*Carbohydrate Research*, 88 (1981) 223–232
Elsevier Scientific Publishing Company, Amsterdam – Printed in The Netherlands

# THE SYNTHESIS OF SPIN-LABELED β-D-GALACTOPYRANOSIDE ANALOGS*

HANS-RICHARD RACKWITZ†
*Department of Chemistry, University of Pennsylvania, Philadelphia, Pennsylvania 19104 (U.S.A.)*
(Received July 23rd, 1979; accepted for publication in revised form, May 23rd 1980)

ABSTRACT

The spin-labeled galactoside analogs 4-(4,4,5,5-tetramethyl-2-imidazolin-2-yl 1-oxide)-2-nitrophenyl β-D-galactopyranoside, 4-(4,4,5,5-tetramethyl-2-imidazolin-2-yl 1-oxide)phenyl β-D-galactopyranoside, and 4-(4,4,5,5-tetramethyl-2-imidazolin-2-yl 1-oxide)-2-nitrophenyl β-D-fucopyranoside were synthesized by condensation of the corresponding 4-formylphenyl galactopyranosides with 2,3-di(hydroxyamino)-2,3-dimethylbutane and subsequent oxidation with lead dioxide, and characterized by u.v. and electron-spin resonance spectroscopy.

## INTRODUCTION

The utility of paramagnetic probes as an aid in the study of structural and dynamic features of proteins in solution has been well documented in recent years[1,2]. The use of stable, organic free radicals with structures similar to natural ligands along with n.m.r. studies has provided information about the geometry and environment of the binding sites for several proteins[3–7]. Lu *et al.*[8] in this laboratory have undertaken an n.m.r. study of the lactose operon repressor. This paper describes the synthesis of several spin-labeled compounds that are analogs of the natural inducer of the *Escherichia coli* lactose operon, allolactose[9], which will be used for n.m.r. studies. These compounds should be useful for studying β-D-galactosidases and related β-D-galactoside-carrier proteins as well.

The analogs of allolactose and their effects on the expression of the structural genes of the *lac* operon have been extensively investigated[10–12]. Analogs that exhibit an effect on the operon are of two types: (a) those that induce the operon, *i.e.*, turn the genes on, and (b) those that prevent induction, *i.e.*, compete with inducers for their binding sites. Initial experiments with (2,2,6,6-tetramethylpiperid-4-yl 1-oxide) β-D-galactopyranoside, synthesized by Struve and McConnell[18], showed no inter-

*This work was supported by grants from the American Cancer Society (VC 136) and the National Institutes of Health (grant GM-22623 to Dr. P. Lu).
†Recipient of a Deutsche Forschungsgemeinschaft Fellowship. Present address: Abteilung Viroidforschung, Max-Planck-Institut für Biochemie, D8033-Martinsried, West Germany.

action with the *lac* repressor. Thus, we have attempted the synthesis of several additional analogs.

The compounds described herein are analogs of *o*-nitrophenyl β-D-galactopyranoside and -fucopyranoside, a neutral effector and an anti-inducer of the *lac* operon, respectively. The convenient introduction of spin labels into β-D-galactopyranosides as described in the present paper can be applied to other sugars as well. In addition, the u.v. and e.s.r. properties and the hydrolysis by β-D-galactosidase is also reported. The interaction with the lactose repressor will be reported elsewhere[19].

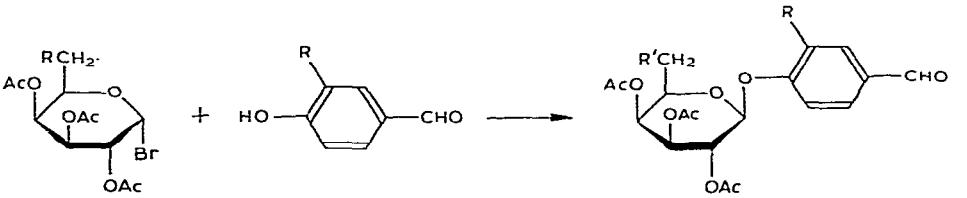
## RESULTS AND DISCUSSION

For coupling an *N*-oxide spin-label to *o*-nitrophenyl β-D-galactopyranoside or -fucopyranoside, it was necessary to introduce another functional group into the aglycon. Ullman and associates[20-24] described the reaction between a carbonyl group and 2,3-di(hydroxyamino)-2,3-dimethylbutane to yield the corresponding di-*N*-oxides. Numerous other exceptionally stable, organic free-radicals that could be obtained by this procedure were characterized by their e.s.r. spectra[25]. Exploiting the versatility of this reaction, we prepared, by Koenigs–Knorr reaction[26] of per-*O*-acetyl-α-D-galactopyranosyl bromides (**1**, **2**) with either 4-hydroxybenzaldehyde (**3**) or 4-hydroxy-3-nitrobenzaldehyde (**4**), respectively, phenyl β-D-galactopyranosides having an aldehyde group in position *para* to the glycosidic bond (**5**–**7**).

The Koenigs–Knorr reaction gave yields between 25% and 85%, depending on the substituents in the 3-position of 4-hydroxybenzaldehydes being used. A nitro group in position *ortho* to the hydroxyl group possibly decreases the yield of the condensation reaction with the bromides for stereochemical reasons. In those cases where the yields were 85% of the starting glycosyl bromides, the corresponding benzaldehyde derivative could be easily crystallized from the reaction mixture. When the yields were less, a preparative purification on a silica gel column with chloroform was performed before crystallization from methanol.

The condensation of the benzaldehyde derivatives **5**–**7** with 2,3-di(hydroxyamino)-2,3-dimethylbutane in benzene could be followed by chromatography on silica gel. After 4–8 h at room temperature, compounds were obtained that moved slower than the corresponding starting-materials. In most cases, these compounds crystallized spontaneously from methanol to give, in good yields, crystals showing n.m.r. data and elementary analyses corresponding to the 4-(1,3-dihydroxy-4,4,5,5-tetramethylimidazolidin-2-yl) derivatives of the phenyl galactosides (**8**–**10**).

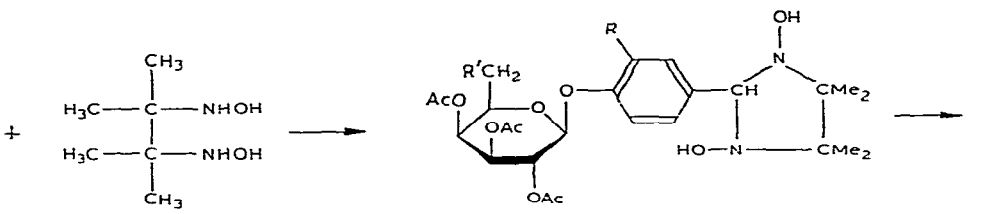
Aliquots of these compounds were further purified by recrystallization from methanol. Otherwise, the crude compounds could be used in the subsequent oxidation step by lead(IV) dioxide without further purification. Contrary to the initial report of Osiecki and Ullman[20], this oxidation did not give the expected 1,3-oxides **11**–**13** but the corresponding unsaturated 1-oxides **14**–**16**, which are known to have, in some cases, greater thermal stability and have been found as byproducts in the synthesis of di-*N*-oxides[25]. Although a violet compound was observed in t.l.c. on silica gel



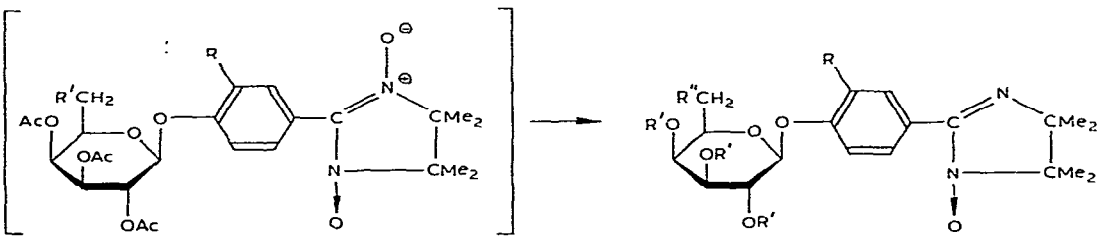
1 R = OAc
2 R = H

3 R = H
4 R = $NO_2$

5 R = $NO_2$, R = OAc
6 R = H, R = OAc
7 R = $NO_2$, R = H



8 R = $NO_2$, R′ = OAc
9 R = N, R′ = OAc
10 R = $NO_2$, R′ = H



11 R = $NO_2$, R′ = OAc
12 R = H, R′ = OAc
13 R = $NO_2$, R′ = H

14 R = $NO_2$, R′ = Ac, R″ = OAc
15 R = H, R′ = Ac, R″ = OAc
16 R = $NO_2$, R′ = Ac, R″ = H
17 R = $NO_2$, R′ = H, R″ = OH
18 R = R′ = H, R″ = OH
19 R = $NO_2$, R′ = R″ = H

shortly after the addition of lead dioxide, no attempt was made to analyze this intermediate which slowly reacted further to give an orange compound. The orange, paramagnetic compounds were purified by p.t.l.c. in silica gel and crystallized easily from methanol. The e.s.r. spectra were in agreement with those reported for similar *N*-oxides and elementary analyses were consistent for 4-(4,4,5,5-tetra-methylimidazolin-2-yl 1-oxide)phenyl per *O*-acetyl glycosides (**14–16**). The *O*-deacetylated *N*-oxides **17–19** were easily obtained by reaction with half-saturated ammonia in metha-

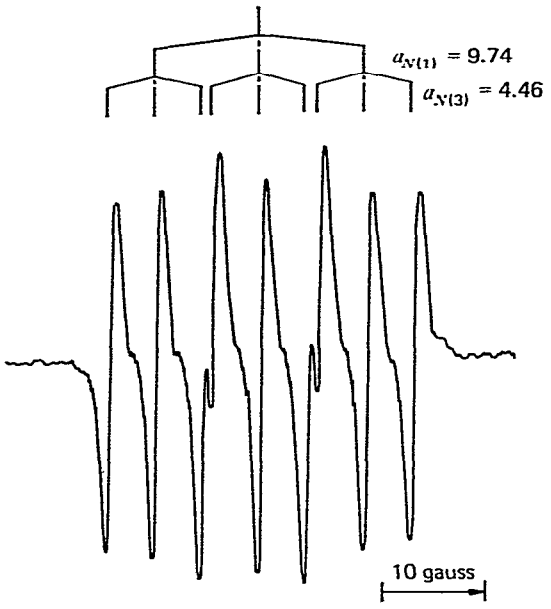



Fig. 1. E.s.r. spectrum at 9.149 GHz of **18** in water. The spectrum was recorded at 25°. The hyperfine splitting by the two nonidentical nitrogen atoms is indicated at the top of the Figure.

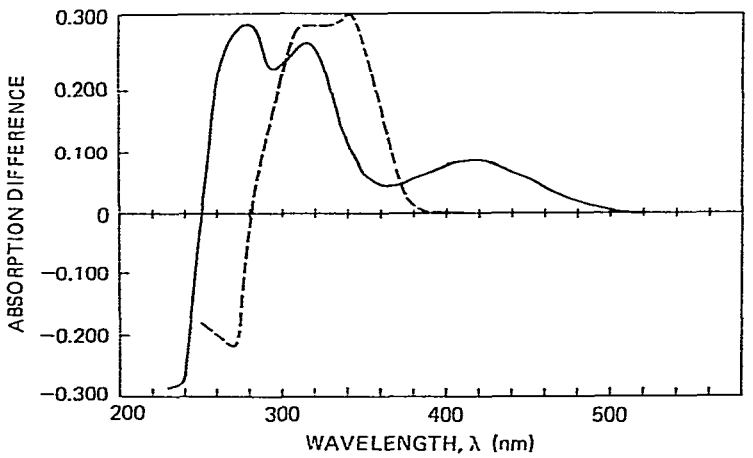



Fig. 2. U.v. absorption difference-spectra of the hydrolysis of equimolar solutions of **17**, **19** (———) and **18** (– – – – –) in M sodium hydroxide. The hydrolysis was performed in a cuvette that was kept at 50°. The u.v. spectra were recorded until no further change in the absorption spectrum could be observed. The difference between the spectrum from the completely hydrolyzed sample and the spectrum from the identical, but unhydrolyzed, compound was then calculated.

nol at 4°. This procedure did not result in the loss of the *N*-oxide spin-label as monitored by the e.s.r. and u.v. spectra of the *O*-acetylated and *O*-deacetylated compounds.

The e.s.r. spectra of **14–17**, **16**, and **19** were identical (see Fig. 1) and present the same characteristics as that of 4,4,5,5-tetramethyl-2-phenylimidazoline 1-oxide,

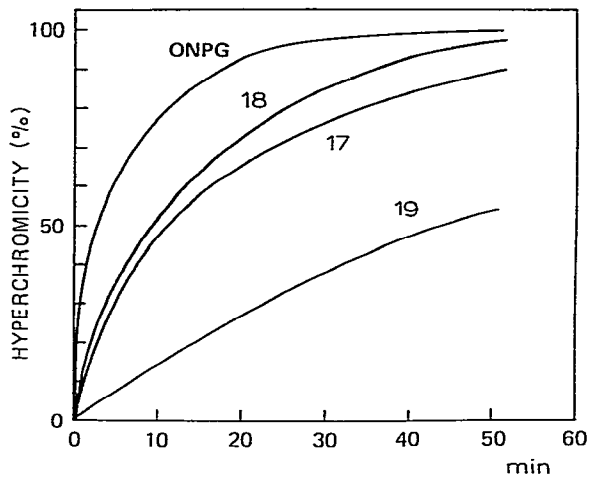



Fig. 3. Hydrolysis of *o*-nitrophenyl β-D-galactopyranoside (ONPG), **17**, **18**, and **19** by β-D-galactosidase from *E. coli*. The hydrolysis was performed in a cuvette containing 20μM substrate and 0.1 mg/mL of β-D-galactosidase in 0.1M Tris buffer (pH 8.0) at 37°. The percentage of increase in absorption at wavelength of maximum hyperchromicity (see Fig. 2) on hydrolysis is plotted *vs.* time.

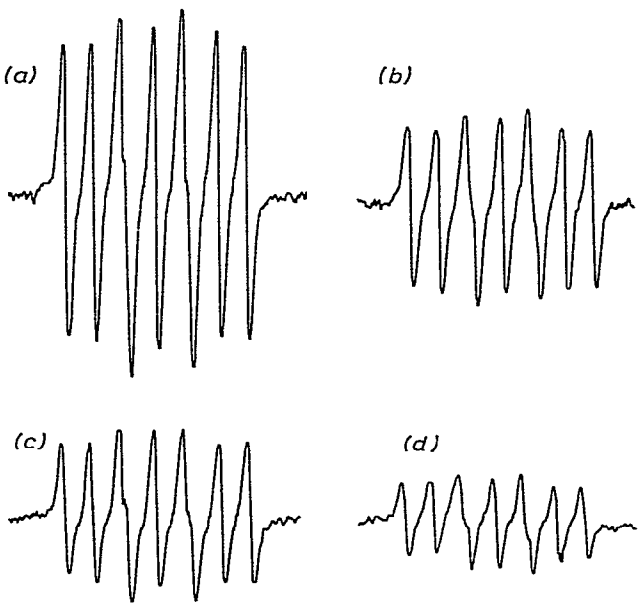



Fig. 4. Reduction of **18** by dithiothreitol as indicated by decrease of spin label. The e.s.r. spectra of 20μM **18** in 0.1M Tris buffer (pH 7.4) containing mM dithiothreitol were recorded at: (a) t, 0 h; (b) t, 5 h; (c) t, 10 h; and (d) t, 24 h. A sample without dithiothreitol showed no significant decrease in the intensity of the e.s.r. spectrum during the same time.

TABLE I

CONDITIONS OF REACTION AND PROPERTIES OF COMPOUNDS OBTAINED (5–10 AND 14–19)

| *Compound* | *Conditions of reaction* (g, mmol)[a] | *Yield* (g, %)[b] | *Formula* | *Anal.* | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | *Calc.* | | | *Found* | | |
| | | | | *C* | *H* | *N* | *C* | *H* | *N* |
| 2,3,4,6-Tetra-*O*-acetyl β-D-galacto-pyranoside | | | | | | | | | |
| 4-Formylphenyl (6) | 1:10 (24.3)<br>3: 8.3 (50) | 9.4 (85) | $C_{21}H_{24}O_{11}$ | 55.8 | 5.4 | | 56.0 | 5.1 | |
| 4-Formyl-2-nitrophenyl (5) | 1:10 (24.3)<br>4: 6.1 (50) | 3.5 (29) | $C_{21}H_{23}NO_{13}$ | 50.7 | 4.7 | 2.8 | 50.8 | 5.0 | 3.0 |
| 4-(1,3-Dihydroxy-4,4,5,5-tetra-methylimidazolidin-2-yl)phenyl (9) | 6: 5 (11)<br>19: 1.9 (13) | 3.5 (55) | $C_{27}H_{38}N_2O_{12}$ | 55.7 | 6.6 | 4.8 | 55.9 | 6.9 | 4.7 |
| 4-(1,3-Dihydroxy-4,4,5,5-tetramethyl-imidazolidin-2-yl)-2-nitrophenyl (8) | 5: 3 (6)<br>19: 1 (7) | 2.3 (62) | $C_{27}H_{37}N_3O_{14}$ | 51.7 | 5.9 | 6.7 | | | |
| 4-(4,4,5,5-Tetramethylimidazolin-2-yl 1-oxide)phenyl (15) | 9: 3 (5.1)<br>$PbO_2$: 12.2 (51) | 1.9 (66) | $C_{27}H_{35}N_2O_{11}$ | 57.7 | 6.3 | 5.0 | 58.0 | 6.7 | 5.0 |
| 2-Nitro-4-(4,4,5,5-Tetramethyl-imidazolin-2-yl 1-oxide)phenyl (14) | 8: 1.5 (2.4)<br>$PbO_2$: 5.74 (24) | 1.2 (82) | $C_{27}H_{34}N_3O_{13}$ | 53.3 | 5.6 | 6.9 | 53.5 | 6.0 | 7.0 |
| β-D-Galactopyranoside | | | | | | | | | |
| 4-(4,4,5,5-Tetramethylimidazolin-2-yl 1-oxide)phenyl (18) | 15: 1.5 (2.66) | 0.97 (90) | $C_{19}H_{27}N_2O_7$ | 56.3 | 6.7 | 6.9 | 56.1 | 6.9 | 6.6 |
| 4-(4,4,5,5-Tetramethylimidazolin-2-yl 1-oxide)phenyl (17) | 14: 1 (1.64) | 0.68 (95) | $C_{19}H_{26}N_3O_9$ | 51.8 | 6.0 | 9.5 | 52.0 | 6.2 | 9.3 |
| 2,3,4,6-Tri-*O*-acetyl-β-D-fuco-pyranoside | | | | | | | | | |
| 4-Formyl-2-nitrophenyl (7) | 2:10 (18.3)<br>4: 9.4 (56) | 3.1 (25) | $C_{19}H_{21}NO_{11}$ | 51.9 | 4.8 | 3.2 | 52.0 | 5.0 | 3.3 |
| 4-(1,3-Dihydroxy-4,4,5,5-tetramethyl-imidazolidin-2-yl)-2-nitrophenyl (10) | 7: 2.5 (5.7)<br>19: 1 (7) | 1.8 (56) | $C_{25}H_{35}N_3O_{12}$ | 52.7 | 6.2 | 7.4 | | | |
| 2-Nitro-4-(4,4,5,5-tetramethyl-idazolin-2-yl 1-oxide)phenyl (16) | 10: 1 (1.8)<br>$PbO_2$: 4.3 (18) | 0.7 (71) | $C_{25}H_{32}N_3O_{11}$ | 54.5 | 5.9 | 7.6 | 54.8 | 6.1 | 7.7 |
| β-D-Fucopyranoside | | | | | | | | | |
| 2-Nitro-4-(4,4,5,5-tetramethyl-idazolin-2-yl 1-oxide)phenyl (19) | 16; 0.5 (0.91) | 0.35 (91) | $C_{19}H_{26}N_3O_8$ | 53.8 | 6.2 | 9.9 | 53.4 | 6.6 | 9.5 |

[a]mmol in parentheses. [b]Percent in parentheses. [c]Silica gel: 17:3 (A), 19:1 (v/v) (B) chloroform–methanol; (C) chloroform; (D) paper chromatography, 7:1:2 (v/v) 2-propanol–25% ammonia–water. [d]Extinction coefficient (ε) in parentheses. Abbreviations: sh, shoulder; max, maximum; and min, minimum. [e]Percent in parentheses.

| $R_F$[c] | *U.v. spectrum* (nm, ε)[d] | *N.m.r. spectrum (δ)* |
|---|---|---|
| 1.0(B)<br>0.50(C) | MeOH: max 263 (16 250), min 230 (2 700), and max 213 (14 400) | $CCl_4$: 9.97 (1 H, CHO), 8.2 (2 H), and 7.5 (1 H, Ph), and 2.2–2.0 (12 H, Ac) |
| 1.0(B)<br>0.52(C) | MeOH: sh 300 (1 840), max 251 (10 700), and min 227 (8 600) | $CDCl_3$: 9.77 (1 H, CHO), 7.7 (2 H) and 7.0 (2 H, Ph), 2.08 (3 H), and 1.9 (9 H, Ac) |
| 0.35(B)<br>0.19(C) | MeOH: max 307 (3 070), min 283 (2 400), max 254 (7 140), min 235 (4 760), and max 220 (9 400) | $Me_2SO$-$d_6$: 7.4 (2 H) and 6.9 (2 H, Ph), 4.55 (1 H, -CH=), 2.15–1.95 (12 H, Ac), and 1.25–1.1 (12 H, Me) |
| 0.38(B)<br>0.18(C) | | |
| 1.0(B)<br>0.06(C)<br>0.42(D) | MeOH: max 460 (800), min 370 (200), sh 270 (7 900), max 245 (22 500), and min 216 (7 300) | |
| 1.0(B)<br>0.06(C)<br>0.41(D) | MeOH: max 460 (800), min 375 (300), sh 300 (3 200), max 231 (21 500), and min 210 (12 200) | |
| 0.31(A)<br>0.01(B)<br>0.86(D) | $H_2O$: max 460 (600), min 365, sh 270 (8 400), max 245 (22 700), and 217 (6 750) | |
| 0.33(A)<br>0.01(B)<br>0.84(D) | $H_2O$: max 460 (750), min 375 (300), sh 300 (3 500), max 231 (21 000), and min 210 (12 500) | |
| 1.0(B)<br>0.53(C) | MeOH: sh 300 (1 750), max 251 (10 900), and min 227 (8 400) | $CCl_4$: 9.97 (1 H, CHO), 2.2–2.0 (9 H, Ac), and 1.3 (3 H, Me) |
| 0.40(B)<br>0.20(C) | | |
| 0.62(B) | MeOH: max 460 (750), min 375 (300), sh 300 (4 000), max 231 (21 300), and min 207 (12 700) | |
| 0.67(A)<br>0.05(B)<br>0.93(D) | $H_2O$: max 460 (800), min 375 (300), sh 300 (4 250), max 231 (21 200), and min 207 (13 000) | |

thus confirming the structure of these compounds[25]. The variations in the u.v. and visible spectra upon hydrolysis of the glycosides with M sodium hydroxide are shown in Fig. 2. Almost all compounds gave a substantial spectral change upon hydrolysis, which enables easy monitoring of enzymic reaction by glycosidases.

When stored in the dark, **14–16** were exceptionally stable with respect to the u.v. and e.s.r. spectra, and chromatographic behavior. Upon reaction with sodium hydroxide, their glycosidic bond was hydrolyzed, as indicated by the u.v. spectra (see Fig. 2). The difference in absorption upon hydrolysis resulting from the change in $\pi-\pi^*$ transition of the phenyl chromophore furnishes an easy assay for hydrolysis with glycosidases. Such chromophore groups are not present in some of the (2,2,6,6-tetramethylpiperid-4-yl 1-oxide) β-D-galactopyranosides previously synthesized[18,27]. Compounds **17–19** were tested as substrates for β-D-galactosidase (see Fig. 3). The amount of hyperchromicity observed in the u.v. spectrum of **17** and **18** was equal to that obtained by hydrolysis with sodium hydroxide. Compound **19** was not readily cleaved by the enzyme, which is in agreement with the observation that β-D-fucopyranosides are not as good substrates as the corresponding β-D-galactopyranosides[28].

The chemical stability of **17–19** in the presence of reducing agents, particularly sulfhydryl groups, which are normally present in buffers for enzyme assays, was also investigated. As indicated by the e.s.r. spectra (Fig. 4), a substantial reduction of the *N*-oxide spin-label was observed, when the concentration of sulfhydryl compounds was ~2 orders of magnitudes higher than that of the spin-label compound. Similar results have been obtained for other *N*-oxides by Morrisett and Drott[29], and by Gaffney[27]. Thus, high concentrations of sulfhydryl groups should be avoided in biochemical reactions where spin-labeled β-D-galactopyranosides are used.

## EXPERIMENTAL

*General.* — All chemicals used in the reactions were as purchased, without further purification. D-Fucose and D-galactose were obtained from Sigma Chemical Co., St. Louis, MO 63178. 2,3-Dihydroxyamino-2,3-dimethylbutane, was prepared as described by Lamchen and Mittag[30]. 2,3,4-Tri-*O*-acetyl-α-D-fucopyranosyl bromide and 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl bromide were prepared as described by Lemieux[31]. β-D-Galactosidase (EC 3.2.1.23) from *E. coli* was obtained from Worthington Biochemical Corp., Freehold, NJ 0778 and had a specific activity of 90 U/mg. U.v. and visible spectra were recorded with a Cary-14 spectrophotometer, n.m.r. data with a Varian A-60 continuous-wave spectrometer, and e.s.r. spectra with a Varian E-3 spectrometer. All spectra were recorded at room temperature. The chemical analyses were performed by Galbraith Laboratories, Inc., Knoxville, TN 37821. All compounds decomposed between 150 and 180°, and were colored from deep yellow to orange; no optical rotation was determined.

*4-Formylphenyl derivatives* **5–7.** — A solution of 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl (**1**) or 2,3,4-tri-*O*-acetal-α-D-fucopyranosyl bromide (**2**) in acetone was introduced dropwise into a solution of the appropriate benzaldehyde derivative (3

or **4**) in M sodium hydroxide (one mol. equiv.) and the mixture was stirred at room temperature (for **2**, the reaction was carried out in suspension because of partial solubility) for 4–8 h. The acetone was evaporated *in vacuo*, and the residue was extracted thrice with chloroform. The combined chloroform extracts were washed three times with 5% sodium hydroxide and then with water until neutral. After being dried ($MgSO_4$), the solution was evaporated and the residue crystallized from 95% ethanol (see Table I). If it failed to crystallize, it was further purified on a preparative silica gel column in chloroform, the main u.v.-absorbing fractions being collected.

*4-(1,3-Dihydroxy-4,4,5,5-tetramethylimidazolidin-2-yl)phenyl β-D-galactopyranosides* (**8–10**). — A solution of the appropriate compound **5–7** in benzene was mixed with an equimol. amount of 2,3-di(hydroxyamino)-2,3-dimethylbutane in benzene and the mixture was kept at room temperature. The reaction course was followed by t.l.c. in silica gel (19 : 1 (v/v) chloroform–methanol). The new compounds formed migrated slower than the corresponding starting material. After 24 h, when the reaction was nearly completed, the reaction mixture was evaporated in *vacuo*, and the residue usually directly crystallized from methanol or purified by preparative silica gel chromatography prior to crystallization (see Table I).

N-*Oxides* **17–19**. — A solution of the appropriate compound **8–10** in benzene was oxidized to the corresponding di-*N*-oxides with an excess of lead(IV) dioxide. The reaction mixture became violet immediately, and then turned to brown and finally to orange. T.l.c. of the reaction mixture initially showed a dark-red compound, which appeared to be unstable, and gave an orange compound when the suspension was stirred for 3–4 h at room temperature. The suspension was filtered, and the filtrate evaporated to dryness. The residue was crystallized from ethanol and gave the corresponding *O*-acetyl derivatives of the imino *N*-oxides **14–16** (see Table I). *O*-Deacetylation of **14–16** was achieved without loss of the *N*-oxide residue by dissolving the compound in methanol, half-saturating with ammonia, and keeping the solution for 12–15 h at 4°. After evaporation, the residue was dissolved in methanol, purified by preparative silica gel chromatography, and lyophylized from water to yield the *N*-oxides **17–19**, as orange to red powders (see Table I).

## REFERENCES

1 I. C. P. SMITH, in M. M. SWARTZ, J. R. BOLTON, AND D. C. BERG (Eds.), *Biological Applications of Electron Spin Resonance*. Wiley-Interscience, New York, 1972, pp. 483–539.
2 L. J. BERLINER, *Spin Labeling*, Academic Press, New York, 1976.
3 M. BASSET, G. DEFAYE, AND E. M. CHAMBAZ, *Biochim. Biophys. Acta*, 491 (1977) 434–446.
4 J. D. MORRISETT, in L. J. BERLINER (Ed.), *Spin Labeling*, Academic Press, New York, 1976, pp. 274–338.
5 T. R. KRUGH, in L. J. BERLINER (Ed.), *Spin Labeling*, Academic Press, New York, 1976, pp. 339–372.
6 T. BUCKMAN, *Biochemistry*, 9 (1970) 3255–3265.
7 R. A. DWEK, S. WAIN-HOBSON, S. DOWER, P. GETTINS, B. SUTTON, S. J. PERKINS, AND D. GIVOL, *Nature (London)*, 266 (1977) 31–37.
8 P. LU, M. A. JAREMA, K. MOSSER, AND W. E. DANIEL, *Proc. Natl. Acad. Sci. U.S.A.*, 73 (1976) 3471–3475.
9 A. JOBE AND S. BOURGEOIS, *J. Mol. Biol.*, 69 (1972) 397–408.

10 J. MONOD AND G. COHEN-BAZINE, *Biochim. Biophys. Acta*, 7 (1951) 585–599.
11 B. MÜLLER-HILL, H. V. RICKENBERG, AND K. WALLENFELS, *J. Mol. Biol.*, 10 (1964) 303–318.
12 S. D. GERO AND C. BURSTEIN, *Biochim. Biophys. Acta*, 117 (1966) 314–418.
13 K. JAYARAMAN, B. MÜLLER-HILL, AND H. V. RICKENBERG, *J. Mol. Biol.*, 18 (1966) 339–343.
14 A. D. RIGGS, D. F. NEWBY, AND S. BOURGEOIS, *J. Mol. Biol.*, 51 (1970) 303–314.
15 R. R. RANDO, *Nature, New Biol.*, 234 (1971) 183.
16 Y. OHSHIMA, T. MIZOKOSHI, AND T. HORIUCHI, *J. Mol. Biol.*, 89 (1974) 127–136.
17 M. D. BARKLEY, A. D. RIGGS, A. JOBE, AND S. BOURGEOIS, *Biochemistry*, 14 (1975) 1700–1712.
18 W. G. STRUVE AND H. M. MCCONNELL, *Biochem. Biophys. Res. Commun.*, 49 (1972) 1631–1637.
19 H.-R. RACKWITZ, M. A. JAREMA, AND P. LU, unpublished results.
20 J. H. OSIECKI, AND E. F. ULLMAN, *J. Am. Chem. Soc.*, 90 (1968) 1078–1079.
21 D. G. B. BOOCOCK AND E. F. ULLMAN, *J. Am. Chem. Soc.*, 90 (1968) 6873–6874.
22 D. G. B. BOOCOCK, R. DAVEY, AND E. F. ULLMAN, *J. Am. Chem. Soc.*, 90 (1968) 5945–5946.
23 E. F. ULLMAN AND D. G. B. BOOCOCK, *Chem. Commun.*, (1969) 1161–1162.
24 P. W. KOPF, R. KREILICK, D. G. B. BOOCOCK, AND E. F. ULLMAN, *J. Am. Chem. Soc.*, 92 (1970) 4531.
25 E. F. ULLMAN, L. CALL, AND J. H. OSIECKI, *J. Org. Chem.*, 35 (1970) 3623–3631.
26 J. CONCHIE AND G. A. LEVVY, *Methods Carbohydr. Chem.*, 2 (1962) 335–336.
27 B. J. GAFFNEY, in L. J. BERLINER (Ed.), *Spin Labeling*, Academic Press, New York, 1976, pp. 183–238.
28 K. WALLENFELS AND R. WEI, in P. D. BOYER (Ed.), *The Enzymes*, 3rd edn., Vol. VII, Academic Press. New York, 1972, pp. 617–663.
29 J. D. MORRISETT AND H. R. DROTT, *J. Biol. Chem.*, 244 (1969) 5083–5086.
30 M. LAMCHEN AND T. W. MITTAG, *J. Chem. Soc. C*, (1966) 2300–2303.
31 R. U. LEMIEUX, *Methods Carbohydr. Chem.*, 2 (1962) 221–222.