Octanol−, Chloroform−, and Propylene Glycol Dipelargonat−Water Partitioning of Morphine-6-glucuronide and Other Related Opiates†

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The pK_a and log *P* values of morphine-6-β-D-glucuronide (M6G) and morphine-3-β-D-glucuronide (M3G) and a range of structurally-related opiates (morphine, normorphine, codeine, norcodeine, 6-acetylmorphine, diacetylmorphine, and buprenorphine) were accurately measured using a potentiometric approach. The measured lipophilicity profiles (pH 2−11, 0.15 M KCl matrix) of M3G and M6G were compared using a proton donor solvent (chloroform) and a proton acceptor solvent (propylene glycol dipelargonate, PGDP), in addition to octanol. The log *P* values and lipophilicity profiles of M6G and M3G determined in octanol−water have confirmed the unexpectedly high lipophilicity of the two glucuronides. These results show the importance of measuring the effect of pH on lipophilicity, since log *D* (pH 7.4) values gave a notably different order of lipophilicity for the opiates compared with log P. M6G, but not M3G, showed significant differences in log *P* between different types of partitioning solvents. The observed order of lipophilicities (log *D*, pH 7.4) was buprenorphine (3.93), diacetylmorphine (0.85), 6-acetylmorphine (0.61), codeine (0.22), morphine (−0.07), M6G (−0.79), M3G (−1.12), norcodeine (−1.26), and normorphine (−1.56).

Introduction

Morphine (**1**) is the analgesic of choice for the control of pain in cancer patients.[1] After oral administration of morphine to adults, the two major metabolites, morphine-3-β-D-glucuronide (**2**, M3G) and morphine-6-β-D-glucuronide (**3**, M6G), attain plasma concentrations exceeding that of the parent drug by significant factors.[2] Also, in long-term morphine treatment, normorphine (**4**) has been reported as a minor metabolite in some patients.[2] Before the 1970s, M3G and M6G were thought to be pharmacologically inactive; however, both M3G and M6G have unexpectedly low clearances and long plasma half-lives.[2] M6G was also observed to be more active as an analgesic than morphine,[3,4] and receptor binding studies[5−8] have indicated that M6G binds to the opioid receptors in the brain and is 50−200 times more potent than morphine when injected directly into the cerebroventricular fluid. M3G, on the other hand, has no intrinsic analgesic activity, but does act as an antagonist at the opioid μ receptor.[2]

In order for drugs to act on the central nervous system (CNS), they must cross the blood−brain barrier. Moderately lipophilic compounds (e.g., diacetylmorphine, nicotine) and small uncharged molecules (e.g., H_2O, O_2, CO_2) can cross the membranes by passive diffusion. On the other hand, ions or otherwise hydrophilic molecules are generally not able to do so without the intervention of an active transport process. However, it has been shown that the zwitterions M3G and M6G can cross the blood−brain barrier.[9−11] Two potential mechanisms have been identified to explain the transport of these polar compounds across the blood−brain barrier. The endothelial wall membranes have several active transport systems, and Polt *et al.*[12] have shown that a number of hexa- and heptapeptides, which ordinarily do not cross the barrier, can penetrate the blood−brain barrier when administered as the hydrophilic β-D-glucoside conjugates. The glucose transporter GLUT-1 is thought to be involved in the active transport of these glycopeptides. It may be postulated that a similar mechanism

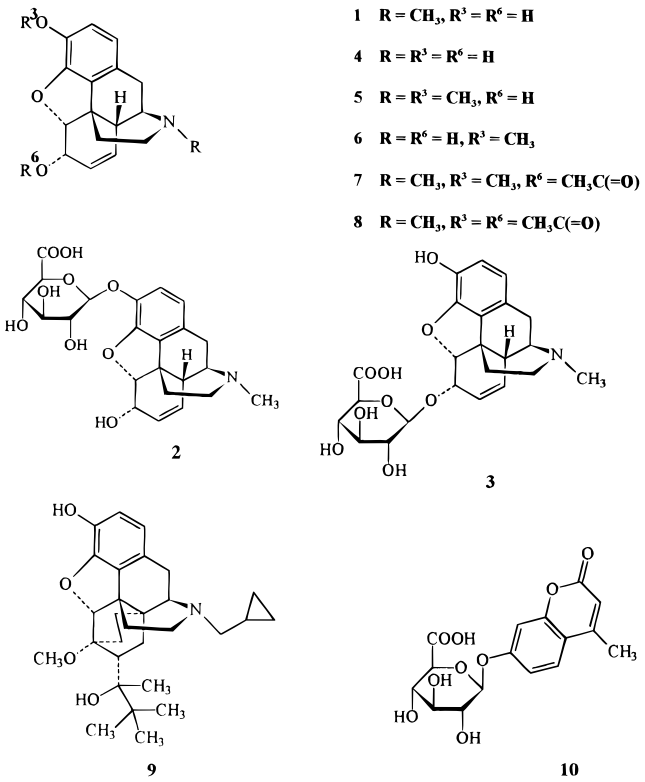

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Table 1. Ionization Constants of Morphine Derivatives (25 °C)[a]

compound	phenolic pK_a	amine pK_a	sugar COOH pK_a	no. of titrations	GOF
morphine	9.26	8.18	none	3	0.5
	9.26[b]	8.17[b]		6	0.8
	9.46[c]	8.13[c]		4	0.8
morphine-6β-D-glucuronide	9.42	8.22	2.77	8	1.0
morphine-3β-D-glucuronide	blocked	8.21	2.86	9	1.1
4-Me-umbelliferyl β-D-glucuronide	none	none	2.82	3	1.2
6-acetylmorphine	9.55	8.19	none	4	0.9
diacetylmorphine (heroin)	blocked	7.95	none	5	0.8
buprenorphine	9.62[d]	8.31[e]	none	8[d]	1.3[d]
				10[e]	2.4[e]
codeine	blocked	8.22	none	3	0.6
norcodeine	blocked	9.23	none	4	0.8
normorphine	8.66	9.80	none	3	0.9

[a] Unless otherwise noted, the ionic strength adjuster is 0.15 M KCl and the estimated standard deviations (esd) of the pK_as, derived from least-squares refinement, are 0.01. [b] 0.15 M NaCl. [c] 0.001 M NaCl. [d] From Yasuda−Shedlovsky *weighted* linear extrapolation, r^2 0.6621, esd = 0.16, slope +166 (acid). [e] Yasuda−Shedlovsky, r^2 0.9235, esd = 0.15, slope −227 (base).

may be involved in the transport of the two morphine glucuronides, but no experimental evidence for this has been reported.

Higher than expected lipophilicities have been observed previously for the zwitterionic *O*-sulfate conjugates of tiaramide and propranolol by Manners *et al.*[13] and were explained by the molecular distance separating the charged groups. A related hypothesis has been proposed by Testa and co-workers[14,15] to explain the enhanced lipophilicity of M6G and M3G. It was shown by conformational energy minimization calculations that both M6G and M3G can exist in stable "extended" and "folded" conformers, with intramolecular hydrogen bonds between the sugar COOH group and either the 3-phenolic OH or the 6-alcoholic OH groups stabilizing the folded form. The latter form was calculated to be more lipophilic than the extended form. Solvents with strong donor or acceptor H-bond properties would favor the hydrophilic extended conformations, whereas nonpolar solvents (e.g., alkanes) would indicate elevated lipophilicity due to intramolecular H-bond formation. According to this hypothesis, M6G and M3G may act as molecular "chameleons", with an increased apparent lipophilicity when in a more lipid-like environment. Direct experimental verification for this view has been difficult to obtain. Murphey and Olsen[16] have applied the standard shake-flask method (2:1 octanol:phosphate buffer) and roughly estimated the apparent partition coefficient at pH 7.4 to be −2.4 for both M3G and M6G. By partition HPLC, Carrupt *et al.*[14] reported isocratic capacity factors indicating a well-separated lipophilicity order: M > M6G > M3G, but with an unexpectedly high HPLC lipophilicity index for M6G and M3G.

In the present study we have measured, with high precision, the log *P* values of M6G and M3G in three partition solvents of varying types of hydrogen-bonding properties: octanol (H-bond donor and acceptor), chloroform (mainly H-bond donor), and propylene glycol dipelargonate (PGDP, H-bond acceptor). In addition, we have characterized the octanol−water partitioning of morphine (**1**), normorphine (**4**), codeine (**5**), norcodeine (**6**), 6-acetylmorphine (**7**), diacetylmorphine (**8**, heroin), and buprenorphine (**9**), a series of opiates representing a 6 order of magnitude span in log *P*. We have also characterized (4-methylumbelliferyl)-β-D-glucuronic acid (**10**). The principal aim of our study was to provide high-precision parameters for testing various hypotheses regarding the membrane transport properties of the morphine-based opiates.

Results

Ionization Constants. Table 1 summarizes the pK_a results. The average GOF ("goodness-of-fit") factor, 0.9, is very close to the statistically expected value of 1.0, indicating excellent overall pK_a refinements for each of the studied compounds. Figure 1a (supporting information) shows the Bjerrum difference plots for morphine (0.15 M NaCl) based on data from six different titrations. The composite plot indicates excellent precision, as judged by the very small scatter of points from different titrations. Since the two pK_as are overlapping, distinct proton dissociation steps in the Bjerrum plot are not visually resolved.

Speciation Plots. Figure 2 (supporting information) shows the aqueous (0.15 M KCl) distributions of species as a function of pH for the morphine derivatives. There are three characteristic classes of plot: (1) *bases*, as in parts f and g of Figure 2, where a cation becomes a neutral species with increasing pH; (2) *ampholytes*, as in Figure 2a,d,e,h, where a cation converts to an anion with increasing pH, *via* a neutral species (zwitterion or uncharged) whose concentration is not greater than about 65−70% of the total drug, maximizing in the pH 8.7−9.3 region; and (3) *acidic glucuronides*, as in parts b and c of Figure 2, characterized by the predominance of a zwitterionic species in the broad pH 3−8 region (>95% of drug in this form in the pH 4−7 region). The lipophilicities of the opiates reflect the changes in the distribution of these charged species with pH.

Yasuda−Shedlovsky Analysis. Figure 3 (supporting information) shows the two Yasuda−Shedlovsky plots[17] for buprenorphine. Ten ethanol−water titrations were performed in an effort to overcome the imprecision of individual p$_s K_a$s (apparent pK_as in co-solvent solutions) determined for this very poorly water soluble molecule. The errors in the extrapolated pK_as are considerably greater than the errors in the ionization constants of water soluble drugs, but remain relatively small in absolute terms.

Partition Coefficients. Table 2 lists the log *P* values of the substances studied. Also listed are the logarithms of the distribution coefficients at pH 7.4 (log $D_{7.4}$). Figure 1b shows the Bjerrum difference plots for morphine based on octanol−water (0.15 M NaCl) titra-

Table 2. Lipid−Water Partition Coefficients of Morphine Derivatives (25 °C)[a]

compound	log $D_{7.4}$	log P			no. of titrations	GOF
		cation	zwitterion or uncharged	anion		
buprenorphine	+3.93	+0.45 ± 0.16	+4.98 ± 0.12	+3.24 ± 0.13	4	2.5
diacetylmorphine	+0.85	−0.94 ± 0.05	+1.58 ± 0.01	none	4	1.9
6-acetylmorphine	+0.61	<−2	+1.55 ± 0.01	−0.42 ± 0.04	4	1.5
codeine	+0.22	<−2	+1.19 ± 0.01	none	2	2.2
morphine	−0.07	−2.1 ± 0.8	+0.89 ± 0.01	<−2	3	2.5
	−0.02[b]	<−2	+0.90 ± 0.01[b]	<−2	6[b]	1.7[b]
	−0.08[c]	<−2	+0.81 ± 0.01[c]	−1.5 ± 0.3[c]	4[c]	2.0[c]
morphine-6β-D-glucuronide	−0.79	<−2	−0.76 ± 0.04	−1.21 ± 0.05	4	1.7
	−0.22[d]	−0.15 ± 0.05	−0.19 ± 0.03[d]	−0.53 ± 0.04[d]	3[d]	2.8[d]
	+0.03[e]	<−2	−0.04 ± 0.1[e]	−0.33 ± 0.08[e]	2[e]	1.9[e]
				+0.37 ± 0.08[e,f]		
morphine-3β-D-glucuronide	−1.12	<−2	−1.10 ± 0.07	−1.45 ± 0.12	5	1.9
	−1.18[d]	−1.12 ± 0.03	−1.12 ± 0.03[d]	<−2	3[d]	0.7[d]
	−1.15[e]	−1.42 ± 0.13	−1.10 ± 0.04[e]	<−2	3[e]	0.7[e]
norcodeine	−1.26	<−2	+0.69 ± 0.01	none	6	2.4
normorphine	−1.56	<−2	−0.17 ± 0.01	<−2	6	1.5
4-Me-umbelliferyl β-D-glucuronide	−1.32	none	−0.39 ± 0.1	−1.3 ± 0.5	3	1.2

[a] Unless otherwise stated, the ionic strength adjuster is 0.15 M KCl, and the partition coefficients refer to the octanol−water medium. The log *P* values for the zwitterions are *macroscopic* constants (as are all constants derived by the pH-metric technique), which means that they refer to the partitioning of the *sum* of the concentrations of the unionized and the zwitterion species.[26] log *P* values are presented with the estimated standard deviation. [b] 0.15 M NaCl. [c] 0.001 M NaCl. [d] Chloroform−water medium. [e] PGDP−water medium. [f] PGDP−water log *P* of $M6G^{2-}$.

tions, with six different ratios of octanol to water. Such plots show the average state of sample protonation, $\bar{n}_H$, as a function of pH. The morphine curves contain an isohydric point at pH 8.7, where there is no dependence of $\bar{n}_H$ on the octanol−water volume ratio, a pattern consistent with the partitioning of the *monoprotonated* species of a diprotic substance.[18] All six curves in Figure 1b indicate higher apparent pK_{a2} (p$_o K_{a2}$) values (pH at $^1/_2 - \bar{n}_H$), compared to the true pK_{a2}, as is expected for a weak acid (phenolic group) titrated in the presence of octanol, and lower apparent pK_{a1} (p$_o K_{a1}$) values (pH at $^3/_2 - \bar{n}_H$), compared to the true pK_{a1}, as is expected for a weak base (amine group).[18] The curve corresponding to the lowest octanol−water ratio (1:20) is only imperceptibly shifted and looks like those in Figure 1a. As more octanol is added, the curves shift more. The highest octanol−water ratio depicted in Figure 1b is 15:5.

Lipophilicity Profiles. Figures 4 (supporting information) shows the M6G lipophilicity profiles in octanol−, chloroform−, and PGDP−water media. Each solvent system has a characteristically different effect on the partitioning behavior of M6G as a function of pH. Figure 5 (supporting information) shows the M3G lipophilicity profiles in the octanol, chloroform, and PGDP systems. Although the different solvents affect M3G ion pair partitioning in different ways (pH > 7 and pH < 4), no effect was observed on the zwitterion partitioning, in contrast to that seen with M6G.

Figure 6 shows the octanol−water lipophilicity profiles of the opiates studied. The ampholytes have characteristic curves with a maximum point corresponding to the partitioning of monoprotonated species at pH 8.7 for morphine, pH 8.9 for 6-acetylmorphine, pH 9.0 for buprenorphine, and pH 9.2 for normorphine. It should be noted that the log *D* value at the maximum point in the lipophilicity curve does not correspond to the log *P* value of the neutral species. At the peak maximum, log *D* is somewhat less than log *P*. This is an effect resulting from the close overlap of the two aqueous pK_as.

The monoprotic bases (diacetylmorphine, codeine, and norcodeine) show characteristic lipophilicity curves

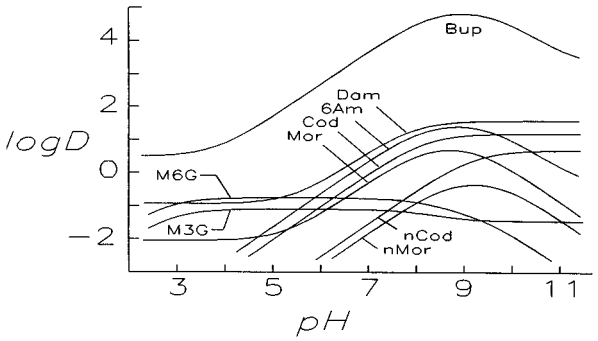

Figure 6. Calculated lipophilicity profiles for morphine and related opiates in octanol−water solutions (0.15 M KCl).

where the region pH > pK_a is that of maximum partitioning. The lipophilicity profiles of the glucuronides are significantly different in their shape from those of the other opiates studied. The curves display a broad region of maximum lipophilicity over the 3−9 pH range. The M6G titrations where 85 mL of octanol and 5 mL of 0.15 M KCl were used revealed a titratable group in the pH 6 region in the Bjerrum difference plot which appeared to arise from a minor impurity in the M6G sample, morphine-6-(Δ^4-dehydroglucuronide),[19] and was accounted for in the titrimetric analysis.

From the lipophilicity profiles of the opiates (Figure 6), it can be seen that the order of lipophilicities at pH 7.4 is buprenorphine > diacetylmorphine > 6-acetylmorphine > codeine > morphine > M6G > M3G > norcodeine > normorphine. However, below pH 5 the order of lipophilicities changes dramatically, with M6G being the second-most lipophilic molecule of the series, as can be seen in Figure 6. For pH > 8, the M6G and M3G become the least lipophilic molecules of the series of opiates.

Discussion

The molecular chameleon hypothesis[14,15] is based on the possible existence of a "folded" conformer (of increased lipophilicity) of M6G and M3G effected by an intramolecular hydrogen bond between the carboxylic acid group and the phenolic OH group in the 3-position

in M6G and the alcoholic OH group in the 6-position in M3G. The high-precision pK_a determinations in the present study (Table 1) give some support to the above hypothesis. However, X-ray diffraction studies of M3G gave no evidence for intramolecular hydrogen bonds between the carboxylic moiety of the glucuronic acid and the alcoholic OH group in the 6-position in M3G, but did indicate the presence of strong intermolecular hydrogen bonds between these groups.[20] The M3G and M6G amine pK_as are virtually identical and are about 0.04 log units higher than that of morphine. 6-Acetylmorphine, which lacks the anionic carboxylate group, has the same amine pK_a value as that of morphine. These observations are consistent with a slight electrostatic interaction between the amine group and the anionic carboxylate group from the conjugated sugar. However, codeine, which does not have a carboxylic group, also has a slightly elevated amine pK_a, which does not support the long-range charge interaction. This may be an indication that morphine may have a slight zwitterionic character in its monoprotonated form.

The phenolic pK_a in 6-acetylmorphine (9.55) is 0.29 log units higher than that in morphine (9.26). This may be due to a hydrogen bond formed between the phenolic proton and the carbonyl oxygen of the acetyl group. In M6G, the phenolic pK_a (9.42) is 0.16 higher than the value in morphine. This may be due to a hydrogen bond of the sort postulated previously.[14,15] It should be noted that the carboxylic pK_a in M6G (2.77) is lower than that of M3G (2.86) and 4-methylumbelliferyl glucuronide (2.82). Such an effect, albeit small, is consistent with the presence of a hydrogen bond. The pK_a of the glucuronide carboxylate group in M3G agrees well with the value reported by Carrupt *et al.*[14] (2.83 ± 0.05).

Diacetylmorphine possesses the least basic amine functionality of the drugs studied, with its pK_a 0.23 log unit lower than that of morphine. The demethylation of the amine group in codeine to produce norcodeine is accompanied by an increased amine pK_a, by 1 log unit. Of the two pK_as in normorphine, it seems reasonable to assign the higher one to the amine group, given the codeine−norcodeine comparison. If this is so, then the phenolic pK_a (8.66) is one of the most acidic of the studied compounds. This can be explained by normorphine being a zwitterion to some extent.

The "relative hydrophilicity", RH, index proposed by Murphey and Olsen[16] can be related to the ratio of the pH 7.4 distribution coefficients ($D_{7.4}$) of morphine to that of another drug. The isocratic capacity factors reported by Carrupt *et al.*[14] can be used to calculate the RH of M6G as 4.5 and of M3G as 13.8 at pH 7. From our data, the RH indices for M6G and M3G are 5.2 and 11.2, respectively. Furthermore, our data indicate M6G RH indices of 1.4 and 0.8 in the partition solvents chloroform and PGDP, respectively; for M3G the two indices are 12.9 and 12.0. Murphey and Olsen[16] characterized the concentrations of morphine, M6G, and M3G in blood and in brain tissue of guinea pigs shortly after subcutaneous injections of the drugs. The blood−brain RH indices for M6G and M3G were found to be 5.9 and 10.0.

Using the MLP method, Gaillard *et al.*[14] calculated the folded-conformer log *P* of M6G and M3G (in the forms where the carboxylic groups are un-ionized) as −1.3 and −1.8, respectively. The distribution coefficients determined by potentiometry at pH 2 for M6G and M3G are −1.7 and −2.1, respectively, in reasonably good agreement with the MLP results. On the basis of the comparisons of the relative hydrophilicity indices, our results are in agreement with those of Carrupt *et al.*[14] and with the *in vivo* measurements of Murphey and Olsen.[16] The advantage of the pH-metric technique is that direct values of the partition coefficients are determined (rather than ratios), along with the lipophilicity profile over an extensive pH range. We have confirmed that both M3G and M6G have higher lipophilicities than would be expected for polar glucuronide conjugates. We have demonstrated that M6G shows differential interactions with partition solvents which possess different types of hydrogen-bonding properties, whereas M3G does not show such effects. This offers partial support for the molecular chameleon hypothesis proposed by Carrupt *et al.*[14] to explain the unexpected partition behavior of these morphine metabolites.

Our data, confirming the unexpectedly high lipophilicity of the morphine glucuronides, provides one possible explanation for the *in vivo* pharmacological activity and pharmacokinetic behavior of these relatively polar metabolites. These observations may have implications for other polar metabolites which are known to be biologically active.

Experimental Procedures

Reagents. The preparations of standard HCl and KOH (Volucon, Rhône Poulenc) are described elsewhere.[18] Water-saturated partition-coefficient grade octan-1-ol (Fisons), and water-saturated HPLC-grade chloroform (Fisons) were used. Propylene glycol dipelargonate, a gift from Rhône-Poulenc Rorer, U.K., was purified[21] by repeated extractions with sodium carbonate solutions, followed by washes with distilled water. Morphine hydrochloride was purchased from May and Baker (Dagenham, UK), morphine-3β-D-glucuronide (crystalline), normorphine hydrochloride monohydrate, codeine phosphate hemihydrate, and norcodeine hydrochloride trihydrate were purchased from Sigma, and were used as received. Morphine-6β-D-glucuronide (M6G) was purchased from UltraFine Chemicals (Salford, U.K.) and was used without further purification. Buprenorphine hydrochloride was purchased from McFarlane Smith (Edinburgh, U.K.).

Apparatus. The computerized titration instrument (Sirius PCA101) used to perform the pK_a and log *P* assays (25.0 ± 0.1 °C, under argon) has been previously described in detail.[17,18,22−26] A Ross-type pH electrode (Orion 8103SC) was used; the operational pH scale was converted to the concentration scale using a four-parameter equation.[17,24]

Potentiometric Titrations. Nearly 120, mostly alkalimetric, sample (0.2−4.5 mM, in 0.15 M KCl) titrations were performed. Buprenorphine was not sufficiently water soluble for normal aqueous titrations. Thus its aqueous pK_as were determined from 25−54 wt % ethanol−water solutions (also 0.15 M in KCl) of the sample (0.08−0.25 mM) using the Yasuda−Shedlovsky procedure.[17] All octanol−water volume ratios were optimized: high ratios (up to 85:5) were used for hydrophilic drugs (morphine metabolites) and low ratios (down to 0.2:20) were used for lipophilic substances. Low buprenorphine concentrations were used (0.08 mM) in dual-phase titrations with low octanol−water volume ratios (0.2/20 and 0.5/20) to avoid possible precipitation at low pH. Care was exercised in not exposing the drug samples to extreme pH conditions during the assays. Diacetylmorphine very quickly hydrolyzes to morphine and acetic acid if the drug is exposed to pH 12 for about 10 min, confirming earlier observations.[27] The weighting scheme, iterative least squares refinement and "goodness-of-fit" (GOF) have been reported previously.[22] The GOF function has the statistical expectation value of unity, given a proper weighting scheme and a valid equilibrium model.

pK_a and log *P* Determination. The ionization constants (aqueous and semiaqueous) were estimated from the Bjerrum difference plots by the $^1/_2 - \bar{n}_H$ method.[18] These were then refined by a nonlinear multititration-set least-squares procedure.[22] The partition parameter, log *P*, where $P = [\text{species}]_{\text{lipid}}/[\text{species}]_{\text{water}}$, was determined from the difference between the aqueous pK_a of the species and the *apparent* pK_a, p$_o K_a$, estimated from a titration in the presence of a partition solvent. The log *P* constants were refined using data from four or more titrations, each with a different lipid−water volume ratio.

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Supporting Information Available: Figures 1−5 (1, Bjerrum plots; 2, calculated distribution of species as a function of pH in aqueous solutions of the opiates studied; 3, Yasuda−Shedlovsky ethanol−water plots for buprenorphine; 4, lipophilicity profiles for M6G in octanol, chloroform, and PGDP; 5, lipophilicity profiles for M3G in octanol, chloroform and PGDP) (5 pages). Ordering information is given on any current masthead page.

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