Pergamon

Bioorganic & Medicinal Chemistry Letters 11 (2001) 1455–1459

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

Hydrophobic Forms of Morphine-6-glucosides

Stephan Schwarzinger,[a,†] Michael Hartmann,[b,‡] Peter Kremminger[b,§] and Norbert Müller[a,*]

[a]*Institut f. Chemie, Johannes Kepler Universität Linz, A-4040 Linz, Austria*
[b]*Hafslund Nycomed Pharma, A-4020 Linz, Austria*

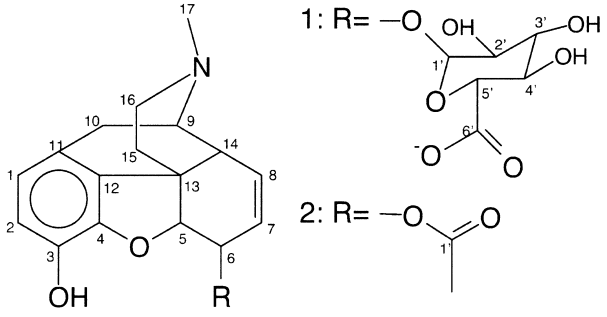
Received 4 August 2000; revised 6 March 2001; accepted 9 April 2001

Abstract—NMR spectroscopy of 6-acetylmorphine (6-AM), a chloroform-soluble model compound for the hydrophilic, highly potent analgesic drug morphine-6-glucoronide (M6G), in a hydrophobic solvent indicates one hydrogen bonded water molecule per molecule of 6-AM. By analysis of nuclear Overhauser enhancements (NOEs) we find a 6-AM dimer in which the monomers are linked by two water molecules. Molecular modeling studies underscore the stability of such dimeric structures involving water molecules for 6-AM and point out their more lipophilic character allowing penetration of the blood–brain barrier.

The action of many drugs depends on their ability to penetrate the blood–brain barrier (BBB). Morphine-6-*O*-β-D-glucuronide (M6G) **1** is a natural metabolite of morphine in humans and animals.[1–3] In apparent contradiction to its polar properties, M6G, which is a more potent analgesic drug than morphine itself,[4–6] is able to penetrate the BBB, although to a lesser extent than morphine.[7–12] Two possible mechanisms for this phenomenon have been proposed: the first possibility is active transport across the BBB involving a glucose transporter similiar to what has been shown for glycopeptide enkephalin analogues.[13] There is also evidence that the P-glycoprotein (Pgp) modulates the penetration of M6G into the brain.[14–16] The second hypothesis based on partitioning experiments and computer simulations assumes that M6G molecules act like 'molecular chameleons' by adopting a different conformation of lower polarity when passing the BBB.[17–19] In order to find more evidence for this hypothesis on a structural basis, we have investigated the structure of morphine-6-glucosides in lipophilic environments both experimentally by NMR spectroscopy and theoretically by molecular modeling.

Since M6G (**1**) is not sufficiently soluble in hydrophobic solvents for extended NMR experiments we used the glycoside 6-acetylmorphine (6-AM) **2**, which readily dissolves in $CDCl_3$ (Scheme 1).

6-AM, the main metabolite of heroin, appears to act in a similar way as M6G.[20] For the passage through a lipophilic barrier only a small fraction of the molecules need to adopt a lipophilic conformation. The concentration gradient across the barrier acts as the driving force and the hydrophobic conformation can be seen as an intermediate state in a kinetic transport model. The conformational energy was mapped as a function of the glycosidic dihedral angles ϕ (C7–C6–O–C1′) and ψ (C6–O–C1′–C2′). The conformational hypersurfaces of 6-AM and M6G are very similar with respect to these dihedral angle variables. For both compounds one finds two main minima corresponding to the extended



Scheme 1.

*Corresponding author. Tel.: +43-732-2468-8746; fax: +43-732-2468-8747; e-mail: norbert.mueller@jk.uni-linz.ac.at
†Present address: Universität Bayreuth Lehrstuhl Biopolymere, Universitätsstraße 30/BGI, D-95440 Bayreuth, Germany.
‡Present address: DSM St-Peter-Straße 25, A-4021 Linz, Austria.
§Present address: Biochemie GmbH, Biochemiestraße 10, A-6250 Kundl, Austria.

0960-894X/01/$ - see front matter
PII: S0960-894X(01)00255-4

($\phi = 157°$, $\psi = 180°$) and the folded ($\phi = -48°$, $\psi = -171°$) conformers of M6G, which have been described earlier by Carrupt et al.[17]

^{1}H NMR spectra of lyophilized 6-AM in dry $CDCl_3$ (Fig. 1A) have a deuterium exchangeable signal with an integral of *three* protons at the chemical shift of 11.1 ppm. This unusual feature is not compatible with a monomeric form of either conformer. This chemical shift would typically be assigned to the phenolic 3-OH proton. However the integral value suggests a water molecule that is in fast exchange with the phenolic proton. The position of this peak varies for different sample preparations between 8.0 and 11.1 ppm, apparently depending on the residual water content and the acidity of the solvent. Upon addition of H_2O to the sample the integral of this peak increases while it is shifted to lower frequency. When the $CDCl_3$ solution is saturated with H_2O the bulk water is observed at about 4.72 ppm while this peak is shifted to 5.93 ppm. None of the other peak positions or integrals are affected significantly by the addition of H_2O. From X-ray crystallography it is known that crystalline morphine binds one water molecule.[22] Therefore, we interpret the NMR data as a chemical exchange process between a water molecule tightly bound to 6-AM and the residual water dissolved in the solvent. This exchange is relatively slow on the NMR time scale (at 200 MHz proton frequency). At the same time the hydrogen exchange process between the phenolic OH and the bound H_2O molecule is fast on this time scale. ^{1}H NMR spectra of morphine-acetate (MA) in $CDCl_3$ exhibit a similar behavior.

Steady-state NOE difference experiments[23] with selective irradiation of these three protons results in intense NOE responses of the signals assigned to protons at positions 2, 9, 14, and 17, and in weaker responses from protons 10 and 16 as shown in Figure 1B. These correlations cannot be explained by any single monomeric hydrated conformation of 6-AM. The simplest molecular arrangement that is compatible with this NOE pattern is a dimeric aggregate consisting of two molecules of 6-AM connected by two water molecules between the phenolic and amino moieties of the two 6-AM molecules. A head–tail oligomer can be excluded because of the positive signs of the nuclear Overhauser enhancements. NOE experiments at 360 and 500 MHz also show that the steady state NOE enhancements

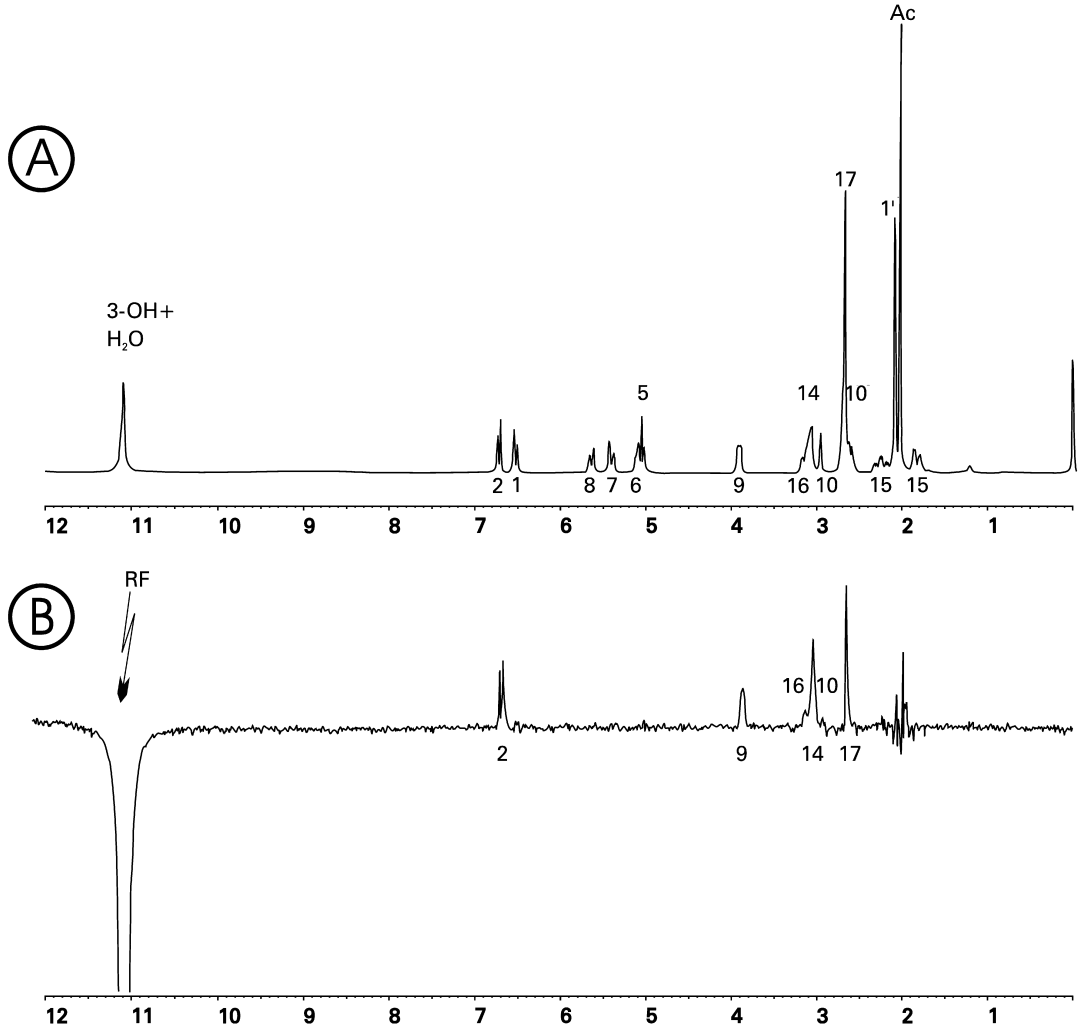


Figure 1. (A) 200 MHz ^{1}H NMR spectrum of 6-AM in $CDCl_3$ (25 °C) showing the broad singlet of the 3-OH phenolic group with an integral of about three protons. (B) Corresponding 200 MHz steady state NOE difference spectrum (1 s irradiation time).

decrease significantly at higher frequency. This behavior is characteristic of molecular species of a molecular mass between 500 and 1000 Da, which further corroborates the existence of a dimer ($M_r = 690.8$ Da). The possibility of the participation of acetate ions in the complex can be ruled out since no NOE enhancements are observed when irradiating the acetate signal at 2.07 ppm.

For comparison, proton NMR spectra of lyophilized morphine-acetate in dry $CDCl_3$ were recorded. They also show an integral of three protons for the very broad phenolic 3-OH peak at about 9.7 ppm. This corroborates that a water molecule is also closely associated with morphine, although the exchange with the remaining OH protons is faster than in the case of 6-AM. Probably the presence of a 6-OH accelerates the exchange. Selective saturation of this peak in steady state NOE difference experiments yielded NOE signals to the protons 1, 2, and 5. These correlations are in accordance with the crystal structure of morphine monohydrate.[22]

In order to extrapolate the experimental results obtained on 6-AM to M6G, which for solubility reasons cannot be examined by the same experimental approach, we performed molecular modeling studies on both molecules including water molecules. For these studies, the force fields MMX from PC-Model and CHARMm 22.2 in combination with Quanta 3.3.1 and Quanta 4.0 were used.[24–26] PC-Model utilizes a semi-empirical π-VESCF method for the calculation of bond orders and atomic charges. Charge templates were used with CHARMm. With both force fields systematic grid searches with respect to the glycosidic dihedral angles ϕ and ψ were performed. We focused on the anionic form of M6G prevailing at physiological pH. For comparisons to M6G, calculations on 6-AM were done for a neutral species employing the CHARMm force field after using HyperChem for MO calculations.[21,27]

6-AM with Bound Water

6-Acetylmorphine spans a conformational space similar to M6G. However, the enthalpy difference between the extended and the folded conformers is much smaller because of the smaller steric requirements of acetate. A water molecule was added to each of the conformers in analogy to the position of the water molecule in the crystal structure of morphine monohydrate. During minimization the water molecule was coordinated to the 6-AM molecule in different positions in the two conformers. In neither case was the water molecule repelled from 6-AM. To screen for other possible binding positions for the water molecule we performed a simulation with PC-Model's docking option, where the position of a water molecule was varied around a 6-AM molecule and subjected to a simulated annealing calculation. It was found that the water molecule can be docked to the phenolic 3-OH group and to the piperidinic nitrogen atom with similar probability. Based on this finding a dimer of the folded 6-AM conformer was constructed in which the 3-OH group of each 6-AM molecule is linked

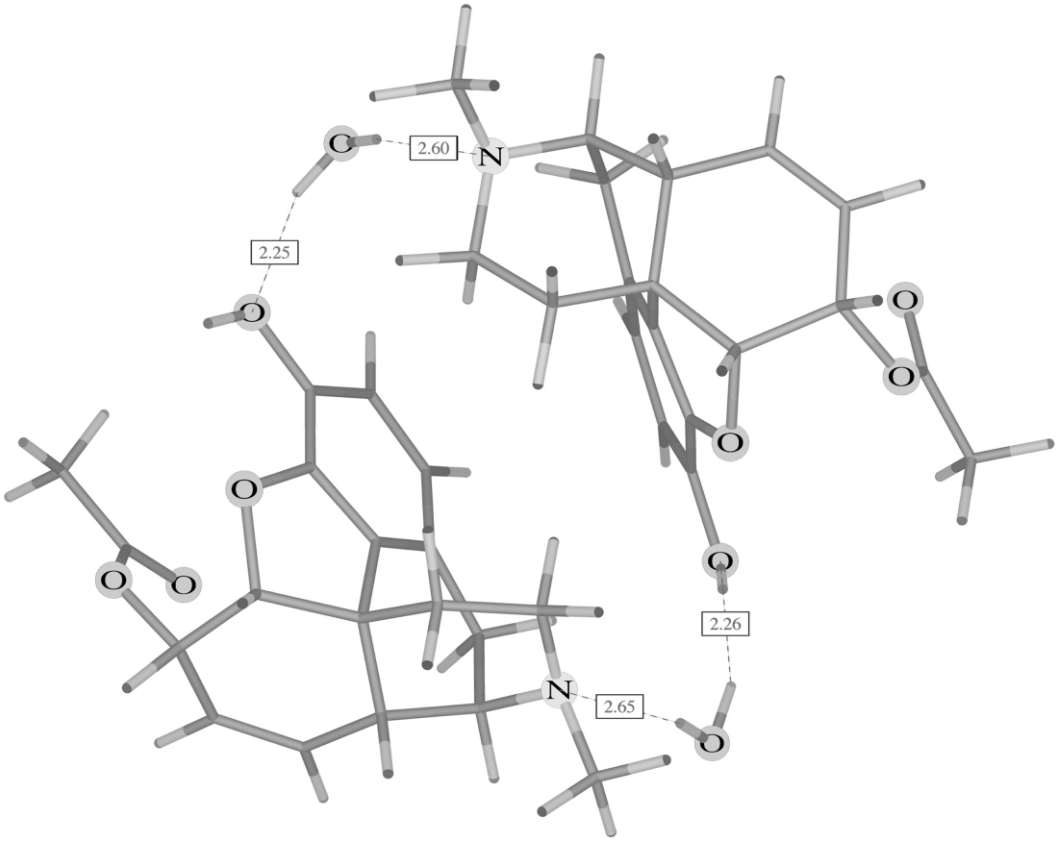


Figure 2. Dimeric 6-AM with two bridging water molecules. Result of CHARMm[25] minimization, displayed by program Ball & Stick.[29]

to the piperidinic nitrogen of the other 6-AM molecule by a water molecule. The energy of this hetero-tetramer was minimized in vacuum as well as in a solvent sphere of water molecules. In both cases a stable complex was obtained. Formation of this dimer (see Fig. 2) is only possible if the methyl group in position 17 flips from an equatorial position (which is preferred in the monomer) into an axial position. It is known that the axial methyl position is an important prerequisite for the agonistic action of morphine and its derivatives.[28] The energy difference between folded conformers with an equatorial and an axial 17-methyl group was calculated to be 2.2 kJ/mol (CHARMm). Therefore the change should occur easily at room temperature. The flipping of methyl group 17 could also be observed in molecular dynamics simulations of M6G.

The axial position of methyl group 17 also has a very strong effect on the dipole moment difference between the folded and the extended conformers of 6-AM, as calculated by CHARMm. If methyl group 17 is in an equatorial position, both conformers have similar dipole moments of 2.2 (extended) and 2.3 Debey (folded). When methyl group 17 is in the axial position, the dipole moment of the folded conformer increases to 3.6 Debye while the extended conformer is nearly unchanged. At the same time the differences in the heat of formation of the individual conformers vary only slightly (Table 1).

Table 1. Effect of the change of the methyl group Me 17 from equatorial to axial position in monomeric 6-AM conformers[a]

	6-AM extended	6-AM folded
Me 17—equatorial		
H_f (kJ/mol)	−415.7	−402.6
μ (Debye)	2.16	2.26
ϕ (°)	160	−48
ψ (°)	176	172
Me 17—axial		
H_f (kJ/mol)	−418.8	−404.6
μ (Debye)	2.24	3.57
ϕ (°)	159	−50
ψ (°)	177	−171

[a]The biggest change is found in the dipole moment of the folded conformer accompanied by a change of 17° in ψ. The data were calculated using Hyperchem/AM1.[27]

This has important consequences on the dipole moment of the water linked dimers. We constructed two hetero-tetramers constituting of two extended 6-AM molecules linked by two H_2O molecules and two folded 6-AM molecules linked by two H_2O molecules, respectively. The complexes will be referred to as 'extended complex' and 'folded complex', respectively. AM1 calculations (HyperChem 3.0) without solvation (starting AM1 minimization from an MM+ minimized structure) gave a dipole moment of 1.74 Debye for the extended complex and 6.88 Debye for the folded complex. This difference is caused by a different orientation of the 6-AM monomers in the complexes: The dipole vectors of the two extended 6-AM monomers in the complex have an angle χ of 130° causing the monomers' dipole moments of about 2.4 Debye to partially cancel out. In contrast, the dipole vectors of the two folded monomers have an angle χ of 49° resulting in a partial addition of the monomer dipoles of about 3.5 Debye. In both cases the dipole moments of the whole complex are increased by the contribution of the dipole moments of the linking water molecules, by about 1 Debye in the extended complex and by almost 2 Debye in case of the folded complex (Table 2).

From these observations we propose that a complex of 6-AM—as described above—can change its dipole

Table 2. Results from molecular modeling studies on the extended 6-AM dimer complex and on the folded dimeric complex of 6-AM[a]

	ϕ (°)	ψ (°)	H_f (kJ/mol)	μ (Debye)
Extended				
Minimized dimeric complex with two water molecules			−1368.1	1.74
Dimer without water molecules			*(−827.4)*	*0.67*
6-AM Monomer 1	*157*	*179*	*(−419.3)*	*2.35*
6-AM Monomer 2	*157*	*180*	*(−419.1)*	*2.42*
Water 1			*(−248.0)*	*1.86*
Water 2			*(−248.0)*	*1.86*
Minimized dimer without water			−838.3	0.54
Minimized 6-AM monomer			−419.9	2.24
Folded				
Minimized dimeric complex with two water molecules			−1338.3	6.88
Complex without water molecules			*(−799.9)*	*5.05*
6-AM Monomer 1	*−48*	*−171*	*(−403.8)*	*3.58*
6-AM Monomer 2	*−51*	*−171*	*(−403.7)*	*3.56*
Water 1			*(−248.0)*	*1.86*
Water 2			*(−248.0)*	*1.86*

[a]Both sandwich complexes have two bound water molecules. The data below were obtained using Hyperchem/AM1. To delineate the contribution of the individual components of the complex, their respective data are shown in *italic* print. In order to obtain these contributions single point calculations only of the indicated parts of the complexes were performed while the remaining parts of the complex were deleted. The single point energies from these non-minimized substructures are given in brackets. The most important difference occurs in the angle ϕ, which causes the dipole moment of the individual 6-AM molecules either to sum up (folded complex, $\phi \approx 50°$) or to cancel out each other (extended complex, $\phi = 157°$). For comparison, we have simulated a minimized dimer of extended 6-AM without the bridging water molecules and a minimized monomer. It can be easily seen that the two water molecules are essential for the formation of the lipophilic complex. While the extended 6-AM dimer without water is slightly destabilized in vacuum (1.5 kJ/mol) compared to two monomeric extended 6-AM molecules, the dimeric complex with two water molecules (−1368.1 kJ/mol) is stabilized by −32.8 kJ/mol compared to the enthalpies of a 6-AM dimer (without water) plus the enthalpies of two water molecules.

moment when it enters a hydrophobic surrounding and vice versa which renders the complex stable in both hydrophobic and hydrophilic environments. This change in lipophilicity is achieved by a very simple rotation of the acetyl moiety which has only a very small energetic barrier that can easily be overcome at room temperature. It also would explain why a relatively hydrophilic molecule such as 6-AM can penetrate the blood–brain barrier. In our opinion, this model can be applied to a wide variety of morphine-6-derivatives. It is important to note that the physico-chemical properties of the substituent in position 6 play a crucial role in determining which conformer is more lipophilic. It should be noted that while for 6-AM the extended conformer seems to be more lipophilic, the situation is reversed for M6G, according to preliminary molecular mechanics calculations.

NMR and molecular modeling investigations on 6-acetylmorphine revealed that a stable complex of two 6-AM molecules linked by two water molecules can be formed. By simply varying the position of the 6-acetyl group, the orientation of the two 6-AM molecules resulting in an altered dipole moment. This in turn influences the solubility in hydrophobic environments, such as biological membranes. Because dimer formation essentially involves only the morphine body of 6-AM we propose this as a general model for morphine-derivatives. Tightly bound water molecules can play a crucial role in cross-membrane transportation of drug molecules by stabilizing oligomeric structures and lipophilic conformers, a notion which is also corroborated by a recent NMR study on dipeptide conformations.[30]

Acknowledgements

The authors thank LIZENS (Linzer Zentrum für Numerische Simulationen) for providing and maintaining the Quanta/CHARMm software packages and for access to the supercomputer resources.

References and Notes

1. Oberst, F. W. *J. Pharmacol. Exp. Ther.* **1940**, *69*, 240.
2. Yoshimura, H.; Oguri, K.; Tsukamoto, H. *Biochem. Pharmacol.* **1969**, *18*, 279.
3. Oguri, K.; Ida, H.; Yoshimura, H.; Tsukamoto, H. *Chem. Pharm. Bull., Tokyo* **1970**, *18*, 2414.
4. Shimomura, K.; Kamatka, O.; Ueki, S.; Ida, S.; Oguri, K.; Yoshimura, H.; Tsukamoto, H. *Tohoku J. Exp. Med.* **1971**, *105*, 45.
5. Paul, D.; Standifer, K. M.; Inturrisi, C. E.; Pasternak, G. W. *J. Pharmacol. Exp. Ther.* **1989**, *251*, 477.
6. Frances, B.; Gout, R.; Monsarrat, B.; Cros, J.; Zajac, J. M. *J. Pharmacol. Exp. Ther.* **1992**, *262*, 25.
7. Yoshimura, H.; Ida, S.; Oguri, K.; Tsukamoto, H. *Biochem. Pharmacol.* **1973**, *22*, 1423.
8. Aasmundstad, T. A.; Morland, J.; Paulsen, R. E. *J. Pharmacol. Exp. Ther.* **1995**, *275*, 435.
9. Stain, F.; Barjavel, M. J.; Sandouk, P.; Plotkine, M.; Scherrmann, J. M.; Bhargava, H. N. *J. Pharmacol. Exp. Ther.* **1995**, *274*, 852.
10. Bickel, U.; Schumacher, O. P.; Kang, Y. S.; Voigt, K. *J. Pharmacol. Exp. Ther.* **1996**, *278*, 107.
11. Wu, D.; Kang, Y. S.; Bickel, U.; Pardridge, W. M. *Drug Metab. Dispos.* **1997**, *25*, 768.
12. Stain-Texier, F.; Boschi, G.; Sandouk, P.; Scherrmann, J. M. *Br. J. Pharmacol.* **1999**, *128*, 917.
13. Polt, R.; Porreca, F.; Szabo, L. Z.; Bilsky, E. J.; Davis, P.; Abbruscato, T. J.; Davis, T. P.; Horvath, R.; Yamamura, H. I.; Hruby, V. J. *Proc. Natl. Acad. Sci. U.S.A.* **1994**, *91*, 7114.
14. Huwyler, J.; Drewe, J.; Klusemann, C.; Fricker, G. *Br. J. Pharmacol.* **1996**, *118*, 1879.
15. Letrent, S. P.; Pollak, G. M.; Brouwer, K. R.; Brouwer, K. L. R. *Drug Metab. Dispos.* **1999**, *27*, 827.
16. Xie, R.; Hammarlund-Udenaes, M.; de Boer, A. G.; de Lange, E. C. M. *Br. J. Pharmacol.* **1999**, *128*, 563.
17. Carrupt, P. A.; Testa, B.; Bechalany, A.; El Tayar, N.; Descas, P.; Perrissoud, D. *J. Med. Chem.* **1991**, *34*, 1272.
18. Gaillard, P.; Carrupt, P. A.; Testa, B. *Bioorg. Med. Chem. Lett.* **1994**, *4*, 737.
19. Avdeef, A.; Barrett, D. A.; Shaw, P. N.; Knaggs, R. D.; Davis, S. S. *J. Med. Chem.* **1996**, *39*, 4377.
20. Rossi, G. C.; Brown, G. P.; Leventhal, L.; Yang, K.; Pasternak, G. W. *Neurosci. Lett.* **1996**, *216*, 1.
21. Autodesk: Hyperchem 3.0, San Rafael, 1992.
22. Bye, E.; *Acta Chem. Scand., Ser. B* **1976**, *30*, 549. Entry MORPHM in Cambridge Crystallographic Structure Database, vers. 5.08, Rel. Oct. 1994, Oxford, 1994
23. Neuhaus, D.; Williamson, M. *The Nuclear Overhauser Effect in Structural and Conformational Analysis*; VCH: New York, 1989.
24. Serena Software: PC-model 386, Bloomington, 1992.
25. Brooks, B. R.; Bruccoleri, R. E.; Olafson, B. D.; States, D. J.; Swaminathan, S.; Karplus, M. *J. Comp. Chem.* **1983**, *4*, 187.
26. Molecular Simulations Inc. Quanta 3.0, Quanta 4.0, CHARMm 22.2, Waltham, 1992.
27. Dewar, J.; Thiel, W. *J. Am. Chem. Soc.* **1977**, *99*, 4899.
28. Snyder, S. H. *Sci. Am.* **1977**, *236*, 44.
29. Müller, N. *Ball & Stick—Molecular Graphics for Macintosh v. 3.8;* Johannes Kepler University Linz, 2000 (http://www.orc.uni-linz.ac.at/mueller/ball_and_stick.html).
30. Poen, C.-D.; Samulski, E. T.; Weise, C. F.; Weisshaar, J. C. *J. Am. Chem. Soc.* **2000**, *122*, 5642.