APPLIED ORGANOMETALLIC CHEMISTRY
Appl. Organometal. Chem. 2002; **16**: 708–712

Synthesis and biological activity of novel *N*-benzoyl-*N*-*tert*-butyl-*N*′-(*β*-triphenylgermyl)propionylhydrazines

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Received 21 November 2001; Revised 14 July 2002; Accepted 14 July 2002

A series of new *N*-benzoyl-*N*-*tert*-butyl-N′-(*β*-triphenylgermyl)propionylhydrazines were synthesized by the condensation reaction of *β*-triphenylgermyl propanoic acid with *N*-benzoyl-*N*-*tert*-butylhydrazines in good yields by using *N*,*N*′-dicyclohexylcorbodiimide as dehydrating agent. These title compounds were evaluated for molting hormone mimicking activity. The results of bioassay showed that the compounds exhibit moderate larvicidal activity, and toxicity assays indicated that the title compounds can induce a premature, abnormal and lethal larval molt. We found that the title compounds possess potential anticancer activities *in vitro*. Copyright © 2002 John Wiley & Sons, Ltd.

KEYWORDS: *N*-benzoyl-*N*-*tert*-butyl-*N*′-(*β*-triphenylgermyl)propionylhydrazine; premature and lethal larval molt; anticancer activity

INTRODUCTION

Recently, a new class of insect growth regulators, the *N*-*tert*-butyl-*N*,*N*′-diacylhydrazines, have been found to mimic the action of 20-hydroxyecdysone to activate the ecdysone receptor, leading to lethal premature molting.[1–3] Relationships between the structure and biological activity of the *N*-*tert*-butyl-*N*,*N*′-dibenzoylhydrazine larvicides have been extensively investigated. The results indicate that molecular hydrophobicity is favorable and that *N*-*tert*-butyl-*N*-benzoylhydrazine is the biologically active unit.[4–6] In addition, both *β*-triphenylgermyl propanoic acid and its derivatives often possess unexpected biological activity.[7–12] The substitution of a phenyl group by a (*β*-triphenylgermyl)propionyl group in the bioactive compound may be expected to induce great changes in molecular properties, such as solubility and hydrophobicity. Hence, in the search for new insect growth regulators with improved profiles, we thought that the preservation of the bioactivity unit and the replacement of the phenyl moiety by (*β*-triphenylgermyl)-propionyl in *N*-*tert*-butyl-*N*,*N*′-dibenzoylhydrazine would change their larvicidial activities to a significant degree. Therefore, we designed and synthesized the series of new title compounds.

EXPERIMENTAL

General

All the melting points were determined with a Thomas-Hoover melting point apparatus; the thermometer was not standardized. IR spectra were recorded with a Shimadzu-435 in KBr discs. ^{1}H NMR spectra were recorded with a Bruker AC-P200 (200 MHz) using tetramethylsilane as an internal standard. Mass spectra were recorded with HP5988A spectrometer using the electron impact method. Elemental analysis was carried out with a Yanaco CHN Corder MT-3 elemental analyzer.

Synthesis

Triphosgene was synthesized by chlorination of dimethylcarbonate;[13,14] it melted at 79 °C, IR (KBr, cm^{-1}): 1820, 1178, 925, 810, 675, 517.

Benzyl chloroformate

A solution of distilled benzyl alcohol (10.92 g, 0.101 mol) and distilled pyridine (11.98 g, 0.150 mol) in methylene dichloride (20 ml) was added dropwise to a solution of triphosgene (15 g, 0.050 mol) in methylene dichloride (30 ml) at −10 °C.

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Contract/grant sponsor: National Natural Science Foundation of China.
Contract/grant sponsor: Research Fund for the Doctoral Program of Higher Education.

Then the resulting mixture was stirred at −10 °C for 2 h, followed by an additional 42 h stirring at room temperature. After the solvent was removed under vacuum, the residue was distilled under reduced pressure, yielding a colorless liquid (16.89 g) in 98.0% yield; b.p.: 42–49/5–6 mmHg, n_D^{20}: 1.5188 (literature,[15] n_D^{20}: 1.5190).

N-tert-Butyl-*N*′-benzyloxycarbonylhydrazine

To a mechanically stirred suspension of *tert*-butylhydrazine hydrochloride (11.50 g, 0.092 mol) in toluene (100 ml) was added dropwise a solution of 10% aqueous sodium hydroxide (36.92 g, 0.092 mol) at room temperature. After 15 min, the reaction mixture was cooled to −15 °C, and solutions of benzyl chloroformate (15 g, 0.088 mol) in toluene (30 ml) and 10% aqueous sodium hydroxide (35.16 g, 0.088 mol) were added dropwise and simultaneously from separate addition funnels, while maintaining the temperature below −10 °C. Following the addition, the reaction mixture was warmed to room temperature and stirred for 2 h. The water phase was extracted three times with 100 ml of chloroform. The extraction solvent was combined with the organic phase, and dried with anhydrous magnesium sulfate and filtered. The solvent was removed by distillation to give a white solid. The solid was then recrystallized from 2-propanol and petroleum ether to obtain a colorless crystalline solid (16.33 g) in 83.6% yield; m.p. 75–77 °C. ^{1}H NMR ($CDCl_3$, ppm) δ1.11 (s, 9H, ^{t}Bu), 5.16 (s, 2H, OCH_2), 5.02 (br, 2H, NHNH), 7.38 (m, 5H, Ph). IR (KBr, cm^{-1}): 3264.0, 3240.0 (NHNH); 1713.4 (C═O); 1521.4, 1491.8, 1466.3(Ph); 1406.2, 1381.8 (^{t}Bu); 1260.8 (C—O); 828.2, 716.1 (Ph).

General procedure for the preparation of *N*-benzoyl-*N-tert*-butyl-*N*′-benzyloxycarbonyl hydrazines 1a and 1b

A solution of benzoyl chloride (0.054 mol) in methylene dichloride (15 ml) was added dropwise to a solution of *N-tert*-butyl-*N*′-benzyloxycarbonylhydrazine (12 g, 0.054 mol) and triethylamine (6.58 g, 0.065 mol) in methylene dichloride (40 ml) under magnetic stirring at 0 °C, then the resulting mixture was stirred at room temperature for 2 h. The solid was then filtered off and the filtrate was washed successively with 2% aqueous hydrochloric acid and 10% aqueous sodium bicarbonate, and then dried with anhydrous magnesium sulfate and filtered. The solvent was removed by distillation to give a white solid. The solid was then recrystallized from ethanol to obtain a colorless crystalline solid.

1a (X = H). Yield: 89.6%; m.p. 150–152 °C. Anal. Calc. for $C_{19}H_{22}N_2O_3$ (%): C, 69.92; H, 6.79; N, 8.58. Found: C, 69.82; H, 6.85; N, 8.78. ^{1}H NMR (DMSO-d_6, ppm) δ1.40 (d, 9H, ^{t}Bu), 4.80–5.05 (m, 2H, OCH_2), 6.84–7.37 (m, 10H, Ph), 9.83 (s, NH). IR (KBr, cm^{-1}): 3219.5 (NH); 1742.5, 1622.0 (C═O); 1578.4, 1531.5, 1498.1 (Ph); 1403.3, 1382.3 (^{t}Bu); 738.6, 712.9 (Ph).

1b (X = 3,5-Me$_2$). Yield: 82.9%; m.p. 134–136 °C. Anal. Calc. for $C_{21}H_{26}N_2O_3$ (%): C, 71.16; H, 7.37; N, 7.90. Found: C, 70.98; H, 7.39; N, 8.14. ^{1}H NMR (DMSO-d_6, ppm) δ1.38 (d, 9H, ^{t}Bu), 2.21 (s, 6H, Ph—Me), 4.80–5.09 (m, 2H, OCH_2), 6.88–7.37 (m, 8H, Ph), 9.77 (s, NH). IR (KBr, cm^{-1}): 3253.5 (NH); 1733.5, 1639.8 (C═O); 1599.6, 1523.6, 1495.6 (Ph); 1399.4, 1365.4 (^{t}Bu); 788.1, 743.9 (Ph).

General procedure for the preparation of *N-tert*-butyl-*N*-benzoylhydrazines 2a and 2b

To a solution of **1a** or **b** (41.40 mmol) in methanol (100 ml) was added 5% Pd–C. Hydrogen gas was then admitted to the solution. The reaction was monitored by thin-layer chromatography and stopped after complete consumption of **1**. The solid was filtered off and the filtrate was concentrated under vacuum to obtain a white powder.

2a (X = H). Yield: 96.4%; m.p. 127–129 °C. ^{1}H NMR ($CDCl_3$, ppm) δ 1.48 (s, 9H, ^{t}Bu), 3.90 (s, 2H, NH_2), 7.28–7.56 (m, 5H, Ph). IR (KBr, cm^{-1}): 3276.0 (NH_2); 1620.5 (C═O); 1573.1, 1529.5, 1508.8 (Ph); 1375.6, 1350.0 (^{t}Bu); 719.6, 696.7 (Ph).

2b (X = 3,5-Me$_2$). Yield: 91.9%; m.p. 128–130 °C. ^{1}H NMR ($CDCl_3$, ppm) δ 1.48 (s, 9H, ^{t}Bu); 2.34 (s, 6H, Ph—Me); 7.00–7.20 (m, 3H, Ph); 4.08 (s, 2H, NH_2).

3-(Triphenylgermyl)propanoic acid (3)

3-(Trichlorogermyl)propanoic acid was synthesized according to the reported procedure.[16] Phenylmagnesium bromide was prepared by reaction between magnesium (0.176 mol) and bromobenzene (0.16 mol) in THF (30 ml) under nitrogen. To the cooled and stirred phenylmagnesium bromide in THF was added dropwise 3-(trichlorogermyl)propanoic acid (0.04 mol) in THF. After the addition, the mixture was refluxed for 4 h. 10% aqueous hydrochloric acid was added. The solution was adjusted to about pH 5. The water phase was extracted three times with methylene dichloride. The methylene dichloride phase was dried with anhydrous magnesium sulfate and filtered. The solvent was removed by distillation to give a white solid. The solid was then recrystallized from methylene dichloride and petroleum ether to obtain a colorless crystalline solid in 81.0% yield; m.p. 130 °C. ^{1}H NMR ($CDCl_3$, ppm) δ 1.86 (t, 2H, $GeCH_2$); 2.51 (t, 2H, CH_2C═O); 7.38–7.53 (m, 15H, Ph). Anal. Calc. for $C_{21}H_{20}GeO_2$ (%): C, 66.91; H, 5.35. Found: C, 66.95; H, 5.96.

General procedure for the preparation of 4

1.56 mmol of *N-tert*-butyl-*N*-benzoylhydrazine and 0.35 g (1.72 mmol) of *N*,*N*′-dicyclohexylcarbodiimide (DCC) were dissolved in 25 ml of dried THF. The reaction mixture was cooled to below 0 °C in an ice–salt bath and stirred for 0.5 h. To the stirred mixture was added dropwise 1.56 mmol of β-triphenylgermyl propanoic acid in dried THF (10 ml). After the addition, the mixture was stirred for approximately 6 h at room temperature. To the reaction mixture was added several drops of glacial acetic acid to destroy excess of DCC. The precipitate was filtered off, the filtrate was washed successively with 2% aqueous hydrochloric acid, distilled water, 10% aqueous sodium bicarbonate and distilled water. The washed solution was dried with anhydrous magnesium sulfate and filtered, and the solvent was removed by distillation to give a white solid. The white solid was purified by vacuum column chromatography on a silica gel using a mixture of petroleum ether (60–90 °C) and ethyl acetate as the eluent. Finally, a colorless crystalline solid **4** was obtained, and compounds **5** and **6** were detected and isolated.

4a (X = H). Yield: 64.0%; m.p. 217–218 °C. ^{1}H NMR ($CDCl_3$, ppm) δ 1.44 (s, 9H, ^{t}Bu); 1.76 (m, 2H, $GeCH_2$); 2.04 (m, 2H, $CH_2C{=}O$); 7.12 (s, 1H, NH); 7.24–7.35 (m, 20H, Ph). Anal. Calc. for $C_{32}H_{34}GeN_2O_2$ (%): C, 69.73; H, 6.22; N, 5.08. Found: C, 69.61; H, 6.25; N, 5.30.

4b (X = 3,5-Me_2). Yield: 88.9%; m.p. 208–209 °C. ^{1}H NMR ($CDCl_3$, ppm) δ 1.44 (s, 9H, ^{t}Bu); 1.71 (m, 2H, $GeCH_2$); 2.00 (m, 2H, $CH_2C{=}O$); 2.20 (s, 6H, Me); 6.89 (s, 1H, NH); 6.93–7.35 (m, 18H, Ph). Anal. Calc. for $C_{34}H_{38}GeN_2O_2$ (%): C, 70.50; H, 6.61; N, 4.84. Found: C, 70.49; H, 6.59; N, 4.87. IR (KBr, cm^{-1}): 3239, 1699, 1621, 1390, 1360, 589.

N,N′-*Dicyclohexyl*-O-*(β-triphenylgermyl)propionylisourea* (**5**):

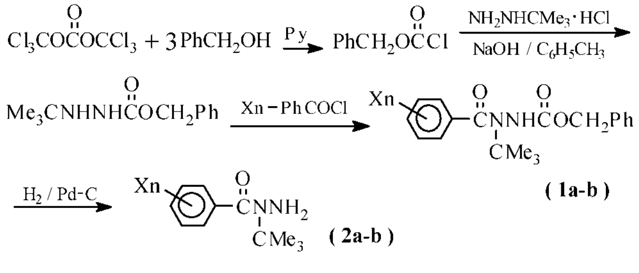
m.p. 172–173 °C. ^{1}H NMR ($CDCl_3$, ppm) δ 0.92–1.90 (m, 22H, $(CH_2)_5$, CH_2Ge); 2.48–2.62 (m, 2H, $CH_2C{=}O$); 3.44–3.80 (m, 2H, NCH); 6.64 (br, 1H, NH); 7.33–7.49(m, 15H, Ph). Anal. Calc. for $C_{34}H_{42}GeN_2O_2$ (%): C, 70.01; H, 7.26; N, 4.80. Found: C, 69.79; H, 7.30; N, 5.01. IR (KBr, cm^{-1}) 3240 (NH), 1695 (C=O), 1647 (N=C), 695 (N−H).

N,N′-*Dicyclohexyl*-N-*(β-triphenylgermyl)propionylurea* (**6**):

m.p. 183–184 °C. ^{1}H NMR ($CDCl_3$, ppm) δ 0.87–1.85(m, 22H, $(CH_2)_5$, CH_2Ge); 2.22–2.36(m, 2H, $CH_2C{=}O$); 3.56–3.76 (m, 1H, NCH); 5.08–5.30 (m, 1H, NCH); 7.34–7.49 (m, 15H, Ph). Anal. Calc. for $C_{34}H_{42}GeN_2O_2$ (%): C, 70.01; H, 7.26; N, 4.80. Found: C, 70.12; H, 7.22; N, 5.05. IR (KBr, cm^{-1}): 3242 (NH), 1625 (C=O), 671 (N−H). MS (EI, 70 eV): 459.40 ($[Ph_3GeCH_2CH_2CONHCH(CH_2)_5]^+$, 17%).

Biology

The larvicidal activities of the title compounds **4** and *N-tert*-butyl-*N,N*′-dibenzoylhydrazine (**RH5849**) were evaluated using a previously reported procedure.[4,17–19] The larvicidal activities were tested against Oriental armyworm (*Mythimna separata* (Walker)) by foliar application. For the foliar armyworm tests, individual corn leaves were placed on moistened pieces of filter paper in Petri dishes. The leaves were then sprayed with the test solution and allowed to dry. The dishes were infested with ten larvae of the fourth instar armyworm. The dishes were then covered with the lid and held for 3 days, at which time the percentage control (mortality) was determined. Percentage mortalities for the armyworm evaluations were determined 4 days after treatment. Evaluations are based on a scale of 0–100% in which 0% equals no activity and 100% equals total kill. For comparative purposes, *N-tert*-butyl-*N,N*′-dibenzoylhydrazine (**RH5849**) was tested under the same conditions.



Xn = H (**a**), 3,5-Me₂ (**b**).

Scheme 1.

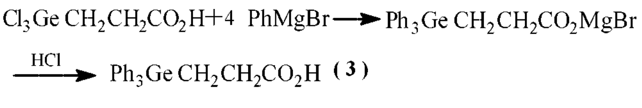
RESULTS AND DISCUSSION

Benzyl alcohol was treated with triphosgene to obtain benzyl chloroformate in good yield. This new and convenient synthesis of benzyl chloroformate can avoid the use of phosgene gas and the presence of the complicated experimental set-up associated with it. Then, benzyl chloroformate was condensed with *tert*-butylhydrazine hydrochloride to give *N-tert*-butyl-*N*′-benzyloxycarbonylhydrazine.

Subsequent acylation with appropriately substituted benzoyl chloride yielded *N-tert*-butyl-*N*′-benzyloxycarbonyl-*N*-substituted benzoylhydrazine. Its further deprotection using 5% Pd–C as a catalyst provided *N-tert*-butyl-*N*-substituted benzoylhydrazine (**2**) in good yield, as shown in Scheme 1.

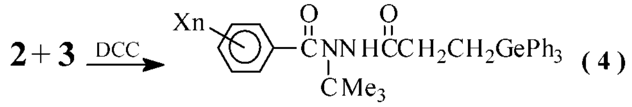
β-Triphenylgermyl propanoic acid (**3**) was prepared by the reaction of *β*-trichlorogermyl propanoic acid with phenylmagnesium bromide in molar ratio 1:4, as shown in Scheme 2.

The transformation of *β*-triphenylgermyl propanoic acid with thionyl chloride into *β*-triphenylgermyl propionyl chloride did not succeed. It is useful to synthesize amides directly from carboxylic acids and amines by using DCC as a dehydrating agent,[20] but the condensation reaction of carboxylic acids and hydrazines by using DCC as a dehydrating agent has seldom been reported. We found that *β*-triphenylgermyl propanoic acid was condensed with *N-tert*-butyl-*N*-substituted benzoylhydrazines successfully to give *N-tert*-butyl-*N*′-(*β*-triphenylgermyl) propionyl-*N*-substituted benzoylhydrazines (**4**) in good yields in THF at room temperature, as shown in Scheme 3.



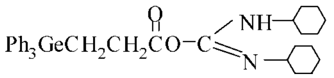
$$Cl_3GeCH_2CH_2CO_2H + 4\ PhMgBr \longrightarrow Ph_3GeCH_2CH_2CO_2MgBr \xrightarrow{HCl} Ph_3GeCH_2CH_2CO_2H\ (3)$$

Scheme 2.



Scheme 3.

During the process of the above condensation reaction, *N,N′*-dicyclohexyl-*O*-(*β*-triphenylgermyl)propionylisourea (**5**) was trapped.

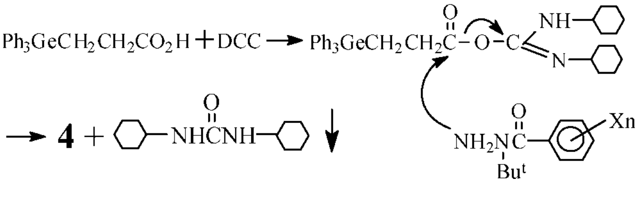


(**5**)

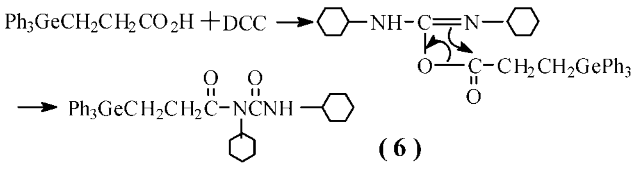
5 According to the existence of intermediate **5**, we proposed the mechanism illustrated in Scheme 4.

β-Triphenylgermyl propanoic acid was condensed with *N*-*tert*-butyl-*N*-substituted benzoylhydrazines to give the title compounds **4** in dioxane at reflux temperature, and at the same time a small amount of *N,N′*-dicyclohexyl-*N*-(*β*-triphenylgermyl)propionylurea (**6**) was obtained as by-product. Thus we have demonstrated that *N,N′*-dicyclohexyl-*O*-(*β*-triphenylgermyl)propionylisourea (**5**) can be converted to *N,N′*-dicyclohexyl-*N*-(*β*-triphenylgermyl)propionylurea (**6**) in dioxane at reflux temperature as shown in Scheme 5.

The larvicidal activities of the title compounds **4** are listed in Table 1. The results of larvicidal activities show that the title compound **4b** exhibits moderate larvicidal activity, but larvicidal activity of product **4a** is low. For example, at 1000 μg ml^{-1}, the percentage mortality of compounds **4b**, **4a** and **RH5849** is 40%, 10% and 100% respectively; however, at 10 μg ml^{-1}, the percentage mortality of compounds **4b**, **4a** and **RH5849** is 0%, 0% and 5% respectively. Toxicity assays indicated that the title compound **4b**, like *N*-*tert*-butyl-*N,N′*-dibenzoylhydrazine (**RH5849**), can induce a premature, abnormal and lethal larval molt at 1000 μg ml^{-1}. Symptoms of toxicity included discoloration, weight loss, cessation of feeding, and developmentally premature, lethal molting at higher rates.



Scheme 4.



Scheme 5.

Table 1. Larvicidal activity of the compounds **4**

	Larvicidal activity (%)	
Compound	1000 μg ml^{-1}	10 μg ml^{-1}
4a	10	0
4b	40	0
RH5849	100	5

At the same time, we found that the title compounds **4a** and **4b** possess potential anticancer activities *in vitro*. The anticancer activity was assayed by the MTT or SRB methods.[12,21–23] The anticancer activities of the title compounds **4** are listed in Table 2. For example, at 10^{-4} mol l^{-1} the inhibitory rate of compound **4a** to HL-60 and BEL-7402 is 28.6% and 36.5% respectively; at 10^{-5} mol l^{-1} the inhibitory rate of compound **4a** to HL-60 and BEL-7402 is 24.5% and 13.5% respectively. At 10^{-4} mol l^{-1} the inhibitory rate of compound **4b** to P388 and A-549 is 42.6% and 52.1% respectively; at 10^{-5} mol l^{-1} the inhibitory rate of compound **4b** to P388 and A-549 is 8.5% and 3.1% respectively.

These results are promising. To the best of our knowledge, this is the first example of an organogermanium compound as an insect growth regulator. Further studies on larvicidal activities and anticancer activities of the title compounds and its derivatives are under way and will be reported in due course.

Acknowledgements

This work was supported by the National Natural Science Foundation of China and the Research Fund for the Doctoral Program of Higher Education.

Table 2. Anticancer activity of the compounds **4** *in vitro*

		Inhibition ratio (%)	
		4a	**4b**
10^{-4} mol l^{-1}	HL-60	28.6	–
	BEL-7402	36.5	–
	P388	–	42.6
	A-549	–	52.1
10^{-5} mol l^{-1}	HL-60	24.5	–
	BEL-7402	13.5	–
	P388	–	8.5
	A-549	–	3.1
10^{-6} mol l^{-1}	HL-60	6.1	–
	BEL-7402	6.0	–
	P388	–	6.0
	A-549	–	0.0

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