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Syntheses of [^{14}C]-detergents: octaethylene-glycol-[1-^{14}C]-dodecylether, [1-^{14}C]-dodecyl-β-D-maltoside and dibromo-analogues

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Summary

Octaethylene-glycol-dodecylether and dodecyl-β-D-maltoside are two widely used detergents in membrane protein studies. We describe here the synthesis of the ^{14}C-labelled brominated analogues, and of the ^{14}C-labelled forms. [1-^{14}C]-5,6-Dibromo-dodecylether was prepared by coupling [1-^{14}C]-(Z)-1-bromo-dodec-5-ene with octaethylene glycol followed by bromination. [1-^{14}C]-5,6-Dibromo-dodecyl-β-D-maltoside was synthesised from [1-^{14}C]-(Z)-dodec-5-en-l-ol via a coupling with α-bromohepta-O-acetyl-maltose followed by a deprotection step and bromination. Following similar methods, octaethylene-glycol-[1-^{14}C]-dodecylether and [1-^{14}C]-dodecyl-β-D-maltoside were also obtained. Copyright © 2001 John Wiley & Sons, Ltd.

Key Words: Barton's bromodecarboxylation; bromination; protein detergents; ^{14}C-detergents

Introduction

Detergents like dodecyl-β-D-maltoside or octaethylene-glycol-dodecyl ether provide a means of delineating the structure of biological membranes in order to purify and characterise their membrane protein

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components.[1–3] ^{14}C-labelling and gel equilibrium chromatography can be used to estimate the binding of the detergent to the protein and may provide a measure of the hydrophobic surface area of the membrane protein.[4]

The bromination of the aliphatic chain of these detergents does not seem to affect their physical properties, and it has been shown that such brominated detergents quench the fluorescence of tryptophan octyl ester,[5] a hydrophobic model of tryptophan in proteins.

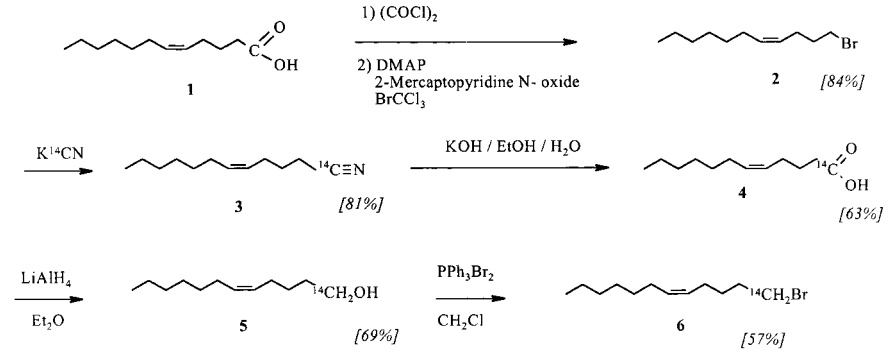
Spectroscopic quenching measurements can therefore provide some topological information, such as the location of the tryptophan residues in the transmembrane protein.[6]

The use of fluorescence quenching and brominated detergents points to a new method of measuring protein binding. This method can be validated by using ^{14}C-labelled detergents both brominated and otherwise to correlate the binding (thanks to radioactivity) with fluorescence quenching.

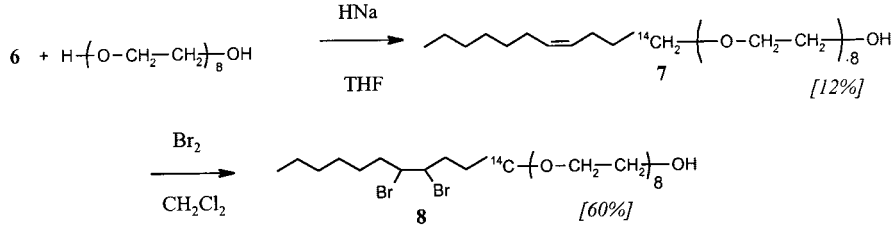
Results and discussion

As the two syntheses were highly similar and presented the same kinds of difficulties, we shall only describe the formation of the brominated form and the last steps of the ordinary detergent. The two brominated detergents we synthesised were characterised by a lateral chain containing 12 carbons. [1-^{14}C]-(Z)-dodec-5-enoic acid was therefore chosen as the key molecule. Its double bond allows the introduction of molecular bromine at the last step. Its carboxylic group can be simply reduced to the corresponding alcohol, which is also easily transformed to its bromo derivative by dibromotriphenylphosphorane.

To obtain [1-^{14}C]-(Z)-dodec-5-enoic acid **4**, we used a method consisting of Barton's bromodecarboxylation[7,8] of commercially available **1**, followed by nucleophilic substitution of the bromine by K^{14}CN in 81% radioactive yield. The hydrolysis of the cyano group afforded the expected [1-^{14}C]-(Z)-dodec-5-enoic acid **4** in 63% radioactive yield. [1-^{14}C]-(Z)-dodec-5-en-l-ol **5** was obtained by the reduction of the acid with lithium aluminium hydride[9] in 69% radioactive yield and dibromotriphenylphosphorane permitted the substitution of the alkoxy group by bromine on the labelled carbon[10] in 57% radioactive yield (Scheme 1).



Scheme 1.

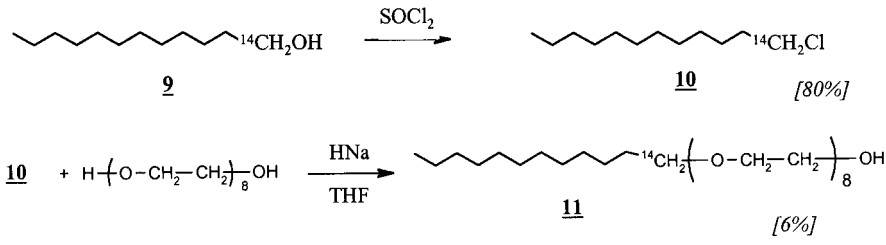


Scheme 2.

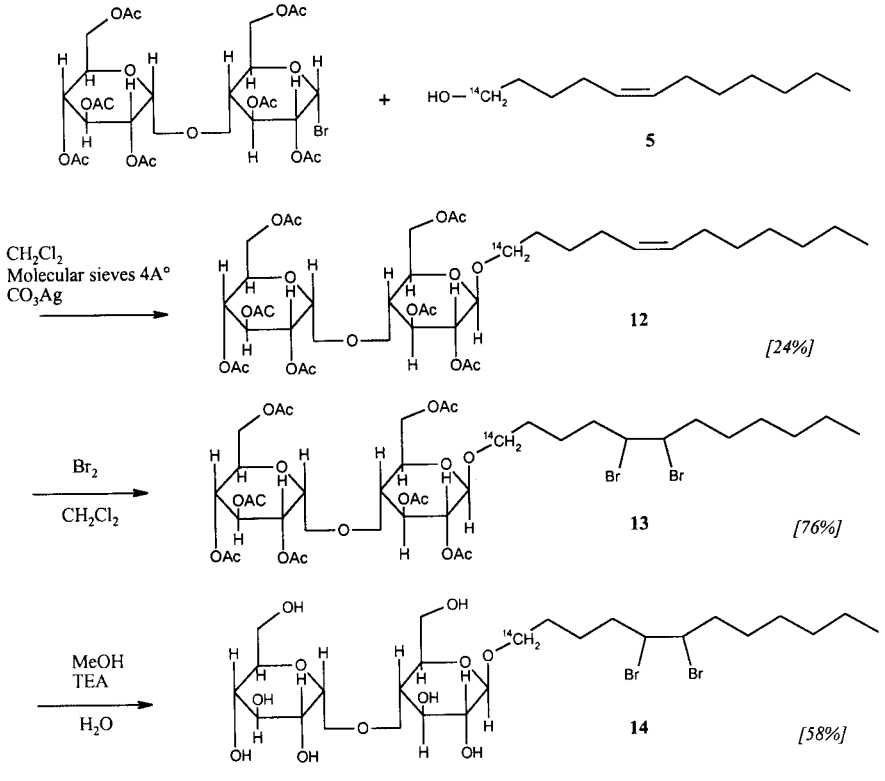
To obtain the octaethylene-glycol-[1-^{14}C]-5-6-dibromododecylether **8**, we adapted a common method of coupling between a bromo derivative and a polyethylene glycol using sodium hydride[11] (Scheme 2). The expected product also reacted with octaethylene glycol giving poly-condensation products, which lowered the yield to 12%.

The similar reaction described in the reference afforded a better yield (32.5%) as two equivalents of polyethylene glycol were used. Some attempts using an unlabelled 1-bromo-dodec-5-ene were conducted, but owing to the excess of octaethylene glycol the purification step was unsuccessful.

The same chemical sequence (Scheme 3) was applied to dodecanoic acid to form [1-^{14}C]-dodecan-1-ol **9** (40% yield). **9** was then submitted to chlorination, instead of bromination, with thionyl chloride to synthesise [1-^{14}C]-l-chlorododecane **10** in 80% yield with the aim of increasing the coupling yield. This saturated chlorinated chain was even less reactive with octaethylene-glycol than its unsaturated brominated analogue and octaethylene-glycol-[1-^{14}C]-dodecylether **11** was obtained in only 6% yield.

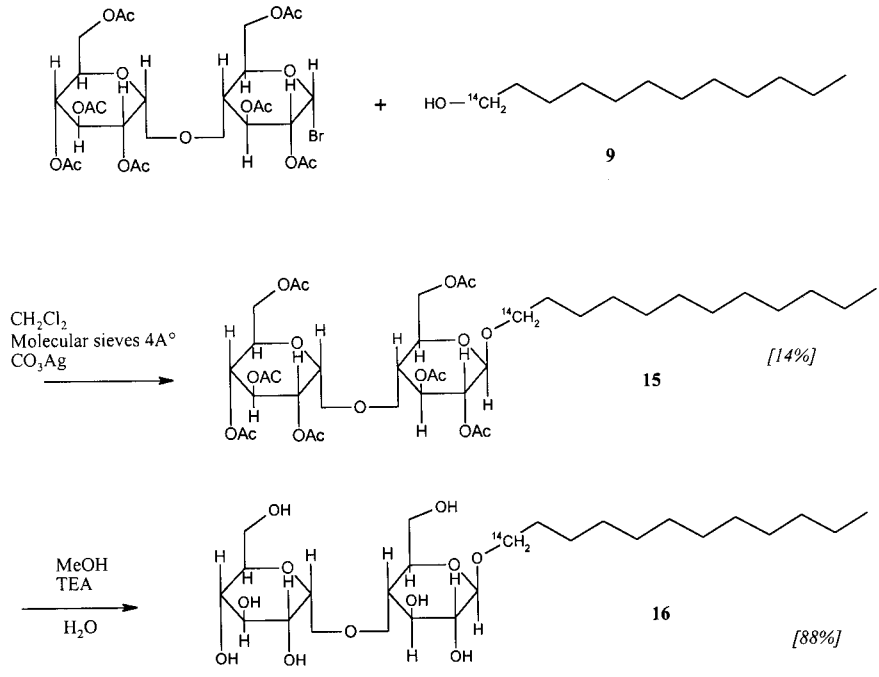


Scheme 3.



Scheme 4.

[1-^{14}C]-5,6-dibromododecyl-β-D-maltoside **14** was prepared from [1-^{14}C]-(Z)-dodec-5-en-1-ol **5** and α-bromohepta-O-acetyl-maltose by a nucleophilic substitution procedure using silver carbonate.[12,13] The low yield (24%) of this step was balanced by a good selectivity, which afforded only the β form of the expected product. After a simple bromination (76% yield) and a deprotection step (58% yield), the expected labelled detergent was obtained as detailed in Scheme 4.



Scheme 5.

The same method was applied to form [1-^{14}C]-dodecyl-β-D-maltoside **16** from [1-^{14}C]-dodecan-1-ol **9** (Scheme 5).

Conclusion

The relatively rapid synthesis of [1-^{14}C]-(Z)-dodec-5-enoic acid and [1-^{14}C]-dodecanoic acid via Barton's bromodecarboxylation of the unlabelled forms yielded the four expected products in only 18 steps. Furthermore, the selectivity of the chosen pathway permitted us, in the case of [1-^{14}C]-5,6-dibromododecyl-β-D-maltoside and [1-^{14}C]-dodecyl-β-D-maltoside, to selectively obtain the β epimer.

Experimental

K^{14}CN was from Amersham Pharmacia Biotech. Other starting materials and chemical reagents were from Aldrich. Sodium hydride was obtained by 60% dispersion in mineral oil after washing with

tetrahydrofuran in a dry glove box. Flash chromatography was performed using silica gel 60 (0.040–0.063 mm) from Merck. All solvents were from SDS. Toluene was dried over 4 Å molecular sieves, diethyl ether and tetrahydrofuran were distilled from sodium benzophenone ketyl and dichloromethane was dried by distillation over calcium hydride. Analytical TLC was performed with silica gel 60F254 (Merck) and visualisation was carried out with iodine and the radioactivity detected using a Berthold's LB 2880-1 automatic TLC-linear analyser fitted with an LB 511-chromatography data system. NMR spectra were recorded on a Bruker AC 300 spectrometer (7.05 T; 300.13 MHz (^{1}H)). Specific activity was determined by mass spectrometry (DCI/NH_3) on a Finnigan instrument (Model 4600). GC/MS analyses were effected using an HP 6890 Series gas chromatograph system coupled to an HP 5973 mass selective detector with the following operating conditions: column: HP-5MS (30 m × 0.25 mm × 0.25 μm). Column temperature: 70°C (5 min) to 300°C (5 min) at 15°C/min. Carrier gas: He (0.9 ml/min). Injector temperature: 250°C.

*(Z)-1-Bromo-undec-4-ene (**2**)*

Oxalyl chloride (10 g, 78.8 mmol) was added to a mixture of **1** (5 g, 25.2 mmol) in 40 ml of dry toluene stirred under argon. The mixture was stirred at room temperature for 15 h under an argon atmosphere, concentrated under vacuum and diluted three times with 30 ml of dry toluene before concentration to remove oxalyl chloride residue. The product was used without further purification. 2-Mercaptopyridine-*N*-oxide, sodium salt hydrate (4.9 g, 33.3 mmol) and 4-dimethylaminopyridine (348 mg, 2.8 mmol) were dried under vacuum overnight and introduced with 75 ml of bromotrichloromethane into a three-necked flask in a dry glove box. Under the ventilated hood, the flask was fitted with a reflux condenser with a system providing a slow flow of argon. The slurry was stirred and heated under reflux (105°C) for 30 min.

In a dry glove box the raw acid chloride was diluted with 25 ml of bromotrichloromethane and stored in a syringe. This mixture was added through a septum to the reaction flask over 30 min. Bubbles and an orange colouration were observed and the reaction was stirred under reflux for 2 h.

A brown product appeared as a side product of the reaction. After cooling at room temperature the reaction mixture was filtered in order to eliminate the brown residue. The crude product was then

concentrated under vacuum and flash chromatographed on silica gel with hexane as eluent to give **2** (4.92 g, 14.77 mmol, 84% yield). The purified product contained a little (1.5%) (Z)-1-chloro-undec-4-ene. No further purification was attempted as this side product reacted with [^{14}C]-potassium cyanide in the same manner as **2**.

GC/MS: (Z)-1-chloro-undec-4-ene (1.5%; rt = 11.17 min; m/z = 188–190 M^+), **2** (98%; rt = 11.99 min; m/z = 232–234 M^+).

*(Z)-Dodec-5-enenitrile (**3**)*

In a 25 ml-flask, 93 mg of **2** (0.4 mmol), 23.3 mg of [^{14}C]-potassium cyanide (55 mCi/mmol, 0.375 mmol, 20 mCi) and 10 ml of dimethyl sulphoxide were stirred for 5 h at 80°C. After cooling, 100 ml of water was added and the aqueous layer extracted two times with 100 ml of diethyl ether. The mixture was dried under vacuum and purified using flash-chromatography on silica gel with hexane/ethyl acetate (90/10) as eluent. 16.3 mCi (55 mCi/mmol, 0.30 mmol) of **3** was obtained (81% yield).

TLC:hexane/ethyl acetate (95/5). Rf = 0.24.

*[1-^{14}C]-(Z)-dodec-5-enoic acid (**4**)*

In a 25 ml-flask, 16.3 mCi of **3** (0.30 mmol), 10 ml of a 30% aqueous potassium hydroxide solution and 10 ml of absolute ethanol were stirred for 14 h at 80°C. The reaction was followed by TLC on silica gel with hexane/diethyl ether/acetic acid (85/15/1). The mixture was then acidified using 20 ml of 5 N HCl and extracted with 100 ml of diethyl ether. The purification by flash chromatography using the same system as the TLC afforded 10.2 mCi (55 mCi/mmol, 0.18 mmol) of pure **4** (63% yield).

TLC:hexane/diethyl ether/acetic acid (85/15/1). Rf = 0.22.

The labelled product was diluted with 189 mg of pure unlabelled compound and the specific activity of the mixture was measured by mass spectrometry.

MS (DCI/NH_3): m/z(%): 216(100), 217(20), 218(18). Specific activity = 9 mCi/mmol.

*[1-^{14}C]-(Z)-Dodec-5-en-1-ol (**5**)*

In a three-necked flask under argon equipped with a magnetic stirrer, 224 mg (9 mCi/mmol, 10.2 mCi, 1.13 mmol) of **4** and 10 ml of anhydrous

diethyl ether were cooled in an ice bath. Gradual addition of 110 mg (2.89 mmol, 2.5 eq.) of lithium aluminium hydride powder produced a bubbling of the mixture which was then stirred overnight at room temperature. Excess reducing agent was eliminated by slow addition of ethyl acetate and solvents were evaporated. The crude product was purified on silica gel with hexane/diethyl ether (66/33) giving 7.07 mCi of **5** (0.69 mg, 69% yield).

GC/MS: rt = 12.17 min; m/z = 166(M-18).

TLC:hexane/diethyl ether (70/30). Rf = 0.23.

*[1-^{14}C]-(Z)-1-Bromo-dodec-5-ene (**6**)*

Dibromotriphenylphosphorane (295 mg, 0.7 mmol, 1 eq.) and 5 ml of dried dichloromethane were placed in an ice-cooled three-necked flask fitted with a rubber septum. 7.07 mCi of **5** was diluted in 5 ml of dried dichloromethane, stored in a syringe and slowly added, with magnetic stirring, to the reaction mixture. After addition, the mixture was stirred at 0°C for 15 min and then 20 ml of water was added. The aqueous layer was extracted twice with 20 ml of dichloromethane and the combined organic layers were concentrated under vacuum. The residue was purified by chromatography on silica gel with hexane/diethyl ether (70/30) giving 2.2 mCi of **6** and 3.9 mCi of **5**. The recycling of the unreacted alcohol afforded 1.85 mCi of the expected product. 57% total yield.

GC/MS: rt = 15.39 min; m/z =246–248 M^+.

TLC:hexane/diethyl ether (70/30). Rf = 0.30.

*Octaethylene-glycol-[1-^{14}C]-(Z)-dodec-5-ene (**7**)*

In an oven-dried three-necked flask, a mixture of octaethylene-glycol (164.5 mg, 0.44 mmol, 1eq), sodium hydride (18 mg, 0.74 mmol, 1.7 eq.) and 10 ml of tetrahydrofuran, was stirred under argon at room temperature. After 1 h, **6** (4 mCi, 0.44 mmol) dissolved in 2 ml of tetrahydrofuran and stored in a syringe was gradually added through a rubber septum to the reaction flask. After 24 h at room temperature the solution was dilulted with 50 ml of water and extracted four times with 20 ml of diethyl ether. The organic solution was concentrated under vacuum and the crude material was flash-chromatographed, eluting with pure acetone. After combination and evaporation of the collected fractions, 476 μCi (0.05 mmol, 12% yield) of **7** was obtained.

TLC:hexane/acetone (60/40). Rf = 0.15.

*Octaethylene-glycol-[1-^{14}C]-5,6-dibromododecylether (**8**)*

To a cooled (0°C) mixture of **7** (476 μCi, 0.05 mmol) in 10 ml of dry dichloromethane, a solution of bromine in dichlormethane (1 mg/ml) was added dropwise over a 15-min period until a yellow colour persisted. The reaction mixture was then concentrated under reduced pressure and the residue purified by flash chromatography on silica gel with pure acetone giving 287 μCi (0.03 mmol, 60% yield) of pure **8**.

TLC:reversed phase (KC18F, Whatman), methanol/water (90/10). Rf = 0.32.

HPLC: column: Zorbax SB C18 (250 × 4.6 mm). Solvent system: acetonitrile/water (70/30).

Flow rate: 1.5 ml/min. Rt = 8.98 min. Chemical and radiochemical purity > 99%.

MS (DCI/NH_3): *m/z* (%): 714 (100), 715 (67), 716 (43), 717 (15).

*[1-^{14}C]-(Z)-Dodec-5-ene-β-*D*-acetyl-maltoside (**12**)*

5 (3 mCi, 0.24 mmol, specific activity = 12.8 mCi/mmol, new synthesis) was dissolved in 5 ml of dry dichloromethane and introduced, under argon, in an oven-dried three-necked flask protected from light with an aluminium film. α-Bromohepta-O-acetyl-maltose (168 mg, 0.24 mmol, 1 eq.) was added and after 5 min 60 mg of powered, freshly activated 4 Å molecular sieves were added to the mixture with magnetic stirring. Silver carbonate (92.6 mg, 0.336 mmol, 1.4 eq.) was introduced after 30 min and the progress of the reaction was followed by TLC. After 12 h, only 40% of the starting material had been consumed and one more equivalent of both α-bromohepta-O-acetyl-maltose and silver carbonate were added. After 36 h no side products appeared and 10% more product had been formed. One more equivalent of each reactant was charged and after 72 h the reaction was stopped by filtration as the TLC control showed a 60% conversion into the expected product. The organic layer was first washed with a saturated solution of sodium hydrogen carbonate and then washed with a saturated solution of sodium chloride.

After evaporation of the solvent, the crude product was flash chromatographed on silica gel with diethyl ether/hexane (80/20), giving 400 μCi of the starting alcohol and 711 μCi (0.05 mmol, 24% yield) of the expected product **12**.

TLC:diethyl ether/hexane (80/20). Rf = 0.20.

*[1-^{14}C]-5,6-Dibromo-dodecyl-β-*D*-acetyl-maltoside (**13**)*

This product was obtained by following the same procedure as described for **8**. The final chromatographic purification was performed on silica gel with diethyl ether/hexane (80/20). 545 μCi (0.04 mmol, 76% yield) of the pure material **13** was recovered.

TLC:diethylether/hexane (80/20). Rf = 0.15.

MS (DCI/NH_3): *m/z* (%): 978 (50), 979 (72), 980 (100), 981 (77), 982 (84).

*[1-^{14}C]-5,6-Dibromo-dodecyl-β-*D*-maltoside (**14**)*

A mixture of methanol/water/triethylamine (20/10/1) was prepared. 20 ml of this solution was inserted, with magnetic stirring, into a flask containing the previous protected product **13**. After 12 h, the reaction mixture was concentrated and flash chromotographed on silica gel with pure acetone. 320 μCi (0.025 mmol, 58% yield) of [1-^{14}C]-5,6-dibromo-dodecyl-β-D-maltoside was obtained.

TLC:reversed phase (KC18F, Whatman), methanol/water (80/20). Rf = 0.25.

HPLC:column: Zorbax SB C18 (250 × 4.6 mm).

Solvent system: acetonitrile/water (75/25). Flow rate: 1.5 ml/min. Rt = 9 min. Chemical and radiochemical purity > 99%.

MS (DCI/NH_3): *m/z* (%): 687 (53), 689 (100), 690 (33), 691 (53).

*[1-^{14}C]-1-Chloro-dodecane (**10**)*

9 (54 mCi) and 10 ml of thionyl chloride were placed in an oven-dried, ice-cooled, one-necked flask equipped with a magnetic stirrer and a reflux condenser. After 5 min of stirring at 0°C, the mixture was heated at 100°C for 4 h. After washing with 30 ml of water, the aqueous layer was extracted twice with 20 ml of diethyl ether and the combined organic layers were concentrated under vacuum. The residue was purified on silica gel with hexane/diethyl ether (60/40) giving 43 mCi of **10**.80% yield.

TLC:hexane/diethyl ether (60/40). Rf = 0.42.

*Octaethylene-glycol-[1-^{14}C]-dodecylether (**11**)*

2.45 MCi (6% yield) of **11** was obtained from 10 as described for its unsaturated analogue **7**.

TLC:hexane/acetone (50/50). Rf = 0.20.

*[1-^{14}C]-dodecyl-β-*D*-acetyl-maltoside (**15**)*

25 MCi (14% yield) of **15** was synthesised from 177 mCi of **9** following the procedure described for **12**.

TLC:diethyl ether/hexane (70/30). Rf = 0.32.

*[1-^{14}C]-dodecyl-β-*D*-maltoside (**16**)*

The same treatment as detailed for **14** afforded 22 mCi (88% yield) of the expected product **16**.

HPLC: column ODS 2 (250 × 4.6 mm). Solvent system: methanol/water (85/15). Flow rate: 1.5 ml/min. Rt = 5.39 min.

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