View Article Online / Journal Homepage / Table of Contents for this issue

COMMUNICATION

OBC
www.rsc.org/obc

Synthesis of glycosyl derivatives as dopamine prodrugs: interaction with glucose carrier GLUT-1 †

Caridad Fernández,[a] Ofelia Nieto,[a] José Angel Fontenla,[c] Emilia Rivas,[c] María L. de Ceballos *[b] and Alfonso Fernández-Mayoralas *[a]

[a] *Instituto de Química Orgánica General, CSIC, Juan de la Cierva, 3, 28006 Madrid, Spain. E-mail: iqofm68@iqog.csic; Fax: +34 91 564 48 53*

[b] *Instituto Cajal, CSIC, Doctor Arce 37, 28002 Madrid, Spain*

[c] *Departamento de Farmacología, Facultad de Farmacia, Universidad de Santiago de Compostela, Campus Sur s/n, 15782 Santiago de Compostela, Spain*

Received 9th December 2002, Accepted 29th January 2003
First published as an Advance Article on the web 7th February 2003

Glucosyl dopamine (DA) derivatives may represent a new class of DA prodrugs that would interact with glucose transporter GLUT-1, present in the blood–brain barrier, and generate DA in the brain. Therefore, compounds bearing the sugar moiety linked to either the amino group or the catechol ring of DA through amide, ester, carbamate, peptide or glycosidic bonds were synthesized. The behavior of the compounds as prodrugs was monitored in different media and the affinity of the glycoconjugates for the glucose carrier GLUT-1 using human erythrocytes was also studied. Most of the compounds were markedly stable in buffer and plasma, and several compounds released DA when incubated with brain extracts and the rate was related to the bond linking DA with glucose. The new glucosyl conjugates substituted at the C-6 position of the sugar were more potent inhibitors of glucose transport when compared to C-1 and C-3 substituted derivatives. This work provides structure–activity information about the interaction of substituted glucose with the GLUT-1 transporter.

Introduction

The blood–brain barrier (BBB) provides an efficient protection of the brain, maintaining the extracellular concentrations of ions, neurotransmitters and growth factors. Indeed hydrophilic compounds are excluded by the BBB and only small lipophilic molecules may cross it. This ability relies on the structure of the brain vessels composed of endothelial cells joined by tight junctions, lined with astrocyte projections. Only a few substances necessary for the brain's proper function are able to cross this barrier.[1–3] Thus, brain transport of essential hydrophilic nutrients is mediated by a number of specific carriers, which are located in the plasma membrane of the BBB endothelial cells.[4] This is the case for glucose, the main energy source of the brain, whose passage is facilitated by the glucose carrier GLUT-1.[5–7]

However, the presence of the BBB hinders pharmacological delivery to the brain thus making difficult the treatment of certain diseases with drugs.[3] This is the case with Parkinson's disease, which is characterized by the dramatic reduction of dopamine (DA) levels in basal ganglia as a consequence of the degeneration of DA neurons in the substantia nigra. The use of DA itself to treat the disease is precluded by the inability of this neurotransmitter to cross the BBB. The most widely employed treatment of this disorder is levodopa,[8] the immediate DA precursor. Levodopa is actively transported into the brain through the neutral amino acid carrier where it is enzymatically converted into DA. However upon continuous levodopa treatment a variety of problems may appear (*e.g.* fluctuations in the clinical response and onset of involuntary movements).[9,10]

We have recently initiated studies on a new approach to deliver DA into the central nervous system (CNS) by linking the neurotransmitter to a sugar molecule so that the resulting glycoconjugate may cross the BBB using the glucose carrier GLUT-1.[11] Once transported into the CNS the prodrug would be hydrolyzed to release DA. In order to reach and provide the brain with the neurotransmitter the glycoconjugates must fulfil several requirements. They must be stable in the periphery, should be recognized by the carrier GLUT-1 and transported into the brain, and, finally, be hydrolyzed by the action of brain enzymes to release DA. Some authors have studied this approach to deliver peptide drugs[12] and cytotoxic agents[13,14] into the CNS.

In the present work we describe the synthesis and biological activities of new glycoconjugates in which DA is attached to different positions of the glucose molecule using a variety of linkages susceptible of enzymatic hydrolysis. A series of compounds had the amino group of DA linked to C-6, C-3 and C-1 of the sugar through a succinyl linker (compounds **1**–**3**, Fig. 1) or a carbamate bond (**4**–**8**). In another series, the sugar was attached to the phenol group of DA through glycosidic (**9** and **10**) and ester (**11**–**13**) bonds. In order to know their properties as prodrugs, *in vitro* stability studies of the compounds have been performed in plasma and rat brain extracts. Finally, the affinity of the glycoconjugates for the glucose carrier GLUT-1 using human erythrocytes was studied as well.

Results and discussion

Synthesis of glycoconjugates

The synthesis of compounds **1**–**3**, **9** and **10** has been previously described.[11] Compounds **4**–**7**, with a carbamate bond linking the sugar and the neurotransmitter were synthesized by activation of the corresponding hydroxyl group of the sugar with *N*,*N*-carbonyldiimidazole (CDI) followed by coupling with the amine group of DA. Thus, the partially benzylated glucoses having free hydroxyl HO-6, HO-3 or HO-1 (compounds **17**, **21**, and **24**, Scheme 1) were first obtained by selective protection/deprotection steps.[11,15]

The reaction of **17** with CDI was performed in dioxane to give **18** in excellent yield (94%). The imidazolylcarbonyl derivative thus obtained was treated with DA derivative **19**[11] in the presence of Et_3N afford the protected glycoconjugate **20** in

† Electronic supplementary information (ESI) available: experimental details for the preparation of all derivatives and biological assays. See http://www.rsc.org/suppdata/ob/b2/b212066f/

DOI: 10.1039/b212066f

Published on 07 February 2003. Downloaded by Christian Albrechts Universitat zu Kiel on 26/10/2014 16:57:45.

View Article Online

Published on 07 February 2003. Downloaded by Christian Albrechts Universitat zu Kiel on 26/10/2014 16:57:45.

Table 1 Half-life (hours) of glycoconjugates in different media

Compound	Type of bond between sugar and DA	Incubation media		
		Buffer (pH 7.0)	Plasma	Brain extract
1	Ester–amide	>48	3	21
4	Carbamate	>48	3	22
6,**7**[a]	Carbamate	>48	1.3	12
8	Peptide–carbamate	30	24	30
9	Glycosidic	>48	>48	134
10	Glycosidic	>48	>48	67
11 + **12**	Ester	0.08	—	—
13	Ester	2.5	0.1	0.4
Dopamine	—	27	2	3

[a] A 2 : 1 mixture of **6** and **7** was used.

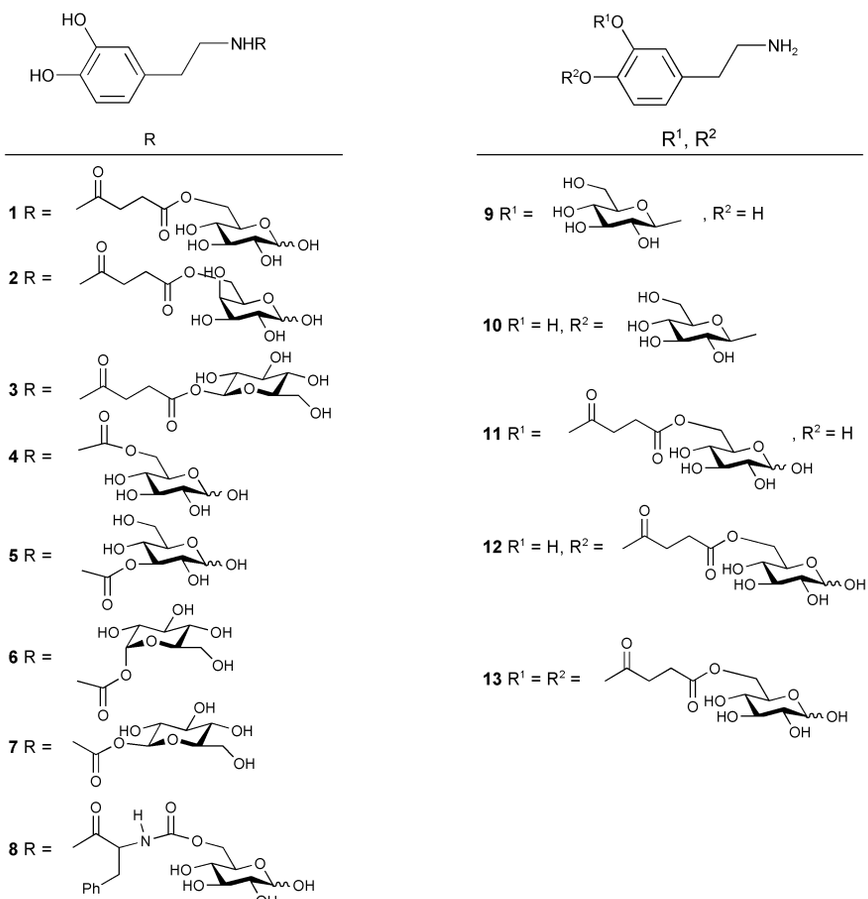


Fig. 1 Glycosyl dopamine derivatives.

87% yield. Removal of the benzyl groups by hydrogenolysis led to **4**. A similar procedure was applied to the alcohol **21**, but using THF instead of dioxane in the reaction with CDI. The subsequent condensation with **19** followed by debenzylation furnished **5**.

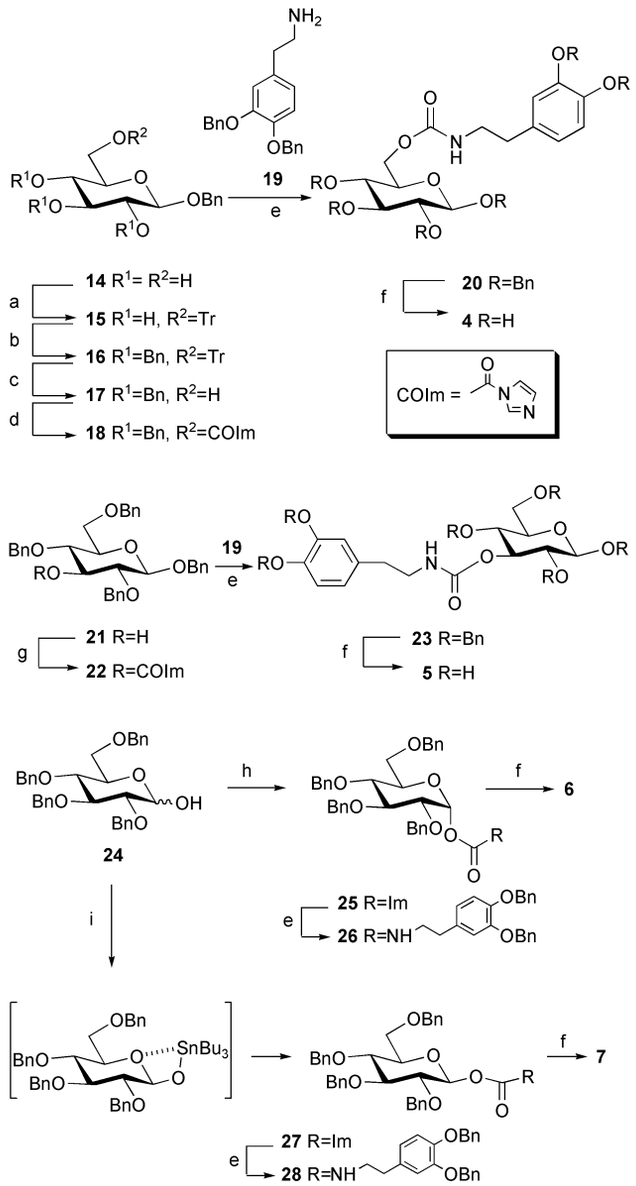
In the case of the activation of alcohol **24** with CDI we faced the problem of the stereoselectivity at the anomeric center.[16–18] We tried the reaction varying solvent (dioxane, THF, CH_2Cl_2, and Et_2O), temperature (80 °C, rt, and −20 °C), and in the presence or absence of a base, giving in all cases anomeric mixtures of imidazolylcarbonyl derivatives **25** (α) and **27** (β) with the α-anomer predominating. The best result was obtained in Et_2O at room temperature to afford a mixture of **25** and **27** in excellent yield and good α-stereoselectivity (98%, α : β, 80 : 20). Treatment of **25** with **19** gave the protected glycoconjugate **26** from which benzyl groups were removed by hydrogenolysis to give **6**.

For the stereoselective synthesis of the β-anomer the activation of hydroxyl groups through the formation of stannyl ethers[19] was used. Our working hypothesis was that coordination of the tin atom with the ring oxygen would be more favorable in the β- than in the α-configured tin ether, allowing the subsequent reaction to proceed selectively towards the formation of the anomeric carbamate with β-configuration. The reaction of **24** with $(Bu_3Sn)_2O$ at 110 °C followed by treatment with CDI at room temperature, gave an anomeric mixture of imidazolylcarbonyl derivatives **25** (α) and **27** (β) in high yield (94%) with the β-anomer as the major compound (α : β, 30 : 70). This mixture without further separation was coupled with the amine **19** and, then chromatographed to give compound **28**. Benzyl groups were removed by hydrogenation to afford **7**.

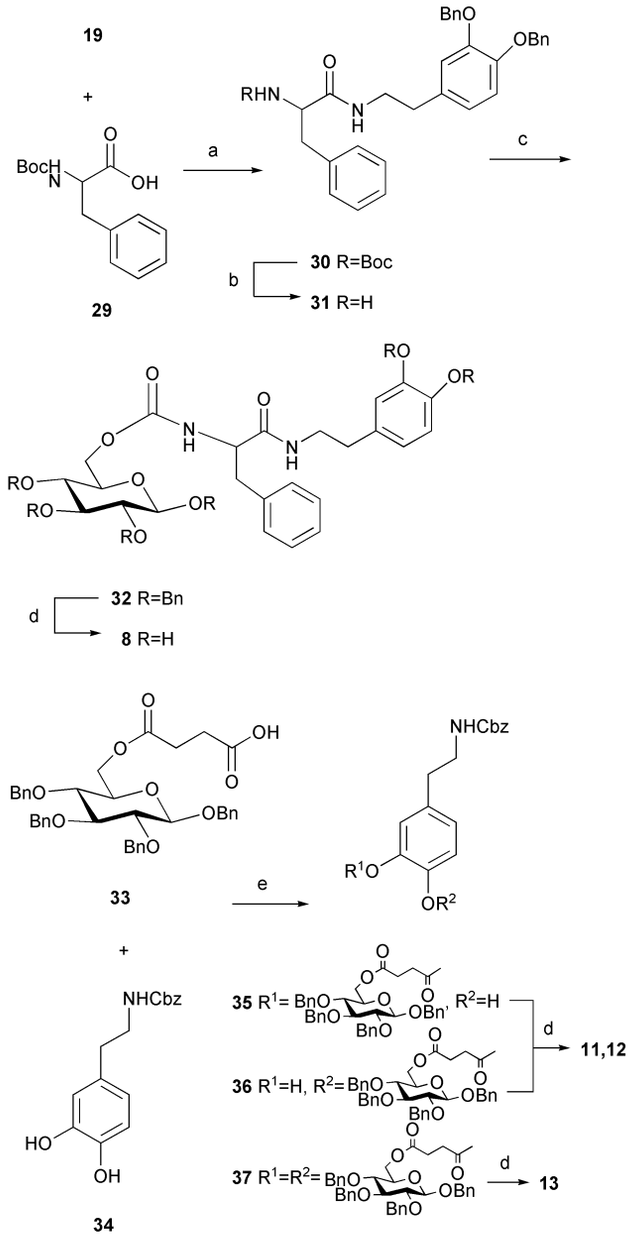
The synthesis of the glycoconjugate containing the phenylalanine unit (compound **8**) was performed using the intermediate **30**, previously obtained by treatment of **19** with *N*-Boc protected amino acid **29** (90%). Deprotection of the Boc group

View Article Online

Published on 07 February 2003. Downloaded by Christian Albrechts Universitat zu Kiel on 26/10/2014 16:57:45.



Scheme 1 (a) TrCl (1.5 equiv), DMAP (0.2 equiv), pyridine, 60 °C; (b) BnBr (3.3 equiv), NaH (3.7 equiv), DMF; (c) *p*-TsOH (0.2 equiv), CH_2Cl_2–MeOH; (d) *N*,*N*-carbonyldiimidazole (1.2 equiv), dioxane; (e) **19** (0.85 equiv), Et_3N, THF, 80 °C; (f) H_2, Pd/C, AcOEt–MeOH–toluene 4 : 3 : 3; (g) *N*,*N*-carbonyldiimidazole (1.2 equiv), THF; (h) *N*,*N*-carbonyldiimidazole, Et_2O; (i) $(Bu_3Sn)_2$ (1 equiv), toluene, 120 °C, 2 h, then, *N*,*N*-carbonyldiimidazole (2 equiv), rt.



Scheme 2 (a) **19** (1 equiv), **29** (1 equiv), Et_3N (4 equiv), BOP (1.1 equiv), THF; (b) TFA, CH_2Cl_2; (c) **18** (1.2 equiv), Et_3N, THF, 80 °C; (d) H_2, Pd/C, AcOEt–MeOH–toluene 4 : 3 : 3; (e) **33** (1 equiv), **34** (0.66 equiv), diisopropylcarbodiimide (DIIC) (1.3 equiv), CH_2Cl_2.

in **30** followed by coupling with the glucose derivative **18** gave **32** (78%), which after debenzylation afforded **8**.

Finally, the phenol esters **11**–**13** were synthesized from the acid **33**, previously obtained by reaction of **17** with succinic anhydride (Scheme 2). Esterification of **33** with the *N*-Cbz protected DA **34** gave the diester **37** and a mixture of monoesters **35** and **36**. Hydrogenolysis of the benzyl groups on **37** gave target **13**, and the same treatment on **35**/**36** afforded a mixture of monoesters **11** and **12**.

Stability studies

To determine the stability in physiological media and the ability to deliver DA after brain uptake, some of the compounds were incubated in buffer (pH 7.0), rat plasma, and rat brain extracts. The progress of the incubations were monitored by HPLC (UV detector, λ = 280 nm) analyzing the disappearance of the glycoconjugate and the formation of DA. Table 1 reports the half-life ($t_{1/2}$) of the compounds in the different incubation media, together with that of DA. For comparative purposes the previously reported[11] results of **1**, **9**, and **10** are also included in the Table. Considering the different linkages established between sugar and DA, the latter should be released after hydrolysis of ester (**11**, **12**, and **13**), amide (**1**), glycosidic (**9**, and **10**), or carbamate (**4**, **6**, and **8**) bonds.

Most of the compounds were more stable in buffer than DA, with the exception of compounds **11**, **12**, and **13** which showed labile phenol ester bonds. The monoesters **11** and **12** were hydrolyzed more rapidly than diester **13**, indicating that the unsubstituted phenol group must accelerate the hydrolysis of the adjacent ester by anchimeric assistance during the reaction. In the case of the succinamate **1** hydrolysis took place first on the ester bond, to give a succinamic acid which accumulated during the incubation period without producing DA. The high stability of the amide bond in **1** is in accordance with literature data on other prodrugs containing this type of bond.[20] Among the glycoconjugates containing a carbamate bond (**4**, **6**, **7**, and **8**), the compounds with the linkage at the anomeric position of the glucose (**6**,**7**) exhibited the highest rate of hydrolysis

View Article Online

Published on 07 February 2003. Downloaded by Christian Albrechts Universitat zu Kiel on 26/10/2014 16:57:45.

in all the media. Carbamate **8**, having an internal phenylalanine unit, was hydrolyzed in the presence of α-chymotrypsin from bovine pancreas (30 units per mL, $t_{½}$ 6.5 h). This result was expected given the primary specificity of this protease for an aromatic amino acid at the amino-terminal unit side of the scissile bond. On the other hand, the disappearance of **4** and **8** in plasma and brain extracts was not accompanied by DA formation. This suggests that **4** and **8** may be cleaved in a different way from hydrolysis of carbamate bond, which does not release DA (for instance, oxidation of the catechol ring).

The glycosides **9** and **10** showed an extraordinary stability in plasma. After 48 h of incubation no appreciable transformation of **9** and **10** was observed. Their high stability in this medium is probably due to the protection of the phenolic groups from oxidation to quinones by the glycosyl substituents, together with the fact that in the blood low levels of β-glucosidases are found. Indeed, β-glucosides of catecholamines occur in nature,[21] and such a conjugation has been interpreted as a protection from oxidation to quinones by the ubiquitous phenoloxidases in tissues. In brain extract, the glycosides were slowly hydrolyzed to give DA.

In conclusion, anomeric carbamates **6** and **7**, glycosides **9** and **10**, and diester **13** released DA after incubation in brain extract, in the following stability order: ester ⩾ carbamate > glycoside. In the case of the compounds **4** and **8** exhibiting a carbamate linkage, formation of DA was not observed after their incubation in plasma and brain extract.

Inhibition of glucose uptake by glycosyl dopamine derivatives

Next we examined the ability of the glycoconjugates to inhibit glucose transport using human erythrocytes, which express the same GLUT-1 transporter present in the BBB. Glucose uptake by erythrocytes was determined using ^{14}C-labeled glucose following a published procedure.[14,22]

In Table 2 the values of IC_{50} obtained for the different glycoconjugates are shown. Monoesters **11** and **12** were not assayed due to their low stability. For comparative purposes the 3-*O*-methyl-D-glucose (3-MG) was also tested.

Compounds with the substitution at the C-1 position of glucose were moderate or weak inhibitors depending on both the anomeric configuration and the type of linker used. The β-configured carbamate **7** was a better inhibitor than the α-anomer **6**, and both were more effective than glucopyranoside **9** and succinamate **3**.

Previous observations suggest that increasing the substituent size at the C-3 position of glucose caused a reduction in affinity.[23] However, compound **5**, in which DA is forming a carbamate linkage with the hydroxyl HO-3 of glucose showed an IC_{50} value of 60.3 mM, similar to that obtained with the less bulky 3-MG. Nevertheless, the presence of an aromatic ring in **5** could produce additional interactions with the protein.

Glucose derivatives substituted at the C-6 position showed the highest affinities for the GLUT-1. Thus, 6-*O*-carbamates **4** and **8** had IC_{50} values of 1.5 and 2.2 mM, respectively, and the succinamate **1** had a value of 12.1 mM. The diester **13**, however, was a poor inhibitor of glucose uptake probably due to its large size. The high affinities of the C-6 modified glucoses are consistent with a proposed model[23,24] of glucose transport that involves a hydrophobic site near the 6-position of bound glucose, which can accommodate relatively bulky and apolar substituents. In our compounds there could be a stabilizing interaction of the aromatic ring of the DA unit with a hydrophobic residue in the active site of the protein. Moreover, the difference of inhibitory effect between carbamate **4** and succinamate **1** suggests that the distance of the aromatic ring to the C-6 position of the sugar is important for activity.

Table 2 IC_{50} values of the glycoconjugates for the inhibition of glucose uptake

Compound	Linkage at the hexose	IC_{50}/mM
3	C-1 (β)	>100.0
6	C-1 (α)	50.0
7	C-1 (β)	31.1
9	C-1 (β)	>100.0
5	C-3	60.3
3-MG	C-3	51.2
1	C-6	12.1
4	C-6	1.5
8	C-6	2.2
13	C-6	76.8
2	C-6	>100.0

The inhibition constant K_i was determined with the best inhibitor, carbamate **4**. The K_i (0.791 ± 0.058 mM) for **4** was almost 15 times lower than the K_M (11.0 mM) of D-glucose. Therefore, it appears that **4** binds more effectively to the carrier than glucose itself.

Glucose uptake inhibition was dependent on the nature of the sugar since the galactose derivative **2** was a poor inhibitor (IC_{50} > 100.0 mM). It has been suggested that HO-4 of glucose establishes a hydrogen bond with the transporter. Given that galactose is the epimer at the C-4 position of glucose, this interaction may not be possible.[23]

Conclusions

For the targeted delivery of dopamine into the CNS using the glucose transport system, the glycosyl dopamine derivatives must show affinity for the GLUT-1 carrier and a good stability balance in the periphery and the brain. The results of glucose uptake inhibition by the glycoconjugates indicate that except for glucose derivatives substituted at position C-6, all other modifications of the sugar gave compounds with moderate or no binding to the carrier. Glycosides **9** and **10**, although fulfilling the prodrug criteria since they exhibited an extraordinarily high stability in plasma and a sustained release of DA in brain extract, can be considered unsuitable candidates due to their lack of affinity for GLUT-1. On the other hand, glycoconjugates with an ester linkage (**10**–**12**) were found too labile and may be hydrolyzed in the periphery. Carbamate derivatives **4**, **6**, and **7** seem to be the prodrugs of choice. Compound **4** showed the best affinity for GLUT-1, even higher than glucose itself. However, when **4** was incubated in brain extract no DA was observed, probably as a consequence of oxidation of the catechol ring occurring more rapidly than hydrolysis of the carbamate bond. Better results gave the anomeric carbamate **6** and **7**. They showed a moderate affinity for GLUT-1, adequate stability in plasma and released DA when incubated in brain extract.

In conclusion, this work presents efficient synthetic routes to new glycosyl dopamine derivatives and has provided structure–activity data about the interaction of substituted glucose to the transporter GLUT-1 that may pave the way for drug delivery for the treatment of CNS or other diseases.

Experimental

Detailed information on the preparation of all derivatives and the biological assays is deposited as Electronic Supplementary Information (ESI). †

Acknowledgements

Financial support by Ministerio de Ciencia y Tecnología (grant BQU2001-1503 and fellowship to C.F.) is gratefully acknowledged.

View Article Online

References

1 Y. Madrid, L. F. Langer, H. Brem and R. Langer, *Adv. Pharmacol.*, 1991, **22**, 299–324.
2 Y. Takakura, K. L. Audus and R. Borchardt, *Adv. Pharmacol.*, 1991, **22**, 137–165.
3 K. L. Audus, P. J. Chikhale, D. W. Miller, S. E. Thompson and R. T. Borchardt, *Adv. Drug Res.*, 1992, **23**, 1–63.
4 I. Tamai and A. Tsuji, *J. Pharm. Sci.*, 2000, **89**, 1371–1388.
5 H. Lund-Andersen, *Physiol. Rev.*, 1979, **59**, 305–349.
6 S. A. Baldwin, *Biochim. Biophys. Acta*, 1993, **1154**, 17–49.
7 M. Mueckler, *Eur. J. Biochem.*, 1994, **219**, 713–725.
8 A. Barbeau, *Trends Pharmacol. Sci.*, 1981, **2**, 297–299.
9 C. D. Marsden and J. D. Parkes, *Lancet*, 1976, **1**, 292–296.
10 D. B. Calne, *New Engl. J. Med.*, 1984, **310**, 523–524.
11 C. Fernández, O. Nieto, E. Rivas, G. Montenegro, J. A. Fontenla and A. Fernández-Mayoralas, *Carbohydr. Res.*, 2000, **327**, 353–365.
12 R. Polt, F. Porreca, L. Z. Szabò, E. J. Bilsky, P. Davis, T. J. Abbruscato, T. P. Davis, R. Horvath, H. I. Yamamura and V. J. Hruby, *Proc. Natl. Acad. Sci. USA*, 1994, **91**, 7114–7118.
13 T. Halmos, M. Santarromana, K. Antonakis and D. Sherman, *Eur. J. Pharmacol.*, 1996, **318**, 477–484.
14 C. Uriel, M.-J. Egron, M. Santarromana, D. Scherman, K. Antonakis and J. Herscovici, *Bioorg. Med. Chem.*, 1996, **4**, 2081–2090.
15 E. Decoster, J.-M. Lacombe, J. L. Strefler, B. Ferrari and A. A. Pavia, *J. Carbohydr. Chem.*, 1983, **2**, 329–341.
16 T. Kappes and H. Waldmann, *Carbohydr. Res.*, 1998, **305**, 341–349.
17 D. B. A. de. Bont, R. G. G. Leenders, H. J. Haisma, I. Van der Meulen-Muileman and H. W. Scheeren, *Bioorg. Med. Chem.*, 1997, **5**, 405–414.
18 R. G. G. Leenders, K. A. A. Gerrits, R. Ruijtenbeek, H. W. Scheeren, H. J. Haisma and E. Boven, *Tetrahedron Lett.*, 1995, **36**, 1701–1704.
19 S. David and S. Hanessian, *Tetrahedron*, 1985, **41**, 643–663.
20 F. M. Menger and M. J. Rourk, *J. Org. Chem.*, 1997, **62**, 9083–9088.
21 T. L. Hopkins, T. D. Morgan, D. D. Mueller, K. B. Tomer and K. J. Kramer, *Insect Biochem. Mol. Biol.*, 1995, **25**, 29–37.
22 A. G. Lowe and A. R. Walmsley, *Biochim. Biophys. Acta*, 1986, **857**, 146–154.
23 J. E. G. Barnett, G. D. Holman and K. A. Munday, *Biochem. J.*, 1973, **131**, 211–221.
24 J. E. G. Barnett, G. D. Holman, R. A. Chalkley and K. A. Munday, *Biochem. J.*, 1975, **145**, 417–429.

Published on 07 February 2003. Downloaded by Christian Albrechts Universitat zu Kiel on 26/10/2014 16:57:45.