122 (26.7), 96 (21.7).

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4-Deoxy-Analogs of *p*-Nitrophenyl β-D-Galactopyranosides for Specificity Study with β-Galactosidase from *Escherichia coli*.

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The synthesis is reported of *p*-nitrophenyl glycosides of D-galactose modified at C-4 with azido- (**5**), amino- (**6**) group and fluorine (**13**). 4-Azido-2,3,6-tri-*O*-benzoyl-4-deoxy-α-D-galactopyranosyl chloride and 2,3,6-tri-*O*-benzoyl-4-deoxy-4-fluoro-α-D-galactopyranosyl bromide were coupled with potassium *p*-nitrophenoxide in the presence of 18-crown-6 giving the corresponding *p*-nitrophenyl 4-azido- and 4-fluoro-4-deoxy-β-D-galactopyranoside derivatives. *p*-Nitrophenyl 4-amino-4-deoxy-β-D-galactopyranoside (**6**) was obtained by selective reduction of *p*-nitrophenyl 4-azido-4-deoxy-β-D-galactopyranoside (**5**) using 1,3-propane dithioltriethylamine. These galactoside analogs were slowly hydrolyzed in the increasing rate order of **5**, **6** and **13** by β-galactosidase from *Escherichia coli*.

Introduction

β-Galactosidase from *Escherichia coli* is a disaccharidase which catalyzes the hydrolysis and transgalactolysis of β-galactopyranosides with overall retention of configuration at the anomeric center.[1~3] Although a great amount of data has been accumulated on β-galactosidase, little is known about the active site.[4,5] The action mechanism of galactosidases has been suggested, in a somewhat analogous way to lysozyme, to involve the formation of a galactosyl-enzyme intermediate by a nucleophile and a general acid catalytic group located at the active site and its subsequent hydrolysis or transglycosidation.[4~10] Efficiency in the formation of a galactosyl-enzyme intermediate relates to substrate's susceptibility to galactosidase *i.e.* satisfaction to a strict glycon specificity is prerequsite for the activity.[8~10]

Amino and fluoro sugars are invaluable analogs[11~20] to assess the role of a sugar OH group as an activity determinant and to elucidate the mechanism of specificity and activity for carbohydrate modifying enzymes. Substrate specificity study using methyl 4-amino- and 4-fluoro-4-deoxy-β-D-galactopyranosides (**7** and **9β**) for β-galactosidase from *Escherichia coli* and their α-anomers for α-galactosidase from *Aspergillus fumigatus* showed that both enzymes have extremely strict specificity for the 4-OH group of D-galactose.[11] The strict glycon specificity of galactosidases was also observed with methyl 5-thio-β-D-galactopyranoside.[11] Those results were contrasting with the relatively loose glycon specificity shown by galactose oxidase.[13]

On the other hand, the activity of β-galactosidase is known

to be substantially affected by aglycons, *i.e.* the increase of V_{max} (10^2 to 10^3) and the decrease of K_m for higher alkyl or aryl β-D-galactopyranosides compared with methyl β-D-galactopyranoside.[2,21] Hence the inactivity of galactosidases previously observed[11] with the 4-deoxy analogs of methyl galactopyranoside could have arisen from the poor methyl aglycon binding of the 4-analogs to the enzyme as well as from the strict glycon specificity of galactosidases. Thus for elucidation of the mechanism of glycon specificity it is desirable to investigate the reactions of β-galactosidase on the 4-deoxy analogs of *p*-nitrophenyl β-D-galactoside. Having enhanced aglycon binding, their enzymic studies can be interpreted more closely related to the accomodating ability for 4-deoxy analogs by β-galactosidase. Furthermore facile quantitations of the liberated *p*-nitrophenol from possible enzymic reactions confer an additional attraction for the 4-deoxy analogs of *p*-nitrophenyl β-D-galactopyranoside.[8,22,23] We report here the syntheses of 4-azido-4-deoxy- (**5**), 4-amino-4-deoxy- (**6**), and 4-deoxy-4-fluoro- (**13**) analogs of *p*-nitrophenyl β-D-galactopyranoside as potential substrates of β-galactosidases.

Their altered hydrogen bond properties and the similiarities in bond length and polarization between C-OH and C-F or C-NH_2 of the 4-fluoro or 4-amino analogs of *p*-nitrophenyl β-D-galactoside[13,16,17] will enable to elucidate the role of the 4-OH group of D-galactose for the recognition of the active site of β-galactosidase and the catalytic mechanism of the enzyme. The result will also enable to envisage other carbohydrate-protein interaction mechanism. In addition, these 4-fluoro or 4-amino analogs having the *p*-nitrophenyl linker arm to couple with proteins have the potential as invaluable intermediates for the syntheses of diagnostic and therapeutic agents.[18~20,24,25]

Results and Discussion

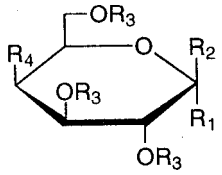
For the synthesis of *p*-nitrophenyl 4-amino-4-deoxy-β-D-galactopyranoside (**6**), methyl 4-azido-2,3,6-tri-*O*-benzoyl-4-deoxy-α-D-galactopyranoside (**1**) was transformed to *p*-nitrophenyl 4-azido-4-deoxy-β-D-galactopyranoside (**4**) by sequential reactions of acetolysis, chlorination and then coupling with *p*-nitrophenoxide. Compound **1** was acetolyzed by treatment of acetic anhydride-acetic acid containing 1% c-sulfuric acid.[26,27] The 1-*O*-actetate **2** was obtained in 96% yield. The ^{1}H NMR spectrum showed a doublet of 4.0 Hz at 6.6 ppm for H-1, suggesting **2** to be the α anomer. Treatment of **2** with titanium tetrachloride in chloroform afforded α-chloride **3** in a quantitative yield.[28] In the ^{1}H NMR spectrum of **3**, a doublet at 6.50 ppm ($J_{1,2}$ 3.2 Hz) was indicative of the α-anomeric configuration. Reaction of α-chloride **3** with potassium *p*-nitrophenoxide and 18-crown-6 in refluxing chloroform for 40 h, and the subsequent column chromatography of the produced mixture yielded crystalline *p*-nitrophenyl 4-azido-2,3,6-tri-*O*-benzoyl-4-deoxy-β-D-galactopyranoside (**4**) in 89% yield. The ^{1}H, and ^{13}C NMR spectra of **4** showed H-1 signal at 5.34 ppm as a doublet of large coupling constant ($J_{1,2}$ 7.8 Hz), and C-1 signal at 98.8 ppm being consistent with the β-configuration for the *p*-nitrophenyl group.[25] Zemplén debenzoylation of **4** furnished crystalline *p*-nitrophenyl 4-azido-4-deoxy-β-D-galactopyranoside (**5**) in 90% yield.

Selective reduction of the azido group of **5** was attempted with $NiCl_2 \cdot 6H_2O$-$NaBH_4$,[29] $Na_2S \cdot 9H_2O$[30,31] and propane-1,3-dithiol.[32] While $NiCl_2 \cdot 6H_2O$-$NaBH_4$, and $Na_2S \cdot 9H_2O$ reduced both the azido and the nitro group indiscriminately giving *p*-aminophenyl 4-amino-4-deoxy-β-D-galactopyranoside,[33] propane-1,3-dithiol-triethylamine in methanol selectively reduced the azido group converting **5** to *p*-nitrophenyl 4-amino-4-deoxy-β-D-galactopyranoside (**6**). Compound **6** was further characterized after acetylation with 2 : 1 pyridine-acetic anhydride and identified as *p*-nitrophenyl 2,3,6-tri-*O*-acetyl-4-*N*-acetyl-4-deoxy-β-D-galactopyranoside (**8**) with an overall yield of 62% from **5**. The IR spectrum of **8** showed strong absorption bands at 3420 and 1655 cm^{-1} for the NH and C=O groups respectively, indicative of secondary amides. In the ^{1}H NMR spectrum of **8**, signals for *p*-nitrophenyl protons appeared at 8.13 and 6.99 ppm. Aromatic proton signals appear as two broad doublets at 7.5 and 6.8 ppm for *N*-acetoamidophenyl group,[33] hence propane-1,3-dithiol-triethylamine in methanol reduced only the azido group of **5** selectively to the amino group of **6**.

Since direct fluorination of *p*-nitrophenyl glycosides with DAST was not possible,[34] *p*-nitrophenyl 2,3,6-tri-*O*-benzoyl-4-deoxy-4-fluoro-β-D-galactopyranoside (**12**) was synthesized by *p*-nitrophenylglycosidation of 2,3,6-tri-*O*-benzoyl-1,4-dideoxy-1-bromo-4-fluoro-α-D-galactopyranose (**11**), which was obtained from the corresponding methyl α-D-galactopyranoside **10** by treatment with HBr. Methyl 4-deoxy-4-fluoro-α-D-galactopyranoside (**9**) was synthesized by the reported method.[11,12] Treatment of compound **9** with benzoyl chloride in pyridine afforded compound **10**, which was then reacted with 30% hydrogen bromide in glacial acetic acid to yield 2,3,6-tri-*O*-benzoyl-4-deoxy-4-fluoro-α-D-galactopyranosyl bromide (**11**) in 86% crude yield. Reaction of α-bromide **11** with potassium *p*-nitrophenoxide and 18-crown-6 in refluxing chloroform for 27 h afforded the *p*-nitrophenyl 2,3,6-tri-*O*-benzoyl-4-deoxy-4-fluoro-β-D-galactopyranoside (**12**) in 90% yield.[25] In the ^{1}H NMR spectrum of **12**, the H-1 appeared with large coupling constant ($J_{1,2}$=7.8 Hz) at 5.40 ppm indicating the *p*-nitrophenyl galactopyranoside **12** being the β-anomer. Coupling constants of 26.6 Hz and 25.9 Hz were observed for ^3J couplings between H-3 and F-4, and H-5 and F-4, respectively.[11,34~37] A ^2J coupling between H-4 and F-4 was 50.3 Hz. These values are close to those of the corresponding derivative of methyl β-D-galactopyranoside.[11] ^{13}C NMR showed the anomeric carbon at 98.7 ppm, indicative of the β-configuration for **12**.[25,35] In addition C-4 at 85.83 ppm splitted by the directly bonded fluorine with a ^1J coupling of 184.4 Hz and C-6 at 61.99 ppm splitted by a ^3J coupling of 4.7 Hz with F-4 were also observed.[11] Treatment of compound **12** with 0.1 N sodium methoxide gave *p*-nitrophenyl 4-deoxy-4-fluoro-β-D-galactopyranoside (**13**) in 93% yield.

Susceptibility to β-galactosidase from *Escherichia coli* was tested with the analogs of *p*-nitrophenyl β-D-galactopyranoside (Table 1 and Figure 2). The reaction mixtures were monitored for release of *p*-nitrophenol as its anion colorimetrically at 420 nm.[8] It was found that β-galactosidase was able to hydrolyze the 4-deoxy analogs of *p*-nitrophenyl β-D-galactopyranoside and the hydrolysis rate was increasing in the order of 4-azido- (**5**), 4-amino- (**6**) and 4-fluoro-analog (**13**). Even though the hydrolysis proceeded very slowly, the pesent result is contrasting to the early finding[11] that not a single one of 4-deoxy analogs of methyl β-D-galactopyranoside was hydrolyzed. However the role of the 4-hydroxyl

Figure 1. Derivatives of Galactopyranoside.



Compound	R_1	R_2	R_3	R_4
1	OMe	H	Bz	N_3
2	OAc	H	Bz	N_3
3	Cl	H	Bz	N_3
4	H	OPNP	Bz	N_3
5	H	OPNP	H	N_3
6	H	OPNP	H	NH_2
7	H	OMe	H	NH_2
8	H	OPNP	Ac	NHAc
9	OMe	H	H	F
10	OMe	H	Bz	F
11	Br	H	Bz	F
12	H	OPNP	Bz	F
13	H	OPNP	H	F
14	H	OPNP	H	OH

Table 1. Substrate specificities of β-D-Galactosidase from *Escherichia coli*

Compound	Formular number	Conc. (mole)	Enzyme (units)	Time (h)	Hydrolysis (%)
p-nitrophenyl β-D-galactopyranoside	**14**	0.1	5	0.2	85
4-azido-4-deoxy	**5**	1.0	50	42	4
4-amino-4-deoxy	**6**	1.0	50	42	9
4-fluoro-4-deoxy	**13**	1.0	50	42	17

group of D-galactose as a determinant in the β-galactosidase hydrolysis is still clearly marked.

The critical role of the 4-hydroxyl group may be evaluated in relation to its ability to form a hydrogen bond as well as its size. The fact that the fluorine atom in **13** cannot act as a proton donor for hydrogen bonding indicates that the 4-OH of D-galactose is not a proton donor for hydrogen bonding.[13,38] It is improbable that the 4-OH of D-galactose is a proton acceptor for hydrogen bonding as the $-NH_2$ group in **6** is more efficient, in that respect, than not only the fluorine atom in **13** but also than the -OH group of "natural" substrates.[13,38] Hence the relatively high reactivity shown by the 4-fluoro-analog **13** indicates that the direct hydrogen bonding between the axial 4-hydroxyl group of D-galactopyranose of "natural" substrates and the enzymic active site surface is not a prerequisite for the hydrolysis or the formation of enzyme-substrate complex of the β-galactosidase from *Escherichia coli*.

It can be proposed that the size of the C-4 substituent is much more critical for the reactivity of β-galactosidase. With the knowledge that the molecular dimensions in terms of bond lengths and subsituent volumes are increasing in

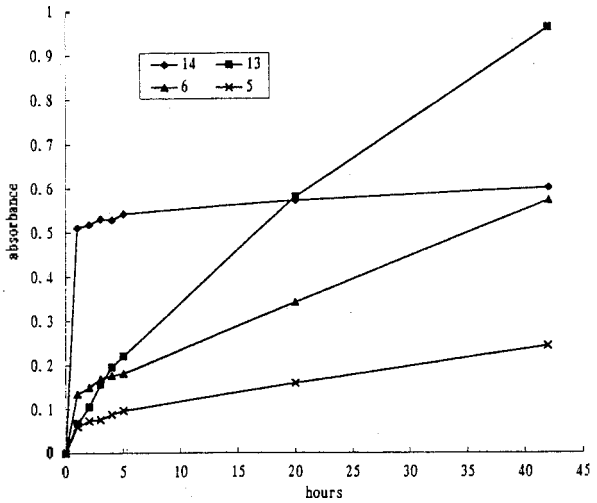


Figure 2. Susceptibility to β-D-galactosidase of 4-deoxy analogs of *p*-nitrophenyl β-D-galactopyranoside.

the order of -F, -OH, $-NH_2$, and $-N_3$,[13] a comparison of the reactivities of 4-azido- (**5**), 4-amino- (**6**), 4-fluoro-analog (**13**) and *p*-nitrophenyl β-D-galactoside relative to the molecular dimension of C-4 substituents shows a correlate, *i.e.* the 4-fluoro-analog (**13**) with the -F, of similiar size to -OH but smaller, shows the highest reactivity. Though the size deviations of $-NH_2$ and -F from -OH are close, the 4-amino-analog (**6**) having $-NH_2$ group which is larger than -OH, showed a lower reactivity than 4-fluoro-analog (**13**) towards the β-galactosidase from *Escherica coli*.

Although it is too hasty to draw a conclusion based only on the present results, the activity of β-galactosidase appears to be on the analogy of galactose oxidase to a certain extent, *i.e.*, the 4-hydroxyl group of D-galactose serves as a filler or an anchor, for a particular cleft on the surface of β-galactosidase.[13] However the accurate filling, or anchoring of D-galactose by 4-OH is far more regorous for the activity of the β-galactosidase than galactose oxidase. This may be related to the proposal that the active site of β-galactosidase extends over a larger part of the enzyme molecule, requiring many amino acids to interact in a concerted way with substrate in the binding process.[4,39] Such interactions could lead to dynamic situations that result in the structural differences in the active site of free enzyme and of the complexes with "natural" substrate or with deoxy-analogs. The strict specificity could be explained in terms of conformational adaptibility of the active site of β-galactosidase from *Escherichia coli*.

Experimental

General. ^1H NMR and ^{13}C NMR spectra were recorded with a Varian VXR-200, a Bruker ARX-400 or a Bruker AW-80 spectrometer on solutions in $CDCl_3$ with tetramethylsilane as the internal standard or in specified solvents. Optical rotations were measured with a Perkin-Elmer 241 polarimeter. IR spectra were recorded on a Mattson 3000 FT-IR spectrometer using thin film on KBr plates. Melting points were determined with an Edmund Buhlen 7400 SPA-1 and are uncorrected. Organic solvents were distilled from appropriate

drying agents (DMF-BaO; Toluene-P_2O_5; EtOAc-K_2CO_3; pyridine-KOH; CH_2Cl_2-$CaCl_2$ and P_2O_5; $CHCl_3$-washed with distilled water, then dried over $CaCl_2$ and P_2O_5; methanol and ethanol- CaO or Mg turnings). Solutions were usually evaporated *in vacuo* below 40 ℃. Thin layer chromatography (tlc) was performed on aluminum sheets, precoated with 0.2 mm layers of silica gel 60F-254 (E. Merck, Darmstadt, Germany), and visualized by charring with 5% sulfuric acid in ethanol. Silica gel (E. Merck, Art 7734, 70-230 mesh) was used for column chromatography.

Enzymic reactions. β-Galactosidase was purchased from Worthington Biochemical Corporation. Enzymic reactions were conducted at 37 ℃ in 50 mM-KH_2PO_4-0.4 M NaOH buffer, pH 7.0, 1 mM $MgCl_2$ and stopped by adding 0.1 M K_2CO_3. *p*-Nitrophenol release was measured as the ionized form with a UV spectrophotometer at 420 nm.

Methyl 4-azido-2,3,6-tri-*O*-benzoyl-4-deoxy-α-D-galactopyranoside (1). A solution of methyl 2,3,6-tri-*O*-benzoyl-4-*O*-methanesulfonyl-α-D-glucopyranoside (3.95 g, 6.9 mmol) and sodium azide (1.34 g, 20.6 mmol) in DMF (40 mL) was heated for 5 h at 130 ℃. The reaction mixture was evaporated under reduced pressure and diluted with methylene chloride, washed with water, dried over sodium sulfate, and evaporated to a syrup. The crude product was purified by chromatography using hexane/EtOAc (7 : 1, v/v) as eluent to give **1** (2.2 g, 61%): IR(KBr) 2100 cm^{-1} (N_3); ^{1}H NMR δ 8.2-7.1 (m, 15H, aromatic H), 6.0, 5.85 (both dd, each 1H, $J_{2,3}$=10.0 Hz, $J_{3,4}$=4.0 Hz, H-2, 3), 5.20 (d, 1H, $J_{1,2}$= 4.0 Hz, H-1), 4.80-3.25 (m, 4H, H-4, 5, 6_a, 6_b), 3.40 (s, 3H, OCH_3).

1-*O*-Acetyl-4-azido-2,3,6-tri-*O*-benzoyl-4-deoxy-α-D-galactopyranose (2). A solution of **1** (2.2 g, 4.2 mmol) in acetic anhydride-acetic acid (7/3, 3 mL) was cooled to 0 ℃ and c-sulfuric acid (1 drop) was added and stirred for 24h at room temperature. When tlc showed the reaction to be complete, sodium acetate trihydrate was added and then stirred for 3 h. The reaction mixture was diluted with methylene chloride, washed with sodium hydrogen carbonate solution and water, dried over sodium sulfate, and concentrated to give **2** (2.2 g, 96%), R_f 0.61 (toluene/EtOAc, 5/1): ^{1}H NMR δ 8.2-7.1 (m, 15H, aromatic H), 6.60 (d, 1H, $J_{1,2}$=4.0 Hz, H-1), 6.15-5.65 (m, 2H, H-2, 3), 4.75-4.15 (m, 4H, H-4, 5, 6_a, 6_b), 2.05 (s, 3H, CH_3CO_2).

4-Azido-2,3,6-tri-*O*-benzoyl-4-deoxy-α-D-galactopyranosyl chloride (3). To a solution of 1-*O*-aectate **2** (2.2 g, 4.0 mmol) in anhydrous chloroform (15 mL) titanium tetrachloride (0.48 mL, 4.4 mmol) in anhydrous chloroform (1 mL) was added. After stirring for 4 h at 80 ℃, the reaction mixture was cooled to room temperature, poured into ice-cracked water (40 mL) and then extracted with methylene chloride. The organic layer was washed with ice water, dried over sodium sulfate, and concentrated under reduced pressure. The obtained syrup (2.1 g, 98%) was used directly in the next step without further purification: R_f 0.6 (toluene/EtOAc, 15/1); ^{1}H NMR δ 8.2-7.1 (m, 15H, aromatic H), 6.50 (d, 1H, $J_{1,2}$=3.2 Hz, H-1), 6.1 (dd, 1H, $J_{2,3}$=10.0 Hz, H-2), 5.8 (dd, 1H, $J_{3,4}$=4.0 Hz, H-3), 4.9-4.15 (m, 4H, H-4, 5, 6_a, 6_b).

***p*-Nitrophenyl 4-azido-2,3,6-tri-*O*-benzoyl-4-deoxy-β-D-galactopyranoside (4).** To a solution of α-chloride **3** (2.1 g, 4.0 mmol) in alcohol-free chloroform (50 mL) potassium *p*-nitrophenoxide (1.4 g, 7.9 mmol), a catalytic amount of 18-crown-6 (0.21 g, 0.8 mmol), and molecular sieve powder (4 Å) were added. The suspension was refluxed with stirring for 40 h. The reaction mixture was cooled and filtered on a Celite pad. The filtrate was washed with diluted sodium hydrogen carbonate solution, successively with cold water several times to remove the excess of phenoxide, dried over anhydrous sodium sulfate, and evaporated to a syrup. The residual syrup was chromatographed by elution with hexane/EtOAc (5/2). *p*-Nitrophenyl β-glycoside **4** was obtained as a white solid 2.2 g (89%), which was crystallized from methanol, mp 103-107 ℃: R_f 0.38 (hexane/EtOAc 5/2); ^{1}H NMR δ 8.1-7.3 (m, 17H, aromatic H, and *p*-NO_2Ph), 7.0 (d, 2H, $J_{H,H}$=10.5 Hz, *p*-NO_2Ph), 6.04 (dd, 1H, $J_{2,3}$=10.2 Hz, H-2), 5.65 (dd, 1H, $J_{3,4}$=3.5 Hz, H-3), 5.34 (d, 1H, $J_{1,2}$=7.8 Hz, H-1), 4.7-4.6 (m, 2H, H-6_a, 6_b), 4.45-4.3 (m, 2H, H-4, 5); ^{13}C NMR δ 161.08, 143.16, 125.63, 116.84 (*p*-NO_2Ph)/ 98.79 (C-1)/ 73.21, 71.70, 68.89 (C-2, 3, 5)/ 62.97 (C-6)/ 59.89 (C-4).

***p*-Nitrophenyl 4-azido-4-deoxy-β-D-galactopyranoside (5).** Compound **4** (394 mg, 0.6 mmol) in methanol (45 mL) was stirred with 0.1 M sodium methoxide in methanol (9 mL) for 2 h at room temperature. The mixture was neutralized by an ion-exchange resin (Dowex-50 H^+ form), and evaporated to a white solid **5** (183 mg, 90%), which was crystallized from methanol, mp 201℃, dec.: R_f 0.79 (toluene/EtOAc/EtOH, 1/1/1); ^{1}H NMR (acetone-d_6) δ 8.2, 7.25 (both d, 2H each, $J_{H,H}$=9.6 Hz, *p*-NO_2Ph), 5.1 (d, 1H, $J_{1,2}$=8.0 Hz, H-1), 4.95-4.8 (m, 1H, H-3), 4.15-3.6 (m, 5H, H-2, 4, 5, 6_a, 6_b).

***p*-Nitrophenyl 4-amino-4-deoxy-β-D-galactopyranoside (6) and *p*-nitrohenyl 4-acetamido-2,3,6-tri-*O*-acetyl-4-deoxy-β-D-galactopyranoside (8).** To a solution of compound **5** (140 mg, 0.4 mmol) in methanol (2.5 mL) propane-1,3-dithiol (86 μL, 0.86 mol) and triethylamine (0.3 mL, 2.2 mmol) were added. The reaction mixture was stirred for 40 h at 50 ℃. When tlc showed complete consumption of **5**, the reaction mixture was concentrated under reduced pressure, diluted with water, and washed with methylene chloride. The aqueous layer was evaporated to give **6** as a syrup which gave a pale yellow solid on further drying, $[\alpha]_D$ −80° (c 1.14, water). Syrupy **6** was dissolved in dry pyridine (2 mL) and acetic anhydride (1 mL) was added, and the mixture was stirred for 4 h at room temperature. When tlc showed the reaction to be complete, the mixture was concentrated and coevaporated with toluene. The residue was column chromatograped using toluene/EtOAc (1/1) as eluent to give **8** (80 mg, 62%): IR(KBr) for **8** 3420 (amide NH), 1655 (amide C=O), 1490, 1340 cm^{-1} (*p*-NO_2Ph); ^{1}H NMR for **8** δ 8.13, 6.99 (both d, 2H each, $J_{H,H}$=9.24 Hz, *p*-NO_2Ph), 6.36 (d, 1H, $J_{1,2}$=9.6 Hz, NH), 5.34 (dd, 1H, $J_{2,3}$=7.9 Hz, H-2), 5.14-5.02 (m, 2H, H-1, 3), 4.83-4.74 (m, 1H, H-4), 4.3-4.05 (m, 3H, H-5, 6_a, 6_b), 2.1, 2.05, 1.98 (CH_3CO_2×4); ^{13}C NMR for **8** 171.2, 170.8, 170.6, 169.9 (C=O)/ 161.6, 143.7, 126.2, 116.9 (*p*-NO_2Ph)/ 99.5 (C-1)/ 72.9, 71.6, 69.1 (C-2, 3, 5)/ 62.7 (C-6)/ 48.0 (C-4)/ 23.5, 21.1 (CH_3CO_2).

Methyl 4-deoxy-4-fluoro-α-D-galactopyranoside (9). Compound **9** was synthesized accordingly to the known procedure and was crystallized from methanol-ethyl acetate, mp 101-103 ℃ (lit.[11] 102-103 ℃): R_f 0.36 (toluene/EtOAc/EtOH, 1/1/1).

Methyl 2,3,6-tri-*O*-benzoyl-4-deoxy-4-fluoro-α-D-galactopyranoside (10). A solution of **9** (60 mg, 0.34 mmol) with benzoyl chloride (0.13 mL, 1.1 mmol) in pyridine (2 mL) was stirred for 3 h at room temperature. To the cool mixture aqueous saturated sodium hydrogen carbonate solution (3 mL) was added slowly. The mixture was extracted with methylene chloride, washed successively with water, 2 N hydrogen chroride solution, sodium hydrogen carbonate solution, and water, dried over sodium sulfate, and then concentrated to give **10** (146 mg, 94%), which was crystallized from chloroform-pet. ether, mp 117-118 ℃: R_f 0.62 (toluene/EtOAc, 5/1); IR (KBr) 1740 cm^{-1} (ester); ^1H NMR δ 8.2-7.1 (m, 15H, aromatic H), 6.05-5.55 (m, 2H, H-2, 3), 5.18 (dd, 1H, $J_{4,F}$=52 Hz, H-4), 5.11 (d, 1H, $J_{1,2}$=3.6 Hz, H-1), 4.7-4.1 (m, 3H, H-5, 6_a, 6_b), 3.42 (s, 3H, OCH_3).

2,3,6-Tri-*O*-benzoyl-4-deoxy-4-fluoro-α-D-galactopyranosyl bromide (11). Reaction of compound **10** (765 mg, 1.5 mmol) with a 30% solution of hydrogen bromide in glacial acetic acid for 7 h afforded α-bromide **11** (720 mg, 86%). The obtained solid was used directly in the next step without further purification: R_f 0.75 (toluene/EtOAc, 5/1); ^1H NMR δ 8.2-7.15 (m, 15H, aromatic H), 6.87 (d, 1H, $J_{1,2}$=4.0 Hz, H-1), 6.15-5.6 (m, 2H, H-2, 3), 5.28 (dd, 1H, $J_{4,F}$=51 Hz, H-4), 4.9-4.3 (m, 3H, H-5, 6_a, 6_b).

***p*-Nitrophenyl 2,3,6-tri-*O*-benzoyl-4-deoxy-4-fluoro-β-D-galactopyranoside (12).** Reaction of compound **11** (393 mg, 0.7 mmol) with potassium phenoxide (249 mg, 1.4 mmol), a catalytic amount of 18-crown-6 (37 mg, 0.14 mmol), and molecular sieve 4 Å in chroloform (5 mL) for 27 h as described procedure for compound **4**, yielded compound **12** as a solid (390 mg, 90%), which was crystallized from methanol, mp 150-152 ℃: R_f 0.53 (hexane/EtOAc, 5/2); ^1H NMR δ 8.07-7.25 (m, 15H, aromatic H), 7.02 (d, 2H, $J_{H,H}$=9.3 Hz, *p*-NO_2Ph), 6.05 (dd, 1H, $J_{2,3}$=10.6 Hz, H-2), 5.48 (dddd, 1H, $J_{3,F}$=26.6 Hz, $J_{3,4}$=2.6 Hz, H-3), 5.40 (d, 1H, $J_{1,2}$=7.8 Hz, H-1), 5.19 (dd, 1H, $J_{4,F}$=50.3 Hz, H-4), 4.7-4.61 (m, 2H, H-6_a, 6_b), 4.34 (dt, 1H, $J_{5,F}$=25.9 Hz, H-5); ^{13}C NMR δ 165.97, 165.74, 164.93 (C=O)/ 161.09, 143.24, 125.58, 116.88 (*p*-NO_2Ph)/ 98.72(C-1)/ 85.83 (C-4, d, $J_{C-4,F-4}$=184.4 Hz)/ 72.05, 71.74 (C-3, 5, d, $J_{C-3,F-4}$=$J_{C-5,F-4}$=8.3 Hz)/ 68.79 (C-2)/ 61.99 (C-6, d, $J_{C-6,F-4}$=4.7 Hz).

***p*-Nitrophenyl 4-deoxy-4-fluoro-β-D-galactopyranoside (13).** Compound **13** was prepared from **12** as described for compound **5** and isolated in 93% yield as a white solid, which was crystallized from methanol, mp 190 ℃, dec.; $[\alpha]_D$ −76° (c 1.26, water); R_f 0.67 (toluene/EtOAc/EtOH, 1/1/1); ^1H NMR in D_2O δ 8.23, 7.25 (both d, each 2H, $J_{H,H}$=9.59 Hz, *p*-NO_2Ph), 7.02 (d, 2H, $J_{H,H}$=9.3 Hz, *p*-NO_2Ph), 5.10 (d, 1H, $J_{1,2}$=7.38 Hz, H-1), 4.56 (dd, 1H, $J_{4,F}$=51.85 Hz, H-4), 4.1-3.72 (m, 5H, H-2, 3, 5, 6_a, 6_b); ^{13}C NMR δ 163.8, 144.3, 127.1, 118.3 (*p*-NO_2Ph)/ 102.2 (C-1)/ 90.4 (C-4, d, $J_{C-4,F-4}$=180.7 Hz)/ 76.0, 73.65 (C-3, 5, d, $J_{C-3,F-4}$=$J_{C-5,F-4}$=17.9 Hz)/ 72.4 (C-2)/ 61.3 (C-6, d, $J_{C-6,F-4}$=5.1 Hz).

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Semiempirical Calculations of Substituent Effects on the Reactions of Cephem-Like β-Lactam Molecules

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Semiempirical PM3 MO calculations are applied to estimate both 1-atom (X=S, O, C) and 3-substituent (Y=R, CH_2R, SR, CH_2SR) effects on the reactions of some 1-atom-replaced and 3-substituted cephem-like β-lactam compounds of thiacephems, oxacephems, and carbacephems. Stabilization energy (SE) of the reaction intermediate for the reaction with a hydroxyl ion can be used to evaluate the facility of a reaction and selected as a chemical reactivity index. With the 1-atom effect only , the SE values obtained imply that thiacephems are generally more reactive than the other two cephem-like molecules and the reactivity order is thiacephems>oxacephems>carbacephems. When it comes to the 3-substituent (Y=R, CH_2R, SR, CH_2SR) effect, chemical reactivity can be best realized by using a 3-substituted thiacephem molecule capable of giving a resonance-stabilized and electron-rich leaving group after the reaction with a nucleophile. SE values, however, decrease in most cases when an additional intervening ethylene group is present (Y=CH_2R, CH_2SR). The overall 3-substituent reactivity tendency is SR>CH_2SR>R>CH_2R.

Introduction

β-Lactam antibiotics are well known to inactivate several enzymes involved in the synthesis of bacterial cell walls by acylating a serine residue and still widely used in the treatment of bacterial diseases. Shortly after the first appearance of benzylpenicillin into the medicinal world at the end of the World War II, almost every effort in an attempt to study the detailed structure-activity relationships of β-lactam antibiotics was made. As a result, it was suggested that the activities of bicyclically fused β-lactam molecules were dominated by the inherent strain of the four-membered ring[1] or by amide resonance resulting from the delocalization of the nitrogen lone pair electrons to the participating atoms.[2] Amide resonance is believed to be responsible for the lower susceptibility of the carbonyl group to nucleophilic attack because it is assumed that the resonance stabilizes amides, and so an inhibition of the amide resonance would lead to increased reactivity. Therefore, synthetic chemists were, and to some extent still are, sure that more active antibiotics could be synthesized by making β-lactam systems more strained or the amide resonance more inhibited by modifying the planarity around the nitrogen. These two proposals, however, failed to explain the activity trends experimentally observed. Alternatives for reactivity indices that have been considered include C-N bond length, ^{13}C NMR chemical shift of the β-lactam carbonyl carbon atom,[3,4] IR stretching frequency of the β-lactam carbonyl group ($\nu_{C=O}$),[5] protonation ability of the nitrogen atom,[6,7] or rate of hydrolysis.[6,7] None of such factors, of course, could explain all the activity trends observed.

Cephalosporins are still being synthesized and widely studied in many drug companies and laboratories over the world. Enamine resonance is of special significance to cephalosporin systems. Many theoretical and experimental investigations on cephalosporin antibiotics have reported that cephalosporins having electron-attracting or good leaving groups at their 3'-positions increase the enamine resonance and lead to good antibacterial activities.[8] It is, however, difficult to quantitatively estimate such activity trends for the substituents at the 3'-position experimentally.

In this context, *ab initio* quantum chemical calculations could be a reliable measure. Unfortunately, because of the computational requirement, most of the molecules containing