C_{21}H_{22}NO_2$: C, 78.72; H, 6.92; N, 4.37. Found: C, 78.80; H, 6.90; N, 4.22. MS (EI) *m/z*: 320 (M^+, 4), 306 (35), 290 (81), 105 (100). **6d**: deep yellow solid, mp 88–91 °C. Anal. Calcd for $C_{13}H_{17}N_2O$: C, 71.86; H, 7.89; N, 12.89. Found: C, 71.84; H, 8.01; N, 12.90. MS (EI) *m/z*: 217 (M^+, 4), 205 (5), 187 (20), 172 (100). **7**: off-white solid, mp 138–140 °C. Anal. Calcd for $C_{24}H_{23}N$: C, 88.57; H, 7.12; N, 4.30. Found: C, 88.45; H, 7.01; N, 4.42. ^{1}H NMR: ($CDCl_3$, 400 MHz) 8.22–7.81 (m, 9H), 5.80 (s, 1H), 2.02 (s, 6H), 1.86 (s, 6H). MS (EI) *m/z*: 325 (M^+, 10), 310 (100), 295 (29), 253 (21). **9**: pale yellow solid, mp 268–270 °C (dec.). Anal. Calcd for $C_{12}H_{16}N_3O_3$: C, 57.59; H, 6.44; N, 16.79. Found: C, 57.62; H, 6.38; N, 16.85. MS (EI) *m/z*: 250 (M^+, 9), 236 (40), 220 (100), 205 (40).