Oriented Synthesis and In Vitro Anticancer Activity of Biquinazoline-2,2′-diones

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The synthesis of a series of biquinazoline-2,2′-diones starting from *o*-nitrobenzaldehydes, anilines, and triphosgene is presented. This general approach features a novel and easy way for access to the target products. The mechanistic course of the reaction suggests the involvement of reduction, coupling, and cyclization by one-pot. These compounds were also investigated in vitro for anticancer activity, and some were found to have good anticancer activity.

Introduction

It is well-known that heterocycles are abundant in nature and are of great significance to life because their structural subunits exist in many natural products such as vitamins, hormones, antibiotics, and alkaloids, as well as pharmaceuticals, herbicides, dyes, and many other compounds. Quinazolinones are an important class of heterocycles[1] with potential pharmacological activities including antibacterial,[2] antihypertensive,[3] antitumor,[4,5] anti-inflammatory,[6] antidiabetic agents[7] and protein kinase inhibitors.[8,9] Moreover, the quinazolinone moiety has been extensively utilized as a druglike scaffold in medicinal chemistry.[10,11] The quinazolinone derivatives can generally be prepared by the reaction of anthranilamides with aldehydes or ketones under either acidic or basic conditions,[12−15] and the typical preparation of anthranilamides, in turn, proceeds by the reaction of anilines with isatoic anhydride. However, this is not an effective protocol if the anilines are substituted with an electron-withdrawing group[16] or present either two *ortho* substituents or one bulky *ortho* substituent. In these cases, the aniline does not react with isatoic anhydride even at elevated temperatures.[17] Yoo reported a novel one-step synthesis of 2,3-dihydro-1*H*-quinazoline-4-ones from 2-nitrobenzamides;[18] however, the reaction must reflux 2 days, which is too long. Reaction of anthranilic acids with carboxylic acids in the presence of $P(OPh)_3$ in pyridine at 150 °C for 10 min under microwave irradiation yielded a library of quinazolinones;[19] the reaction temperature limited the application of the method. Additionally, the synthesis of biquinazoline-2,2′-diones (Figure 1) is rather scarce, therefore, a novel and general approach would be desirable and of high value.

Previously, we described a facile synthesis of quinazolinone derivatives by the novel reductive cyclization of 2-nitrobenzamide and triphosgene or isothiocyanates promoted by low-valent titanium reagent.[20,21] As our earlier work goes, in this paper, we would like to report a novel access to the construction of the biquinazoline-2,2′-dione skeleton (**1**). For our target structure **1**, the most efficient route would require the reductive coupling of *N*-(2-nitrobenzylidene)aniline derivatives **2** with triphosgene (Scheme 1). *N*-(2-Nitrobenzylidene)aniline can be obtained from corresponding *o*-nitrobenzaldehydes and anilines. Therefore, we now report for the first time an efficient strategy for the synthesis of biquinazoline-2,2′-diones from *o*-nitrobenzaldehydes, anilines, and triphosgene.

The synthesis of **1** is depicted in Scheme 2. *N*-(2-Nitrobenzylidene)aniline **2** was prepared from *o*-nitrobenzaldehyde and aniline in EtOH, which is simple, cheaply priced, and readily available. With compound **2** in hand we

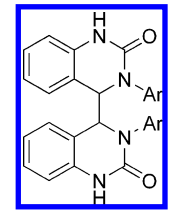
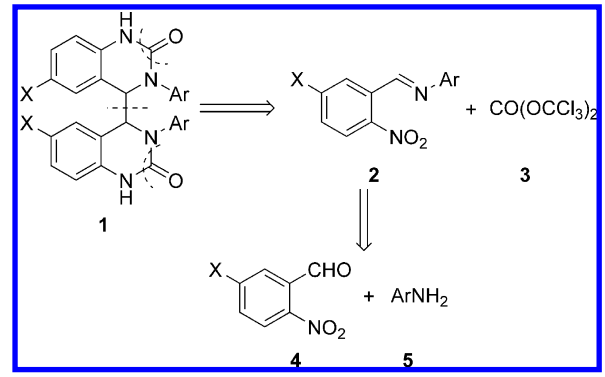


Figure 1. Biquinazoline-2,2′-diones.

Scheme 1. Retrosynthetic Pathway for the Synthesis of Biquinazoline-2,2′-diones (**1**)



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Scheme 2. Reagents and Conditions: (a) EtOH, Reflux 2 h; (b) $TiCl_4$/Sm, Triphosgene, THF, Reflux 2 h

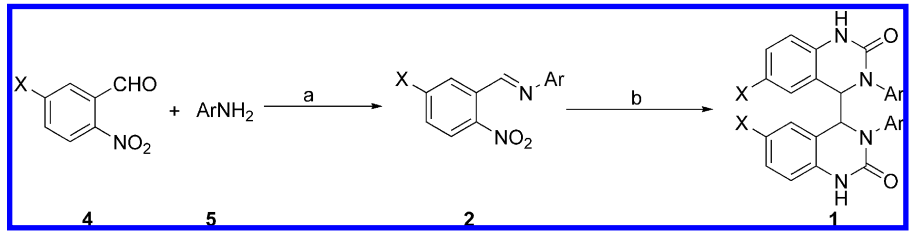
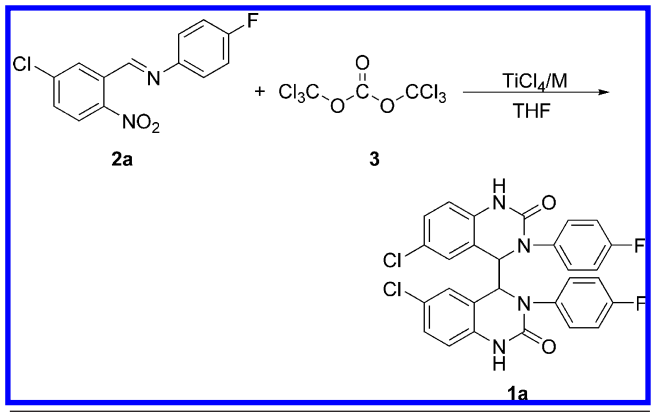


Table 1. Optimization of Reaction Conditions for Compound **1a**



entry	$TiCl_4$/M	ratio[a]	yield/%
1	$TiCl_4$/Zn	1:4	85
2	$TiCl_4$/Mg	1:4	69
3	$TiCl_4$/Al	1:4	62
4	$TiCl_4$/Fe	1:4	57
5	$TiCl_4$/Sm	1:4	94
6	$TiCl_4$/Sm	1:1	72
7	$TiCl_4$/Sm	1:2	78
8	$TiCl_4$/Sm	1:3	89
9	$TiCl_4$/Sm	1:5	93

[a] This ratio is the ratio of **2a** and low-valent titanium reagent.

then looked at the reductive coupling of double linkage, nitro group of the Schiff base **2** and triphosgene to give biquinazoline-2,2′-diones **1**.

Initially, the reaction of *N*-(5-chloro-2-nitrobenzylidene)-4-fluoroaniline **2a** and triphosgene at reflux was examined. As a preliminary study, low-valent titanium system and the ratio of **2a** and low-valent titanium reagent were screened in the model reaction. The results are shown in Table 1.

It was observed that among the screened low-valent systems, $TiCl_4$/Sm was superior to other low-valent titanium systems (Table 1, entries 1−4). To further optimize reaction conditions, a similar test was carried out at ratios of **2a** and low-valent titanium reagent ranging from 1:1 to 1:5. The yield of product **1a** was increased as the ratio was increased from 1:1 to 1:4 (Table 1, entries 5, 6−8). However, a further increase of the ratio to 1:5 failed to improve the yield of product **1a** (Table 1, entry 9). Therefore, 1:4 was chosen for all further reactions.

With the optimized conditions in hand, we then performed the reaction of a variety of *N*-(2-nitrobenzylidene)aniline **2** and triphosgene **3** via low-valent titanium reagent ($TiCl_4$/Sm). The results are summarized in Table 2.

As shown in Table 2, we were pleased to find that the method was applicable to a broad substrate scope on *N*-(2-nitrobenzylidene)anilines. Anilines containing various electron-donating and electron-withdrawing substituents were reacted under the optimized conditions, and the corresponding products were obtained in good yields; therefore, no remarkable electronic effects on the reaction were observed. We were also pleased to find that no significant steric effects were observed for the *ortho*-, *meta*-, and *para*-substituted anilines, especially, the anilines presenting two *ortho* substituents can also give moderate to good yields (Table 2, entry 19). Good yield was also obtained when *N*-(2-nitrobenzylidene)naphthalene-1-amine reacted with triphosgene (Table 2, entry 20).

In our study, we ran the reaction of **2n** with the absence of triphosgene. However, tetraamine **6** can not be obtained and the reductive coupling product 2-(4-methylphenyl)-2*H*-indazole **7** was surprisingly obtained in 30% yield (Scheme 3). Therefore, we think that nitro group reduction precedes reductive coupling.

Although the mechanism of the reaction is presently unclear, a few possible sequences of events are outlined in Scheme 4. First of all, $TiCl_4$ is reduced by Sm dust to give low-valent titanium species. In the initial step, because nitro group reduction precedes reductive coupling, nitro compound was reduced by low-valent titanium to amine **8**, which was then reacted with triphosgene to give compound **9**. Then compound **9** was reductive coupled and produce intermediate **10** by low-valent titanium reagent.[22] Then **11** was obtained by addition, and product **1** was obtained by hydrolysis.

All the products were characterized by ^{1}H NMR, ^{13}C NMR, IR, and HRMS spectra. The structure of **1e** was further confirmed by X-ray diffraction analysis. The molecular structure of the product **1e** is shown in Figure 2.

Finally, selected compounds were screened for anticancer activity by the sulforhadamine B (SRB) method. The cells used in this study were Human liver cancer Bel7402. The results of the prescreening were listed in Figure 3.

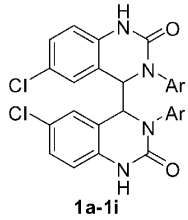
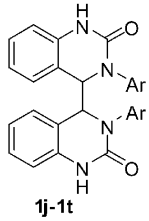
Then we chose **1b**, **1h**, and **1k** to determine the IC_{50} further. The results are shown in Table 3.

Among the compounds synthesized, the most active compound was **1b**. The IC_{50} values for **1b**, **1h**, and **1k** were 0.33 μM, 2.13 μM, and 1.04 μM, respectively. Good to improved anticancer activity was observed for them. However, the activity of compounds **1d** and **1i** were lower than that of other compounds, and the reason is presently unclear.

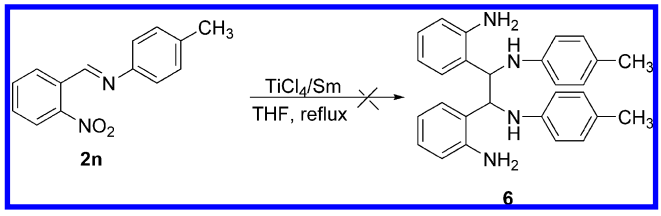
Conclusions

In conclusion, we have developed an efficient and convenient method for the preparation of biquinazoline-2,2′-diones. The process was carried out in tetrahydrofuran (THF) via $TiCl_4$/Sm to generate diversity on the biquinazolindiones. A variety of substrates can participate in the process with good yields. The procedure used commercially available

Table 2. Synthesis of Biquinazoline-2,2′-diones **1** Induced by $TiCl_4$/Sm System

Entry	Product	Ar	Yield/%	m.p./°C
1	 **1a-1i**	4-FC_6H_4 (**1a**)	94	> 300
2		4-ClC_6H_4 (**1b**)	75	> 300
3		4-$CH_3C_6H_4$ (**1c**)	74	295-296
4		4-$CH_3OC_6H_4$ (**1d**)	76	> 300
5		3-Cl-4-FC_6H_3 (**1e**)	94	> 300
6		3-Cl-4-$CH_3C_6H_3$ (**1f**)	82	> 300
7		3-$CH_3C_6H_4$ (**1g**)	92	291-292
8		2-$CH_3C_6H_4$ (**1h**)	83	> 300
9		C_6H_5 (**1i**)	95	> 300
10		C_6H_5 (**1j**)	83	> 300
11	 **1j-1t**	4-ClC_6H_4 (**1k**)	83	> 300
12		4-FC_6H_4 (**1l**)	90	> 300
13		4-BrC_6H_4 (**1m**)	80	> 300
14		4-$CH_3C_6H_4$ (**1n**)	79	> 300
15		4-$CH_3OC_6H_4$ (**1o**)	78	> 300
16		3-$CH_3OC_6H_4$ (**1p**)	82	285-287
17		3-Cl-4-FC_6H_3 (**1q**)	73	> 300
18		2-$CH_3C_6H_4$ (**1r**)	73	> 300
19		2,6-$Cl_2C_6H_3$ (**1s**)	86	> 300
20		$C_{10}H_7$ (**1t**)	76	> 300

Scheme 3. Reaction with the Absence of Triphosgene



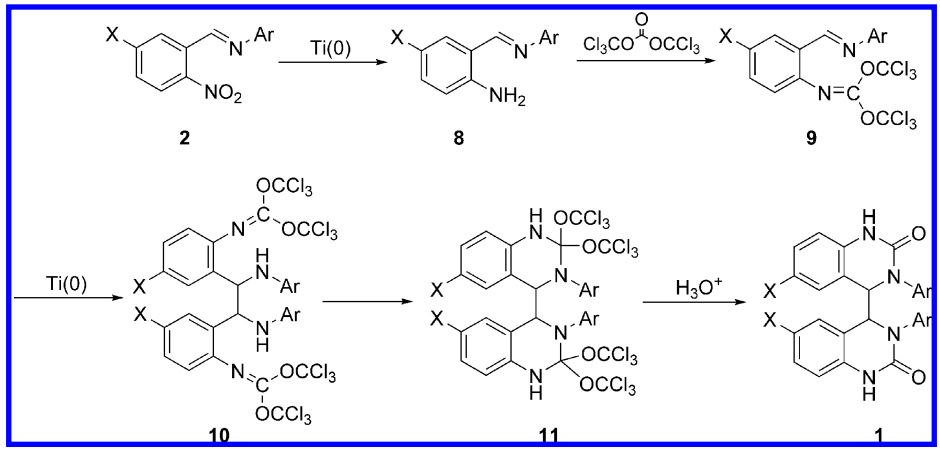
o-nitrobenzaldehydes and anilines and is suitable for library synthesis in drug discovery efforts. In particular, the product could easily be collected by recrystallization. The short reaction time and easily available materials render this method particularly attractive for the efficient preparation of biologically and medicinally interesting molecules. Importantly, almost all of the compounds exhibited good anticancer activity. It should be pointed out that this is the first report of anticancer activity among these compounds.

Experimental Section

General Information. THF was distilled from sodium-benzophenone just prior to use. All the reactions were conducted under N_2 atmosphere. Melting Points are uncorrected. IR spectra were recorded on a Tensor 27 spectrometer in KBr with absorptions in cm^{-1}. ^1H NMR (400 MHz) and ^{13}C NMR (100 MHz) spectra were recorded on a Bruker DPX-400 MHz spectrometer in DMSO-d_6 solution. *J* values are in hertz. Chemical shifts are expressed in parts per million downfield from internal standard TMS. The exact mass measurements were obtained by high resolution mass instrument (GCT-TOF instrument).

General Procedure for the Synthesis of Biquinazoline-2,2′-diones 1. $TiCl_4$ (0.5 mL, 4 mmol) was added dropwise using a syringe to a stirred suspension of samarium powder (0.6 g, 4 mmol) in freshly distilled anhydrous THF (10 mL) at room temperature (r.t.) under a dry N_2 atmosphere. After completion of the addition, the mixture was refluxed for 2 h. The suspension of the low-valent titanium reagent formed was cooled to r.t., and a solution of *N*-(2-nitrobenzylidene) anilines (1 mmol) and triphosgene (1 mmol) in THF (5 mL) was added dropwise. The reaction mixture was then refluxed for 2 h under N_2 atmosphere. After this period, the thin-layer chromatography (TLC) analysis of the mixture showed the completion of this reaction. The mixture was then quenched with 5% HCl (30 mL) and extracted with $ClCH_2CH_2Cl$ (3 × 50 mL). The extracts were washed with water (3 × 50 mL) and dried over anhydrous Na_2SO_4. After evaporation of the solvent under reduced pressure, the crude products were purified by recrystallization from 95% ethanol and *N*,*N*-dimethylformamide (DMF).

Scheme 4. Supposed Reaction Mechanism



6,6′-Dichloro-3,3′-bis(4-fluorophenyl)-3,3′,4,4′-tetrahydro-4,4′-biquinazoline-2,2′-dione (1a). ^{1}H NMR (400 Hz, DMSO-d_6) (δ, ppm): 9.53 (br, s, 2H, 2 × NH), 7.40−7.36 (m, 4H, ArH), 7.23 (d, J = 2.4 Hz, 2H, ArH), 7.16 (dd, J_1 = 2.4 Hz, J_2 = 8.4 Hz, 2H, ArH), 7.06 (t, J = 8.8 Hz, 4H, ArH), 6.59 (d, J = 8.8 Hz, 2H, ArH), 5.25 (s, 2H, 2 × CH). ^{13}C NMR (100 Hz, DMSO-d_6) (δ, ppm): 161.9, 158.7, 152.1, 137.9, 137.8, 137.6, 129.4, 129.4, 129.2, 127.2, 125.3, 119.4, 115.9, 115.4, 65.3 (4 C).

IR (KBr) ν: 3197, 3054, 2931, 1684, 1597, 1507, 1447, 1392, 1306, 1218, 1156, 1091, 941, 857, 831, 817, 743, 716, 663 cm^{-1}.

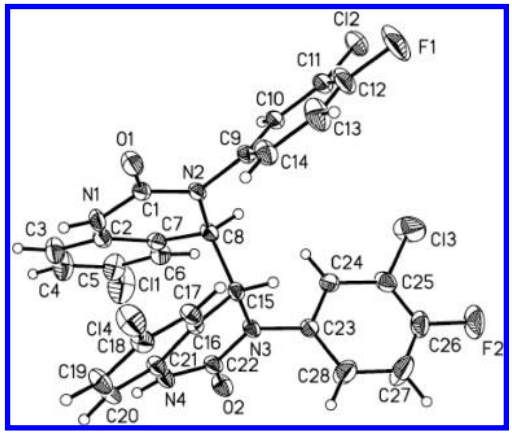


Figure 2. Molecular structure of product **1e**.

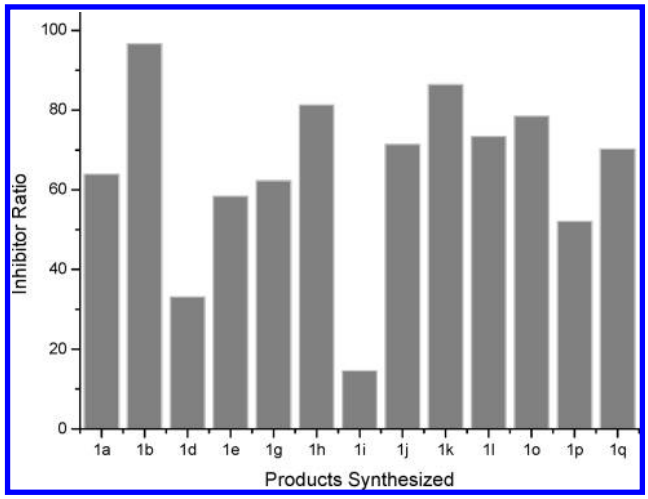
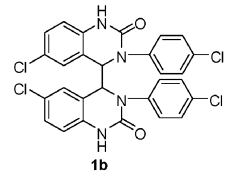
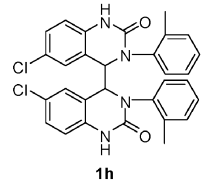
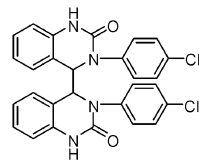


Figure 3. Inhibitor ratio of the products by prescreening.

Table 3. IC_{50} of Compounds **1b**, **1h**, and **1k**

Compound	IC_{50}/μM
 **1b**	0.33
 **1h**	2.13
 **1k**	1.04

HRMS (ESI): m/z calcd for: 573.0673 [M+Na]$^+$, found: 573.0674.

6,6′-Dichloro-3,3′-bis(4-methylphenyl)-3,3′,4,4′-tetrahydro-4,4′-biquinazoline-2,2′-dione (1c). ^{1}H NMR (400 Hz, DMSO-d_6) (δ, ppm): 9.35 (br, s, 2H, 2 × NH), 7.25 (d, J = 8.4 Hz, 4H, ArH), 7.22 (d, J = 2.4 Hz, 2H, ArH), 7.19 (dd, J_1 = 2.4 Hz, J_2 = 8.4 Hz, 2H, ArH), 7.07 (d, J = 8.4 Hz, 4H, ArH), 6.58 (d, J = 8.4 Hz, 2H, ArH), 5.15 (s, 2H, 2 × CH), 2.28 (s, 6H, 2 × CH_3).

^{13}C NMR (100 Hz, DMSO-d_6) (δ, ppm): 152.2, 139.1, 137.7, 135.8, 129.8, 129.3, 127.3, 127.2, 125.1, 119.1, 115.2, 64.6 (4 C), 21.2.

IR (KBr) ν: 3197, 3052, 2925, 1679, 1598, 1501, 1447, 1389, 1312, 1212, 1165, 1132, 1111, 1091, 1018, 995, 935, 821, 741, 717, 669 cm^{-1}.

HRMS (ESI): m/z calcd for: 565.1174 [M+Na]$^+$, found: 565.1235.

3,3′-Bis(4-chlorophenyl)-3,3′,4,4′-tetrahydro-4,4′-biquinazoline-2,2′-dione (1l). ^{1}H NMR (400 Hz, DMSO-d_6) (δ, ppm): 9.31 (br, s, 2H, 2 × NH), 7.40 (d, J = 8.8 Hz, 4H, ArH), 7.29 (d, J = 8.8 Hz, 4H, ArH), 7.15 (d, J = 7.2 Hz, 2H, ArH), 7.11 (t, J = 7.6 Hz, 2H, ArH), 6.85 (t, J = 7.6 Hz, 2H, ArH), 6.56 (d, J = 7.6 Hz, 2H, ArH), 5.19 (s, 2H, 2 × CH).

^{13}C NMR (100 Hz, DMSO-d_6) (δ, ppm): 151.0, 137.1, 136.7, 136.7, 128.5, 128.4, 128.1, 126.1, 120.3, 116.0, 114.7, 114.5, 112.5, 108.8, 63.9 (4 C).

IR (KBr) ν: 3212, 3068, 2918, 1674, 1600, 1491, 1448, 1419, 1389, 1311, 1286, 1247, 1218, 1155, 1092, 1035, 1014, 872, 847, 828, 744, 659 cm^{-1}.

HRMS (ESI): m/z calcd for: 537.0861 $[M+Na]^+$, found: 537.0877.

3,3′-Bis(3-methoxyphenyl)-3,3′,4,4′-tetrahydro-4,4′-biquinazoline-2,2′-dione (1q). ^{1}H NMR (400 Hz, DMSO-d_6) (δ, ppm): 9.14 (br, s, 2H, 2 × NH), 7.23−7.16 (m, 4H, ArH), 7.11 (t, J = 7.2 Hz, 2H, ArH), 7.00 (d, J = 7.6 Hz, 2H, ArH), 6.91−6.85 (m, 4H, ArH), 6.75 (dd, J_1 = 2.0 Hz, J_2 = 8.0 Hz, 2H, ArH), 6.53 (d, J = 8.0 Hz, 2H, ArH), 5.20 (s, 2H, 2 × CH), 3.65 (s, 6H, 2 × CH_3O).

^{13}C NMR (100 Hz, DMSO-d_6) (δ, ppm): 152.1, 151.9, 139.1, 138.9, 138.2, 130.3, 129.6, 129.6, 129.3, 129.3, 127.8, 127.7, 127.5, 127.3, 121.7, 121.5, 119.6, 119.3, 119.3, 117.9, 117.4, 116.8, 116.8, 116.5, 114.1, 113.9, 67.4 (4 C), 66.2 (4′ C).

IR (KBr) ν: 3184, 3061, 2994, 2937, 1679, 1602, 1496, 1443, 1421, 1302, 1265, 1226, 1204, 1169, 1045, 815, 773, 760, 743, 697 cm^{-1}.

HRMS (ESI): m/z calcd for: 529.1852 $[M+Na]^+$, found: 529.1883.

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Supporting Information Available. Detailed descriptions of experimental procedures and spectroscopic and analytical data are available for compounds **1**. This material is available free of charge via the Internet at http://pubs.acs.org.

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