Synthesis of some *N*- and *S*-glycosides of D-galactose bearing hydrazinocarbonyl and diazomethylcarbonyl functions in the aglycon

GILLES DUPUIS AND BENOÎT LECLAIR

Département de Biochimie, Université de Sherbrooke, Sherbrooke (Qué.), Canada J1H 5N4

Received March 12, 1982

GILLES DUPUIS and BENOÎT LECLAIR. Can. J. Chem. **60**, 2531 (1982).

We describe the synthesis of *N*- and *S*-glycosides derived from D-galactopyranose in which the aglycon bears certain reactive groups. In a first series, the anomeric carbon is linked to an amino group that is acylated by a functionalized succinic acid chain. The terminal group of the aglycon moiety is a hydrazide function which can be converted by ultraviolet light irradiation into an azide and a nitrene. Alternatively, the terminal group is a diazoketone function which can be converted into a carbene, by ultraviolet light irradiation. A second series comprises glycosides of 1-thio-β-D-galactopyranose. The aglycon consists of a 6-carbon chain with a carboxylic end group. The latter has been converted into a hydrazide and diazoketone function. We show that the diazo group of the diazoketones (compounds **5** and **12**) is susceptible to decomposition by ultraviolet irradiation, being nearly quantitatively decomposed after 3 minutes. These compounds add to a growing list of hexose derivatives which can be used in the field of photoaffinity-labeling of the sugar binding sites of certain lectins and of hexose transport systems, or to prepare modified proteins or ligands for affinity chromatography.

GILLES DUPUIS et BENOÎT LECLAIR. Can. J. Chem. **60**, 2531 (1982).

Nous décrivons la synthèse de *N*- et *S*-glucosides dérivés du D-galactopyranose chez lesquels la portion aglycone porte certains groupements réactifs. Dans la première série de dérivés, le carbone anomérique est lié à un groupement aminé qui est acylé par un acide succinique fonctionnalisé. D'une part, la partie terminale de la portion aglycone est une fonction hydrazide qui peut être convertie en azide et en nitrène, après irradiation par la lumière ultraviolette. D'autre part, le groupement terminal de l'aglycone est une diazocétone qui peut être convertie en un dérivé carbène, par irradiation sous lumière ultraviolette. Dans la deuxième série de dérivés, nous avons préparé des glycosides du 1-thio-β-D-galactopyranose. La portion aglycone est une chaîne de six unités de carbone se terminant par un groupement carboxyle. Ce dernier a été converti en hydrazide ou en diazocétone. Nous montrons que le groupement cétodiazo des composés **5** et **12** est décomposé par irradiation ultraviolette. Trois minutes d'irradiation suffisent à décomposer les produits. Les composés décrits ici s'ajoutent à la liste de dérivés d'hexoses déjà décrits dans la littérature, lesquels peuvent être utilisés comme photo-marqueurs, du site de fixation de glucides chez des lectines et dans le système de transport des hexoses ou pour préparer des protéines modifiées ou des colonnes d'affinité ayant des glucides comme affinants.

Introduction

The method of affinity-labeling has achieved success for determination of the nature of the amino acid residues located in the environment of the "active site" of biologically active proteins such as enzymes, antibodies, and others (1–3). This method is based on the synthesis of suitable reactive derivatives of the natural ligand or of molecules structurally related to it. The choice of derivatives is dictated by several factors such as reactivity, specificity of labeling, and stability in the medium used for reaction.

The design of affinity-labeling reagents has been extended to light-sensitive reagents (photoaffinity-labeling). This approach was pioneered by Westheimer and his group in their study of the active site of chymotrypsin (4). These workers used a diazo derivative which yielded a highly reactive nitrene after irradiation by light (5, 6).

Other types of derivatives have also been prepared. The ones used mainly are diazo and chloromethyl ketone derivatives or other activated forms of the carbonyl function (7, 8).

The choice of ligand is dictated by the properties of the protein to be modified. For instance in the case of lectins, a class of proteins which can specifically recognize monosaccharides or polysaccharidic structures (9, 10), synthesis of labeling probes would involve preparation of suitably modified glycosides. From a synthetic standpoint, the synthesis of monosaccharide derivatives appears to be the simplest route for affinity-labeling of lectins.

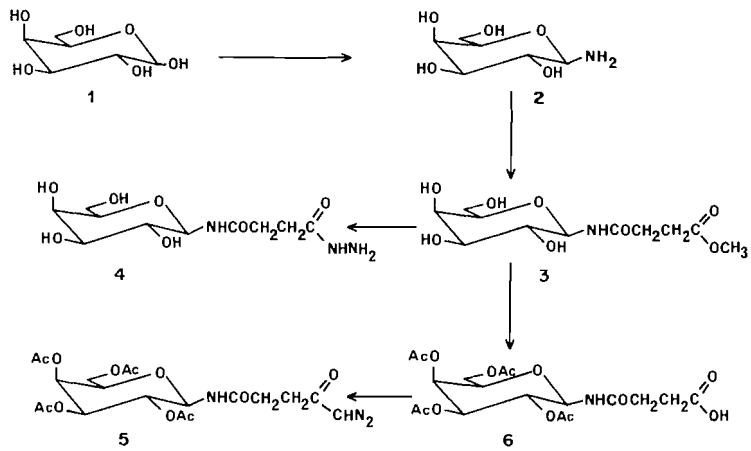
We have selected D-galactose as a model hexose and report the synthesis of derivatives wherein the anomeric carbon bears an aminoacyl chain or a *S*-alkyl chain. The compounds possess a hydrazide group which should be easily transformable into the corresponding azide (11) and nitrene groups (12), or a diazoketone function which can be transformed into the corresponding carbene, by ultraviolet irradiation (13).

Results and discussion

Synthesis of 1-amino-β-D-galactopyranosides

β-D-Galactopyranosylamine **2** (14) was *N*-acylated to give the methyl succinamido derivative **3**. This was done in a manner similar to the one described by Gordon *et al.* (15) for the synthesis of the ε-aminocaproyl derivative of β-D-galactopy-

0008-4042/82/202531-06$01.00/0



SCHEME 1

ranosylamine. Briefly, the mixed anhydride prepared from monomethyl succinate and isobutyl chloroformate constituted the necessary acylating agent which was allowed to react with compound **2** suspended in DMF.[1] Compound **3** was obtained crystalline in 70% yield. Attempts to prepare the analogous derivative of α-D-galactopyranosylamine failed. Treatment of compound **3** for two hours with hydrazine in refluxing methanol afforded a nearly quantitative yield of the crystalline hydrazide **4**.

In order to prepare the free acid from compound **3**, the latter was saponified by treatment with a methanolic solution of KOH, under reflux, for a brief period of time. Under these conditions, the methyl ester group was quantitatively cleaved, as evidenced by ^{1}Hmr and ir spectra, whereas the amide bond remained intact. The oily compound was peracetylated with acetic anhydride in pyridine to afford compound **6**.

Several methods have been used to introduce the diazoketone function. These include conversion of the free acid into an acyl halide (16–20) and subsequent reaction with diazomethane or reaction of a mixed anhydride with diazomethane (21). We selected the mixed anhydride method because of simplicity of its application to **6**. The triethylammonium salt of **6** was treated with isobutyl chloroformate, and the ensuing mixed anhydride was mixed with an ether solution of freshly prepared diazomethane. Stirring the mixture at room temperature (overnight) gave only a low yield of conversion of the anhydride, as evidenced by the strong ir band at 1760 cm^{-1}. The mixture was therefore gently heated under reflux for 18 h, in the dark. An ir spectrum revealed complete disappearance of the 1760 cm^{-1} absorption band and appearance of the characteristic diazo band at 2200 cm^{-1} and a band at 1640 cm^{-1} for the diazo ketone structure. These spectral features suggested a successful conversion of **6** into **5**.

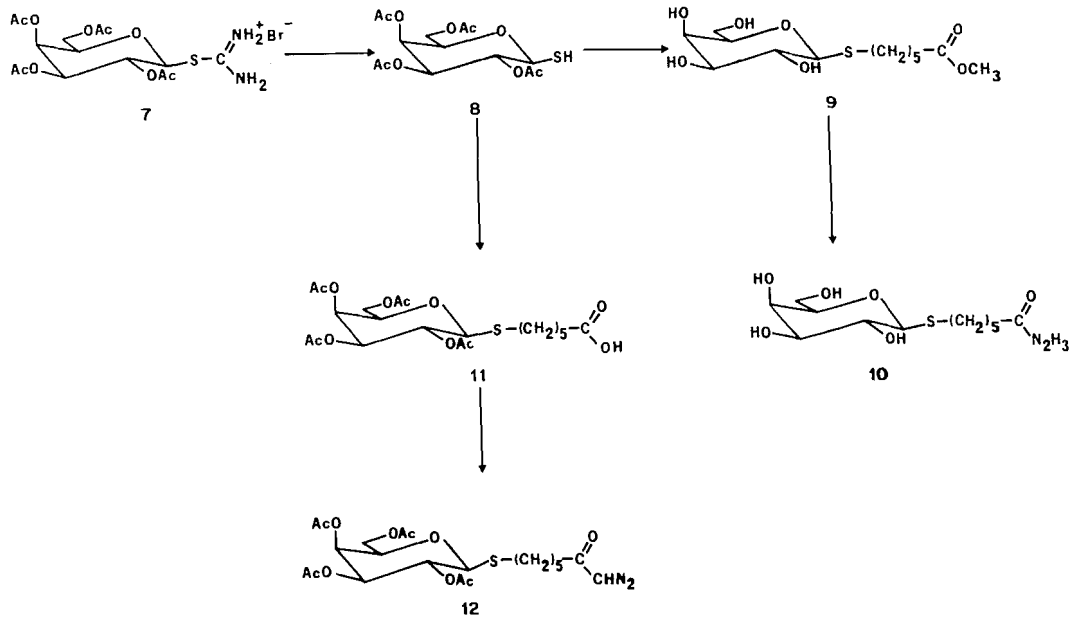
The light sensitivity of diazo derivative **5** was investigated by irradiation of the compound with an ultraviolet lamp. The disappearance of the band at 254 nm was followed spectrophotometrically. A fifty percent decrease in optical density was observed after less than one minute, and nearly quantitative decomposition occurred after 3 minutes of irradiation.

Synthesis of 1-thio-β-D-galactopyranosides

Alkyl 1-thioaldopyranosides containing an amino, imido ester, aldehyde, or ester group at the terminal position of the aglycon have previously been reported (22–26). These compounds can be prepared by *S*-alkylation of 1-thioglycoses or by reaction of ethyleneimine with 1-thioaldose derivatives. Because of the demonstrated facility of the first approach (23), this method was selected in the present study.

A series of 1-thio-β-D-galactopyranose derivatives was generated by introduction of a medium size alkyl chain of six carbon units bearing the appropriate reactive functions. The starting material was 2,3,4,6-tetra-*O*-galactopyranosyl bromide (27) which was allowed to react with thiourea, as described by Bonner and Kahn (28). The ensuing crystalline hydrobromide salt **7** was quantitatively reduced to 2,3,4,6-tetra-*O*-acetyl-1-thio-β-D-galactopyranose **8** by treatment with sodium bisulfite at alkaline pH (29). The potassium thiolate of **8** was allowed to react with methyl 6-bromohexanoate, as

[1]Abbreviations used: DMF, *N*,*N*-dimethylformamide; ^{1}Hmr, proton magnetic resonance; ir, infrared; THF, tetrahydrofuran; m, multiplet; t, triplet; s, singlet.



SCHEME 2

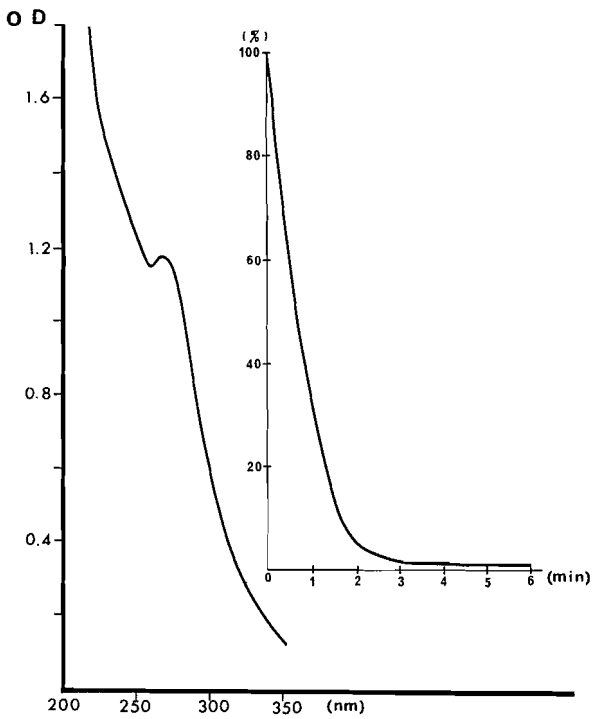


FIG. 1. Ultraviolet spectrum of 1-diazo-7-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosylthio)-2-heptanone (**12**) in ethanol. OD is the optical density. *N*-(2,3,4,6-Tetra-*O*-acetyl-β-D-galactopyranosyl)-5-diazo-4-oxopentanamide **5** had a spectrum identical to that of **12**. The insert shows the remaining proportion (%) of compound **12** as a function of time (min) of irradiation with a polychromatic ultraviolet lamp. The quantity of remaining compound was determined at 254 nm. The decomposition of compound **5** gave a similar curve.

described (25), and the oily product was deacetylated with sodium methoxide in methanol, to give the crystalline thioglycoside **9**. Reaction of **9** with hydrazine in refluxing methanol gave the crystalline hydrazine **10** (30).

The diazo ketone **12** was obtained in two steps from the peracetylated thiogalactose **8**. First, the free acid intermediate **11** was prepared by reaction of the potassium salt of compound **8** with potassium 6-bromohexanoate, followed by acidification. Compound **11** was then converted into the desired diazo derivative **12** by allowing the mixed anhydride prepared from **11** and isobutyl chloroformate to react with an excess of ethereal diazomethane, under reflux. The diazo derivative **12** was decomposed by ultraviolet irradiation as shown in Fig. 1. The kinetic course of decomposition was identical to that of compound **5**.

Conclusions

Compounds prepared in this study should prove to be useful for affinity-labeling of lectins possessing a specificity for galactose such as those obtained from jequirity beans, peanuts, osage oranges, castor beans, and from *Bandeiraea simplicifolia* (10). It may be pointed out that the hydrazides **4** and **10** can probably be converted into the corresponding azides (11) affording the possibility of generating nitrenes, by irradiation (12). Furthermore, diazo derivatives such as compounds **5** and **12** should yield highly reactive carbenes, after irradiation (13), but they can also be

transformed into halomethyl ketone derivatives by treatment with HBr or HCl (31). This transformation provides an additional tool for affinity-labeling studies. It is to be noted that compounds **5** and **12** are peracylated glycosides; however, the acetyl groups can be removed by treatment with sodium methoxide/methanol, without affecting the diazo function.

Sugar derivatives such as those described here can be used in biological systems apart from the study of the nature of lectin sugar binding sites. These may include the study of sugar transport systems in prokaryote and eukaryote cells (32, 33), the nature of the components involved in galactose-induced "puffing" of *Drosophila* giant chromosomes (34), etc. In addition, they represent further agents which can be used to prepare affinity columns (15, 35–37), to modify proteins by reaction with alkyl glycosides (11, 30, 38, 39), and to study cell adhesion to glycoside-modified solid surfaces and proteins (40).

Experimental

D-Galactose was obtained from Sigma Chemical Co. (St. Louis, MO); other reagents were from Aldrich Chemical Co. (Milwaukee, WI) or from Fisher Scientific Co. or Canlab (Montreal).

All evaporations were performed under reduced pressure at 20–45°C, with a rotary evaporator. Melting points were determined in capillary tubes on a Tottoli apparatus (Brinkman Instruments, Rexdale, Ontario) and are not corrected. Thin layer chromatography was performed on silica gel G-coated glass plates. Plates were developed in the following system (v/v): I, chloroform – acetic acid – water (6:7:1); II, methanol–chloroform (1:1); III, acetone – petroleum ether 65–110°C (9:1); IV, benzene – ethyl acetate (5:1). Compounds were revealed by spraying the plates with dilute sulfuric acid followed by heating. Optical rotations were determined by Galbraith Laboratories (Knoxville, TN). The ^{1}Hmr spectra were recorded on a Varian T-60 spectrometer; chemical shifts are presented in parts per million (δ) with reference to tetramethylsilane as internal standard. Infrared spectra were determined with a Perkin–Elmer spectrometer, model 457. Ultraviolet spectra were recorded on a Cary model 118 instrument.

N-(β-D-Galactopyranosyl)-3-methoxycarbonylpropanamide (3)

In a three-neck flask protected from moisture, monomethyl succinate (3.96 g; 30 mmol) was dissolved in DMF (50 mL), triethylamine (4.16 mL; 30 mmol) was added, and the solution was cooled at −5°C. Isobutyl chloroformate (3.94 mL; 30 mmol) was then added slowly, under stirring. The mixture was stirred for 20 min at −5°C, and then filtered, by suction, into a chilled (−5°C) flask containing a suspension of β-D-galactopyranosylamine (R_f^I, 0.21; mp 138°C (lit. (14) m.p. 137–138°C) (4.50 g; 25 mmol) in DMF (250 mL). The reaction mixture was stirred for 30 min at −5°C. It was then placed in an ice bath and stirred for 16 h at ambient temperature without removal of the bath. The solvent was evaporated and the oily product was crystallized from 1-butanol. Yield, 5.1 g (70%); mp 167°C; R_f^I, 0.33; $[\alpha]_D^{20}$ +11.3° (*c* 1, H_2O); ^{1}Hmr (D_2O): 2.65 s (4H, $2CH_2$), 3.60 s (3H, CO_2—CH_3). *Anal.* calcd. for $C_{11}H_{19}NO_8$ (293.2): C 45.05, H 6.53, N 4.78; found: C 44.69, H 6.24, N 5.08.

N-(β-D-Galactopyranosyl)-3-hydrazinocarbonylpropanamide (4)

Compound **3** (0.5 g, 1.7 mmol) was dissolved in anhydrous methanol (3 mL) and absolute hydrazine (0.2 mL; 6.1 mmol) was added. The solution was heated under reflux for two hours. Cooling afforded crystals which were recrystallized from methanol. Yield, 0.48 g (95%); mp 236°C (dec.); R_f^I 0.14; $[\alpha]_D^{20}$ = +7.8° (*c* 1, H_2O). *Anal.* calcd. for $C_{10}H_{19}N_3O_7$ (293.3): C 40.95, H 6.53, N 14.33; found: C 40.92, H 6.53, N 14.12.

N-(2,3,4,6-Tetra-O-acetyl-β-D-galactopyranosyl)-3-carboxypropanamide (6)

Compound **3** (1 g; 3.4 mmol) was dissolved in absolute methanol (5 mL) and a solution of KOH (2 g; 35 mmol) in anhydrous methanol (10 mL) was added. The mixture was heated under reflux for 8–10 min, cooled, acidified to pH 2.5 with 1 *N* HCl, and evaporated to dryness. Two successive evaporations of added methanol gave an oily residue which was dissolved in freshly distilled pyridine (5 mL), and acetic anhydride (5 mL) was added. The mixture was left at room temperature for 6 h. The suspension was filtered and the solvents were evaporated. Two successive evaporations of added methanol afforded a viscous material which was purified on a silicic acid column (2 × 30 cm) and equilibrated with chloroform. The desired product was eluted with 5% methanol in chloroform. The product was precipitated from chloroform by the addition of ether. Yield, 1.1 g (70%); mp 118–120°C; R_f^{II} 0.3; $[\alpha]_D^{20}$ +27.8° (*c* 1, $CHCl_3$); ^{1}Hmr ($CDCl_3$): 2.05 m (12 H, 4 CH_3—*CO*), 2.60 m (4H, 2 CH_2), 4.10 m (3H, $CHCH_2$—*OAc*), 5.20 m (3H, 3 CH—*OAc*), 11.50 s (1H, CO_2H). *Anal.* calcd. for $C_{18}H_{25}NO_{12}$ (447.39): C 48.32, H 5.63, N 3.13; found: C 48.56, H 5.66, N 3.44.

N-(2,3,4,6-Tetra-O-acetyl-β-D-galactopyranosyl-5-diazo-4-oxopentanamide (5)

Compound **6** (1.8 g; 4 mmol) was dissolved in a 4:1 mixture of THF/DMF (10 mL), the temperature was lowered to −5°C, and triethylamine (0.57 mL; 4 mmol) was added. Isobutyl chloroformate (0.53 mL; 4 mmol) was then added dropwise to the stirred mixture which was filtered after 15 min. the filtrate was transferred to a solution of diazomethane in ether (alcohol-free) (70 mL) contained in a polished-glass flask (Aldrich). The reaction mixture was protected from light and gently heated under reflux for 18 h. The solvent was evaporated and a slightly colored, oily residue was obtained. The ir spectrum showed the presence of the diazo function (2200 cm^{-1}), and of the carbonyl group of the acetyl (1740 cm^{-1}) and diazoketone (1640 cm^{-1}) functions, indicating that compound **5** was formed.

S-(2,3,4,6-Tetra-O-acetyl-β-D-galactopyranosyl)-pseudothiouronium bromide (7)

2,3,4,6-Tetra-*O*-acetyl-α-D-galactopyranosylbromide, mp, 88°C (lit. (27) mp 84–85°C) (13.2 g; 32 mmol), was dissolved in acetone (13 mL) and recrystallized thiourea (2.7 g; 35 mmol) was added. The mixture was boiled under reflux for 15 min and then allowed to cool. The crystals of **7** were filtered off and recrystallized from acetone and from 2-propanol. Yield, 12.9 g (83%); mp 172°C (lit. (22) mp 171–171.5°C); R_f^{III}, 0.24.

5-(Methoxycarbonyl)pentyl-1-thio-β-D-galactopyranoside (9)

2,3,4,6-Tetra-*O*-acetyl-1-thio-β-D-galactopyranose **8** was obtained by reduction of **7** according to the procedure of Černý *et al.* (29). The thio sugar (1.5 g; 4.12 mmol) was dissolved in acetone (20 mL) and an aqueous solution of K_2CO_3 (0.57 g; 4.12 mmol) was added. After 30 s the mixture was lyophilyzed. The rest of the procedure was done in a manner similar to that of Connolly *et al.* (25). The dry potassium salt of **8** was suspended in DMF (25 mL) and methyl 6-bromohexanoate (prepared from 6-bromohexanoic acid and etheral diazomethane) (0.8 g; 4

mmol) in DMF (5 mL) was added. The reaction mixture was stirred for 2 h at room temperature. The precipitate was removed by filtration and the solvent evaporated. The residue was dissolved in chloroform and the solution was washed with water. The organic phase was dried and evaporated. The product was an oil, as previously reported (25). For deacetylation, the product was dissolved in anhydrous methanol (5 mL) and 0.5 mL of 0.43 *M* $NaOCH_3$ in methanol was added. The mixture was stored in the dark for 18 h, at room temperature, then neutralized to pH 7.0 with *N* HCl and evaporated to dryness. The solid was dissolved in boiling ethyl acetate and the filtered solution deposited cyrstals on cooling. Yield, 0.67 g (50%, based on compound **8**); mp 96°C; R_f^1 0.66; $[\alpha]_D^{20}$ −24.1° (*c* 1, H_2O); ^{1}Hmr (D_2O): 1.50 m (6H, inner methylene groups of aglycon), 2.35 t (2H, CH_2—CO_2CH_3), 2.65 t (2H, CH_2—*S*), 3.55 m (7H, pyranosic protons), 3.60 s (3H, CO_2—CH_3). *Anal.* calcd. for $C_{13}H_{24}SO_7$ (324.4): C 48.13, H 7.46; found: C 48.42, H 7.36.

6-(β-D-Galactopyranosylthio)hexanoic acid hydrazide (10)

A solution of compound **9** (0.5 g; 1.54 mmol) in anhydrous methanol (3 mL) containing absolute hydrazine (0.2 mL; 6.1 mmol) was heated under reflux for 2 h and evaporated. Compound **10** was crystallized from ethanol. Yield, 460 mg (92%); mp 180°C (lit. (30) mp 182–183°C); R_f 0.31; $[\alpha]_D^{20}$ − 26.7° (*c* 1, H_2O). *Anal.* calcd. for $C_{12}H_{24}N_2O_6S$ (324.4): C 44.43, H 7.46, N 8.64; found: C 44.32, H 7.36, N 8.44.

1-Diazo-7-(2,3,4,6-tetra-O-acetyl-β-D-galactopyranosylthio)-2-heptanone (12)

The carboxylic acid **11** was prepared from the potassium salt of **8** in a manner similar to that of compound **9**. Thus, the potassium salt of **8** (1.5 g; 4.1 mmol) was allowed to react with the potassium salt of 6-bromohexanoic acid (0.8 g; 4.1 mmol) in acetone–water (1:1 v/v, 20 mL). The mixture was stirred for two hours at room temperature, then acidified to pH 3 with *N* HCl and extracted with chloroform. The oil obtained after evaporation was purified on a silicic acid column (2 × 30 cm) equilibrated with chloroform. The desired compound **11**, an oil, was eluted with chloroform. Yield, 1.4 g (70%); R_f^V 0.20; $[\alpha]_D^{20}$ − 8.6° (*c*, 1, $CHCl_3$); ^{1}Hmr ($CDCl_3$): 1.50 m (6H, inner methylene groups of aglycon), 2.05 four s (12H, $4CO_2CH_3$), 2.30 t (2H, CH_2—CO_2H), 2.70 t (2H, *S*—CH_2), 4.00 s (2H, CH_2—*OAc*), 5.10 m (3H, CH—*OAc*), 11.20 s (1H, CO_2H).

A mixture of compound **11** (1.72 g; 3.6 mmol) and triethylamine (0.5 mL: 3.6 mmol) in THF (9 mL) was cooled to −5°C, and isobutyl chloroformate (0.47 mL; 3.6 mmol) was added in portions, with stirring. After 15 min, the mixture was filtered and added to an ethanol-free ether solution of diazomethane (24.5 mmol), in a "Vitro" flask (Aldrich). The reaction mixture was gently boiled under reflux for 18 h in the dark. Evaporation of the solvents gave **12** as an oil; ir (neat): 2100 (CH=N=N), 1640 (ketonic CO), and 1745 (ester CO); ^{1}Hmr ($CDCl_3$): 1.60 m (6H, inner methylene groups of the aglycon), 2.10 four s (12H, 4 CO_2—CH_3), 2.50 t (2H, CH_2—*COCHN*$_2$), 2.89 t (2H, *S*—CH_2), 3.40 s (1H, CHN_2), 4.10 s (2H, CH_2—*OAc*), 5.20 m (CH—OAC).

Deacetylation of compound 12

Compound **12** (40 mg; 0.085 mmol) was dissolved in a minimum amount of anhydrous methanol and $NaOCH_3$ (0.010 mmol) in anhydrous methanol (25 μL) was added. The solution was stored in the dark for 8 h at room temperature, carefully neutralized to pH 4.5 with dilute acetic acid, and evaporated. The syrupy residue was dissolved in a small volume of anhydrous methanol and the suspension was centrifuged. Evaporation of the organic layer afforded an oily product which showed (ir) the characteristic bands of the diazo (2100) and the ketonic carbonyls (1640). In addition, the ultraviolet spectrum showed the characteristic maximum of absorption at 254 nm.

Ultraviolet spectra and kinetics of decomposition of compounds 5 and 12

The ultraviolet spectra of compounds **5** and **12** in ethanol (Fig. 1) showed an absorption maximum at 254 nm. Each compound was irradiated with an ultraviolet lamp ("Mineral light" UVSL 25, Ultraviolet Products, Inc., San Gabriel, CA) placed at 10 cm from the solutions. Spectra were recorded at various intervals, and the percentage of untransformed compound is plotted as a function of time of irradiation (Fig. 1). After 1 min of irradiation, approximately 70% of compounds **5** and **12** were decomposed. Complete photolysis was observed after 3 min of irradiation.

Acknowledgements

The authors are indebted to the Medical Research Council of Canada for financial support (grant MT 6343) and to the Université de Sherbrooke for a "Subvention Institutionnelle à la Recherche Libre".

1. S. J. Singer. Adv. Protein Chem. **22**, 1 (1967).
2. D. Givol. Int. Rev. Biochem. Defence and Recognition II B, **23**, 71 (1979).
3. D. S. Sigman and G. Mooser. Annu. Rev. Biochem. **44**, 889 (1975).
4. J. Shafer, P. Baronowsky, R. Laursen, F. Finn, and F. H. Westheimer. J. Biol. Chem. **241**, 421 (1966).
5. H. Bayley and J. R. Knowles. Methods Enzymol. **46**, 69 (1977).
6. V. Chowdhry and F. H. Westheimer. Annu. Rev. Biochem. **48**, 293 (1979).
7. H. Nakayama and Y. Kanaoka. FEBS Lett. **37**, 200 (1973).
8. C. A. Converse and F. F. Richards. Biochemistry, **8**, 4431 (1969).
9. H. Lis and N. Sharon. Annu. Rev. Biochem. **42**, 541 (1973).
10. I. J. Godlstein and C. E. Hayes. Adv. Carbohydr. Chem. Biochem. **35**, 127 (1978).
11. R. U. Lemieux, D. A. Barker, and D. R. Bundle. Can. J. Biochem. **55**, 507 (1977).
12. W. Lwowski. Angew. Chem. Int. Ed. Engl. **6**, 897 (1967).
13. W. Kirmse. Carbene chemistry. Academic Press, New York. 1964.
14. R. Barker, C.-K. Chiang, I. P. Trayer, and R. L. Hill. Methods Enzymol. **34B**, 317 (1974).
15. J. A. Gordon, S. Blumberg, H. Lis, and N. Sharon. FEBS Lett. **24**, 193 (1972).
16. W. Reid and H. Mengler. Fortschr. Chem. Forsch. **5**, 1 (1965).
17. F. Weygand and H. J. Bestmann. Newer Prep. Methods Org. Chem. **3**, 451 (1964).
18. H. O. House and C. J. Blankley. J. Org. Chem. **33**, 53 (1968).
19. E. Shaw, M. Mares-Guia, and W. Cohen. Biochemistry, **4**, 2219 (1965).
20. D. P. Bloxham, R. A. Chalkley, S. J. Coghlin, and W. Salam. Biochem. J. **175**, 999 (1978).
21. C. G. Rasool, S. Nicolaidis, and M. Akhtar. Biochem. J. **157**, 675 (1976).
22. S. Chipowsky and Y. C. Lee. Carbohydr. Res. **31**, 339 (1973).

23. R. T. LEE and Y. C. LEE. Carbohydr. Res. **77**, 149 (1979).
24. Y. C. LEE, C. P. STOWELL, and M. J. KRANTZ. Biochemistry, **15**, 3956 (1976).
25. D. T. CONNOLLY, S. ROSEMAN, and Y. C. LEE. Carbohydr. Res. **87**, 227 (1980).
26. R. T. LEE and Y. C. LEE. Carbohydr. Res. **34**, 151 (1974).
27. W. T. HASKINS, R. M. HANN, and C. S. HUDSON. J. Am. Chem. Soc. **64**, 1852 (1942).
28. W. A. BONNER and J. E. KAHN. J. Am. Chem. Soc. **73**, 2241 (1951).
29. M. ČERNÝ, J. STANĚK, and J. PACÁK. Monatsh. **94**, 290 (1963).
30. K. KAWAGUCHI, M. KUHLENSCHMIDT, S. ROSEMAN, and Y. C. LEE. J. Biol. Chem. **256**, 2230 (1981).
31. C. SCHOELLMANN and E. SHAW. Biochemistry, **2**, 252 (1963).
32. H. R. KABACK. Annu. Rev. Biochem. **39**, 561 (1970).
33. M. HOSANG, E. M. GIBBS, D. F. DIEDRICH, and G. SEMENZA. FEBS Lett. **130**, 244 (1981).
34. W. BEERMANN. Chromosoma, **41**, 297 (1973).
35. K. FILKA, J. ČOUPEK, and J. KOCOUREK. Biochim. Biophys. Acta, **539**, 518 (1978).
36. J. PIPKOVA, V. HOREJSI, and J. KOCOUREK. Biochim. Biophys. Acta, **541**, 515 (1978).
37. H. LIS, R. LOTAN, and N. SHARON. Ann. NY Acad. Sci. **234**, 232 (1974).
38. R. T. LEE and Y. C. LEE. Biochemistry, **19**, 156 (1980).
39. D. H. BUSS and I. J. GOLDSTEIN. J. Chem. Soc. C, 1457 (1968).
40. P. H. WEIGEL, R. L. SCHNAAR, M. S. KUHLENSCHMIDT, E. SCHMELL, R. T. LEE, Y. C. LEE, and S. ROSEMAN. J. Biol. Chem. **254**, 10830 (1979).