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Phosphonic acid analogs of GABA through reductive dealkylation of phosphonic diesters with lithium trialkylborohydrides

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Abstract—Lithium trialkylborohydrides were found to effect rapid monodealkylation of phosphonic diesters, and this reaction was applied to the synthesis of alkylphosphonic acid 2-aminoethyl esters [$H_2N(CH_2)_2OP(OH)R$, **4**], a little-explored class of analogs of the inhibitory neurotransmitter γ-aminobutyric acid (GABA). Compound **4a** (R = Me) proved to be a potent antagonist at human ρ1 $GABA_C$ receptors (expressed in *Xenopus laevis* oocytes), with an IC_{50} of 11.1 μM, but is inactive at $\alpha_1\beta_2\gamma_2$ $GABA_A$ receptors.

γ-Aminobutyric acid (GABA) is a major inhibitory neurotransmitter in the central nervous system and has three major classes of receptors, designated $GABA_A$, $GABA_B$, and $GABA_C$.[1] Effectors of these receptors (agonists, antagonists, and allosteric modulators) are an important class of compounds as pharmaceuticals and pharmacological probes. Compounds targeting $GABA_A$ and $GABA_B$ have been extensively studied,[2] and $GABA_C$ effectors are attracting increased interest.[3] For this reason, and because of the important role of $GABA_C$ receptors in vision,[3d,4] we have sought to develop new $GABA_C$ effectors. We report here the discovery of a new reaction of lithium trialkylborohydrides, the reductive monodealkylation of phosphonic diesters, and its application to the synthesis of 2-aminoethyl alkylphosphonates (**4**), a previously unexplored class of $GABA_C$ receptor antagonists.

Phosphinic acids are the most prominent class of $GABA_C$ antagonists.[5] In the course of pursuing new synthetic approaches to 3-aminopropyl alkyl phosphinates (e.g., **2a**–**c**, Fig. 1), we surveyed metal hydrides for their ability to reduce phosphonic diesters to the corresponding H-phosphinates. Among the reagents tested, only lithium trialkylborohyrides reacted cleanly, but monodealkylation, rather than reduction at phosphorus, was observed. Partial conversion was observed with sodium tri(*s*-butyl)borohydride, while little conversion was observed with the corresponding potassium reagent, suggesting a specific role for the lithium counterion.

The dealkylation reaction, which likely occurs via S_N2 nucleophilic attack at carbon, was surprising in that

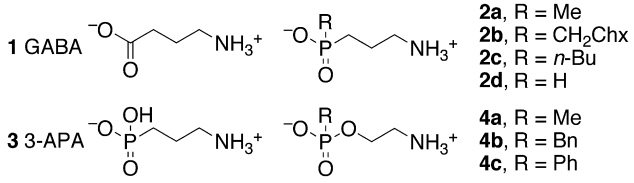

Figure 1. Structures of GABA and phosphorus oxyacid analogs.

Keywords: GABA antagonists; $GABA_C$ receptors; Dealkylation; Phosphonate esters; Lithium trialkylborohydrides.
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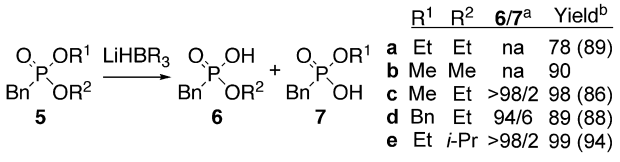
other metal hydride reagents, such as lithium aluminum hydride,[6] lithium bis(methoxyethoxy)aluminum hydride,[7] sodium bis(methoxyethoxy)aluminum hydride,[8] and sodium diethyl aluminum hydride,[8] are known to attack phosphonates at phosphorus. The examples described herein are the first in which nucleophilic attack on phosphonate esters by metal hydride reagents occurs preferentially at carbon, leading to dealkylation.

The initial substrate tested was diethyl difluorobenzylphosphonate,[9] which was subjected to reaction with 1.5 equiv of $LiHBEt_3$ or $LiHB(s\text{-}Bu)_3$ in THF at room temperature. With both reagents, the diester was consumed within 30 min, and the sole product was the monoethyl ester, which could be isolated in 89 or 82% yield, respectively. The reaction was repeated with diethyl benzylphosphonate (**5a**), and again, clean monodealkylation was observed, though in this instance, the reaction required an hour to go to completion.

A set of additional diesters **5b–e** was examined to test the scope and selectivity of the reaction (Scheme 1). In each case, treatment with 1.5–2.2 equiv of $LiHBR_3$ led to clean monodealkylation with high yield, and pure products were obtained through a simple workup involving only repeated evaporation from methanol to remove borates and protonation via aqueous extraction or ion exchange.

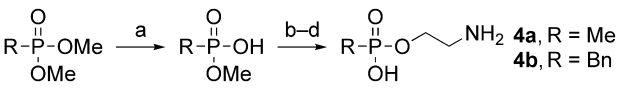
Lithium triethylborohydride has been noted as an exceptionally potent S_N2 nucleophile that rapidly reduces primary alkyl sulfonates and halides.[10] Consistent with our postulate of an S_N2 mechanism for the phosphonate dealkylation, the selectivity for methyl over ethyl, and ethyl over isopropyl, was complete as judged by ^1H NMR, while selectivity for benzyl over ethyl was 94/6 with both reagents.

Observations from preliminary ^1H NMR experiments are also consistent with an S_N2 mechanism. When the dealkylation of **5a** with $LiHBEt_3$ was performed in a sealed NMR tube fitted with a J. Young valve, a singlet at δ 0.81 ppm, consistent with ethane, appeared and grew over the course of the reaction. No olefinic signals were observed, excluding E2 elimination as the primary mechanism. In the analogous reduction of dimethyl methylphosphonate, a singlet at δ 0.18 ppm, consistent with methane, likewise emerged. Our findings therefore suggest that lithium trialkylborohydrides can displace substantially more basic leaving groups than has been observed previously.



	R^1	R^2	6/7[a]	Yield[b]
a	Et	Et	na	78 (89)
b	Me	Me	na	90
c	Me	Et	>98/2	98 (86)
d	Bn	Et	94/6	89 (88)
e	Et	*i*-Pr	>98/2	99 (94)

Scheme 1. Monodealkylation of phosphonic diesters. [a]Selectivities were the same for $LiHBEt_3$ and $LiHB(s\text{-}Bu)_3$. [b]Isolated yields (**6** + **7**) for $LiHBEt_3$ and, in parentheses, $LiHB(s\text{-}Bu)_3$.



Scheme 2. Synthesis of phosphonic analogs of GABA. Reagents and conditions: (a) $LiHBEt_3$, THF, rt, 90% (R = Me), 90% (R = Bn); (b) $BocNH(CH_2)_2OH$, $EtO_2CN{=}NCO_2Et$, PPh_3, 75% (R = Me), 94% (R = Bn); (c) $LiHB(s\text{-}Bu)_3$ THF, rt, 87% (R = Me), 74% (R = Bn); (d) TFA, 92% (**4a**) 94% (**4b**).

Nucleophilic displacement of alkyl groups in phosphonate esters occurs with a variety of other nucleophiles, and reactions of this type are useful for preparative deprotection reactions. Boron[11] and silicon[12] halides are widely employed for complete dealkylation, though recent work has led to the development of binuclear boron complexes that catalyze removal of a single alkyl group by BBr_3.[13]

Monodealkylation of phosphonic diesters is commonly effected with heteroatom-based nucleophilic reagents in the absence of a strong Lewis acid. Methyl and benzyl esters are cleaved most easily, and reagents used for cleaving these groups include sodium iodide in refluxing acetone or 2-butanone,[14] lithium bromide in acetonitrile,[15] *tert*-butylamine,[16] quinuclidine or DABCO in refluxing toluene,[17] and potassium cyanide in DMF at 70 °C.[18] With these reagents, it is often possible to cleave a methyl group preferentially over benzyl[16,18] or ethyl,[19] and potassium cyanide appears to be effective only on methyl groups.

Cleavage of ethyl groups requires more potent nucleophiles, higher temperatures, or both. Sodium thiophenoxide and thioethoxide in ethanol at 70 °C are effective,[20] and refluxing morpholine has been used with one substrate.[21] More commonly employed are alkali metal halides, such as lithium bromide in higher ketone solvents (e.g., 2-hexanone or 2-pentanone at 80–110 °C)[22] or refluxing pyridine.[23] At 100 °C in DMF, both iodide and azide (as their lithium or sodium salts) are effective, and azide also cleaves isopropyl groups.[24]

The nucleophilicity of lithium trialkylborohydrides is such that dealkylations proceed quickly to completion at room temperature even with ethyl phosphonoesters and modest substrate concentrations (e.g., 0.1–0.2 M). For preparative applications, this high reactivity may be advantageous with refractory substrates and when short reaction times or lower reaction temperatures are desired. The use of THF in place of more toxic, higher-boiling solvents may also be a benefit in some cases. High reactivity is also the principal drawback of the reagents, as it makes them incompatible with easily reduced groups. In other respects, such as high yield, selectivity, and simplicity of workup, the lithium trialkylborohydride procedure compares favorably with the alternatives.

To demonstrate the suitability of the dealkylation reaction for slightly more complex substrates in a multi-step reaction sequence, we employed it in the synthesis of two

2-aminoethyl alkylphosphonate analogs (**4a** and **4b**, Scheme 2) of GABA which had not been studied as GABA effectors. Our approach exploited the high selectivity of the reaction for methyl over primary substituents to allow preparation of the targets from readily available dimethyl phosphonates. These were first mono-demethylated with $LiHBEt_3$ and then realkylated via the Mitsunobu reaction[25] with $BocNH(CH_2)_2OH$. The remaining methyl groups were cleaved (with $\leqslant 3\%$ attack at the primary carbon) using $LiH(s\text{-}Bu)_3$, and the Boc group was removed by treatment with TFA to afford **4a** and **4b** in 92 and 94% yields, respectively. The stability of the Boc urethane to the hydride reagent may result from protective deprotonation at nitrogen.

The biological activity of the compounds was assessed by measuring chloride ion currents at homopentameric human ρ_1 $GABA_C$ receptors expressed in *Xenopus laevis* oocytes. Neither compound activated the receptor, and benzylphosphonate **4b** was also inactive as an antagonist. However, methylphosphonate **4a** proved to be a full antagonist of GABA-induced currents, reducing them in a dose-dependent fashion to near-baseline values (Fig. 2). Hill analysis of averaged data from six different oocytes led to the determination of an IC_{50} of 11.1 μM and a Hill coefficient n_H of 1.94 (Fig. 3).

For reference, two well-characterized $GABA_C$ receptor antagonists, (1,2,5,6-tetrahydropyridine-4-yl)methylphosphinic acid (TPMPA) and (3-aminopropyl)-*n*-butylphosphinic acid (**2c**), were examined under the same conditions. The IC_{50} values determined for these compounds were in good agreement with literature values (IC_{50} of 0.67 μM vs. a reported binding constant K_b of 2.1 μM for TPMPA[5b] and IC_{50} of 68.2 μM vs. a reported value of 62.5 μM for **2c**[5d]).[26] Compound **4a**

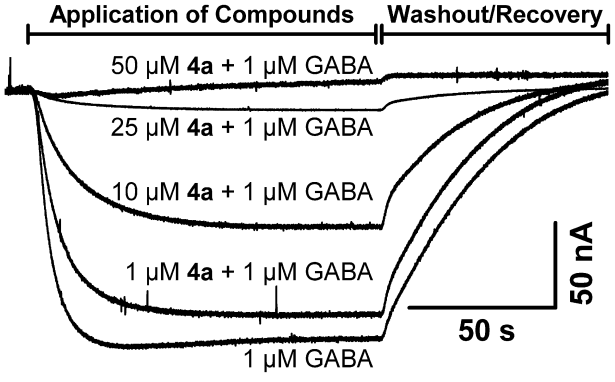


Figure 2. Representative responses of a *X. laevis* oocyte expressing homopentameric human ρ1 $GABA_C$ receptors to 1 μM GABA in the presence of different concentrations of **4a**. Oocytes were superfused with Ringer solution at a flow rate of approximately 1 mL/min while chloride ion currents across the membrane were monitored with a two-microelectrode voltage clamp. Over a fixed period, denoted by the bar labeled Application of Compounds, the superfusing medium was switched to Ringer solution supplemented with compounds as indicated. After this treatment period, the superfusing medium was switched back to Ringer solution, leading to washout of the compounds and recovery of the membrane current, as denoted by the bar labeled Washout/Recovery.

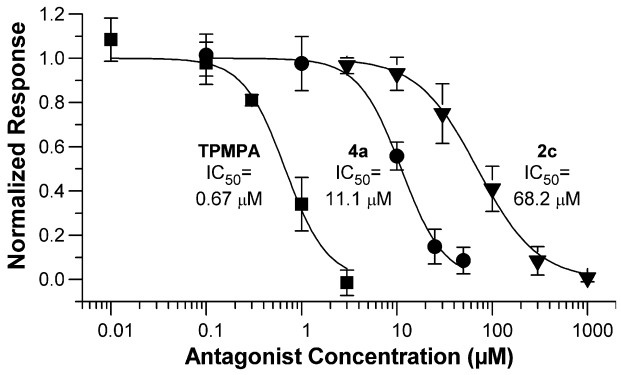


Figure 3. Dose–response curves of antagonists versus 1 μM GABA at homopentameric ρ_1 $GABA_C$ receptors expressed in *X. laevis* oocytes. Data are shown for TPMPA (squares), **4a** (circles), and **2c** (triangles), and reflect the averages obtained from two determinations in each of 6–8 oocytes. Curves show the best fit of the Hill equation to the data.

was also tested at heteropentameric ($\alpha_1\beta_2\gamma_2$) $GABA_A$ receptors expressed in oocytes. At 400 μM, it neither activated the receptor nor substantially reduced the current elicited by 40 μM GABA in four different oocytes.

The closest analogs of **4a** which have been studied at $GABA_C$ receptors are (3-aminopropyl)methylphosphinic acid **2a** ($IC_{50} = 0.75$ μM, $K_b = 0.58$ μM)[5d] and phosphonic acid **3** ($K_b = 10$ μM).[5a] The closest analog of **4b** is phosphinic acid **2b**, which is likewise inactive at $GABA_C$.[5d] Comparison with the present results suggests that structure–activity relationships at $GABA_C$ are conserved between the isosteric 3-aminopropyl phosphinates and 2-aminoethyl phosphonates. Almost all known phosphonic analogs of GABA incorporate terminal phosphono groups. We have found only one other example of a 2-aminoethyl phosphonate that has been studied at GABA receptors. Cates et al. demonstrated that phenylphosphonic acid derivative **4c** weakly inhibited the binding of [^{3}H]GABA to $GABA_A$ and $GABA_B$ receptors but did not assess effects (agonism or antagonism) on receptor function.[27]

Because of their potent activity, the 2-aminoethyl phosphonates are a promising new class of $GABA_C$ antagonists. In this regard, the ease with which additional analogs with varying side chains at phosphorus can be made from readily available phosphonic diesters facilitates a further exploration of structure–activity relationships. The novel dealkylation described should prove of value in the synthesis of these and other asymmetrically substituted phosphonic acid derivatives.

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Supplementary data

Experimental procedures, ^{1}H NMR spectra for all products from Schemes 1 and 2, and representative electrophysiological recordings (for TPMPA and **2c** at $GABA_C$ receptors along with **4a** at $GABA_A$ and $GABA_C$ receptors). Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2007.04.026.

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