Pergamon

Bioorganic & Medicinal Chemistry Letters 10 (2000) 1125–1127

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

Syntheses and Properties of Photoactivatable Sugar Derivatives Designed to Probe the Sugar-Binding Site of Melibiose Permease

Yves Ambroise,[a] Charles Mioskowski,[a] Gérard Leblanc[b] and Bernard Rousseau[a,*]

[a]*Service des Molécules Marquées, Département de Biologie Cellulaire et Moléculaire, CEA/Saclay, 91191 Gif sur Yvette cedex, France*
[b]*Laboratoire de Physiologie des Membranes Cellulaires, LRC-CEA 16V, Université de Nice Sophia-Antipolis and CNRS (ERS 1253), 06238, Villefranche sur Mer cedex, France*

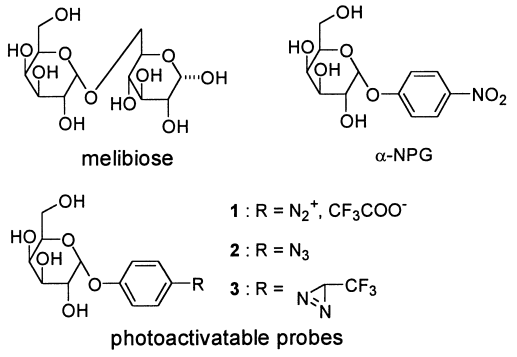
Received 31 January 2000; accepted 16 March 2000

Abstract—Three new photoreactive sugar analogues bearing an azido, a diazonium salt or a diazirine group as the photophore as well as a tritium atom were developed. Two of these new photoactivatable compounds gave excellent preliminary results, with a high affinity for the melibiose permease of *Escherichia coli*.

The melibiose permease (MelB) of *Escherichia coli*, encoded by melB, catalyzes the accumulation of a variety of α-galactosides (melibiose, *p*-nitrophenyl-α-D-galactopyranoside (α-NPG), Scheme 1) in association with Na^+, H^+ or Li^+ by a cation/sugar symport mechanism.[1,2] MelB is a highly hydrophobic membrane transporter of 473 amino acids[3,4] which is among the best studied co-transporters of the Na^+/solute symporter family.[5,6] A topological model of MelB consisting of 12 transmembrane α-helices has received independent support from a variety of experimental approaches, including phoA fusion analysis[7,8] and proteolytic digestion experiments.[9] The purification of large amounts of homogeneous MelB[4] has enhanced the prospects for structural studies using, for example, spectroscopy[10,11] and crystallography.[12]

Another potential means of studying MelB carrier structure is to label the protein in situ. Active-site-directed labeling of proteins, and particularly photo-dependent labeling, is a strategy of choice to identify the domains and/or residues directly participating in the substrate-protein interaction.[13]

This approach requires chemical engineering of photoreactive substrates displaying good affinity. Among compounds transported by MelB, *p*-nitrophenyl-α-D-galactopyranoside (α-NPG) is a high-affinity ligand (K_d = 0.6 μM).[14] Examination of the structural requirements for ligand binding suggests that photoactivatable probes should have an α-galactopyranoside unit linked to a hydrophobic part. We chose to modify the structure of the high-affinity ligand α-NPG by replacing the nitro moiety by a photoactivatable group. Three different compounds were designed bearing a diazonium salt unit **1**, an azido group **2** or a 3-(trifluoromethyl)-3-phenyldiazirine moiety **3** (Scheme 1).



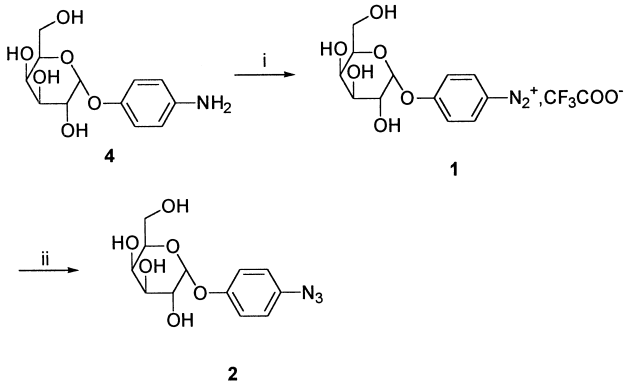
Scheme 1. Structures of melibiose, α-NPG and photoactivatable probes.

In the present study, we described the syntheses of these three photoactivatable compounds and their tritiated form. We also used radioactive probes to study their binding to melibiose permease. The photochemical properties of these compounds are also described.

*Corresponding author. Fax: +33-1-69-08-79-91; e-mail: bernard.rousseau@cea.fr

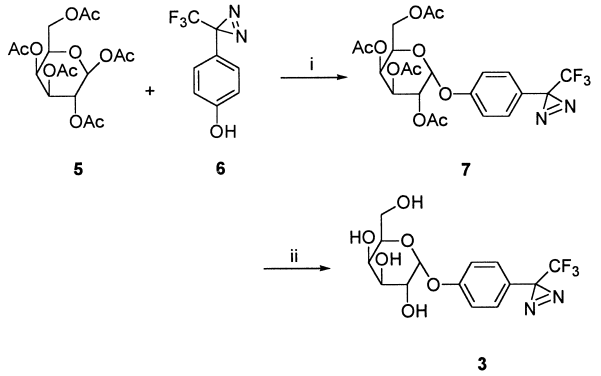
0960-894X/00/$ - see front matter
PII: S0960-894X(00)00180-3

Commercially available aniline **4** was used as the starting material for the synthesis of probes **1** and **2** (Scheme 2). The diazonium salt **1** was obtained from the amine by reaction of sodium nitrite in trifluoroacetic acid/water solution.[15] Diazonium salt was converted to aryl azide by the addition of sodium azide to the acidic diazonium salt solution.[16,17]



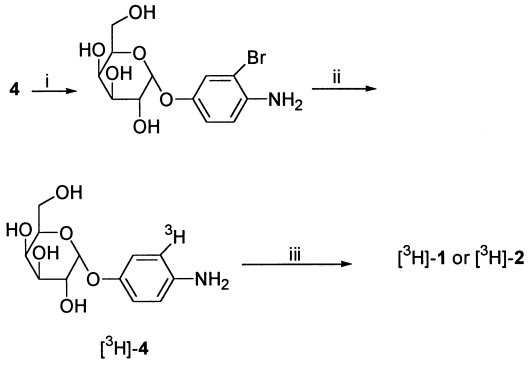
Scheme 2. (i) $NaNO_2$, H_2O/CF_3COOH 1/2, 0 °C, 30 min, 95%; (ii) NaN_3, room temperature, 2 h, 77%.

3-(Trifluoromethyl)-3-phenyldiazirine (**3**) was obtained by stannic tetrachloride-catalyzed glycosylation of β-D-galactose pentaacetate **5** by phenol **6** (Scheme 3).[18] This reaction was highly diastereoselective (de = 92%). The two isomers were easily separated by chromatography to provide the α isomer in 43% yield. Phenol **6** was synthesized in 6 steps as described by Hatanaka et al.[19] The acetylated α-galactoside **7** was de-*O*-acetylated by sodium methoxide to furnish the photoactivatable probe **3** in 90% yield.[20]



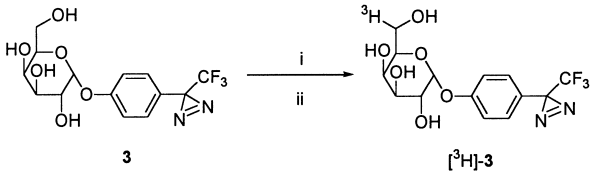
Scheme 3. (i) $SnCl_4$, CH_2Cl_2, room temperature, 16 h, 43%; (ii) CH_3ONa, CH_3OH, room temperature, 5 h, 90%.

The synthesis of the tritium-labeled photoactivatable probes follows a similar sequence of reactions. First, amine **4** was brominated by treatment with bromine in an acetic acid solution to provide the monobrominated compound in 91% yield (Scheme 4). The tritium was introduced by catalytic dehalogenation using tritium gas and Pd/C catalyst in water/methanol/triethylamine.[21] Under these condition, the incorporation of tritium was high (specific activity of 25 Ci/mmol). Tritiated aniline [^{3}H]-**4** was converted to tritiated photoactivatable probes [^{3}H]-**1** and [^{3}H]-**2** as described for unlabeled compounds.



Scheme 4. (i) Br_2, CH_3COOH, room temperature, 2 h, 91%; (ii) Pd/C, CH_3OH:H_2O 1:1, triethylamine, tritium gas, 1 atm, room temperature, 2 h, 50% after an HPLC purification; (iii) $NaNO_2$ or $NaNO_2$ followed by NaN_3.

[^{3}H]-**3** was prepared by chemoenzymatic synthesis (Scheme 5). Galactose analogue **3** was enzymatically oxidized to the 6-aldehyde in an incubation mixture containing phosphate buffer pH 7, catalase and galactose oxidase.[22] The crude aldehyde was reduced by [^{3}H]-KBH_4 to furnish, after HPLC purification, the labeled compound with a specific activity of 15 Ci/mmol.



Scheme 5. (i) Galactose oxidase, catalase, pH = 7, room temperature, 1 h; (ii) KB[^{3}H]$_4$, NaOH 0.01 N, 5 °C, 1 h, 74%.

The photoaffinity analogues **1**, **2** and **3** were evaluated for their binding to inverted membrane vesicles containing over-expressed histidine-tagged recombinant MelB under non-energized conditions by flow dialysis.[14] Binding constants, i.e., dissociation constant *K*d and maximal number of binding sites (Bmax), were calculated as described by Damiano et al.[14] The *K*d values for the photoactivatable compounds were 60 μM for compound **1**, 1 μM for azido **2** and 2 μM for diazirine **3**. Diazonium salt **1** exhibited moderate affinity for the transporter. Interestingly, both azido **2** and diazirine **3** are high affinity ligands with *K*d in the micromolar range comparable to the *K*d value of the high-affinity ligand α-NPG.

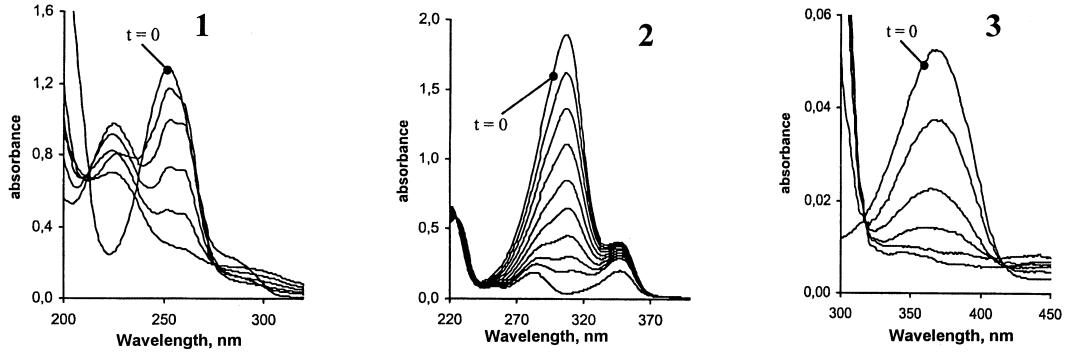


Figure 1. UV-spectral recording of the photoirradiation of **1**, **2** and **3** at 18 °C. 100 μM of **1** or **2** or **3** in 50 mM Tris buffer, 50 mM NaCl, pH = 8. **1** λ = 250 nm, E = 5.10^{-5} mW/cm^2, 0, 10, 30, 60, 90 and 120 s. **2** λ = 300 nm, E = 10^{-5} mW/cm^{-2}, 0,30, 60, 90, 120, 150, 180, 210, and 240 sec. **3** λ = 366 nm, E = 1.5 mW/cm^{-2}, 0, 120, 300, 720, 1200, 2000 s.

These probes must have favorable photochemical properties if they are to be used as irreversible photochemical markers. Figure 1 shows the absorption spectra of probes **1**, **2** and **3** as well as their photodecomposition by irradiation in buffered medium. Irradiation of each compound led to its complete disappearance, and the observed isobestics are illustrative of a unique photodecomposition process.

In summary, three new photoaffinity analogues and their tritiated form have been prepared, in order to map the binding site of melibiose permease. Two of these photoactivatable compounds gave excellent preliminary results with a high affinity for the melibiose permease of *Escherichia coli*. These probes should also be useful for mapping the binding site of other sugar transporters that have a substrate specificity similar to that of MelB (e.g., lactose permease). Photoaffinity labeling experiments with MelB are currently underway and will be reported elsewhere.[23]

Acknowledgements

We are grateful to Morgane Le Bastard, Raymonde Lemonnier and Alain Valleix for skillful technical assistance.

References and Notes

1. Pourcher, T.; Bassilana, M.; Sarkar, H. K.; Kaback, H. R.; Leblanc, G. *Phil. Trans. R. Soc. Lond. B.* **1990**, *326*, 411.
2. Poolman, B.; Knol, J.; Van der Does, C.; Henderson, P. J.; Liang, W. J.; Leblanc, G.; Pourcher, T.; Mus-Veteau, I. *Mol. Microbiol.* **1996**, *19*, 911.
3. Yazyu, H.; Shiota-Niiya, S.; Shimamoto, T.; Kanazawa, H.; Futai, M.; Tsuchiya, T. *J. Biol. Chem.* **1984**, *259*, 4320.
4. Pourcher, T.; Leclercq, S.; Brandolin, G.; Leblanc, G. *Biochemistry* **1995**, *34*, 4412.
5. Reizer, J.; Reizer, A.; Milton, H. S. *Biochim. Biophys. Acta* **1994**, *1197*, 133.
6. Poolman, B.; Konings, W. N. *Biochim. Biophys. Acta* **1993**, *1183*, 5.
7. Pourcher, T.; Bibi, E.; Kaback, H. R.; Leblanc, G. *Biochemistry* **1996**, *35*, 4161.
8. Botfield, M. C.; Naguchi, K.; Tsuchiya, T.; Wilson, T. H. *J. Biol. Chem.* **1992**, *267*, 1818.
9. Gwizdek, C.; Leblanc, G.; Bassilana, M. *Biochemistry* **1997**, *36*, 8522.
10. Mus-Veteau, I.; Pourcher, T.; Leblanc, G. *Biochemistry* **1995**, *34*, 6775.
11. Maehrel, C.; Cordat, E.; Mus-Veteau, I.; Leblanc, G. *J. Biol. Chem.* **1998**, *273*, 33192.
12. Lacapere, J. J.; Stokes, D. L.; Mosser, G.; Rank, J. L.; Leblanc, G.; Rigaud, J. L. *Ann. N.Y. Acad. Sci.* **1997**, *834*, 9.
13. Kotzyba-Hibert, F.; Kapfer, I.; Goeldner, M. *Angew. Chem., Int. Ed. Engl.* **1995**, *34*, 1296.
14. Damiano-Forano, E.; Bassilana, M.; Leblanc, G. *J. Biol. Chem.* **1986**, *261*, 6893.
15. Characterization data for compound **1**: ^{1}H NMR (300 MHz, D_2O) δ 3.75 (m, 2H), 3.94 (dd, J = 2, 9 Hz, 1H), 4.12–4.15 (m, 3H), 6.08 (d, J = 2 Hz, 1H), 7.64 (d, J = 9 Hz, 2H), 8.58 (d, J = 9 Hz, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) 61.3, 68.0, 69.3, 69.6, 73.4, 98.2, 104.0, 116.5, 120.2, 162.5, 167.3; MS (electrospray) m/z 283 (M^+); HPLC, Zorbax-ODS, H_2O/CF_3COOH 100/0.1; V_R = 6.4 min.
16. Lamotte, H.; Dégeilh, F.; Rousseau, B.; Neau, P.; Ripoche, P. *Bioorg. Med. Chem. Lett.* **1993**, *3*, 2587.
17. Characterization data for compound **2**: ^{1}H NMR (300 MHz, D_2O) δ 3.90 (d, J = 7 Hz, 2H), 4–410.28 (m, 4H), 5.80 (d, J = 2 Hz, 1H), 7.27 (d, J = 9 Hz, 2H), 8.38 (d, J = 9 Hz, 2H); ^{13}C NMR (75 MHz, D_2O) 61.5, 68.6, 69.7, 69.9, 72.2, 98.4, 119.7, 120.6, 134.9, 153.9 MS (CI, NH_3) m/z 315 (MNH_4^+); HPLC, Zorbax-ODS, H_2O:CH_3OH:CF_3COOH 80/20/0.1; V_R = 15.8 min.
18. Banoub, J.; Bundle, D. R. *Can. J. Chem.* **1979**, *57*, 2085.
19. Hatanaka, Y.; Hashimoto, M.; Kurihara, H.; Nakayama, H.; Kanaoka, Y. *J. Org. Chem.* **1994**, *59*, 383.
20. Characterization data for compound **3**: ^{1}H NMR (300 MHz, CD_3OD) δ 3.62 (d, J = 7 Hz, 2H), 3.78 (t, J = 7 Hz, 1H), 3.90 (m, 3H), 5.50 (d, J = 2 Hz, 1H), 7.11–7.21 (m, 4H); ^{13}C NMR (75 MHz, $CDCl_3$) 55.0, 114.0, 114.1, 120.7, 122.0, 127.9, 160.4; MS (CI, NH_3) m/z 382 (MNH_4^+); HPLC, Zorbax-ODS, H_2O:CH_3CN:CF_3COOH 80:20:0.1; V_R = 18.3 min.
21. Hadj-Hamdri, A.; Vidal-Cros, A.; Gaudry, M.; Sobrio, F.; Rousseau, B. *J. Labelled Compd. Radiopharm.* **1995**, *36*, 795.
22. Kennedy, E. P.; Rumley, M. K.; Armstrong, J. B. *J. Biol. Chem.* **1974**, *249*, 33.
23. Ambroise, Y.; Leblanc, G.; Rousseau, B. *Biochemistry* **2000**, *39*, 1338.