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Note

Synthetic, spectroscopic and antitumor activity studies on phosphacyanoboranes

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Abstract

Triphenylphosphinecyanoborane (**1**), diphenyl(2-methoxyphenyl)phosphinecyanoborane (**2**), diphenyl(*p*-tolyl)phosphinecyanoborane (**3**), methyldiphenylphosphinecyanoborane (**4**) were synthesized either by Lewis-base exchange reaction or by the reaction of the sodium cyanoborohydride with the corresponding phosphine hydrochloride in 54–88% yields. All the products were characterized by spectroscopic techniques and elemental analyses. These compounds were evaluated for their antitumor activity.

Keywords: Lewis-base exchange reaction; Antitumor activity; Substituted borane adducts; BNCT; Phosphacyanoboranes

1. Introduction

A number of interesting, and potentially useful, compounds of phosphorus and boron have been synthesized by the coupling of electron donor phosphorus groups with electron acceptor borane fragments. The observation that a number of boron analogues of the phosphonoacetates functioned as effective hypolipidaemic agents [1] has led us to extend our studies to other P–B linked compounds, such as the phosphineboranes. The first phosphineboranes, PPh_3BH_2X (X = CN, COOH, COOEt and CONHEt), was reported by Martin and coworkers using a somewhat cumbersome, and low yield, procedure involving the synthesis, and subsequent solvolysis, of PPh_3BH_2CN [2]. Moreover, the synthesis of substituted-phenylphosphineboranes has not yet been reported. Our recent report of a new and high-yield procedure for the synthesis of triphenylphosphinecarbomethoxyborane, which exhibited good antitumor activity [3], has encouraged us to extend our studies on the syntheses, properties and antitumor activities of the phosphineboranes. A parallel interest in studying such compounds is their potential application in boron neutron capture therapy (BNCT) [4–9]. Herein, we report the syntheses, spectroscopic characterization and antitumor activity studies of triphenylphosphinecyanoborane (**1**), diphenyl(2-methoxyphenyl)phosphinecyanoborane (**2**), diphenyl(*p*-tolyl)phosphinecyanoborane (**3**) and methyldiphenylphosphinecyano-borane (**4**).

2. Experimental

2.1. Materials

Me_3NBH_2CN was prepared by literature methods [10]. Triphenylphosphine, methyldiphenylphosphine, diphenyl(2-methoxyphenyl)phosphine, diphenyl(*p*-tolyl)-phosphine were obtained commercially (Aldrich) and used without further purification. Monoglyme (1,2 dimethoxyethane, DME) and tetrahydrofuran (THF) were distilled from CaH_2 and stored over molecular sieves. All other solvents and reactants were of reagent grade and were used as received.

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2.2. Spectroscopic and analytical procedures

Proton, boron-11, phosphrus-31 and carbon-13 NMR spectra were obtained on Brucker Fourier-Transform NMR spectrometer at 200, 64.2, 80.2 and 50.3 MHz, respectively. Infrared spectra were recorded using a Nicolet Magna 550 FT-IR spectrophotometer with OMNIC software. Elemental analyses were obtained in house using Perkin–Elmer 2400 CHN elemental analyser. All NMR samples were prepared in d^6-DMSO with $BF_3{\cdot}OEt_2$ as an internal standard.

2.3. Synthetic procedures

All experiments were carried out in 250 ml Pyrex-glass two-necked round-bottom flasks, each containing a magnetic stirring bar and nitrogen inlet. All known compounds were identified by comparing their IR and NMR spectra with those of authentic samples. The hydrochloride salts of methyldiphenylphosphine, diphenyl(2-methoxyphenyl)phosphine and diphenyl(*p*-tolyl)phosphine were prepared by dissolving the particular phosphine in Et_2O and then bubbling HCl gas into the solution until saturation was obtained. The hydrochloride salts were then recrystallized from 95% EtOH solution.

2.4. Triphenylphosphinecyanoborane (*1*)

Equimolar amounts of triphenylphosphine (1.00 g, 3.81 mmol) and trimethylaminecyanoborane (0.50 g, 3.83 mmol) were dissolved in anhydrous DME (50 ml) under N_2 atmosphere. The mixture was heated to reflux and the extent of the reaction was monitored by ^{11}B NMR spectroscopy. After 18 h, the solvent was removed under reduced pressure and the crude white solid product was then recrystallized from CH_2Cl_2–C_5H_{12} (9:1) to produce off-white needles of PPh_3BH_2CN (0.75 g, 65% yield, soluble in polar and slightly soluble in nonpolar organic solvents; m.p. 170–172 °C). Spectroscopic and analytical data for **1**: ^1H NMR (DMSO, relative to Me_4Si) δ 7.42–7.66 [m, aromatic H]; ^{11}B NMR (DMSO, relative to $BF_3{\cdot}OEt_2$) δ −31.90 [d of t, BH_2, $J_{(BH)} = 107$ Hz, $J_{(BP)} = 109$ Hz]; ^{13}C NMR (DMSO, relative to Me_4Si) δ 127.00–129.00 [phenyl C's]; ^{31}P NMR (DMSO, relative to H_3PO_4) δ 10.81 [q, $J_{(BP)} = 109$ Hz]; IR (KBr pellet, cm^{-1}) 2415, 2389 [ν(B–H)]; *Anal.* Calc. for $C_{19}H_{17}PBN$: C, 75.78; H, 5.64; N, 4.65. Found: C, 75.42; H, 5.72; N, 4.69%.

2.5. Diphenyl(2-methoxyphenyl)phosphinecyanoborane (*2*)

Equimolar amounts of diphenyl(2-methoxyphenyl)phosphine hydrochloride (1.00 g, 3.04 mmol) and sodium cyanoborohydride (0.19 g, 3.04 mmol) were dissolved in anhydrous DME (35 ml) under N_2 atmosphere. The mixture was heated to reflux and the extent of the reaction was monitored by ^{11}B NMR spectroscopy. After 11 h, the reaction was complete and the reaction mixture was filtered to remove a white precipitate (NaCl). The solvent was removed from the filtrate under reduced pressure to give the product as a creamy solid, which was subsequently recrystallized from CH_2Cl_2 to obtain white crystals of $(C_6H_5)_2P(C_6H_4OCH_3)BH_2CN$ (0.83 g, 73% yield, slightly soluble in polar organic solvents; m.p. 110–112 °C). Spectroscopic and analytical data for **2**: ^1H NMR (DMSO, relative to Me_4Si) δ 3.85 [s, OCH_3], 7.10–7.63 [m, aromatic H]; ^{11}B NMR (DMSO, relative to $BF_3.OEt_2$) δ −36.20 [d of t, BH_2, $J_{(BH)} = 111$ Hz, $J_{(BP)} = 117$ Hz]; ^{13}C NMR (DMSO, relative to Me_4Si) δ 58.30 [OCH_3], 122.70–128.30 [phenyl C's]; ^{31}P NMR (DMSO, relative to H_3PO_4) δ 9.07 [q, $J_{(BP)} = 115$ Hz]; IR (KBr pellet, cm^{-1}) 2422, 2380 [ν(B–H)]; *Anal.* Calc. for $C_{20}H_{19}POBN$: C, 72.43; H, 5.73; N, 4.23. Found: C, 72.66; H, 5.92; N, 4.31%.

2.6. Diphenyl(p-tolyl)phosphinecyanoborane (*3*)

Equimolar amounts of diphenyl(*p*-tolyl)phosphine hydrochloride (1.00 g, 3.20 mmol) and sodium cyanoborohydride (0.20 g, 3.20 mmol) were dissolved in anhydrous DME (50 ml) under N_2 atmosphere. The mixture was heated to reflux and the reaction monitored by ^{11}B NMR spectroscopy. After completion (8.5 h), the mixture was filtered to remove a white precipitate, identified as NaCl. The solvent was then removed from the filtrate under reduced pressure to give a white solid that was recrystallized from CH_2Cl_2–C_6H_{14} (1:1) to produce off-white crystals of $(C_6H_5)_2P(C_6H_4CH_3)BH_2CN$ (0.54 g, 54% yield, sparingly soluble in polar solvents; m.p. 117–119 °C). Spectroscopic and analytical data for **3**: ^1H NMR (DMSO, relative to Me_4Si) δ 2.15 [unresolved, CH_3], 7.20–7.80 [m, aromatic H]; ^{11}B NMR (DMSO, relative to $BF_3{\cdot}OEt_2$) δ −38.00 [d of t, BH_2, $J_{(BH)} = 102$ Hz, $J_{(BP)} = 112$ Hz]; ^{13}C NMR (DMSO, relative to Me_4Si) δ 40.10 [CH_3], 123.00–131.50 [phenyl C's]; ^{31}P NMR (DMSO, relative to H_3PO_4) δ 10.45 [q, $J_{(BP)} = 110$ Hz]; IR (KBr pellet, cm^{-1}) 2453, 2372 [ν(B–H)]; *Anal.* Calc. for $C_{20}H_{19}PBN$: C, 76.11; H, 6.03; N, 4.44. Found: C, 75.96; H, 6.10; N, 4.37%.

2.7. Methyldiphenylphosphinecyanoborane (*4*)

Methyldiphenylphosphine hydrochloride (5.00 g, 21.14 mmol) and sodium cyanoborohydride (1.33 g, 21.16 mmol) were dissolved in anhydrous DME (50 ml) under N_2 atmosphere. As in the syntheses of **2** and **3**, the mixture was heated to reflux and the reaction followed by ^{11}B NMR spectroscopy. After completion (9 h) the

mixture was filtered to remove NaCl. Solvent was removed from the filtrate under reduced pressure and the resulting crude colorless oil product was purified by flash chromatography on silica using Et_2O–C_6H_{14} (1:1) eluent to obtain $(C_6H_5)_2P(CH_3)BH_2CN$ (4.5 g, 88% yield, soluble in polar solvents). Spectroscopic and analytical data for **4**: ^1H NMR (DMSO, relative to Me_4Si) δ 2.02 [s, CH_3], 7.44–7.93 [m, aromatic H]; ^{11}B NMR (DMSO, relative to $BF_3{\cdot}OEt_2$) δ −37.50 [d of t, BH_2, $J_{(BH)} = 99.1$ Hz, $J_{(BP)} = 102.9$ Hz]; ^{13}C NMR (DMSO, relative to Me_4Si) δ 20.10 [CH_3], 126.00–130.80 [phenyl C's]; ^{31}P NMR (DMSO, relative to H_3PO_4) δ 3.90 [q, $J_{(BP)} = 103.5$ Hz]; IR (KBr pellet, cm^{-1}) 2478, 2395 [ν(B–H)]; *Anal.* Calc. for $C_{14}H_{15}PBN$: C, 70.26; H, 6.27; N, 5.85. Found: C, 70.33; H, 6.15; N, 6.03%.

2.8. Antitumor activity studies

In vitro tests for anti-tumor activity against a number of human cancer cells were conducted on compounds **1**–**4** through the National Cancer Institute's (NCI) Developmental Therapeutics Program [11]. Preliminary screening results, in terms of the concentrations that cause 50% growth inhibition (GI_{50}), total growth inhibition (TGI), and death of 50% of the cell population (LC_{50}) are listed in Table 1.

3. Results and discussion

3.1. Syntheses

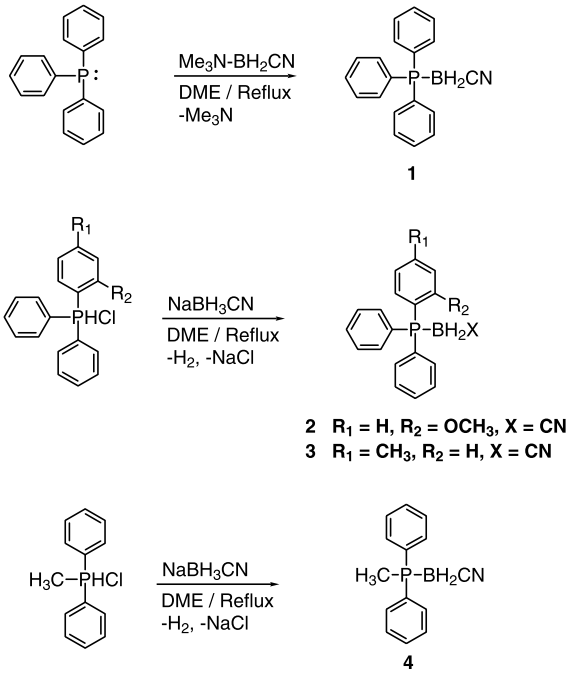
The phosphineboranes were synthesized using two different methods as outlined in Scheme 1. Triphenylphosphinecyanoborane (**1**) was synthesized by the direct Lewis-base exchange reaction between triphenylphosphine and trimethylaminecyanoborane in a 1:1 molar ratio using DME as a solvent. Although a yield of 65% was an improvement over the method of Martin and coworkers [2], the long reaction time of 18 h is somewhat of a disadvantage if the method is to be generally applied. Therefore, an alternative, two-step method was developed for the syntheses of **2**–**4**; this is also shown in Scheme 1. The new route consisted of first preparing the particular phosphine hydrochloride salt and then reacting it with sodium cyanoborohydride, $NaBH_3CN$, to give the phosphineborane and sodium chloride. The overall yields of 54–88% are comparable with those obtained from the phosphine/amine exchange reactions, but the reaction times were about halved. Purification of the solid products (**1**–**3**) was achieved by recrystallization, while **4** was purified by chromatography.

Table 1
GI_{50}, TGI and LC_{50} values (mol dm^{-3}) of the phosphineboranes

Compound panel/cell line affected	$GI_{50} \times 10^5$	TGI $\times 10^5$	$LC_{50} \times 10^5$
Non-small cell lung cancer			
A549/ATCC	4.9	> 10.0	> 10.0
NCI-H226	7.7	> 10.0	> 10.0
NCI-H23	3.3	> 10.0	> 10.0
NCI-H522	3.0	> 10.0	> 10.0
Small cell lung cancer			
DMS 114	6.9	> 10.0	> 10.0
DMS 273	3.9	> 10.0	> 10.0
CNS cancer			
SF-268	2.3	7.18	> 10.0
SF-295	1.9	4.53	> 10.0
SNB-75	3.5	3.83	9.74
SNB-78	1.3	5.79	> 10.0
U251	2.8	6.36	> 10.04
XF 498	1.4	2.82	5.88
Leukemia			
RPMI-8226	0.0047	> 10.0	> 10.0
Renal cancer			
RXF-393	0.14	> 10.0	> 10.0
Leukemia			
CCRF-CEM	4.5	> 10.0	> 10.0
HL-60 (TB)	2.5	9.91	> 10.0
Melanoma			
LOX IMBI	6.6	> 10.0	> 10.0
MALME-3M	4.9	> 10.0	> 10.0
Ovarian cancer			
IGROV1	6.6	> 10.0	> 10.0
OVCAR-3	4.9	> 10.0	> 10.0
Renal cancer			
ACHN	8.1	> 10.0	> 10.0
CAKI-1	2.2	8.0	> 10.0
Prostate cancer			
PC-3	3.1	> 10.0	> 10.0
Breast cancer			
MCF7	4.4	> 10.0	> 10.0
MCF7/ADR-RES	3.5	> 10.0	> 10.0

3.2. Characterization

All the compounds were characterized by their IR spectra, ^1H, ^{11}B, ^{13}C, and ^{31}P NMR spectra and elemental analyses. All of these data are consistent with the formulations given in Scheme 1. The proton-decoupled ^{31}P NMR spectra of **1**–**4** showed a quartet in the range of $\delta = 3.9$–12.45 ppm, corresponding to a phosphorus atom bonded to a boron atom. The proton-decoupled ^{11}B NMR spectrum of each phosphineborane showed a doublet in the range of $\delta = -29$ and −38 ppm, while their proton-coupled spectra showed doublet of triplet patterns, consistent with a boron being split by hydrogen and phosphorous coupling. These spectra are quite similar to the ^{31}P and ^{11}B resonances of $\delta = 12.45$



Scheme 1.

and −29.5 ppm, respectively, found for $PPh_3BH_2COOCH_3$ that has been structurally characterized [3]. The IR spectra of **1**–**4** showed all the expected bands consistent with the structures, as did their ^{13}C NMR spectra.

3.3. Antitumor activity studies

Table 1 summarizes the results obtained from National Cancer Institute's Developmental Therapeutics Screening Program [11]. The table lists the experimental values of the concentrations of the phosphineboranes that cause 50% growth inhibition (GI_{50}), total growth inhibition (cytostatic) (TGI), and the concentration that is lethal to 50% of the cell population (LC_{50}). The results show that all the compounds exhibit some anti-tumor activity and that triphenylphosphinecyanoborane (**1**) shows a high degree of specificity within the central nervous system (CNS) tumor cell lines. However, the effective concentrations ($>10^{-5}$ M) are generally too high to be practical in a clinical setting. The exception is diphenyl(2-methoxyphenyl)phosphinecyanoborane (**2**), which exhibited a clinically acceptable GI_{50} value of 4.74×10^{-8} M against the leukemia RPMI-8226 cell line.

4. Conclusions

This work reports the synthesis and spectroscopic characterizations of four new phosphineboranes. The two types of synthetic methods described, direct base exchange or hydrochloride/sodium cyanoborohydride reaction, are general ones that should be applicable to other phosphineboranes. The compounds were screened for anti-tumor activity against a number of human cancer cell lines. All showed some anti-tumor activity and triphenylphosphinecyanoborane (**1**) demonstrated a high selectivity. However, with the exception of diphenyl(2-methoxyphenyl)phosphinecyanoborane (**2**), all compounds possessed GI_{50}, TGI, and LC_{50} concentrations that were too large to be of clinical interest.

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