Morphine 6-Glucuronide and Morphine 3-Glucuronide as Molecular Chameleons with Unexpected Lipophilicity

Pierre-Alain Carrupt,† Bernard Testa,*,† Antoine Bechalany,† Nabil El Tayar,† Patrick Descas,‡ and Daniel Perrissoud‡

Institut de Chimie Thérapeutique, Ecole de Pharmacie, Université de Lausanne, BEP, CH-1015 Lausanne, Switzerland, and Laboratoires Sarget, F-33701 Mérignac, France. *Received July 23, 1990*

Morphine 6-glucuronide, but not morphine 3-glucuronide, is a highly potent opiate receptor agonist. In fact, there is converging evidence that much of the analgesic effect occurring after morphine treatment in humans is due to this metabolite rather than to the parent drug. Yet glucuronides as a rule are considered as highly polar metabolites unable to cross the blood–brain barrier and rapidly excreted by the urinary and/or biliary routes. Here, we report that morphine 6-glucuronide, and to a lesser extent morphine 3-glucuronide, are far more lipophilic than predicted, and in fact not much less lipophilic than morphine itself. Force-field and quantum mechanical calculations indicate that the two glucuronides can exist in conformational equilibrium between extended and folded forms. The extended conformers, because they efficiently expose their polar groups, must be highly hydrophilic forms predominating in polar media such as water; in contrast, the folded conformers mask part of their polar groups, thus being more lipophilic and likely to predominate in media of low polarity such as biological membranes.

Morphine in animals and humans undergoes a variety of metabolic pathways, in particular glucuronidation of the 3-OH phenolic group and of the 6-OH alcoholic group to yield morphine 3-*O*-β-D-glucuronide (M3G) and morphine 6-*O*-β-D-glucuronide (M6G), respectively (see Chart I). Both reactions occur in human liver microsomes, the 3-glucuronidation predominating over 6-glucuronidation for the natural (−)-morphine, while the reverse is true for the unnatural (+)-morphine.[1] Following oral administration of morphine to humans, the M6G and M3G plasma AUC (area under the curve) exceeded that of the parent drug by factors of 9:1 and 50:1, respectively.[2] But while M3G is inactive as an μ-opiate receptor ligand, affinities have been reported for M6G that range from identical with that of morphine to 10 times smaller.[3,4] In addition, M6G appears to have enhanced affinity for δ-opiate receptors and high μ versus κ receptors selectivity as compared to morphine.[4,5]

When injected to rats intracerebroventricularly[6] or into the periaqueductal gray,[7] M6G was found to be considerably more potent as an analgesic than morphine, from 60 to 200 times in the former study. More important, M6G in rats was 1–4 times more active as an analgesic than morphine when the two compounds were administered subcutaneously,[6] due in part to its longer duration of action.[8] That M6G can penetrate into the brain despite its "high polarity" has been proven unambiguously.[9] In addition, the finding of significant amounts of M3G both in the brain and plasma of rats at 45 min after intraperitoneal injection of [^{14}C]morphine has provided indirect evidence that M3G penetrates the blood–brain barrier.[9] In patients, intravenous M6G afforded high plasma levels and marked pain relief without morphine or M3G being detectable in the plasma at any time.[10] In fact, Osborne et al.[10] go as far as suggesting that most of the analgesic effect occurring after morphine treatment is due to the 6-glucuronide metabolite rather than to morphine itself.

Within current knowledge, the far from negligible brain penetration of M3G and mainly M6G are difficult to reconcile with the expected pharmacokinetic behavior of highly polar, hydrophilic conjugates. To examine this discrepancy, we have measured the lipophilicity of morphine and its glucuronide conjugates by using reversed-phase high-performance liquid chromatography (RP-HPLC). Under applied conditions, this method yields log k_i values (k_i being isocratic capacity factors) that have been repeatedly shown to be highly correlated with log P values (P being the octanol/water partition coefficient), the ad-

Chart I

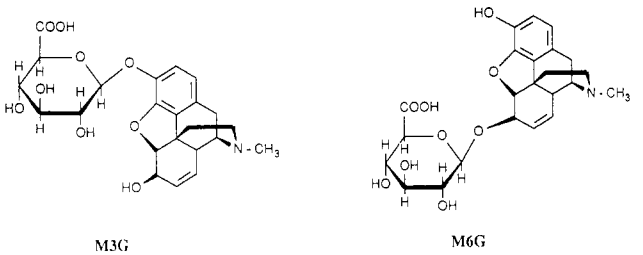

Table I. Measured or Calculated Lipophilic Indices (log k_i values) of Morphine (M), Morphine 6-Glucuronide (M6G), and Morphine 3-Glucuronide (M3G) at pH Values of 3–8

compd	log k_i values at various pH						pK_a[a]
	3	4	5	6	7	8	
M[b]	−0.17	−0.12	−0.09	0.29	0.97	1.50	
M6G[b]	−0.28	−0.34	−0.37	−0.22	0.32	0.87	3.23 ± 0.05
M3G[b]	−0.77	−0.93	−1.04	−0.79	−0.17	0.37	2.83 ± 0.05
G[c]	−2.62	−3.29	−4.23	−4.25	−4.25	−4.25	3.20 ± 0.03
MG[d]	−1.79	−2.41	−3.32	−2.96	−2.28	−1.75	

[a] pK_a values (± SD) of the carboxylic group. [b] Measured values, SD = 0.03. [c] Glucuronic acid was too polar to be measurable by the RP-HPLC method. The log k_i value of the neutral form was calculated from the octanol–water partition coefficient reported by Lüllmann et al.[31] by using a previously established[18] Collander-type conversion equation.[31] The log k_i value was then corrected for ionization at the various pH values according to the pH-partition theory.[14] [d] The expected log k_i values of morphine glucuronide (the method does not discriminate positional isomers) were calculated according to the additivity rule:[33] log k_i(MG) = log k_i(M) + log k_i(G) − f(OH$_{al}$) − f(H attached to O atom) where f is the fragmental constant of the stated functional group.[15]

* To whom correspondence should be addressed.

(1) Coughtrie, M. W.; Ask, B.; Rane, A.; Burchell, B.; Hume, R. *Biochem. Pharmacol.* **1989**, *38*, 3273.
(2) Osborne, R.; Joel, S.; Trew, D.; Slevin, M. *Clin. Pharmacol. Ther.* **1990**, *47*, 12.
(3) Christensen, C. B.; Jørgensen, L. N. *Pharmacol. Toxicol.* **1987**, *60*, 75.
(4) Oguri, K.; Yamada-Mori, I.; Shigezane, J.; Hirano, T.; Yoshimura, H. *Life Sci.* **1987**, *41*, 1457.
(5) Frances, B.; Gout, R.; Campistron, G.; Panconi, E.; Cros, J. *Prog. Clin. Biol. Res.* **1990**, *328*, 477.
(6) Abbott, F.; Palmour, R. *Life Sci.* **1988**, *43*, 1685.
(7) Pasternak, G. W.; Bodnar, R. J.; Clark, J. A.; Inturrisi, C. E. *Life Sci.* **1987**, *41*, 2845.
(8) Paul, D.; Standifer, K. M.; Inturrisi, C. E.; Pasternak, G. W. *J. Pharmacol. Exp. Ther.* **1989**, *251*, 477.

0022-2623/91/1834-1272$02.50/0

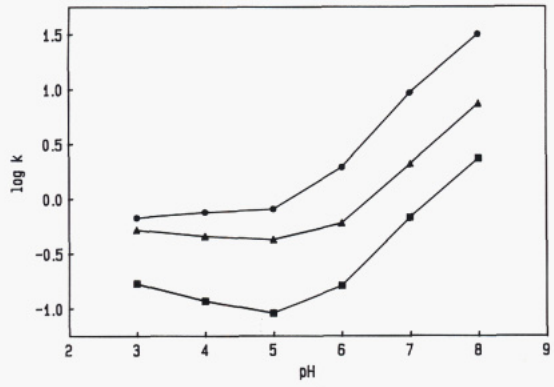

Figure 1. Chromatographic lipophilicity indices, as a function of pH, of morphine (●), morphine 6-glucuronide (▲), and morphine 3-glucuronide (■).

vantages of the RP-HPLC method being a high sensitivity and a considerably increased precision.[12-14]

Results and Discussion

Lipophilicity. The influence of pH on the lipophilicity of morphine and its two glucuronides is reported in Table I and illustrated in Figure 1. The lipophilicity index of morphine is seen to be constant between pH 3 and 5 and to increase beyond this value due to the progressive appearance of the neutral species. The two glucuronides display a comparable if somewhat more complex behavior due to the carboxylic group which is partly in the neutral form in the acidic range. The greater acidity of M3G compared to M6G (see Table I) accounts for its log k_i increasing when going from pH 5 to pH 3.

Particularly remarkable in Figure 1 is the demonstration that M3G and especially M6G are only slightly less lipophilic than morphine itself over the entire pH range investigated. At physiological pH, the decrease in lipophilicity is only 0.5 log k_i unit for M6G and 1 log k_i unit for M3G. By using the fragmental method[14,15] to calculate the lipophilicity of morphine glucuronide from the log k_i values of morphine and glucuronic acid yields values (Table I) that at physiological pH are 2.5 and 2 log k_i units smaller than measured. In fact, the two glucuronides are indeed predicted to be highly hydrophilic compounds, a prediction denied by the present experiment. At this stage, it appears that the two glucuronides display a lipophilicity compatible with their pharmacokinetic behavior, yet the reasons for their unexpectedly high lipophilicity remains unexplained. This problem was approached by a theoretical conformational study of the two glucuronides to search for intramolecular interactions.

Theoretical Conformation Study. Selected results of this study are presented in Table II. At physiological pH, the two glucuronides exist mainly as zwitterions, but the conformational calculations showed no detectable differences between zwitterions and anions, the positive and negative charges being too far removed to interact detectably. In contrast, the anionic and neutral forms displayed different conformational behaviors, and results for these two forms are reported in Table II. It can first be seen that the extended form is preferred for M3G in both neutral and anionic states, whereas for M6G it is the folded form which is preferred in both electric states. The reason for this difference is presumably due to the existence in the folded conformer of M6G of a strong hydrogen bond between the 3-phenolic group and the carboxylic/

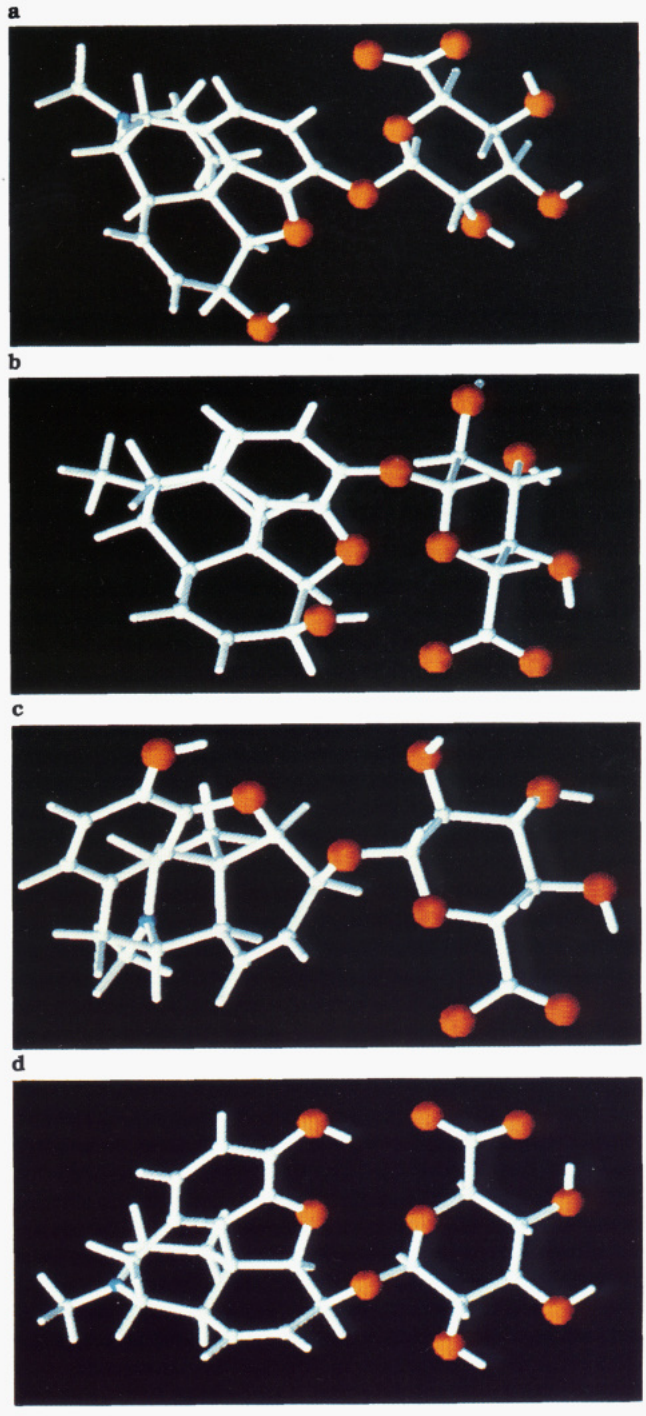

Figure 2. Molecular graphic representation of M3G and M6G (anionic forms) in their extended and folded conformers of lowest energy. These figures were generated by the QUANTA software (Polygen Corporation) on a Silicon Graphics Iris 4D/7D workstation. The parts to the figure are as follows: a, M3G in the extended conformation; b, M3G in the folded conformation; c, M6G in the extended conformation; d, M6G in the folded conformation.

(9) Yoshimura, H.; Ida, S.; Oguri, K.; Tsukamoto, H. *Biochem. Pharmacol.* **1973**, *22*, 1423.
(10) Osborne, R.; Joel, S.; Trew, D.; Slevin, M. *Lancet* **1988**, i, 828.
(11) El Tayar, N.; Van de Waterbeemd, H.; Testa, B. *J. Chromatogr.* **1985**, *320*, 305.
(12) Braumann, T. *J. Chromatogr.* **1986**, *373*, 191.
(13) Bechalany, A.; Röthlisberger, T.; El Tayar, N.; Testa, B. *J. Chromatogr.* **1989**, *473*, 115.
(14) Rekker, R. F. *The Hydrophobic Fragmental Constant. Its Derivation and Applications. A Means of Characterizing Membrane Systems*; Elsevier: Amsterdam, 1977.
(15) Rekker, R. F.; de Kort, H. M. *Eur. J. Med. Chem.* **1979**, *14*, 749.

Table II. Calculated Enthalpy of Formation of Conformers Corresponding to Local Minima on the Energy Hypersurface and Their Water-Accessible Surface Areas

conformer	ΔH_f,[a] kcal/mol	relative energy	S_{tot},[b] Å^2	S_{apolar},[b] Å^2	S_{polar},[b] Å^2	ΔS_{tot}[c]	ΔS_{polar}[d]
			Anionic Form				
3-A-E (M3G, extended)	-357.08	0.00	664.5	421.4	243.1		
3-A-F (M3G, folded)	-349.63	7.45	615.6	414.4	202.2	48.9	40.9
6-A-E (M6G, extended)	-352.14	2.35	662.3	448.4	213.9		
6-A-F (M6G, folded)	-354.49	0.00	637.4	448.9	188.5	24.9	25.4
			Neutral Form				
3-N-E (M3G, extended)	-322.80	0.00	674.9	421.0	253.9		
3-N-F (M3G, folded)	-321.66	1.14	637.0	417.1	219.9	37.9	34.0
6-N-E (M6G, extended)	-319.00	0.81	666.8	407.8	259.0		
6-N-F (M6G, folded)	-319.81	0.00	651.9	424.3	227.6	14.9	31.4

[a] Enthalpy of formation of conformers. [b] Total water-accessible surface area. [c] Difference in total surface area between extended and folded conformer. [d] Difference in polar surface area between extended and folded conformer.

carboxylate group. This internal H bond can be seen in Figure 2 part d.

For each glucuronide in both electric states, the extended and folded conformer of lowest energy are presented in Figure 2. Figure 2 parts a and c clearly shows that in the extended conformers the morphine and glucuronide moieties interact minimally by through-space interactions and that they are maximally exposed to the solvent. In sharp contrast, the folded conformers (Figure 2 parts b and d) mask a significant portion of their polar regions from the solvent. These changes are expressed in a quantitative fashion by the water-accessible surface areas (Table II); while the water-accessible surface area of the apolar regions changes very little upon folding (it even increases in some cases), the water-accessible surface area of the polar regions (in fact the ether, hydroxyl, and carboxylic groups) is shown to undergo a marked decrease.

The differences in energy between the extended and folded conformers are of the order of a few kilocalories/mole, and likely to be influenced by solvent effects not taken into account in the calculations. In fact, the calculated conformational behavior of the two glucuronides strongly suggests that they will exist predominantly as extended, polar conformers in water, and as folded, more lipophilic conformers in media of low polarity such as biological membranes. In other words, the present work suggests the possibility for morphine glucuronides to behave as *molecular chameleons*, i.e. to adapt their polarity to that of the medium. This behavior could account for the comparatively good brain penetration and slow urinary excretion of morphine glucuronides. Jiang[16] has cogently discussed the importance of self-coiling of organic molecules on their lipophilic interactions.

The pK_a values of M3G and M6G (Table I) are compatible with this interpretation. A priori, two intramolecular effects can be expected to influence the acidity of the glucuronate moiety, namely an inductive effect enhancing the acidity of M3G, and an internal carboxylate–phenol hydrogen bond enhancing the acidity of M6G. The results (Table I) show that only in M3G is the acidity enhanced, thus confirming the inductive effect in M3G but not the existence of a H bond between the carboxylate and phenolic group in M6G. In other words, the pK_a of M6G expresses the predominance in water of the extended conformer.

A larger than expected lipophilicity of a few zwitterionic sulfate conjugates is documented, e.g. tiaramide and propranolol *O*-sulfates, and is ascribed to the proximity of the opposite charges.[17,18] However, effects of proximity on lipophilicity have been shown to decrease markedly with distance,[19] and in the case of morphine glucuronides should have a negligible influence, in line with the failure of our calculations to detect any interaction between the two opposite charges. The present study documents the higher than expected lipophilicity of morphine *O*-glucuronides and suggests self-coiling as the underlying mechanism.

Experimental Section

Chemistry. All melting points (uncorrected) were determined on a Kofler melting point apparatus. Infrared (IR) spectra were recorded on a FTIR Nicolet 5DXC spectrometer (KBr tablets). UV spectra were recorded on a Unicam PU 8800 spectrometer. Optical rotations were measured at 589 nm with a AA10-Optical Activity polarimeter. ^{13}C NMR spectra were measured at 50.523 MHz on a Bruker AC 200 spectrometer. All compounds were analyzed for C, H, and N, and the analytical results were within ±0.4% of the theoretical values. Preparative HPLC was carried out on a Jobin Yvon Modulprep with refractive index detector. HPLC analyses were performed at 35 °C and a flow rate of 1 mL/min by using a modular isocratic system (Waters Model 510). A variable-wavelength UV detector (Waters Model 481) and a Bondapack C_{18} column (10 μm, 15 cm) were used. Mobile phase was prepared volumetrically from a combination of 13% acetonitrile with 87% aqueous buffer solution (0.01 M KH_2PO_4 and heptanesulfonic acid 0.005 M, pH was adjusted to 2.2 with phosphoric acid). Fast atomic bombardment (FAB) mass spectra were obtained on a Finnigan-MAT 8230 spectrometer with nitrobenzyl alcohol as matrix.

Morphine 3-Glucuronide (Made According to Ref 20 with Slight Modifications). To a solution of morphine (3.98 g, 0.014 mol), and LiOH·H_2O (0.5 g) in methanol (20 mL) was added methyl 2,3,4-tri-*O*-acetyl-1α-bromo-1-deoxy-D-glucopyranuronate[21] as a solid (4 g, 0.0125 mol). After the addition, the mixture was allowed to stand at room temperature for 0.5 h. Then a solution of LiOH·H_2O (1.4 g) in H_2O (20 mL) was added with stirring for 30 min. The mixture was concentrated to a small volume and brought to pH 8 with acetic acid. Unreacted morphine (7 g) precipitated at this point and was removed by filtration. The filtrate was concentrated to a syrup and taken up in MeOH (50 mL). The glucuronide started to crystallize spontaneously at room temperature. Crystallization was completed at −10 °C overnight. Filtration, followed by drying, afforded a solid which was crystallized again in ethanol–water leading to 1.9 g (29%) of chromatographically pure morphine 3-glucuronide: mp 240 °C dec; HPLC t_R = 4.96 min (100%); IR 1603 cm^{-1}; UV (H_2O) λ_{max} 276–282 nm, ϵ = 1533, (NaOH 0.1 N) λ_{max} 276–282 nm, ϵ = 1505; $(\alpha)^{29}_D$ = −142.6° (c = 0.55, H_2O); ^{13}C NMR (DMSO-d_6) δ 171.26, 147.01, 138.35, 133.84, 130.80, 127.15, 118.59, 116.28, 99.83, 91.50,

(16) Jiang, X.-K. *Acc. Chem. Res.* **1988**, *21*, 362.
(17) Manners, C. N.; Payling, D. W.; Smith, D. A. *Xenobiotica* **1988**, *18*, 331.
(18) Manners, C. N.; Payling, D. W.; Smith, D. A. *Xenobiotica* **1989**, *19*, 1387.
(19) Abraham, D. J.; Leo, A. J. *Proteins Struct. Funct. Gen.* **1987**, *2*, 130.
(20) Berrang, B.; Twine, C. E.; Hennessee, G. L.; Caroll, F. *Synth. Commun.* **1975**, *5*, 231.
(21) Bollenback, G. N.; Long J. W.; Benjamin D. G.; Lindquist J. A. *J. Am. Chem. Soc.* **1955**, *77*, 3310.

75.94, 74.91, 72.97, 71.50, 66.00, 58.16, 45.50, 42.20, 41.44, 38.98, 33.62, 21.13 ppm/TMSD. FABMS 462 $(M + H)^+$. Anal. ($C_{23}H_{27}NO_9 \cdot 2.5H_2O$) C, H, N.

Morphine 6-Glucuronide (Made According to Reference 22 with Slight Modifications). To a solution of sodium carbonate (30 g) in 300 mL of water, 3 g of morphine were added. Dropwise addition of acetic anhydride (45 mL) was achieved in three parts with a mechanically stirrer. The reaction mixture was stirred for 20 min at room temperature and then ice was added. The reaction mixture was extracted with chloroform. The combined organic phases were dried over Na_2SO_4 and then filtered. The filtrates were concentrated in vacuo to afford 3 g of 3-acetylmorphine.

In a flask protected from moisture, 3-acetylmorphine (2.5 g, 0.0076 mol) dissolved in 250 mL of dried benzene was condensed in presence of 7 g of dry $AgCO_3$ with methyl 3,4,5-tri-*O*-acetyl-lα-bromo-1-deoxy-D-glucopyranuronate[21] added in several portions (4.5 g, 0.014 mol) and stirred for 10 h and the mixture heated to reflux. In the meantime, benzene was gradually distilled off. Stirring was continued for 11 h then the reaction mixture was filtered. The filtrate was concentrated in vacuo to 100 mL then extracted with ice-cooled HCl 0.5% (100 mL three times). Combined extracts were adjusted to pH 8.0 with $NaHCO_3$ and extracted with 200 mL of chloroform three times. The $CHCl_3$ layer was dried over $NaSO_4$ and evaporated to dryness in vacuo. The resulting viscous residue was chromatographed on silica gel column eluted with chloroform and methanol. Recrystallization in methanol afforded 2.85 g of a solid.

To a suspension of this solid (1.5 g, 0.002 mol) in 30 mL of absolute MeOH was added 11 mL of 1% NaOMe. After allowing to stand overnight the reaction mixture was evaporated to dryness in vacuo. The residue was dissolved in 11 mL of 0.43 N $Ba(OH)_2$ and left aside for 1 h. Then the pH was adjusted to 6.0 with 2 N oxalic acid and the barium salt which precipitated was filtered off. The filtrate was evaporated to dryness in vacuo, and the residue was recrystallized from H_2O–EtOH to a crude product which was chromatographed on a column Polygosyl C8 25–40 μm eluted with H_2O–MeOH (95/5 v/v). Combined fractions of pure product were evapored in vacuo to afford 1.65 g (38%) of chromatographically pure morphine 6-glucuronide: mp 253–255 °C dec; HPLC t_R = 6.16 min (100%); IR 1603 cm^{-1}; UV (H_2O) λ_{max} 281–284 nm, ϵ = 1474, (NaOH 0.1 N) λ_{max} 294–296 nm, ϵ = 2684; $(\alpha)^{29}_D$ = −266° (c = 0.56, H_2O); ^{13}C NMR (D_2O) δ 178.44, 148.42, 140.86, 134.28, 131.90, 128.87, 126.05, 123.30, 120.59, 104.39, 91.08, 79.13, 78.36, 75.96, 75.74, 74.62, 63.34, 60.26 (EtOH), 49.70, 44.55, 43.55, 41.02, 34.99, 24.08, 19.65 (EtOH) ppm/sodium 3-(trimethylsilyl)propionate-d_4; FABMS 462 $(M + H)^+$. Anal. ($C_{23}H_{27}NO_9 \cdot 2H_2O$, 0.7 EtOH) C, H, N.

Physicochemical Measurements. The log k_i values were measured by RP-HPLC with use of previously described equipment and protocol[13] with the following modifications. The lipophilic stationary phase was an octadecyl copolymer gel (ODP column 15 cm × 6 mm i.d., particle size 5 μm, Asahi Chemicals, Kawasaki, Japan). The mobile phases were prepared volumetrically from a combination of 10% methanol and 90% aqueous phosphate buffers 0.01 M.[23] All solutions were purified by filtration by using a Milli-Q system (Millipore). All measurements were performed at 37 ± 1 °C by thermostating the column and the eluent reservoir. The flow rate (1.00 mL/min) was strictly controlled by using a digital flowmeter (Phase Separation Ltd., Queensferry, GB). The logarithm of isocratic capacity factors, log k_i, is defined as log k_i = log $(t_r - t_0)/t_0$, where t_r is the retention time of the solute and t_0 is the column dead time determined by using methanol as the nonretained compound.

The pK_a values of the carboxylic group were measured by titrimetry in distilled, degassed water of ionic strength 0.1, as previously described.[24]

Theoretical Conformational Studies. Local minima were searched on the energy hypersurface by the following procedure. First, electron distributions were calculated by the semiempirical MNDO procedure,[25,26] and the conformational space explored twice by using the SYBYL force field[27] and the DISCOVER force field.[28] This exploration led to the identification of a small number of local minima corresponding to extended and folded conformers. These were selected for full geometry optimization by using the AM1 semiempirical method (MOPAC 4.0, QCPE 453).[29] The calculations were run on the following computers: VAX 8550 (SYBYL), Silicon Graphics workstation (INSIGHT or DISCOVER), Cray-XMP (MOPAC 4.0).

The surface areas accessible to a molecule of water (taken as a sphere of radius 1.5 Å) were calculated by the MOLSV software (QCPE 509) (working on a SUN 386i workstation) by using published van der Waals radii.[30] Table II reports the water-accessible surface area of the total molecule, of its apolar regions, and of its polar regions (restricted here to the hydroxyl, ether, and carboxylate groups).

Acknowledgment. B.T., P.A.C., N.E.T., and A.B. are indebted to the Swiss National Science Foundation for research grants No. 3.508–0.86 and No. 31–27531.89. Prof. G. Chapuis and Prof. M. Mutter kindly allowed access to a Silicon Graphics Iris workstation. ^{13}C NMR spectra were provided by Cesamo (Bordeaux), elemental analyses by Laboratories Wolf (Clichy), and FAB Mass Spectra by Asta Pharma (Frankfurt).

(22) Yoshimura, H.; Oguri, K.; Tsukamoto, H. *Chem. Pharm. Bull.* **1968**, *16*, 2114.
(23) Elving, P. J.; Markowitz, J. M.; Rosenthal, I. *Anal. Chem.* **1956**, *28*, 1179.
(24) Walther, B.; Carrupt, P.-A.; El Tayar, N.; Testa, B. *Helv. Chim. Acta* **1989**, *72*, 507.
(25) Dewar, M. J. S.; Thiel, W. *J. Am. Chem. Soc.* **1977**, *99*, 4899.
(26) Dewar, M. J. S.; Thiel, W. *J. Am. Chem. Soc.* **1977**, *99*, 4907.
(27) Clark, M.; Cramer, R. D., III; Van Opdenbosch, N. *J. Comput. Chem.* **1989**, *10*, 982.
(28) Mackay, D. H. J.; Berens, P. H.; Wilson, K. R.; Hagler, A. T. *Biophys. J.* **1984**, *46*, 229.
(29) Dewar, M. J. S.; Zoebisch, E. G.; Healy, E. F.; Stewart, J. P. P. *J. Am. Chem. Soc.* **1985**, *107*, 3902.
(30) Gavezzotti, A. *J. Am. Chem. Soc.* **1983**, *105*, 5220.
(31) Lüllmann, H.; Timmermans, P. B. M. W. M.; Ziegler, A. *Eur. J. Pharmacol.* **1979**, *60*, 277.
(32) Collander, R. *Acta Chem. Scand.* **1951**, *5*, 774.
(33) Fujita, T.; Iwasa, J.; Hansch, C. *J. Am. Chem. Soc.* **1964**, *86*, 5175.