Synthesis and Use of the *n*-Bromododecane-1,12-diols as Conformational Probes for General Anesthetic Target Sites

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The *n*-bromododecane-1,12-diols with bromine on carbons 2, 3, 5, and 6, respectively, were synthesized and found to be potent general anesthetics. They were also found to be potent inhibitors of firefly luciferase, a protein model for the primary target sites underlying general anesthesia. However, their effects on lipid bilayers were small, lowering the chain-melting phase transition temperature by less than 1 °C at their EC_{50} concentrations for general anesthesia. A large dependence upon the position of the bromine atom was found for both *n*-hexadecane/water partition coefficients and inhibition constants for firefly luciferase; a much smaller positional dependence was found for induction of general anesthesia and for disrupting lipids. These results are consistent with the bulky bromine atom inhibiting the conformational flexibility of the diol hydrocarbon chain, making these bromo diols useful probes for ascertaining the shapes of apolar binding sites. In particular, our measurements suggest that these novel anesthetics produce general anesthesia by binding to long and relatively narrow apolar target sites in the central nervous system.

Introduction

General anesthesia, which can be defined as the reversible, drug-induced loss of consciousness, is unique among pharmacological phenomena in that it can be produced by an extremely wide range of chemicals having no common structure or chemical groupings (for a review, see ref 1). Although the traditional view has been that the primary target sites are lipids in nerve cell membranes,[2,3] more recent evidence[4] strongly suggests that the targets are proteins. Positive evidence for direct protein/anesthetic interactions has come largely from studies on certain lipid-free soluble proteins—the luciferase enzymes—which have been found to be particularly sensitive to general anesthetic inhibition. These studies support the view that general anesthetic molecules bind to apolar pockets on critical proteins in the central nervous system (CNS).

In an attempt to discern the dimensions and polarity profiles of anesthetic-binding pockets in the unknown target sites underlying general anesthesia, a number of investigations in this laboratory have been directed at mapping and comparing both luciferase and CNS target sites, by studying the actions of homologous series of *n*-alkanes, *n*-alcohols, *n*-alkane-α,ω-diols, and cycloalcohols on both luciferase enzymes and animals.[5-10] In the present investigation we have extended this program by synthesizing, characterizing, and then studying the effects of members of the homologous series of *n*-bromododecane-1,12-diols on both firefly luciferase and tadpoles. This homologous series of agents differ from the previously studied[8] series of *n*-alkane-α,ω-diols in that they are structural isomers all having the same size, each having on its methylene chain a bulky bromine atom which might be expected to affect localized chain bending.

Chemistry

The first strategy for the synthesis of the *n*-bromododecane-1,12-diols involved the preparation of a 1,12-diprotected triol, conversion of the free hydroxyl group into a bromo function, and deprotection of the terminal alcohol groups. Although this strategy proved successful for the synthesis of 5-bromododecane-1,12-diol (4) (Scheme I), it suffered as a general route for three reasons. First, the need to protect the terminal alcohols in the precursor molecules added extra steps to the synthesis. Second, the Grignard reaction in the middle of the synthesis proved capricious when applied to the preparation of the diprotected 1,6,12-triol, with reduction of the precursor 8-(benzyloxy)octanal (1) to 8-(benzyloxy)octanol becoming a serious side reaction. Third, the final deprotection step was messy (*N*-bromosuccinimide proved even less clean than bromine for the removal of the benzyl groups).

A better approach seemed to be the use of masked, rather than protected, hydroxy groups in the precursors. Terminal double bonds were chosen for this role since hydroboration–oxidation should cleanly effect terminal hydroxylation without any problems of reduction of the internal bromide. Indeed, this method proved eminently suitable to the synthesis of the 3- and 6-bromododecane-1,12-diols (**8a,b**, respectively) (Scheme II).

Attempts to make the 4-bromo congener by any method were thwarted by the tendency of the final product to cyclize to a tetrahydrofuran even under almost neutral conditions.

The 2-bromododecane-1,12-diol (11) was prepared by a Hell–Vollhard–Zelinsky monobromination of dodecane-1,12-dioic acid (**9**), followed by diborane reduction of the product (Scheme III).

All the bromo diols were very viscous oils which occluded solvent molecules and required evacuation under oil pump vacuum for at least 2 weeks before they gave satisfactory microanalyses.

Results

All four of the newly synthesized *n*-bromododecane-1,12-diols were found to induce general anesthesia in tadpoles of *Rana temporaria*, with EC_{50} concentrations that were on average 4% of their aqueous solubilities C_{sat} (Tables I and II). In this sense they are much more potent

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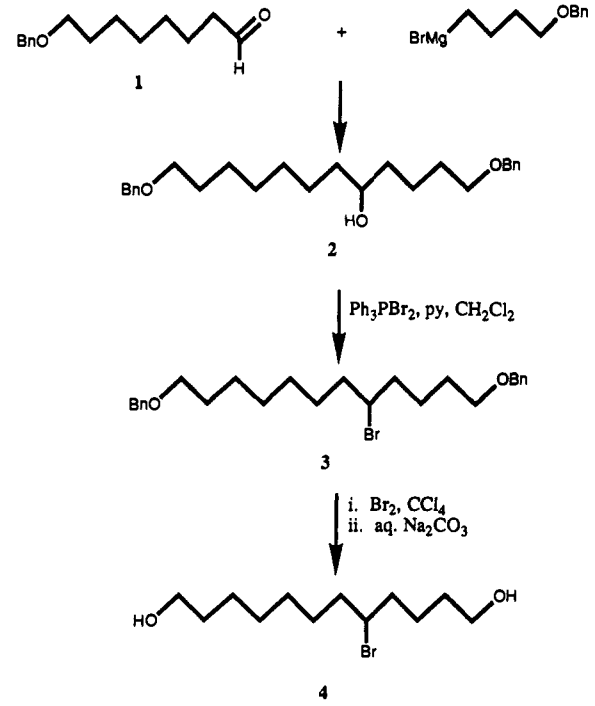
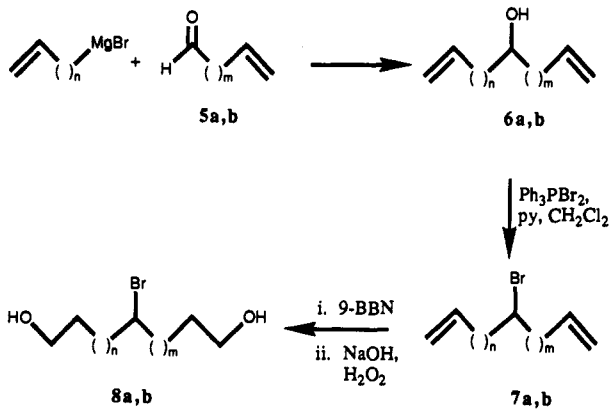
Scheme I[a]



a: Bn = CH_2Ph

Scheme II



a) n = 0, m = 7

b) n = 4, m = 3

anesthetics than the parent compound, *n*-dodecane-1,-12-diol, whose EC_{50} is almost half of its aqueous solubility. In fact, the brominated diols behave similarly to most simple anesthetic agents, whose EC_{50}/C_{sat} ratios are generally[10,11] about 0.02–0.04. In absolute terms, however, the EC_{50} concentrations of all of the dodecanediols, brominated or otherwise, are similar, the maximum ratio between EC_{50} concentrations being only about 3-fold.

On the other hand, both the hexadecane/water partition coefficients K_{hd} (Table I) and the inhibition constants K_i for inhibiting firefly luciferase (Table II) were found to be strongly dependent upon the position of the bromine atom. For example, K_{hd} and $(K_i)^{-1}$ of 2-bromododecane-1,12-diol (11) are 41 (±11) and 33 (±3), respectively, times greater than those of the 6-bromo congener (8b).

Inhibition of firefly luciferase by the *n*-bromododecane-1,12-diols was found to be competitive with respect to the natural substrate firefly luciferin, as has previously been

Scheme III

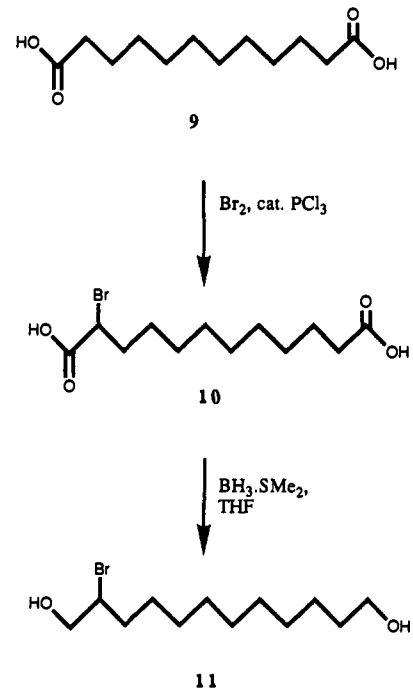


Table I. Physical Properties of Native and Br-Substituted *n*-Dodecane-1,12-diols

compound	C_{sat},[a] μM	K_{hd}[b]
dodecane-1,12-diol	95 ± 9	4.7 ± 0.3
2-bromo-dodecane-1,12-diol (11)	725 ± 66	13 ± 2
3-bromo-dodecane-1,12-diol (8a)	800 ± 40	3.1 ± 0.3
5-bromo-dodecane-1,12-diol (4)	2340 ± 150	0.9 ± 0.1
6-bromo-dodecane-1,12-diol (8b)	2260 ± 130	0.32 ± 0.07

[a] Aqueous solubility (mean ± SE), in 25 mM *N*-glycylglycine and 10 mM $MgSO_4$ (pH 7.8). [b] Hexadecane/water partition coefficient (mean ± SE), expressed as the equilibrium ratio of the molar concentration of the compound in *n*-hexadecane to that in the above buffer.

Table II. EC_{50} Concentrations for Producing General Anesthesia and EC_{50} Concentrations and Inhibition Constants K_i for Inhibiting Firefly Luciferase[a]

compound	general anesthesia: EC_{50},[b] μM	firefly luciferase	
		EC_{50},[c] μM	K_i,[d] μM
dodecane-1,12-diol	39 ± 3	1.54 ± 0.08	0.77 ± 0.04
2-bromo-dodecane-1,12-diol (11)	34 ± 8	0.88 ± 0.06	0.44 ± 0.03
3-bromo-dodecane-1,12-diol (8a)	56 ± 14	1.74 ± 0.08	0.87 ± 0.04
5-bromo-dodecane-1,12-diol (4)	29 ± 8	7.38 ± 0.44	3.69 ± 0.22
6-bromo-dodecane-1,12-diol (8b)	97 ± 11	29.2 ± 1.4	14.6 ± 0.7

[a] Values are means ± SE. [b] Concentration which anesthetizes 50% of a population of *Rana temporaria* tadpoles at 20 °C. [c] Concentration which inhibits luciferase activity by 50% when the luciferin concentration is at its K_m. [d] Inhibition constant for luciferase.

demonstrated for a large number of anesthetic agents,[5] including *n*-alkane-α,ω-diols.[8] This is illustrated for the 6-bromo congener (8b) in Figure 1, where it can be seen that double-reciprocal plots of activity versus luciferin concentration at different bromo diol concentrations share a common intersection on the ordinate axis. Moreover, inhibition involves only one bromo diol molecule, as judged by the linearity of plots of f(I) versus [I], where [I] is the inhibitor concentration and f(I) is the factor by which the apparent Michaelis constant for luciferin increases as a function of [I]. A typical result is illustrated in Figure 2 for the 3-bromo congener (8a). It can be seen that the f(I) plot is linear while the $\sqrt{f(I)}$ plot is not, which is diagnostic of the involvement of only a single inhibitor molecule.[5]

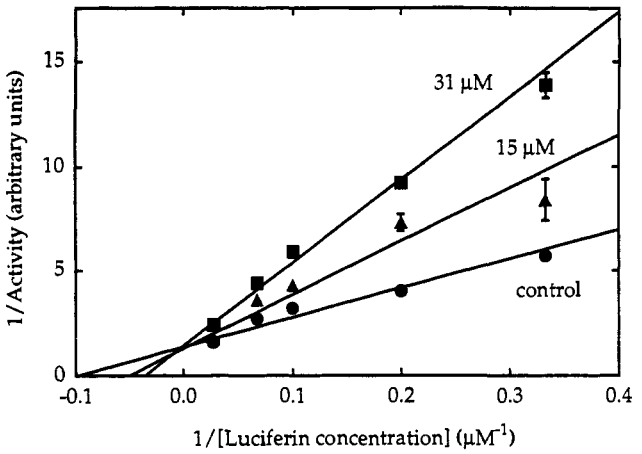


Figure 1. Competitive nature of the inhibition of firefly luciferase. Double-reciprocal plots of enzyme activity versus luciferin concentration are shown at different concentrations of 6-bromododecane-1,12-diol (**8b**): (●) control, (▲) 15 μM, (■) 31 μM. The error bars are standard errors and where not shown are smaller than the symbol. The lines were drawn using the method of weighted least squares, with weighting factors proportional to the squares of the enzyme activities.

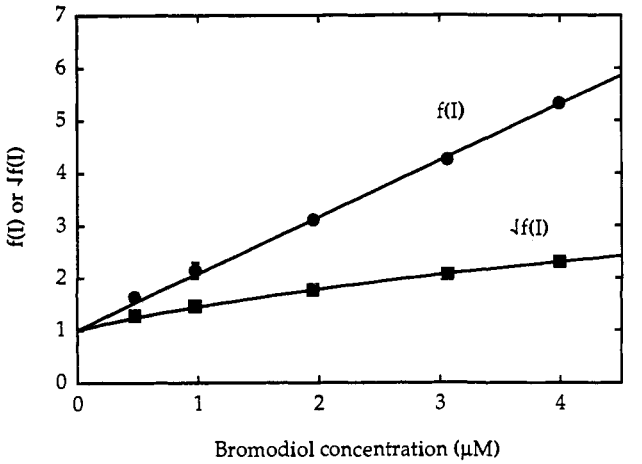


Figure 2. Only one bromododecanediol molecule is involved in the inhibition of firefly luciferase. The factor f(I) and its square root are plotted against the concentration of 3-bromododecane-1,12-diol (**8a**). Notice that the f(I) plot (●) is linear while the $\sqrt{f(I)}$ plot (■) is not. The error bars are standard errors and where not shown are smaller then the symbol. The lines are the predictions of a simple binding model[5] for competitive inhibition by only one molecule of 3-bromododecane-1,12-diol (**8a**).

The EC_{50} concentrations (Table II) for inhibiting firefly luciferase are considerably lower than those for producing general anesthesia, consistent with the finding[8] that the anesthetic-binding sites on luciferase are on average more apolar than the target sites underlying general anesthesia.

All of the n-bromododecane-1,12-diols (but not the unbrominated n-dodecane-1,12-diol) shifted the main chain-melting phase transition of dipalmitoyllecithin bilayers to lower temperatures (Table III). A typical set of melting curves are shown in Figure 3, for 2-bromododecane-1,12-diol (**11**). For all of the brominated diols, the depression ΔT in the melting temperature was found to be linearly related to diol concentration; a typical dose–response relationship is shown in Figure 4, for the 3-bromo congener (**8a**). From the linear dose–response characteristics, values of ΔT were calculated for melting temperature depressions produced by the EC_{50} concentrations of the n-bromododecane-1,12-diols for general anesthesia. These values and their standard errors are listed in Table III, where it can be seen that in all cases they are significantly less than 1 °C (average value = 0.45 °C).

Table III. Depression (ΔT) of Chain-Melting Phase Transition Temperature for Lipid Bilayers of Dipalmitoyl-L-α-phosphatidylcholine[a]

compound	concentration for ΔT = 1 °C,[b] μM	ΔT at anesthetic EC_{50},[c] °C
dodecane-1,12-diol	*d*	*d*
2-bromo-dodecane-1,12-diol (**11**)	77 ± 6	0.44 ± 0.11
3-bromo-dodecane-1,12-diol (**8a**)	85 ± 5	0.66 ± 0.17
5-bromo-dodecane-1,12-diol (**4**)	157 ± 9	0.18 ± 0.05
6-bromo-dodecane-1,12-diol (**8b**)	184 ± 10	0.53 ± 0.07

[a] Values are means ± SE. [b] Concentration of compound which reduces phase transition temperature by 1 °C. [c] Depression produced at EC_{50} concentration for general anesthesia. [d] The phase transition temperature was unaffected even by a saturated solution of the parent compound.

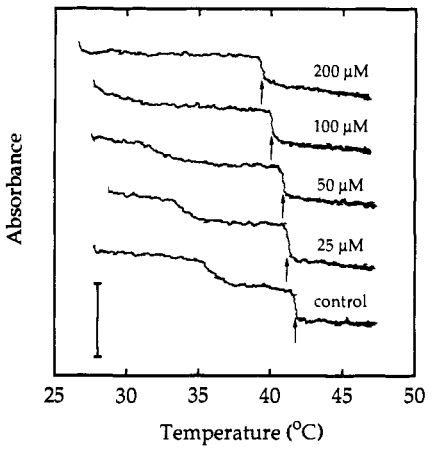


Figure 3. The bromododecanediols reduce the main chain-melting phase transition temperature of lipid bilayer vesicles of dipalmitoyllecithin. The data shown here are for 2-bromododecane-1,12-diol (**11**). The optical absorbance at 450 nm undergoes a sharp change at the transition temperature, T_m, indicated by the arrows. As the concentration of diol is increased, T_m is shifted to lower temperatures. The traces have been offset in the vertical direction for clarity. The calibration bar represents a change in absorbance of 0.1 unit.

Discussion

The position of the bromine atom on the n-dodecane-1,12-diol backbone had a profound influence on the hexadecane/water partition coefficients K_{hd} and on the inhibition constants K_i for firefly luciferase, but not on the EC_{50} concentrations for general anesthesia. The data (Tables I and II) are perhaps best visualized by plotting, as a function of bromine position, the apparent incremental standard Gibbs free energies $\Delta(\Delta G_n^{\ominus})$ for substituting a bromine atom for a hydrogen atom on carbon number n on the dodecane-1,12-diol backbone. It can be seen from Figure 5 that $\Delta(\Delta G_n^{\ominus})$ is similar for both partitioning into n-hexadecane and for inhibiting firefly luciferase, increasing monotonically as the position of the bromine atom moves from the end to the center of the diol backbone. Because of the chain distances involved, this is unlikely to be due to an inductive effect of the bromine atom on the properties (e.g. hydrogen bonding) of the terminal hydroxyl groups. It seems more likely to be due to the presence of the bulky bromine atom inhibiting the conformational flexibility of the diol molecules. Indeed, although other explanations may be possible, a reasonable and consistent explanation of both the partitioning and luciferase results can be provided by assuming that the bromine atom restricts chain bending.

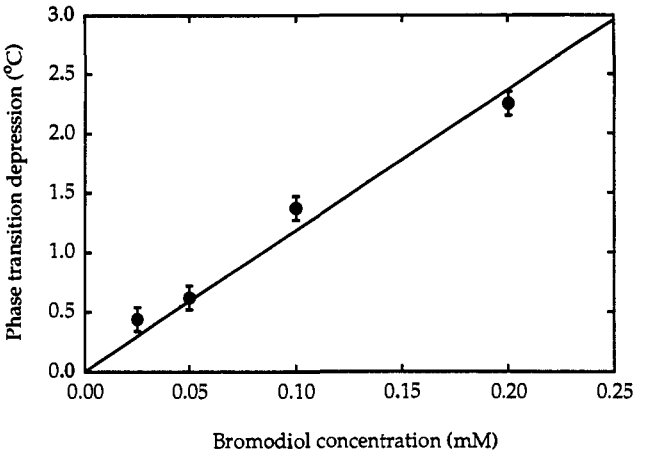


Figure 4. The depression (ΔT) of the lipid phase transition temperature is linearly related to the concentration of the brominated dodecanediol. The data shown are for 3-bromododecane-1,12-diol (**8a**). The line (constrained to pass through the origin) was fitted by the method of weighted least squares, with weighting factors proportional to the reciprocals of the standard errors. The error bars are standard errors for three determinations.

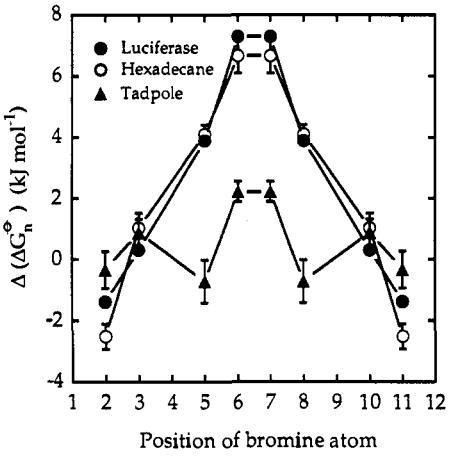


Figure 5. The apparent incremental standard Gibbs free energy change $\Delta(\Delta G_n^\ominus)$ for addition of a bromine atom to carbon atom number n on the dodecane-1,12-diol backbone is shown for partitioning into n-hexadecane (○), for inhibiting firefly luciferase (●), and for producing general anesthesia (▲). The data have been plotted to emphasize the symmetry of the molecules. The error bars are standard errors and where not shown are smaller than the symbols.

Consider first the hexadecane/water partition coefficients K_{hd}. In the aqueous phase, both terminal hydroxyl groups are presumably well satisfied by hydrogen bonding to water molecules, regardless of diol conformation. In the hexadecane phase, however, there is a very large free energy cost associated with exposing hydroxyl terminals to alkane. [Using values[12] of the standard Gibbs free energy change for the transfer of *n*-alcohols and *n*-alkanes with 1–8 carbon atoms from water to hexadecane, one can calculate the average (±SE) free energy cost to be 20.9 ± 0.2 kJ/mol for a single terminal hydroxyl group.] In hexadecane, therefore, there will be a strong tendency for the dodecanediols to adopt U-shaped conformations which allow the two terminal hydroxyl groups on a single molecule to hydrogen bond with each other. However, this tendency will be resisted by the unfavorable steric energy required to bend the diol chain into a U-shaped conformation. There will thus be an equilibrium between intramolecularly hydrogen-bonded, U-shaped structures and more extended structures in which the terminal hydroxyl groups are not hydrogen bonded to each other. [For dodecane-1,12-diol a value (mean ± SE) of $K_{hd} = 1.0 \pm 0.2$ for these "extended" structures can be estimated from the measured[13] K_{hd} = 2000 ± 400 value for undecan-1-ol, by taking into account the factors of 4.07 ± 0.08 for adding a methylene group[13] and (1.22 ± 0.09) × 10^{-4} for simultaneously adding a terminal hydroxyl group and removing a terminal hydrogen atom (using the above K_{hd} data[12] for *n*-alkanes and *n*-alcohols). This is less than our experimental $K_{hd} = 4.7 \pm 0.3$ for dodecane-1,12-diol by a factor of 4.7 ± 1.0, suggesting that most of the unbrominated dodecanediol molecules exist in U-shaped forms in hexadecane.] This equilibrium will be shifted away from U-shaped forms toward "extended" structures by any factor that increases the steric energy required to produce U-shaped conformations.

The introduction of a bulky bromine atom into the diol hydrocarbon chain will tend to increase this steric energy, by inhibiting localized bending. Now it is clear from simple geometrical considerations, or from manipulating molecular models (e.g. CPK space-filling models), that the formation of the requisite U-shaped forms for intramolecular hydrogen bonding is critically dependent upon bending of the diol chain near its center. Thus the addition of a bromine atom might be expected to have its maximal steric effect when added to the center of the chain, with progressively less effect as it moves away from the center. Since the hydrogen-bonded U-shaped forms have a higher intrinsic K_{hd} than the "extended" forms, the consequence of these considerations is that in a series of monobrominated α,ω-diols one would expect the experimentally measured values of K_{hd} to progressively increase as the bromine atom moves from the center toward the end of the diol chain. This is just what we have observed (see Table I and Figure 5) for the positional effect of the bromine atom upon partitioning between hexadecane and water.

Next consider the inhibition of the enzyme firefly luciferase by the dodecanediols. It is clear from Figure 5 that the free energies $\Delta(\Delta G_n^\ominus)$ calculated from inhibition constants K_i mirror rather closely those for partitioning into hexadecane. This is likely to also be related to the effect of the bulky bromine atom on hydrocarbon chain bending. Indeed, mapping of the anesthetic-binding pocket on firefly luciferase with the homologous series of *n*-alkane-α,ω-diols, *n*-alcohols, and *n*-alkanes suggests that it is so shallow that it can accommodate a straight hydrocarbon chain of at most seven carbons but sufficiently wide as to be able to accommodate two chains side by side.[8] Thus in order to accommodate the long-chain dodecanediols, it is essential that the chains bend back upon themselves, with the terminal hydroxyl groups probably being anchored at the polar mouth of the binding pocket. The number of such U-shaped conformations is reduced by the presence of a bromine atom, as discussed above for K_{hd}, with the maximum effect occurring when the bromine is near the center of the diol backbone.

Table III lists the results for the *n*-bromododecane-1,-12-diols depressing the phase transition temperature of bilayers of dipalmitoyllecithin, a sensitive test of lipid bilayer disruption.[4,14] It was not possible to calculate apparent values of $\Delta(\Delta G_n^\ominus)$, because no effect was found for *n*-dodecane-1,12-diol even at saturation. Although temperature depressions (ΔT) were observed with all of

the brominated diols, the actual depressions at EC_{50} concentrations for general anesthesia averaged only 0.45 °C and were all significantly less than 1 °C (see Table III). Since such tiny temperature changes do not cause general anesthesia, these results argue against lipid target sites. Although the effect of bromine position on ΔT was small, the concentrations necessary to produce ΔT = 1 °C increased monotonically as the bromine position shifted toward the center of the diol chain. If one considers how the brominated diols insert into lipid bilayers, it is clear that their terminal hydroxyl groups will tend to reside in the polar headgroup regions, with their methylene chains suspended in the lipid hydrocarbon region. Since the lipid hydrocarbon region is too thick for traversal by a single dodecane chain, both diol hydroxyls will insert at the same side of the bilayer, with their backbones adopting U-shaped conformations. However, as it is not necessary for the terminal hydroxyls to hydrogen bond to each other, the degree of bending need not be as pronounced as in hexadecane. Since the bulky bromine inhibits the requisite chain bending progressively more as it approaches the center of the diol, one can thus understand why the lipid results are qualitatively in the same direction as for K_{hd} but much less pronounced.

Finally, having considered solvent, lipid, and protein sites, what picture emerges when one considers the induction of general anesthesia by these dodecane-1,12-diols? All of these diols, brominated or not, are potent general anesthetics, in that their EC_{50} concentrations are in the tens of micromolar range. Figure 5 shows, however, that the pattern of apparent free energies $\Delta(\Delta G_n^{\ominus})$ calculated from EC_{50} concentrations for general anesthesia differ substantially from those for partitioning into hexadecane and for inhibiting the luciferase enzyme. No longer is there a monotonic increase in $\Delta(\Delta G_n^{\ominus})$ as the bromine atom approaches the center of the diol molecule. Instead, the free energies fluctuate around 0 kJ/mol except for carbon number 6, and even there $\Delta(\Delta G_n^{\ominus})$ is less than one-third of the values for K_{hd} and K_i. What this suggests is that it is not necessary for the diol chains to bend back upon themselves in order to bind to the primary (unknown) sites underlying general anesthesia.

This conclusion is consistent with the results of a recent investigation[8] using another species of tadpole (*Xenopus laevis*), in which the animal sites were mapped by measuring and analyzing general anesthesia EC_{50} data for the homologous series of *n*-alkane-α,ω-diols and *n*-alcohols. The data suggested that the sites to which straight-chain anesthetics bind to produce general anesthesia are long and narrow apolar pockets extending from the aqueous solution, sufficiently long to accommodate hydrocarbon chains of about 12 carbons but not sufficiently wide to accommodate chains bent back on themselves.

Conclusions

This study has shown that the newly synthesized *n*-bromododecane-1,12-diols are potent general anesthetics which can be used as probes to discriminate between different types of anesthetic-binding sites. Sites which require sharp bending of these long-chain anesthetic probes about their centers, either for intramolecular hydrogen bonding of the terminal hydroxyls (as in hexadecane) or for packing into a shallow protein pocket (as in firefly luciferase), take up progressively less of the probes from water as their bromine atom is positioned nearer the center of the dodecanediol backbone. Sites which require only moderate bending of these probes (such as lipid bilayers) show the same effect, but it is much reduced in magnitude. On the basis of these findings with known sites, the measured potencies of the *n*-bromododecane-1,12-diols for producing general anesthesia support the view that the unknown animal target sites to which they bind require little chain bending. From these and other[1,4-10] considerations, together with the small effects observed with lipids, we conclude that these sites are long and relatively narrow apolar pockets extending from the surfaces of anesthetic-sensitive proteins in the central nervous system.

Experimental Section

Infrared spectra were recorded on a Perkin-Elmer 881 spectrophotometer as thin films. ^1H NMR spectra were recorded on a JEOL FX 90Q instrument using tetramethylsilane or chloroform as internal standards in $CDCl_3$. ^{13}C NMR spectra were recorded on Bruker WM-250, JEOL GSX 270, and Bruker AM-500 instruments. Coupling constants are in hertz and signals are quoted as singlet (s), triplet (t), quintet (quint), and multiplet (m). Mass spectra were determined on a VG Micromass 7070B machine by the electron impact (EI) or fast atom bombardment (FAB) (thiodiethanol matrix) method.

2-Bromododecane-1,12-diol (11). Dodecane-1,12-dioic acid (9) (2.3 g, 10 mmol) was treated with dry bromine (0.51 mL, 1.6 g, 10 mmol) (previously washed once with concentrated sulfuric acid). The mixture was cautiously treated with phosphorus trichloride (0.05 mL) and then heated to 65–70 °C for 4 h. During this time the color of the mixture gradually decreased. Finally the mixture was heated to 100 °C over 15 min and then allowed to cool and stand overnight at room temperature. The resultant yellow solid was washed with water three times to leave a pale yellow solid, mp shr 95–115, 118 °C (2.53 g). The ^1H NMR spectrum of this solid, which was a mixture of 2-bromo and 2,11-dibromo acids as well as starting material, showed a triplet at 4.2 ppm due to the $CHBr{\cdot}CO_2H$ proton.

The above solid was dissolved in dry tetrahydrofuran (THF) (50 mL) under argon and treated with a solution of $BH_3{\cdot}SMe_2$ in ether (Aldrich, 2 M, 9.0 mL, 18 mmol). Toward the end of the addition, a gelatinous solid formed which stopped the stirrer. After completion of the addition the solid slowly dissolved, and after 23 h the reaction was quenched by the addition of methanol (9 mL) dropwise. The mixture was stirred for 1.5 h and then concentrated on a rotary evaporator. The residue was treated with methanol (50 mL) and the solution evaporated again to leave a white, waxy solid (2.4 g). This was triturated with ethyl acetate–ether (1:1, 20 mL) and filtered to remove insoluble dodecane-1,12-diol (480 mg). The filtrate was concentrated to an oily solid which still contained some carboxylic acid impurities by IR spectroscopy. This material was dissolved in dry THF (50 mL) and treated with a further portion of $BH_3{\cdot}SMe_2$ (Aldrich, 2 M in ether, 4 mL, 8 mmol). After reaction for 24 h, the mixture was worked up with methanol as before. The resultant yellow, viscous oil was chromatographed on silica gel using ether as eluent to give the required diol (11) as a colorless, viscous oil (760 mg, 27% overall): IR ν_{max} 3360 (broad, OH stretch), 1050 cm^{-1} (broad, CO stretch); ^1H NMR δ 1.2–1.9 (18 H, m, 9 × CH_2), 3.70 (4 H, m, 2 × CH_2O), 4.10 (1 H, m, CHBr); ^{13}C NMR δ 26, 27.5, 29, 29.5 (3 peaks visible), 33, 35, 60, 63, 67; MS *m/z* (FAB) 283 + 281 (M^+ + 1), 265 + 263 (M^+ + 1 − H_2O), 201 (M^+ + 1 − Br), 183, 165, 109, 95, 83, 69, 55 (100%), 41. Anal. ($C_{12}H_{25}BrO_2$) C, H; Br: calcd, 28.41; found, 27.70.

3-Bromododecane-1,12-diol (8a). To a solution of decen-10-ol (1.56 g, 10.0 mmol) in dichloromethane (75 mL) was added pyridinium chlorochromate (6.05 g, 30.0 mmol). The mixture was stirred at room temperature for 4 h. Ether (150 mL) was added to the mixture and the resultant slurry was filtered through a pad of Celite (lower layer) and silica gel (upper layer). The residue in the reaction vessel was washed with a further portion (50 mL) of ether, and this was used to rinse the filter pad. The combined filtrate and rinsings were concentrated to a yellow oil which showed little OH by IR spectroscopy. The oil was dissolved

in dry THF (30 mL) and the solution was treated with a solution of vinylmagnesium bromide (Aldrich, 1 M in THF, 15 mL, 15 mmol). The mixture was stirred at room temperature for 1 h and then treated with saturated aqueous ammonium chloride solution (40 mL). The mixture was poured into ether (100 mL), the layers were separated, and the ether layer was washed with water (3 × 20 mL). After drying ($MgSO_4$), the ethereal solution was concentrated to a pale yellow oil (**6a**) (1.53 g): IR ν_{max} 3400 (OH stretch), 3040 (=CH stretch), 1640 cm^{-1} (C=C stretch); ^1H NMR δ 1.40 (12 H, m, 6 × CH_2), 2.00 (2 H, m, allylic CH_2), 4.10 (1 H, m, CHO), 4.8–5.3 (4 H, m, 2 =CH_2), 5.6–6.0 (2 H, m, 2 CH=).

The pale yellow oil **6a** (1.53 g, 8.41 mmol) was dissolved in dry dichloromethane (20 mL) containing dry pyridine (0.680 mL, 714 mg, 9.00 mmol). This solution was added dropwise to a solution of triphenylphosphine (2.22 g, 8.47 mmol) and bromine (0.43 mL, 1.36 g, 8.50 mmol) in dry dichloromethane (120 mL) over 10 min. The reaction mixture was stirred at room temperature for 1 h and then concentrated. The residue was triturated with a mixture of light petroleum and ether (1:1, 30 mL), the triturate was filtered, and the solid was washed with a portion of the solvent mixture (20 mL). The combined filtrates were concentrated to a yellow oil containing a little solid. This was chromatographed on silica gel using light petroleum/ether (1:1) as eluent to give the bromo diene **7a** as a yellow oil (1.325 g): IR ν_{max} 3077 cm^{-1} (=CH stretch); ^1H NMR δ 1.2–1.6 (12 H, m, 6 × CH_2), 1.8–2.2 (2 H, m, allylic CH_2), 4.5 (1 H, m, CHBr), 4.9–5.3 (4 H, m, 2 =CH_2), 5.6–6.2 (2 H, m, 2 CH=).

To a solution of the bromo diene **7a** above (1.325 g, 5.430 mmol) in dry THF (50 mL) was added under argon a solution of 9-borabicyclo[3.3.1]nonane (9-BBN) (Aldrich, 0.5 M in THF, 21.7 mL, 10.9 mmol). The mixture was stirred at room temperature overnight. A solution of *m*-chloroperbenzoic acid (mCPBA) (5.60 g, 32.6 mmol) in THF (15 mL) was added carefully (exothermic reaction) over 15 min and the resultant mixture was left to stir for a further 1.5 h. The solution was concentrated and the solid residue was dissolved in methanol (80 mL) and the solution concentrated again. The solid residue (5.47 g) was dissolved in ether (100 mL) and the ethereal solution was washed with saturated sodium bicarbonate solution (4 × 20 mL), 4 N potassium hydroxide solution (1 × 20 mL), and water (3 × 20 mL), and dried ($MgSO_4$). After filtration of the drying agent, the ethereal solution was concentrated to a viscous oil (1.06 g). This oil was chromatographed on silica gel using ethyl acetate/ether (1:1) as the eluent to give the bromo diol **8a** as a golden yellow oil (300 mg, 10.6% overall): IR ν_{max} 3350 (broad, OH stretch), 1055 cm^{-1} (CO stretch); ^1H NMR δ 1.2–2.0 (18 H, m, 9 × CH_2), 3.60 (2 H, t, CH_2O), 3.82 (2 H, t, CH_2O), 4.20 (1 H, quint, CHBr); ^{13}C NMR δ 26, 27.5, 28.9, 29.3, 29.5, 33, 39, 41, 55, 61, 63; MS *m/z* (FAB) 283 + 281 (M^+ + 1), 265 + 263 (M^+ + 1 − H_2O), 201 (M^+ + 1 − Br), 183, 149, 123, 109, 95, 81, 69, 55 (100%), 41. Anal. ($C_{12}H_{25}BrO_2$) H; C: calcd, 51.25; found, 52.06; Br: calcd, 28.41; found, 32.41.

5-Bromododecane-1,12-diol (4). To dry magnesium (720 mg, 30 mg atom) in dry ether (5 mL) was added a small portion of 4-(benzyloxy)-1-bromobutane plus a crystal of iodine. After the reaction had initiated (about 5 min), the suspension was diluted with ether (10 mL) and then treated with a solution of 4-(benzyloxy)-1-bromobutane (6.8 g, 28 mmol) in dry ether (25 mL) at such a rate as to maintain reflux. After the addition was complete, the resultant light gray solution was heated to reflux for a further 5 min. Then a solution of 8-(benzyloxy)octanal (**1**) (5.8 g, 25 mmol) in dry ether (25 mL) was added at such a rate as to maintain reflux. After the addition, the mixture was heated to reflux for a further 5 min. The reaction was allowed to cool and 1 N HCl (45 mL) was added slowly. The two layers were separated, and the ethereal layer was washed with water until the washings were neutral (3 × 10 mL) and then dried ($MgSO_4$) and evaporated to a pale yellow oil (10.5 g). This oil was chromatographed on silica gel using light petroleum/ether (1:1) as eluent to give 1,12-bis(benzyloxy)dodecan-5-ol (**2**) as a colorless oil (6.34 g): IR ν_{max} 3420 (broad, OH stretch), 1100 (broad, CO stretch), 740, 700 cm^{-1}; ^1H NMR δ 1.2–1.8 (18 H, m, 9 × CH_2), 3.3–3.6 (5 H, m, CHOH + CH_2O), 4.45 (4 H, s, 2 benzylic), 7.3 (10 H, s, 2 Ph); MS *m/z* 399 (M^+), 381 (M^+ − H_2O), 289, 271, 213, 201, 181, 91 (100%, $PhCH_2^+$).

The alcohol **2** (6.0 g, 15 mmol) was dissolved in dry dichloromethane (50 mL) containing dry pyridine (1.30 g, 1.33 mL, 16.5 mmol). This solution was added dropwise over 15 min to a solution of triphenylphosphine (4.32 g, 16.5 mmol) and bromine (0.85 mL, 2.64 g, 16.5 mmol) in dry dichloromethane (50 mL) at room temperature. The reaction was stirred at room temperature overnight, then evaporated, and triturated with a mixture of light petroleum and ether (1:1) (50 mL). The insoluble yellow solid was filtered and washed with a further portion of the same solvent mixture (20 mL). The combined filtrate and washing was evaporated to a cream-colored suspension. This was chromatographed on silica gel using light petroleum/ether (1:1) as eluent to give 5-bromo-1,12-bis(benzyloxy)dodecane (**3**) as a pale yellow oil (6.37 g): IR ν_{max} 1100 (broad, CO stretch), 750, 700 cm^{-1}; MS *m/z* 371 + 369 (M^+ − $PhCH_2$), 107, 91 (100%).

The bromo diether **3** (5.44 g, 11.7 mmol) was dissolved in carbon tetrachloride (110 mL) under $CaCl_2$ and treated with bromine (0.60 mL, 1.92 g, 12.0 mmol). The mixture turned from brown to yellow at room temperature over a period of 4 min. More bromine (0.30 mL, 0.96 g, 6.0 mmol) was added, and again the mixture was left to turn yellow (4 min). A final portion of bromine (0.30 mL, 0.96 g, 6.0 mmol) was added, and the mixture was stirred at room temperature for 10 min. To the resultant orange-brown solution was added a solution of sodium carbonate (5.25 g, 50.0 mmol) in water (20 mL) over 2 min. The suspension was stirred at room temperature for 30 min to give an orange organic phase and a yellow, cloudy aqueous suspension. The whole mixture was poured into ethyl acetate (500 mL), and the organic layer was separated, washed with water (2 × 50 mL), and dried ($MgSO_4$). After filtration of the drying agent and evaporation of the solvent, the residue was chromatographed on silica gel using ethyl acetate/ether (1:1) as eluent to give an orange oil (3.6 g). This oil still seemed to contain α-bromohydrin ethers (^1H NMR), and therefore, it was dissolved in methanol/water (5:1) (18 mL) and stirred with K_2CO_3 (1.38 g) for 2.5 h. The mixture was poured into ethyl acetate (60 mL) and worked up as above to give an orange oil (2.41 g). This was chromatographed three times on silica gel using ethyl acetate/ether (1:1) as eluent to give the bromo diol **4** as a yellow oil (1.04 g, 15% overall): IR ν_{max} 3350 (broad, OH stretch), 1060 cm^{-1} (broad, CO stretch); ^1H NMR δ 1.2–1.9 (18 H, m, 9 CH_2), 2.6 (2 H, s, 2 OH), 3.60 (4 H, distorted t, 2 CH_2OH), 4.05 (1 H, quint, CHBr); ^{13}C NMR δ 24, 25.5, 27.5, 29, 29.2, 32, 32.5, 38.8, 39, 58.5, 62.5, 62.8; MS *m/z* (FAB) 283 + 281 (M^+ + 1), 265 + 263 (M^+ + 1 − H_2O), 201 (M^+ + 1 − Br), 165, 81, 67, 55, 41, 39, 31, 27 (100%). Anal. ($C_{12}H_{25}BrO_2$) C, H; Br: calcd, 28.41; found: 32.62.

6-Bromododecane-1,12-diol (8b). A small portion of 6-bromo-1-hexene was added to dry magnesium (768 mg, 32.0 mg atom) in dry ether (5 mL). The reaction began without difficulty and was diluted with dry ether (20 mL). A solution of 6-bromo-1-hexene (4.0 mL, 32 mmol) in dry ether (10 mL) was then added at such a rate as to maintain reflux. After completion of the addition, a solution of 6-hexenal (**5b**) [from 4.0 g, 40 mmol of 6-hexen-1-ol and pyridinium chlorochromate (PCC)] in ether (100 mL) was added dropwise at such a rate as to maintain gentle reflux. After the addition, the mixture was stirred at room temperature overnight. Saturated aqueous ammonium chloride solution (80 mL) was added dropwise, and the two layers were separated. The aqueous layer was dried ($MgSO_4$), filtered, and evaporated to give a very pale, yellow oil (5.2 g). Since this oil still contained some aldehyde which was very close to the desired dienol **6b** (TLC), it was dissolved in ethanol (20 mL) and treated with a portion (1 g) of sodium borohydride. The mixture was left to stir overnight and then poured onto ice and 2 N HCl (100 mL). The suspension was extracted with ether (3 × 20 mL), and the combined extracts were washed with water (3 × 10 mL) and dried ($MgSO_4$). Filtration and evaporation gave a pale yellow oil (2.9 g) which was chromatographed on silica gel using light petroleum/ether (1:1) as eluent to give dodeca-1,11-dien-6-ol (**6b**) as an almost colorless oil (2.12 g): IR ν_{max} 3380 (broad, OH stretch), 3080 (=CH stretch), 1640 (C=C stretch), 905 cm^{-1}; ^1H NMR δ 1.25–1.65 (10 H, m, 5 CH_2), 2.0–2.2 (4 H, m, 2 allylic CH_2), 3.4 (1 H, m, CHOH), 4.85–5.15 (4 H, m, 2 =CH_2), 5.6–6.05 (2 H, m, 2 =CH); MS *m/z* 181 (M^+ − 1), 164 (M^+ − H_2O), 149, 95, 81 (100%), 69, 67, 57, 55, 41.

To a solution of triphenylphosphine (3.06 g, 11.7 mmol) and bromine (0.60 mL, 1.87 g, 11.7 mmol) in dry dichloromethane (100 mL) was added dropwise at room temperature a solution of the dienol **6b** (2.12 g, 11.6 mmol) and pyridine (0.90 mL, 0.924 g, 11.7 mmol) in dry dichloromethane (50 mL). The mixture was stirred at room temperature for 1 h and then concentrated to a white solid. This was triturated with light petroleum/ether (2:1), and the triturate was filtered and concentrated to a pale yellow oil (2 g) containing a little solid. This was chromatographed on silica gel using light petroleum/ether (2:1) as eluent to give the bromo diene **7b** as a colorless oil (2.15 g): IR ν_{max} 3080 (=CH stretch), 1640 (C=C stretch), 905 cm^{-1}; ^1H NMR δ 1.2–2.2 (14 H, m, 5 CH_2 and 2 allylic CH_2), 4.0 (1 H, m, CHBr), 4.85–5.15 (4 H, m, 2 $=CH_2$), 5.6–6.0 (2 H, m, 2 =CH).

To a solution of the bromo diene (**7b**) (1.95 g, 7.99 mmol) in dry THF (50 mL) under argon was added 9-BBN (Aldrich, 0.5 M in THF, 32 mL, 16 mmol). The mixture was stirred at room temperature for 5 h, and then ethanol (16 mL) and 6 N NaOH (5.28 mL) were added, followed cautiously by the addition of hydrogen peroxide (30%, 10.5 mL) over 15 min. An exothermic reaction took place on addition of the peroxide, and after addition was complete, the mixture was allowed to return to room temperature (20 min) and then stirred at this temperature for 50 min. The mixture was poured into ethyl acetate (500 mL), and the layers were separated. The organic layer was washed with water until neutral (5 × 50 mL) and dried ($MgSO_4$). After filtration and evaporation there remained a viscous, almost colorless oil. This was chromatographed three times on silica gel using ethyl acetate/ether (1:1) as eluent to give the bromo diol **8b** as a pale yellow oil (1.25 g, 14% overall): IR ν_{max} 3350 (broad, OH stretch), 1050 cm^{-1} (broad, CO stretch); ^1H NMR δ 1.2–1.9 (18 H, m, 9 CH_2), 2.3 (2 H, s, 2 OH), 3.6 (4 H, t, 2 CH_2OH), 4.0 (1 H, quint, CHBr); ^{13}C NMR δ 25, 25.5, 27, 27.2, 28.6, 32.3, 32.4, 39, 58, 62.3, 62.4; MS *m/z* (FAB) 283 + 281 ($M^+ + 1$), 265 + 263 ($M^+ + 1 - H_2O$), 201 ($M^+ + 1 - Br$), 183, 165, 123, 109, 95, 81, 67, 55 (100%), 41. Anal. ($C_{12}H_{25}BrO_2$) C, H; Br: calcd, 28.41; found, 29.38.

Purification and Assay of Firefly Luciferase. Pure crystals of the enzyme firefly luciferase were obtained from desiccated firefly lanterns (Sigma Chemical Co.) of the North American firefly *Photinus pyralis* using the affinity chromatography purification procedure described earlier.[5,15] Light-emitting luciferase assays were performed as described previously[5,7] under conditions [2 mM adenosine 5′-triphosphate (ATP)] where the ATP-binding site was effectively saturated and anesthetic inhibition was competitive with respect to the substrate firefly luciferin. The bromo diols were applied as ethanolic solutions such that the final concentration of ethanol was 23 mM, which is less than 3% of the K_i for ethanol;[6] nonetheless, the same amount of ethanol was included in the control assays. All luciferase assays were performed at room temperature (25 ± 1 °C).

Analysis of Firefly Luciferase Inhibition Data. For the determination of the inhibition constants K_i for the various diols, the inhibited activities v_i were determined in triplicate at (typically) five diol concentrations; each individual inhibitor assay was paired with a control assay for the activity v_0 in the absence of inhibitor. From each (v_i, v_0) data pair, an estimate of the inhibition constant was made using $K_i = [I]/\{([S]/K_m + 1)(v_0/v_i - 1)\}$, where K_m is the Michaelis constant for luciferin in the absence of inhibitor, and [I] and [S] are the inhibitor and substrate (luciferin) concentrations, respectively. Using the same data, the factor f(I), by which the apparent Michaelis constant for luciferin increases as a function of [I], was calculated as described previously[7] using the relationship $f(I) = (v_0/v_i) + ([S]/K_m)(v_0/v_i - 1)$. The mean value and standard error of the inhibition constant K_i was calculated from the (typically 15) individual estimates of K_i using the method of weighted least squares, with weighting factors proportional to $[I]^2(v_i/v_0)^2/(K_i)^4$. The EC_{50} concentration, defined as that concentration [I] which half-inhibits luciferase activity when luciferin is present at its K_m concentration, was calculated using[5] $EC_{50} = 2K_i$.

Lipid Phase Transitions. Depressions (ΔT) in the main chain-melting phase transition temperature of aqueous suspensions of vesicles of dipalmitoyllecithin (dipalmitoyl-L-α-phosphatidylcholine) due to the presence of varying concentrations of diols were determined as described previously.[10] The diols were applied as ethanolic solutions such that the final concentration of ethanol was 57 mM; the controls also contained 57 mM ethanol. For each diol, 15 melting curves were typically measured: three controls and three diol curves at each of four different diol concentrations. Plots of melting-point depression (ΔT) versus diol concentration gave straight lines (see, for example, Figure 4), whose slopes were determined by the method of weighted least squares with the line constrained to pass through the origin. In order to check for depletion of the diols from the aqueous phase, measurements of ΔT were also made as a function of lipid concentration at constant diol concentration for 2-bromododecane-1,12-diol (11). Assuming that ΔT is proportional to the concentration of diol in the membrane, it is straightforward to show that the molar lipid/water partition coefficient (K_{lipid}) can be obtained as the ratio of the slope to the y intercept of a plot of total diol concentration divided by ΔT versus lipid concentration. This procedure gave (mean ± SE) $K_{lipid} = 670 \pm 30$ for 2-bromododecane-1,12-diol (11), corresponding to a depletion from the aqueous phase of only 9.1 ± 0.4%. Since this depletion was small and is likely to represent an upper limit for the series [since 2-bromododecane-1,12-diol (11) gave $\Delta T = 1$ °C at the lowest concentration of all the diols, and it also has the largest hexadecane/water partition coefficient K_{hd}], no corrections were made for depletion in the tabulated results.

Determination of Aqueous Solubilities. Aqueous solubilities of the diols at 25 ± 1 °C were determined in a buffer (pH 7.8) consisting of 25 mM *N*-glycylglycine and 10 mM $MgSO_4$ using a modification of the method of Curry et al.,[10] with diol concentrations determined by their inhibition of firefly rather than bacterial luciferase.

Determination of Hexadecane/Water Partition Coefficients. Molar partition coefficients (K_{hd}) between *n*-hexadecane and the buffer described immediately above were determined by the luciferase method described previously.[13] At least five independent determinations of K_{hd} were made for each diol. All determinations were made at 25 ± 1 °C.

Determination of General Anesthetic Potencies. General anesthetic EC_{50} concentrations were determined for tadpoles of *Rana temporaria* (lengths between 20 and 25 mm) at 20 ± 1 °C. For each diol, six tadpoles were placed in each of 7–10 beakers containing 300 mL of tap water and various concentrations of the diol. The anesthetic endpoint was defined as the lack of a reflex response of the tadpole to a gentle tap on its side or snout with a smooth-ended glass rod. Each tadpole was tested once every 15 min for up to 2 h, even though a steady state was always reached in less than 1 h. Dose–response data were then analyzed by the method of Waud.[16] Some tadpoles exposed to the 5-bromo congener 4 showed erratic "convulsing" behavior and were not included in the analysis. All of the tadpoles tested recovered when returned to pure tap water.

Calculation of Apparent Incremental Standard Gibbs Free Energies $\Delta(\Delta G_n^{\ominus})$. These are the free energies for adding a bromine atom (strictly, for substituting a bromine atom for a hydrogen atom) to carbon atom number n on the unbrominated parent compound (dodecane-1,12-diol). A positive value indicates that adding a bromine atom to dodecanediol reduces its hexadecane/water partition coefficient K_{hd}, its association ($1/K_i$) with luciferase, or its potency ($1/EC_{50}$) as a general anesthetic. They were calculated as $RT \ln (K_{hd}^0/K_{hd}^n)$ for the hexadecane/water partition coefficients, $RT \ln (K_i^n/K_i^0)$ for the luciferase inhibition constants, and $RT \ln (EC_{50}^n/EC_{50}^0)$ for the animal EC_{50} concentrations, where R is the gas constant, T is the absolute temperature, and the superscripts 0 and n refer respectively to the unbrominated *n*-dodecane-1,12-diol and to the dodecanediol brominated at carbon number n.

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