Potential Antitumor Agents. 12. 2-Formyl-4-aminophenylpyridine Thiosemicarbazones†

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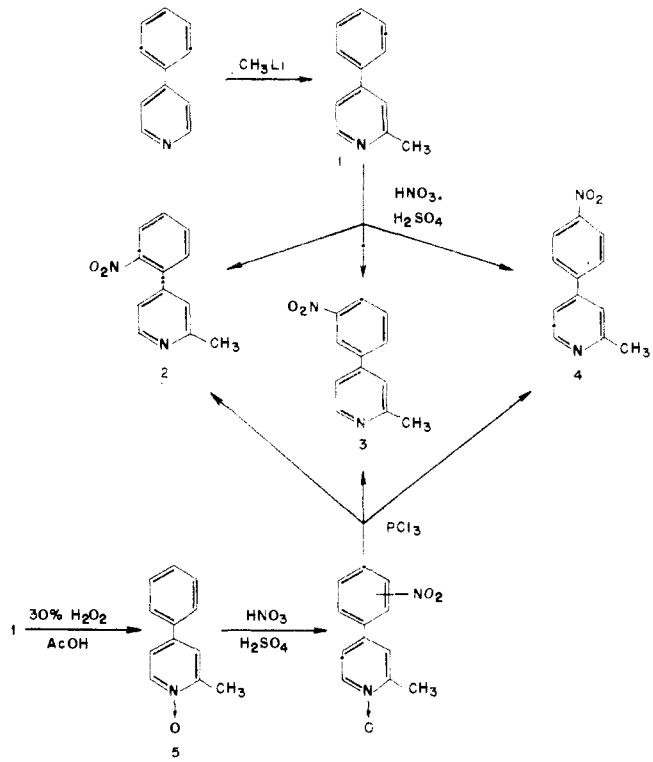
The antitumor agent 2-formyl-4-(*m*-amino)phenylpyridine thiosemicarbazone (4-APPT) has been synthesized by a new route to give significantly better overall yields than previously reported. 4-Phenyl-2-picoline was formed by methylation of 4-phenylpyridine with CH_3Li which upon nitration produced a mixture of *o*-, *m*-, and *p*-nitro-substituted derivatives. These isomers were separated by the solubility differences of their hydrochloride or nitrate salts in 10, 27, and 40% yields, respectively. Identification and confirmation of the structure of these isomers were carried out by nmr. Each isomer was individually subjected to a series of reactions to oxidize the 2-CH_3 group to the corresponding carboxaldehyde and to reduce the NO_2 function to an amino group. These agents were tested for antineoplastic activity in mice bearing Sarcoma 180 ascites cells; while the *o*- *and p*-amino-substituted derivatives were inactive, the *m*-amino-substituted agent (4-APPT) proved to be an extremely potent antineoplastic agent.

2-Formyl-4-(*m*-amino)phenylpyridine thiosemicarbazone (4-APPT),[1] designed in an effort to develop an agent of this series with efficacy against cancer in man, has been reported to be one of the most active of the antineoplastic α-(N)-heterocyclic carboxaldehyde thiosemicarbazones. 2-Formyl-5-hydroxypyridine thiosemicarbazone (5-HP), a drug of this class which was clinically evaluated against human cancer,[2,3] proved to have little utility in man due in part to rapid inactivation and excretion.[2] The objective of this study was to design and synthesize an agent which (a) was not susceptible to the enzymatic degradation which limited the utility of 5-HP and (b) had greater inhibitory potency for the target enzyme ribonucleoside diphosphate reductase than did 5-HP. 4-APPT has shown strong inhibitory potency *in vivo* as an antineoplastic agent against the murine ascitic neoplasms Sarcoma 180, Ehrlich carcinoma, and Hepatoma 129.[4] Furthermore, this agent was found to be the most potent known α-(*N*)-pyridinecarboxaldehyde thiosemicarbazone inhibitor of ribonucleoside diphosphate reductase. Thus, for example, 4-APPT was about 30-fold more potent than 5-HP as an inhibitor of this enzyme.[1] Since 4-APPT appeared to possess the necessary requisite properties for consideration as an analog of the α-(N)-heterocyclic carboxaldehyde thiosemicarbazone class for clinical trial, it was deemed necessary to devise a more suitable method of preparation of this agent than previously described. Previous methodology coupled diazotized *m*-nitroaniline with 2-picoline, presumably a free-radical condensation, to yield a mixture of four different isomers, the 3-, 4-, 5-, and 6-substituted *m*-nitrophenyl-2-picolines, which presented difficulties in both separation and purification procedures.[1] These difficulties resulted in an overall yield of only about 4% of the intermediate, 4-(*m*-nitro)phenyl-2-picoline (**3**), making the method essentially unsuitable for large-scale synthesis of this material. We now report a superior method of preparation of **3** that can be utilized on a relatively large scale. In addition, two new compounds were synthesized in which the NH_2 group of 4-APPT was inserted at the ortho or para positions instead of meta in the phenyl ring. Another compound with no substitution in the phenyl ring was also synthesized. These new derivatives were tested for their antitumor activity against the murine neoplasm, Sarcoma 180.

Chemistry. 4-Phenyl-2-picoline (**1**) was prepared by methylation of 4-phenylpyridine with methyllithium by a published procedure,[5] which was modified to give 63% yield instead of 42% as reported. Nitration of **1** in HNO_3 and H_2SO_4 produced a mixture of *o*-, *m*-, and *p*-nitro derivatives (**2**, **3**, and **4**, respectively, Scheme I). The three isomers were conveniently separated by the solubility differences of their hydrochloride or nitrate salts in 10, 27, and 40% yields of ortho, meta, and para isomers, respectively, as described in the Experimental Section. Since it has been reported that nitration of 2- or 4-phenylpyridine 1-oxides yields significantly greater proportions of the *m*-nitro isomer than of nitration of the parent 2- or 4-phenylpyridines,[6] we attempted the nitration of 4-phenyl-2-picoline 1-oxide (**5**) in the anticipation of obtaining larger amounts of 4-(*m*-nitro)phenyl-2-picoline (**3**). Nitration of **5** was carried out either at room temperature using KNO_3 and concentrated H_2SO_4 or by heating at 100° for 2 hr in a mixture of concentrated H_2SO_4 and HNO_3. Both procedures produced similar yields (about 90%) of a mixture of three isomers, which could not be separated individually as was possible with nonoxygenated derivatives. Therefore, this mixture was first deoxygenated with PCl_3 and then followed for separation of ortho, meta, and para isomers as described in the Experimental Section. The results with **5** were different than those of the earlier findings[6] of nitration of 4-phenylpyridine 1-oxide, the yield of the three isomers being 24, 22, and 45% for the ortho, meta, and para

Scheme I



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isomers, respectively. Thus, the presence of the 1-oxide in **5** did not appear to influence significantly the orientation of the nitration reaction with respect to the other isomers. However, the overall total yield of the mixture of the three isomers was significantly greater. This difference between 4-phenylpyridine *N*-oxide and **5** may be due to the presence of an activating 2-CH_3 group in **5** which would tend to orient the nitration reaction at ortho and para positions, thus negating the influence of the 1-oxide. Nitration of the same species of either **1** or **5** (probably as the conjugate acid[7]) might be responsible in each series for producing a similar set of isomers.

The synthesis of compound **4** was also investigated by coupling diazotized *p*-nitroaniline with 2-picoline, employing the procedure for meta isomer formation described previously by this laboratory.[1] However, the separation of 3-, 4-, 5-, and 6-substituted *p*-nitrophenyl-2-picolines was found to be much more difficult than described for the meta isomers. Separation of these isomers could not be achieved utilizing chromatography (silica gel and alumina) or fractional crystallizations. Smaller quantities of the 4-isomer (**4**) were obtained by the latter technique; this material was found to be identical with that obtained by the nitration of 4-phenyl-2-picoline.

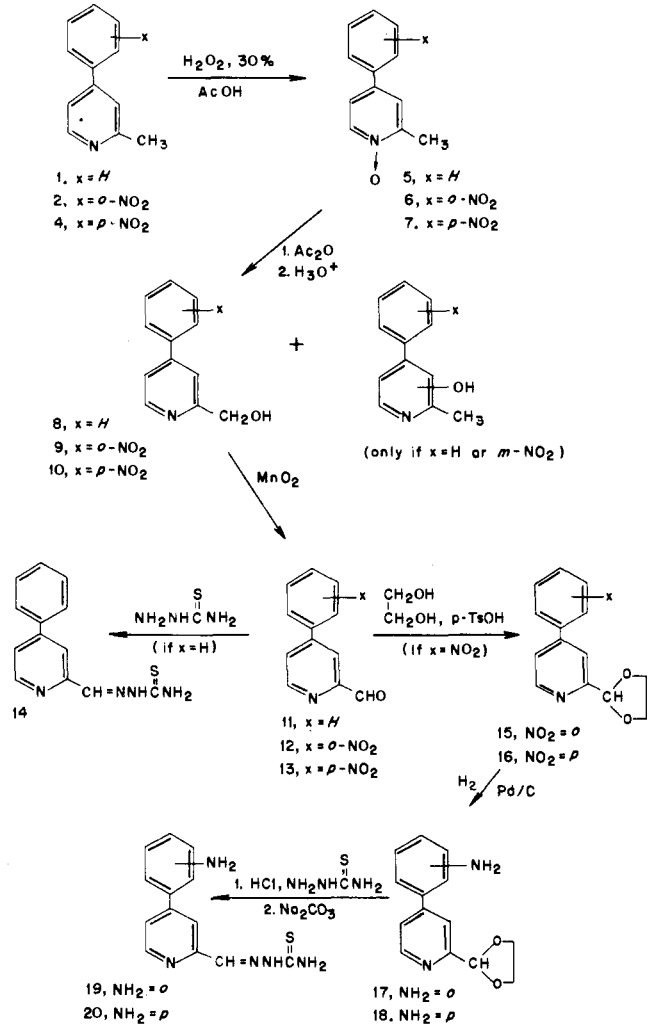
Identification and confirmation of the structure of the three isomers **2**, **3**, and **4** were carried out by nmr. Compound **4** containing a *p*-nitro substituent produced a symmetrical spectrum of the AA'BB' type, the phenyl protons appearing in two doublets at δ 6.7 and 6.1 with the same coupling constant (J = 8 Hz) resulting from the sum of $J_{ortho} + J_{para}$. The 3 and 5 H of the pyridine ring appeared at δ 5.7. The most downfield proton at δ 7.0 was a doublet (J = 4 Hz) due to the 6 H of pyridine; the 2-CH_3 group appeared as a singlet at δ 2.65. In compound **2** with an o-NO_2 group the phenyl protons gave a complex spectrum appearing around δ 6.0 as expected. The nmr spectrum of the *m*-NO_2 derivative **3** has been described earlier.[1]

Compounds **1**, **2**, and **4** were individually subjected to a series of reactions, shown in Scheme II, to oxidize the 2-CH_3 group to the corresponding carboxaldehyde. These reactions are similar to those described for 4-(*m*-nitro)-phenyl-2-picoline (**3**) (*i.e.*, N-oxidation, rearrangement with Ac_2O, acid hydrolysis of the resulting ester, and MnO_2 oxidation of the carbinol). One significant deviation which occurred with **2** and **4** was that no phenolic compound could be isolated after acid hydrolysis of the esters obtained from the rearrangement of the *N*-oxide, whereas with **3** we have found that a 7% yield of the phenolic compound was produced.[1] A probable explanation for this difference is that activation of the 2-CH_3 group occurs due to the electron-withdrawing effect of the o- or *p*-NO_2 group which results in conformational stability of the intermediate, the anhydro base, during intramolecular rearrangement.[8]

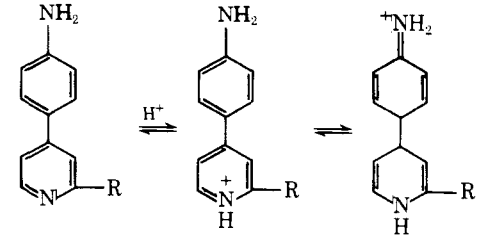
Reduction of the NO_2 group to an NH_2 function was carried out by converting the carboxaldehydes **12** and **13** to their cyclic ethylene acetals **15** and **16** which were reduced by catalytic hydrogenation using Pd/C and then allowed to react with thiosemicarbazide in the presence of HCl to yield **19** and **20**, respectively. The experimental details of these reactions have been described earlier;[1] therefore, only the relevant data for each compound are listed in Table I.

Biological Results and Discussion. The aim of this work was to synthesize the intermediate **3** in larger quantities by a more facile procedure than originally described for the preparation of 4-APPT. However, in addition to having described such a simplified procedure, we have also synthesized and tested for antineoplastic activity the unsubstituted 4-phenyl-2-formylpyridine thiosemicarbazone

Scheme II

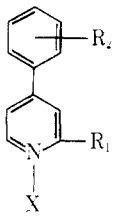


14 and its ortho- and para-substituted amino derivatives **19** and **20**, respectively. The results of these tests are shown in Table II; antineoplastic activity was determined by measuring the effect of these agents on the survival time of mice bearing Sarcoma 180 ascites cells. Only the maximum prolongation of life produced by the administration of each material is listed, although a relatively wide range (10–60 mg/kg/day) of daily dosage levels was tested for each agent. As expected, 4-APPT was the most effective derivative in this series in increasing the average life span of tumor-bearing mice. Thus, animals bearing Sarcoma 180 survived to an average of 32.5 days in comparison to untreated tumor-bearing controls which lived for only 12.4 days. The 4-phenyl derivative **14** showed only marginal activity but was more toxic as evidenced by a 12% decrease in body weight at the maximum effective dose of 20 mg/kg/day. The inactivity of the ortho- and para-substituted amino derivatives **19** and **20**, respectively, could possibly be explained by the mesomeric stabilization of the cationic species as shown below for the para substituent. Similar



R = $-CH{=}NNHC(=S)NH_2$

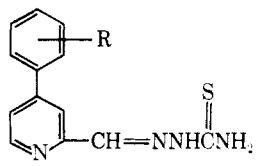
Table I



Compd	R_1	R_2	X	Mp, °C	Recrystn solvent	Yield, %	Formula	Analyses
2	CH_3	*o*-NO_2		69–70	Hexane	40	$C_{12}H_{10}N_2O_2$	C, H, N
4	CH_3	*p*-NO_2		156–157	Me_2CO–cyclohexane	10	$C_{12}H_{10}N_2O_2$	C, H, N
6	CH_3	*o*-NO_2	O	131–133	C_6H_6–cyclohexane	51	$C_{12}H_{10}N_2O_3$	C, H, N
7	CH_3	*p*-NO_2	O	229–231	EtOH–H_2O	82	$C_{12}H_{10}N_2O_3$	C, H, N
8	CH_2OH	H		Oil		66	$C_{12}H_{11}NO$	
9	CH_2OH	*o*-NO_2		99–100	C_6H_6–cyclohexane	65	$C_{12}H_{10}N_2O_3$	C, H, N
10	CH_2OH	*p*-NO_2		180–181	EtOH	82	$C_{12}H_{10}N_2O_3$	C, H, N
11	CHO	H		48–49	Petr ether	62	$C_{12}H_9NO$	C, H, N
12	CHO	*o*-NO_2		106–107	Et_2O	95	$C_{12}H_8N_2O_3$	C, H, N
13	CHO	*p*-NO_2		168–169	C_6H_6–hexane	71	$C_{12}H_8N_2O_3$	C, H, N
14	$CH{=}NNHCSNH_2$	H		220–222 dec	EtOH	91	$C_{13}H_{12}N_4S$	C, H, N
15		*o*-NO_2		89–90	Et_2O	67	$C_{14}H_{12}N_2O_4$	C, H, N
16		*p*-NO_2		139–141	EtOH	90	$C_{14}H_{12}N_2O_4$	C, H, N
17		*o*-NH_2		Oil		80	$C_{14}H_{14}N_2O_2$	
18		*p*-NH_2		100–101	C_6H_6–cyclohexane	52	$C_{14}H_{14}N_2O_2$	C, H, N
19	$CH{=}NNHCSNH_2$	*o*-NH_2		133–135 dec[a]	EtOH–H_2O	58	$C_{13}H_{13}N_5S \cdot H_2O$	C, H, N
20	$CH{=}NNHCSNH_2$	*p*-NH_2		245–246 dec[b]	DMF–H_2O	84	$C_{13}H_{13}N_5S$	C, H, N

[a] HCl salt mp 238-240° dec. [b] HCl salt mp 253-255° dec.

Table II. Effect of 4-Substituted 2-Formylpyridine Thiosemicarbazones on the Survival Time of Mice Bearing Sarcoma 180 Ascites Cells



Compd	R	Max effective daily dose, mg/kg[a]	Av Δ wt, %[b]	Av survival time, days + S.E.	No. of 50-day survivors[c]	% T/C[d]
Control			+22.6	12.4 ± 0.6	0/20	
14	H	20	−12.0	18.6 ± 7.9	0/10	150
19	*o*-NH_2	40	+0.6	14.2 ± 1.1	0/5	115
4-APPT	*m*-NH_2	40	−7.2	32.5 ± 2.4	3/15	262
20	*p*-NH_2	10	+15.6	15.0 ± 3.2	0/5	121

[a] Administered once daily for 6 consecutive days, beginning 24 hr after tumor implantation; each value represents the results obtained with 5-15 animals; dose levels were administered in a range of 10–60 mg/kg/day for each compound. [b] Average weight change from onset to termination of drug treatment. [c] Mice that survived more than 50 days were calculated as 50-day survivors in determination of the average survival time. [d] % T/C = (treated/control) × 100.

structures can also be formed with the ortho-substituted compound. Formation of these species would tend to hinder the coordination with metals of a pair of free electrons at the ring nitrogen. Since evidence is available that such coordination of the ring nitrogen with iron might be involved in the mechanism of the tumor-inhibitory action of this class of agents,[9] the formation of cationic species would appear to explain their inactivity.

Experimental Section

Melting points were taken on a Thomas-Hoover capillary melting point apparatus and are uncorrected. The ir absorption spectra were obtained with a Perkin-Elmer Model 257 spectrophotometer with thin films of liquids and KBr pellets of solids. Uv spectra were obtained using a Perkin-Elmer Model 402 ultraviolet–visible spectrophotometer with solutions made in absolute ethanol. Nmr spectra were determined with a Varian T-60A spectrophotometer with TMS as an internal standard. The spectral data were as expected; therefore, routine data are not included. Elemental analyses were performed by the Baron Consulting Co., Orange, Conn. Where analyses are indicated only by symbols of the element, the analytical results for those elements were within ±0.4% of the theoretical values.

Antitumor Activity. Experiments were performed on female CD-1 mice. Transplantation of Sarcoma 180 ascites cells was carried out using a donor mouse bearing a 7-day tumor growth. The experimental details have been described earlier.[10] The percentage change in body weight from onset to termination of therapy was used as an indication of drug toxicity. Dose levels were administered in the range of 10–60 mg/kg/day for 6 consecutive days for each compound. Determination of the sensitivity of Sarcoma 180 ascites cells to these agents was based on the prolongation of survival time afforded by the drug treatment.

2-Methyl-4-phenylpyridine (1). To a solution of 31 g (0.2 mol) of 4-phenylpyridine in 200 ml of Et_2O was added slowly 100 ml (2 *M* solution in Et_2O, 0.2 mol) of methyllithium while nitrogen was bubbled through the mixture. After the addition was complete, Et_2O was removed by distillation and was continuously replaced with 200 ml of toluene. The mixture was heated at 110° for 12 hr, the reaction was then decomposed cautiously by the addition of water, and the mixture was extracted with Et_2O. The Et_2O extracts were dried over KOH and evaporated under vacuum to leave an oil which was vacuum distilled: bp 102–103° (0.2 mm) [lit.[5] 99–100° (0.7 mm)]; yield 21.4 g (63%).

2-Methyl-4-nitrophenylpyridine (2–4). 2-Methyl-4-phenylpyridine (20.35 g, 0.122 mol) was added slowly to a solution of 7.8 ml (0.122 mol) of HNO_3 (70%; *d* 1.42) and 33 ml of concentrated H_2SO_4 at 0° with stirring. The mixture was heated at 100° for 2 hr, cautiously added to 200 g of crushed ice, made alkaline with concentrated NH_4OH, and extracted with $CHCl_3$. The $CHCl_3$ extracts were dried ($MgSO_4$) and evaporated under vacuum to leave a mixture of three isomers, *o*- (**2**), *m*- (**3**), and *p*-nitro (**4**) derivatives. The mixture was crystallized in HCl salt form from 5 *N* HCl to give 13.05 g of predominantly **4** which was purified by further recrystallization from 5 *N* HCl to yield 11.23 g (40%), mp 234–237°. The filtrate was made alkaline with Na_2CO_3 solution, extracted with $CHCl_3$, and dried ($MgSO_4$), the solvent was removed under vacuum, and the residue consisting of mainly meta and ortho isomers crystallized from 5 *N* HNO_3. The nitrate salt, consisting predominantly of **3**, was filtered and recrystallized from 5 *N* HNO_3 to yield 7.6 g (27%), mp 199–201°. The filtrate containing mostly the nitrate of **2** was evaporated to dryness under vacuum and recrystallized two times from a mixture of CH_3OH and EtOAc (1:1) to yield 2.8 g (10%), mp 151–152°.

The hydrochloride or nitrate salts were converted to the free base and were recrystallized from appropriate solvents as listed in Table I.

4-Phenyl-2-picoline *N*-Oxide (5). 4-Phenyl-2-picoline (16.9 g, 0.1 mol) was heated with a mixture of 15 ml of 30% H_2O_2 and 45 ml of glacial AcOH at 80°. After 3 hr, an additional amount (15 ml) of 30% H_2O_2 was added and the heating was continued for an additional 21 hr. The mixture was evaporated to a thick syrup, dissolved in 150 ml of $CHCl_3$, and treated with K_2CO_3 and a small amount of water to form a paste. The mixture was triturated thoroughly, the $CHCl_3$ layer dried (K_2CO_3), the solvent evaporated under vacuum, and the residue washed with ether to yield 14.1 g (76%) of **5** which was recrystallized from C_6H_6 and Et_2O: mp 101–105°. This material was used without further purification.

References

(1) K. C. Agrawal, A. J. Lin, B. A. Booth, J. R. Wheaton, and A. C. Sartorelli, *J. Med. Chem.*, **17**, 631 (1974).
(2) R. C. DeConti, B. R. Toftness, K. C. Agrawal, R. Tomchick, J. A. R. Mead, J. R. Bertino, A. C. Sartorelli, and W. A. Creasey, *Cancer Res.*, **32**, 1455 (1972).
(3) I. H. Krakoff, E. Etcubanas, C. Tan, K. Mayer, V. Bethune, and J. H. Burchenal, *Cancer Chemother. Rep.*, **58**, (1), 207 (1974).
(4) K. C. Agrawal, B. A. Booth, E. C. Moore, and A. C. Sartorelli, Abstracts of the International Cancer Congress, 2–138, Florence, Italy, Oct 1974.
(5) J. M. Bonner, J. Court, and T. Fay, *Bull. Soc. Chim. Fr.*, 1204 (1967).
(6) A. R. Hand and A. R. Katritzky, *J. Chem. Soc.*, 1754 (1958).
(7) A. R. Katritzky and M. Kingsland, *J. Chem. Soc. B*, 862 (1968).
(8) S. Oae, S. Tamagaki, T. Negoro, K. Ogino, and S. Kozuka, *Tetrahedron Lett.*, **8**, 917 (1968).
(9) K. C. Agrawal, B. A. Booth, E. C. Moore, and A. C. Sartorelli, *Proc. Amer. Ass. Cancer Res.*, **15**, 73 (1974).
(10) K. C. Agrawal, B. A. Booth, and A. C. Sartorelli, *J. Med. Chem.*, **11**, 700 (1968).

Synthesis and Tumor-Uptake Study of Phosphate Esters of Polyhedral Hydroxyboranes

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The phosphorylations of $B_{12}H_{11}OH^{2-}$, $B_{12}H_{10}(OH)_2^{2-}$, and $B_{20}H_{17}OH^{4-}$ with $POCl_3$ and $(C_6H_5O)_2POCl$ were investigated and the following derivatives were isolated: $B_{12}H_{11}OPO_3H^{3-}$, $B_{12}H_{11}OPO_3H_2^{2-}$, $B_{12}H_{11}OPO(OC_6H_5)_2^{2-}$, $B_{12}H_{11}OPO(OC_6H_5)OH^{2-}$, $B_{12}H_{10}(OP_2O_6H_2)_2^{4-}$, $B_{12}H_{10}(OPO_3H_2)_2^{2-}$, $B_{12}Br_{10}(OPO_3H)_2^{4-}$, $B_{12}H_{10}[OPO(OC_6H_5)_2]_2^{2-}$, $B_{20}H_{18}OP_2O_6H_2^{4-}$, $B_{20}H_{18}OPO_3H_2^{3-}$. The B–O–P bonds proved very resistant to hydrolysis and the phosphates were administered in the form of Na^+ salts at pH 7.2 to rats bearing subcutaneous glioma. The boron concentrations in tumors and the tumor/blood concentration ratios were compared with those of parent hydroxy derivatives. Except when the POH function was blocked by phenyl groups the phosphorylation invariably resulted in a greatly enhanced uptake of the borane into tumors and improved the tumor/blood boron ratio. The phosphate function appears to be one of the most effective handles for the incorporation of boron into brain tumors and the compounds show considerable promise for use in the neutron capture therapy of brain tumors.

The attributes which boron compounds should possess if they are to be useful in the treatment of brain tumors by neutron capture therapy have been discussed in a number of articles.[1–6] Chief among these is the ability to accumulate in the tumor at higher levels than in normal tissue, so that when the neutron flux is applied the tumor will be selectively irradiated. Recent calculations[7] indicate that if radiation damage to healthy adjacent tissue is to be kept to a minimum, no less than 10 μg of ^{10}B per gram of tumor is needed, while concentrations of 30–40 μg might be needed to ensure complete destruction of the tumor. Moreover, the quantity of boron in blood at the time of irradiation should have declined to a sufficiently low level so that radiation fibrosis of small blood vessels does not result in an ischemic necrosis of normal brain. Recently Hatanaka and coworkers[6] reported satisfactory medical results even at low tumor/blood boron ratios when the boron compounds were accompanied by certain steroids. One possible way of making compounds with the properties desired for successful neutron capture therapy[1,2,5] involves attaching a functional group to the boron compound capable of binding the latter covalently to some component of tumor tissue. Such functional groups have often been referred to as handles.[1]

The search for compounds of the type described above