Pharmacokinetic Analysis of 6-Monoamino-β-cyclodextrin after Intravenous or Oral Administration to Rats Using a Specific Enzyme Immunoassay

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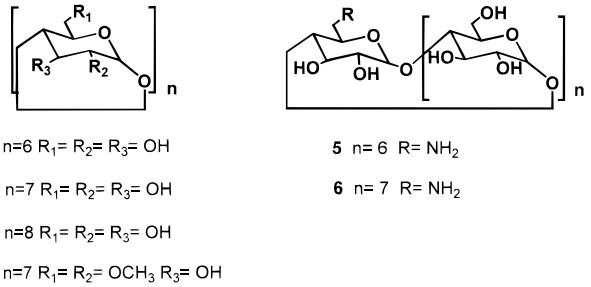
Abstract □ We have developed a highly sensitive enzyme immunoassay for 6-monoamino-β-CD (mono(6-amino-6-deoxy)cyclomaltoheptaose) and its parent compound (β-CD) with a detection limit in the 100 pg/mL range. The polyclonal antibodies obtained are highly specific for the β-cyclodextrin core and do not recognize other cyclic cyclodextrins (i.e., α- and γ-CD) or linear analogues. This enzyme immunoassay can be used to quantify 6-monoamino-β-CD in rat urine and plasma. Using this immunoassay, we have evaluated the main pharmacokinetic parameters of 6-monoamino-β-CD after iv administration to the rat of a 25 mg/kg dose. Since this method is strictly specific to the native β-CD form, we have demonstrated that the molecule rapidly disappeared from plasma but is probably distributed in the tissues. The urinary route appears as the predominant way of elimination since almost all the administered drug is recovered in urine. Finally, analysis of the same molecule after oral administration to the rat (25 mg/kg) demonstrates low plasma levels and that about 1% of the administered dose is excreted in urine. These experiments demonstrate the high stability of the β-CD core irrespective of the method of administration. This immunological method could provide relevant information on the fate of β-CD and some derivatives for drug delivery using different modes of administration (oral, parenteral, transmucosal, or dermal).

Introduction

Cyclo-malto-oligosaccharides (cyclodextrins or CDs) are natural molecules with the unique feature of trapping small organic compounds in their hydrophobic cavity. Such inclusion complexes induce modifications of the physical properties of the "guest" molecules, particularly in terms of their solubility and stability.[1] Pharmaceutical applications of cyclodextrins and derivatives are aimed at increasing the bioavailability and reducing the side-effects of drugs.[2] CDs can overcome formulation problems encountered with some drugs using classical pharmaceutical excipients, which explains the increasing number of CD-based formulations appearing on the market. Some CD derivatives may prove useful in delivery of hydrophilic compounds such as peptides[3] or oligonucleotides.[4,5]

However, the use of CD molecules as drug carriers or stabilizers requires safety evaluation. Investigation of the biological fate of CDs as well as toxicological issues must be considered before approval of these new excipients by regulatory agencies. Nephrotoxicity of α- and β-CD[6] as well as the hemolytic character of β-CD[7] severely hamper their potential use in pharmaceutical applications, at least for parenteral administration. However, the availability of different substituted analogues with better safety profiles (as recently reviewed[8]) broadens the range of administration. We recently described the decreased hemolytic properties of 6-monoamino-β-CD compared with the parent compound.[9] Using a competitive enzyme immunoassay which is both sensitive and specific for the cyclic structure of 6-monoamino-β-CD, we have investigated the fate of this compound after iv and oral administration to the rat (both at a single 25 mg/kg dose). After parenteral administration, the main pharmacokinetic parameters of 6-monoamino-β-CD were determined and indicated little metabolism and major elimination by the urinary route. It was shown that in the rat, after oral intake, a small but not inconsiderable proportion of 6-monoamino-β-CD is detected in plasma and eliminated in urine.

The data presented support the potential use of 6-monoamino-β-CD and demonstrate the efficiency of immunological methods in specifically monitoring such compounds for safety evaluation.



1 n=6 R_1= R_2= R_3= OH
2 n=7 R_1= R_2= R_3= OH
3 n=8 R_1= R_2= R_3= OH
4 n=7 R_1= R_2= OCH_3 R_3= OH
5 n= 6 R= NH_2
6 n= 7 R= NH_2

Scheme 1—Structure of the Linear and Cyclic Maltooligosaccharides Used in the Present Study: **1**, α-CD; **2**, β-CD; **3**, γ-CD; **4**, (2,6)-Dimethyl-β-CD; **5**, 6-Monoamino-β-CD; **6**, 6-Monoamino-γ-CD

Materials and Methods

Reagents and Buffer—Unless otherwise stated, all reagents were of analytical grade, from Sigma (St Louis, MO). The linear and cyclic oligosaccharides used are presented in Scheme 1. β-CD and α-, γ-CD were gifts from Roquette Frères (Lestrem, France) and Wacker (Germany), respectively. 6-Monoamino-β-CD[10] was prepared from 6-*O*-*p*-tolylsulfonyl cyclomaltoheptaose[11] according to a modified procedure.[12,13] 6-Monoamino-γ-CD was obtained in

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three steps from the parent CD as described elsewhere.[14] Maltoheptaose was obtained from Fluka AG (Basel, Switzerland). Heptakis-(2,6-di-*O*-methyl)cyclomaltoheptaose was obtained from Janssen (Beerse, Belgium) and was recrystallized five times in MeOH to over 99% purity. The chemical and optical purity of the different compounds was checked by ^{1}H NMR, mass spectroscopy, and chemical analysis.

Acetylcholinesterase (AChE, EC 3.1.1.7) from the electric organs of the electric eel *Electrophorus electricus* was purified by affinity chromatography[15] and used in its G4 form for 6-monoamino-β-CD labeling. AChE activity was measured using the colorimetric method of Ellman et al.[16]

All reagents used for immunoassays were diluted in the following buffer (EIA buffer): 0.1 M potassium phosphate buffer pH 7.4 containing 0.9% NaCl, 1 mM EDTA, 0.1% bovine serum albumin (BSA), and 0.01% sodium azide. The washing buffer was a 10 mM phosphate buffer containing 0.05% Tween 20.

Apparatus—Solid-phase EIA was performed in 96-well microtiter plates (Immunoplate Maxisorb with certificate, Nunc, Denmark) using automatic Titertek microtitration equipment (washer, dispenser and reader) from Labsystems (Helsinki, Finland).

Antiserum Production—For the production of antibodies, 6-monoamino-β-CD was covalently linked to bovine serum albumin (BSA) by means of glutaraldehyde as previously described.[17]

Preparation of the Enzymatic Tracer—6-Monoamino-β-CD was covalently coupled to AChE using the heterobifunctional reagent *N*-succinimidyl-4-(*N*-maleimidomethyl)-cyclohexane-1-carboxylate (SMCC) as previously described.[17] This method involved the reaction of thiol groups previously introduced into 6-monoamino-β-CD (by reaction of its primary amino group with *N*-succinimidyl-*S*-acetylthioacetate (SATA) in alkaline medium) with maleimido groups incorporated into the enzyme.

Competitive EIA Procedure—Competitive EIA was performed as described elsewhere for various molecules.[18−20] Microtiter plates were coated with mouse monoclonal anti-rabbit IgG antibodies in order to ensure separation of bound and free moieties of the enzymatic tracer during the immunological reaction. Before use, the plates were washed with 0.01 M phosphate buffer (pH 7.4) containing 0.05% Tween 20. The total volume of the immunological reaction was 150 μL, each component (enzymatic tracer, diluted rabbit polyclonal antisera, and cyclodextrin standard) being added in a 50 μL volume. 6-Monoamino-β-CD-AChE enzyme conjugate was used at a concentration of 2 Ellman units/mL (for Ellman unit definition, see ref 19). The working dilution for the rabbit antiserum was determined by performing serial dilution experiments.

After 18-h incubation at 4 °C, the plates were washed and the enzyme activity of the bound immunological complex was revealed by addition of 200 μL of enzymatic substrate and chromogen (Ellman's reagent[16]) to each well. After 1 h of gentle shaking in the dark at room temperature, the absorbance at 414 nm in each well was measured automatically. Results are given in terms of $B/B_0 \times 100$ as a function of the dose (logarithmic scale), B and B_0 representing the bound enzyme activity in the presence or absence of competitor, respectively. A linear log−logit transformation was used to fit the standard curve.[21] The sensitivity of the assay was characterized by the dose of 6-monoamino-β-CD inducing a 50% lowering of the binding observed in the absence of competitor (B/B_0 50%). Nonspecific binding represented less than 0.1% of the total enzyme activity. Finally, the minimum detectable concentration (MDC) was taken as the concentration of competitor inducing a significant decrease (three standard deviations in B_0). All determinations were made in duplicate and quadruplicate for B_0.

To characterize the specificity of the antibodies, standard curves were plotted using various derivatives of malto-oligosaccharides. The results are expressed in terms of percentage cross-reactivity (CR) [(dose of 6-monoamino-β-CD B/B_0 50%/dose of analogue B/B_0 50%) × 100].

For rat plasma samples, a methanol precipitation procedure was performed before the assay. Four volumes of cold methanol were added to the sample before centrifugation (2500 rpm, 10 min). The pellet was discarded and the supernatant dried under vacuum using a SpeedVac apparatus (Savant, Farmingdale, NY) before reconstitution with EIA buffer. Standard curves were plotted by spiking control plasma samples with known amounts of 6-monoamino-β-CD and demonstrated more than 95% recovery of the molecule in the supernatant.

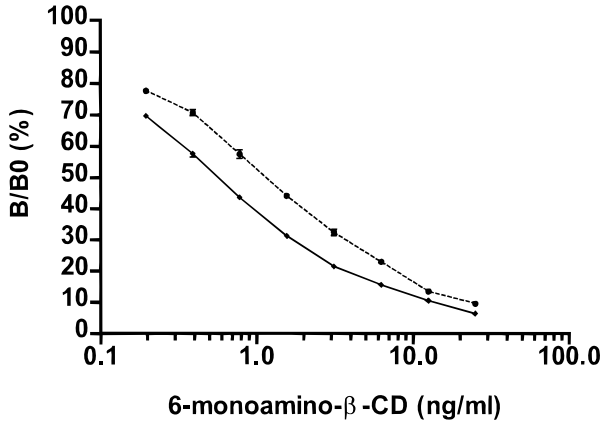

Figure 1—Standard curve for 6-amino-β-cyclodextrin enzyme immunoassay performed in rat urine (full line) and EIA buffer after methanol extraction of rat plasma (dotted line).

Animal Experiments—Experiments were performed on 10-week-old (325 ± 60 g) male Sprague−Dawley rats (Iffa Credo, Les Oncins, France) which throughout the study had free access to water and to a commercial feed (DO4, UAR, Villemoisson, France). The rats were acclimatized for 4 days in individual metabolic cages before experiments. Rats were anaesthetized (one-third O_2, two-thirds NO_2, and 4% fluothane) and catheters were placed in the right femoral artery and vein, passed up into the abdominal vessels, and their free extremity exteriorized at the back of the neck. One day after surgery, a single intravenous dose of 6-monoamino-β-CD (25 mg/kg) in saline solution (47.4 mg/mL) was injected into four rats via the venous catheter. Oral administration of 6-monoamino-β-CD (25 mg/kg) in saline solution (50.1 mg/mL) was performed on four other rats using a 1 mL tuberculin syringe and a 16 gauge gavage needle.

Blood (200 μL) was sampled at various times (0, 5, 10, 15, 20, 30 min, 1 h, 2 h, 4, 8, and 24 h) at the arterial catheter. The blood was centrifuged, and the resulting plasma kept frozen at −20 °C until assay. The pellets containing red blood cells were reinjected after dilution (1/2) with saline dilution through the venous catheter. During the experiment, the rats were housed individually in glass metabolic cages which allowed separate collection of urine and feces. Urine was collected at 4, 8, and 24 h.

Pharmacokinetic Evaluation—A specific computer program (Siphar from SIMED, Créteil, France), designed for nonlinear regression of pharmacokinetic data, was used to fit plasma concentrations. Pharmacokinetic parameters were evaluated using a noncompartmental approach as previously described.[22] The area under the plasma concentration curve (AUC) was determined using the trapezoidal lin-log rule and extrapolated to infinity. The apparent elimination rate constant (k_e) was determined by the decay of the plasma concentration at the four last values (between 30 min and 4 h). Clearance was calculated from the dose divided by the AUC. The apparent volume of distribution was determined using the relation Cl/k_e. Quantities eliminated in urine (Q_e) were evaluated over 0−24 h. Urinary clearance was calculated on the basis of $Cl_u = Q_e/AUC$. Values are expressed as mean ± SD.

Results

Sensitivity and Specificity of the Assay—Using the 6-amino-β-CD-AChE tracer, we optimized the antiserum dilution for the assay. A typical routine standard curve for this assay in EIA buffer using antiserum (no. L1076SAB) at 1/50000 initial dilution is presented in Figure 1. The sensitivity at B/B_0 50% is about 550 pg/mL (25.4 fmol/well) with a minimum detectable concentration close to 110 pg/mL (4.8 fmol/well). The specificity of this assay was demonstrated by testing against different compounds. Parent cyclodextrin (β-CD) gave a full response (90% of cross-reactivity), whereas neither substituted β-CD (i.e. heptakis(2,6-di-*O*-methyl)-β-CD) nor other native or modified CD (i.e. α-CD, γ-CD, and 6-monoamino-γ-CD) displayed any significant cross-reactivity (<0.1%). Linear maltoheptaose was not recognized at all by the antibodies.

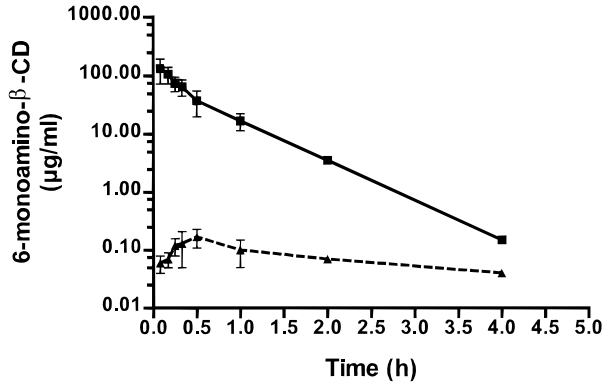

Figure 2—Analysis of plasma levels using specific immunoassay after administration of 6-monoamino-β-cyclodextrin (25 mg/kg) to rats by iv (full line) or oral intake (dotted line). Each value represents the mean ± SD of four rats.

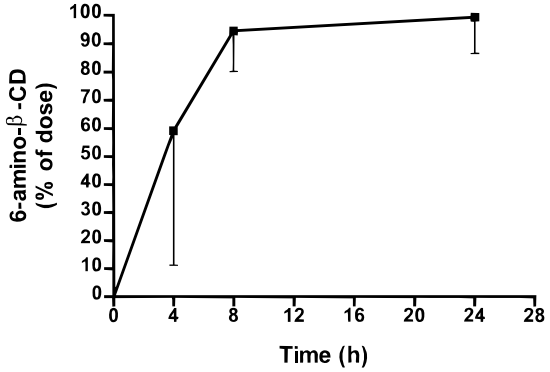

Figure 3—Cumulative urinary excretion of 6-monoamino-β-cyclodextrin after iv administration (25 mg/kg) to rats. Each value represents the mean ± SD of four rats.

All these experiments demonstrated the strict specificity of this assay for the cyclic core of β-CD. Recognition of the molecule by the antibodies seems to involve the secondary hydroxyl rim since the 2,6-di-methyl-β-CD derivative was not detected.

Before performing the pharmacokinetic experiments, we tested the analytical characteristics of the immunomethod in different biological fluids. Rat urine and plasma samples were spiked with known concentrations of 6-monoamino-β-CD. While the assay works properly for urine samples, we observed a lack of reproducibility for undiluted plasma samples. We thus applied a methanol precipitation protocol (see Methods), which provided good reproducibility with only a small change in sensitivity (Figure 1; minimum detectable concentration close to 180 pg/mL). Under these conditions, the precision of the assay is very satisfactory since the coefficients of variation (%) were 8.7, 5.6, and 9.6 within assays and 9.1, 6.1, and 9.8 between assays, for 10, 2, 0.3 ng/mL of 6-amino-β-CD, respectively.

Pharmacokinetic Measurements for 6-Monoamino-β-CD after iv Administration—After a single injection at 25 mg/kg, 6-monoamino-β-CD was detected for up to 4 h in plasma. The 8 and 24 h plasma samples presented no detectable immunoreactivity (except for a single rat for which we measured values of 60 ± 20 ng/mL and 50 ± 30 ng/mL of 6-monoamino-β-CD in the 8 and 24 h samples, respectively). In urine, meaningful concentrations were observed, remaining higher than 20 μg/mL in the 8−24 h collection period. The mean plasma concentration curve and cumulative urinary excretion of iv administered 6-monoamino-β-CD are presented in Figures 2 and 3, respectively. The main pharmacokinetic parameters are listed in Table 1. 6-Monoamino-β-CD disappears rapidly from plasma, as demonstrated by a short half-life (21 min) and MRT (28 min). The apparent volume of distribution (V_d) corresponds to 20% of the total body weight and is close to the plasma and the interstitial volume. This indicates that 6-monoamino-β-CD probably penetrates into tissues to some extent. Most if not all administered compound is eliminated in urine (100% at 24 h) with an apparent urinary clearance close to the systemic clearance. This clearance value is close to the renal glomerular filtration rate.[23]

Table 1—Main Pharmacokinetic Parameters[a] of 6-Monoamino-β-CD after Intravenous Administration (25 mg/kg) to Rats

parameters		rat 1	rat 2	rat 3	rat 4	mean ± SD
$T_{1/2}$	min	20	24	20	19	21 ± 2
MRT	min	28	34	19	29	28 ± 5
Cl_s	mL·min^{-1}·kg^{-1}	5.3	6.3	5.4	10.5	6.9 ± 2.1
AUC (0–inf)	μg·mL^{-1}·h	83.9	72.2	80.8	40.4	69.3 ± 17.2
V_d	mL·kg^{-1}	149	220	153	287	202 ± 56
Q_{el} (0–24 h)	% dose	85	103	95	115	100 ± 11
Cl_u (0–24 h)	mL·min^{-1}·kg^{-1}	4.5	6.4	5.1	12.2	7.1 ± 3.1

[a] Abbreviations: $T_{1/2}$, half-life; MRT, mean residence time; Cl_s, systemic clearance; AUC (0–inf), area under the curve from 0 to infinity; V_d, apparent volume of distribution; Q_{el} (0–24 h), eliminated quantity during 24 h; Cl_u (0–24 h) urinary clearance.

Table 2—Main Pharmacokinetic Parameters[a] of 6-Monoamino-β-CD after Oral Administration (25 mg/kg) to Rats

parameters		rat A	rat B	rat C	rat D	mean ± SD
C_{max}	μg·mL^{-1}	0.15	0.14	0.25	0.13	0.17 ± 0.06
T_{max}	min	30	30	30	30	30
AUC (0–inf)	μg·mL^{-1}·h	0.15	0.25	0.58	0.40	0.35 ± 0.19
Q_{el} (0–24 h)	% dose	0.76	0.68	1.80	0.12	0.84 ± 0.70

[a] Abbreviations: C_{max}, maximal concentration; T_{max}, time of maximal concentration; AUC (0–inf), area under the curve from 0 to infinity; Q_{el} (0–24 h), eliminated quantity during 24 h.

Oral Administration of 6-Monoamino-β-CD—We detected 6-monoamino-β-CD in plasma and urine of all the rats who had received a single oral 25 mg/kg dose. The plasma kinetics measurements are presented in Figure 2 (dotted line), and the main pharmacokinetic parameters are listed in Table 2. The plasma concentration exhibited a maximal value of 170 ± 60 ng/mL at 30 min after administration. 6-Monoamino-β-CD data were presented over the 4 h after oral intake, since it was undetectable in the 8 and 24 h samples. Cumulative urinary elimination of 6-monoamino-β-CD represented 0.84 ± 0.7% of the initial dose. 6-Monoamino-β-CD could still be assayed in the 8−24 h urine (concentration and amount ranging from 0.16 to 11.53 μg/mL and from 1.5 to 71.8 μg, respectively). The bioavailability of 6-monoamino-β-CD was 0.5% and 0.89% as calculated from AUC(0−inf) and Qel (0−24 h), respectively.

Discussion

We have determined the main pharmacokinetic parameters of a β-CD derivative, 6-monoamino-β-CD, which was shown to be less hemolytic than the parent compound, after iv administration at a single 25 mg/kg dose. These results were obtained using a sensitive and specific enzyme immunoassay. Due to the high specificity of this method toward the intact β-CD core, we have demonstrated that little metabolism of this compound occurs. Indeed, 6-monoamino-β-CD is rapidly eliminated from the systemic circulation and is almost totally eliminated via the urinary route, rather like the parent compound.[24] However, in contrast with previous observations for β-CD,[24] 6-monoamino-β-CD has an apparent volume of distribution of 202 mL/

kg, pointing to a probable extravascular distribution, in select tissues or storage pools. The urinary clearance is close to the systemic clearance and appeared close to the glomerular filtration rate.

Using the same immunoanalytical method, we also analyzed the fate of 6-monoamino-β-CD in the rat after oral intake (25 mg/kg). We demonstrated the presence of detectable amounts of the intact molecule in plasma, with a maximal concentration observed 30 min after administration. The molecule is then eliminated via the urinary route. Although 6-monoamino-β-CD was detected in both plasma and urine of the 4 treated rats, large variations were observed; the extent of gastrointestinal absorption of this molecule depends on the individual animal. The cumulative amount of intact CD excreted represents 0.84 $\pm$ 0.7% of the dose. These observations are in agreement with previous reports on orally administered β-CD[25,26] using either radiolabeled compound or a sensitive HPLC technique.[27] Thus, chemical modification does not seem to induce major changes in the pharmacokinetic behavior of the molecule compared with the parent compound. Finally, we have calculated a low oral bioavailability for this molecule, indicating only minimal absorption.

To our knowledge, this is the first full description of the pharmacokinetic analysis of this β-cyclodextrin derivative after iv and oral administration to the rat. A specific analytical method such as the presently described enzyme immunoassay may yield valuable information on the fate of cyclodextrin(s) used for pharmaceutical applications, irrespective of the mode of administration (parenteral and oral for the present study but dermal or transmucosal applications can also be investigated). Moreover, we have observed that this assay retains its analytical characteristics when applied to human urine and plasma samples (data not shown). We are currently developing similar specific immunoassays for methyl derivatives of β-CD, i.e., heptakis-(2, 6-di-*O*-methyl)cyclomaltoheptaose (DIMEB) and heptakis-(2,3,6-tri-*O*-methyl)cyclomaltoheptaose (TRIMEB) which may provide further important data for pharmaceutical applications.

References and Notes

1. Loftsson, T.; Brewster, M. Pharmaceutical applications. 1. Drug solubilization and stabilization. *J. Pharm. Sci.* **1996**, *85*, 1017−1025.
2. Stella, V.; Rajewski, R. Cyclodextrins: their future in drug formulation and delivery. *Pharm. Res.* **1997**, *14*, 556−567.
3. Schipper, N.; Verhoef, J.; De Lannoy, L.; Romeijn, S.; Brakkee, J.; Wiegant, V.; Gispen, W.; Merkus F. Nasal administration of an ACTH(4−9) peptide analogue with dimethyl-beta-cyclodextrin as an absorption enhancer: pharmacokinetics and dynamics. *Br. J. Pharmacol.* **1993**, *110*, 1335−1340.
4. Zhao, Q.; Temsamani, J.; Agrawal, S. Use of cyclodextrin and its derivatives as carrier for oligonucleotide delivery. *Antisense Res. Rev.* **1995**, *5*, 185−192.
5. Zhao, Q.; Temsamani, J.; Iadarola, P.; Agrawal, S. Modulation of oligonucleotide-induced immune stimulation by cyclodextrin analogues. *Biochem. Pharmacol.* **1996**, *52*, 1537−1544.
6. Frank, D.; Gray, J.; Weaver, R. Cyclodextrin nephrosis in the rat. *Am. J. Pathol.* **1976**, *83*, 367−382.
7. Shiotani, K.; Uehata, K.; Irie, T.; Uekama, K.; Thompson, D.; Stella, V. Differential effects of sulfate and sulfobutyl ether of beta-cyclodextrin on erythrocyte membranes in vitro. *Pharm. Res.* **1995**, *12*, 78−84.
8. Irie, T.; Uekama, K. Pharmaceutical applications of cyclodextrins. III. Toxicological issues and safety evaluation. *J. Pharm. Sci.* **1997**, *86*, 147−162.
9. Bost, M.; Lainé, V.; Pilard, P.; Gadelle, A.; Defaye, J.; Perly, B. The hemolytic properties of chemically modified cyclodextrins. *J. Inclus. Phenom. Mol. Recogn.* **1997**, *29*, 57−63.
10. Petter, R.; Salek, J.; Sikorski, C.; Kumaravel, G.; Lin, F. Cooperative binding by aggregated mono-6-(alkylamino)-β-cyclodextrins. *J. Am. Chem. Soc.* **1990**, *112*, 3860−3868.
11. Takahashi, K.; Hattori, K.; Toda, F. Monotosylated α and β-cyclodextrins prepared in an alkaline aqueous solution. *Tetrahedron Lett.* **1984**, *25*, 3331−3334.
12. Djedaïni-Pilard, F.; Desalos, J.; Perly, B. Synthesis of a new molecular carrier: N(Leu-enkephalin)yl-6-amido-6-deoxycyclomaltoheptaose. *Tetrahedron Lett.* **1993a**, *34*, 2457−2460.
13. Djedaïni-Pilard, F.; Perly, B. Utilisation de cyclodextrines aminées pour la solubilisation aqueuse de composés hydrophobes, en particulier de molécules pharmaceutiquement actives. French Patent 93-15472, 1993b.
14. Djedaïni-Pilard, F.; Azaroual-Bellanger, N.; Gosnat, M.; Vernet, D.; Perly, B. Potential formation of intramolecular inclusion complexes in peptido-cyclodextrins as evidenced by NMR spectroscopy. *J. Chem. Soc., Perkin Trans. 2* **1995**, 723−730.
15. Massoulié, J.; Bon, S. Affinity chromatography of acetylcholinesterase: the importance of hydrophobic interactions. *Eur. J. biochem.* **1976**, *68*, 531−539.
16. Ellman, G.; Courtney, K.; Andres, V.; Featherstone, R. A new and rapid colorimetric determination of acetylcholine esterase activity. *Biochem. Pharmacol.* **1961**, *7*, 88−95.
17. Créminon, C.; Pilard, F.; Grassi, J.; Perly, B.; Pradelles, P. A sensitive and specific enzyme immunoassay for cyclomaltoheptaose and some derivatives. *Carbohydr. Res.* **1994**, *258*, 179−186.
18. McLaughlin, L.; Wei, Y.; Stockman, P.; Leahy, K.; Needleman, P.; Grassi, J.; Pradelles, P. Development, validation and application of an enzyme immunoassay (EIA) of atriopeptin. *Biochem. Biophys. Res. Commun.* **1987**, *144*, 469−476.
19. Pradelles, P.; Grassi, J.; Chabardes, D.; Guiso, N. Enzyme immunoassays of adenosine cyclic 3′,5′-monophosphate and guanosine cyclic 3′,5′-monophosphate using acetylcholinesterase. *Anal. Chem.* **1989**, *61*, 447−453.
20. Grassi, J.; Frobert, Y.; Pradelles, P.; Chercuitte, D.; Gruaz, D.; Dayer, J.-M.; Pradelles, P. Production of monoclonal antibodies against interleukin-1α and -1β; Development of two enzyme immunoassays (EIA) using acetylcholinesterase and their application to biological media. *J. Immunol. Methods* **1989**, *123*, 193−210.
21. Rodnard, D.; Lewald, J. Computer analysis of radioligand assay and radioimmunoassay data. *Acta Endocrinol.* **1970**, *64*, 79−103.
22. Gibaldi, M.; Perrier, D. In *Pharmacokinetics in drugs and the pharmaceutical sciences*; Marcel Dekker: New York, 1982; pp 15−48.
23. Bivin, W.; Crawford, M.; Brewer, N. In *The laboratory rat: biology and diseases*; Barker, H., Lindsey, J., Weisbroth, S., Eds; Academic Press: New York, 1979; Vol. 1, pp 90−95.
24. Frijlink, H.; Visser, J.; Hefting, N.; Oosting, R.; Meijer, D.; Lerk, C. The pharmacokinetics of beta-cyclodextrin and hydroxypropyl-beta-cyclodextrin in rat. *Pharm. Res.* **1990**, *7*, 1248−1252.
25. Gerloczi, A.; Fonagy, A.; Keresztes, P.; Perlaky, L.; Szejtli, J. Absorption, distribution, excretion and metabolism of orally administered 14C-beta-cyclodextrin in rat. *Arzneim. Forsch.* **1985**, *35*, 1042−1047.
26. Kubota, Y., Fukuda, M.; Muroguchi, M.; Koizumi, K. Absorption, distribution and excretion of beta-cyclodextrin and glucosyl-beta-cyclodextrin in rats. *Biol. Pharm. Bull.* **1996**, *19*, 1068−1072.
27. Kubota, Y., Fukuda, M.; Ohtsuji, K.; Koizumi, K. Microanalysis of beta-cyclodextrin and glucosyl-beta-cyclodextrin in human plasma by high-performance liquid chromatography with pulsed amperometric detection. *Anal. Biochem.* **1992**, *201*, 99−102.

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