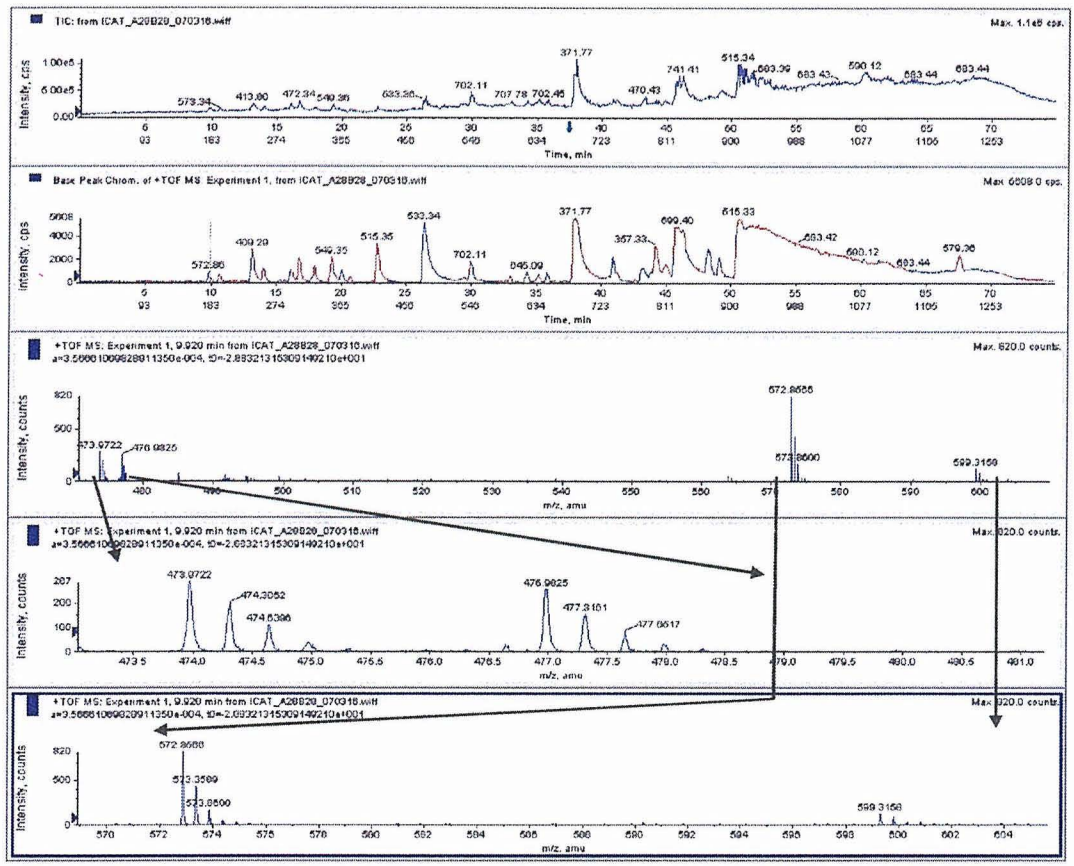
図 8　ICAT による発現差が確認されたものの MS/MS 測定が行われなったピークの例




Showing TOF MS peaks of cICAT labeled samples with peak with light and heavy label pair and peaks with out its pair (possible biomarker candidate). Even though the signal are of good quality it could not be identified by the standard cICAT method of data processing (identify and quantify) .

図 9　ProICAT ソフトウエアによる同定、定量結果

**Pro Group Report**

**Report Parameters:**
**ProtScore threshold: 1.30; Show competitor proteins within ProtScore: 2.00; Software version: 1.0.2**

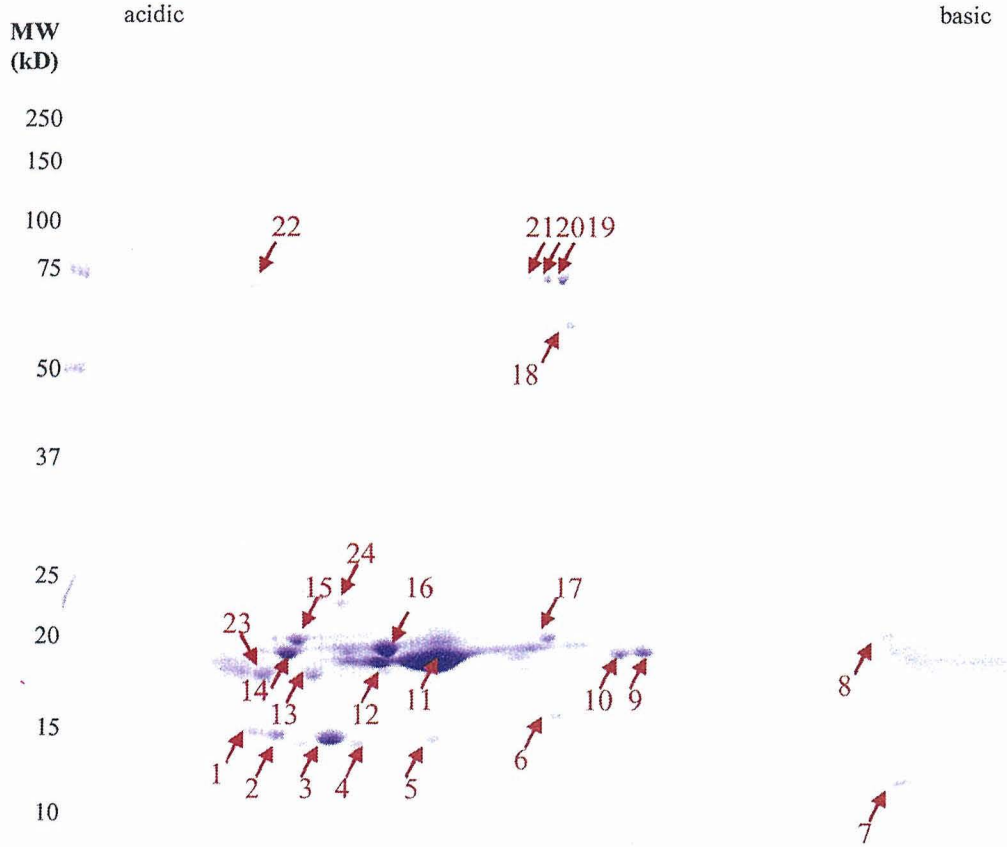
**Report Statistics (2009 total spectra):**

| Confidence (ProtScore) Cutoff | Proteins Identified | Proteins before Grouping | Distinct Peptides | Spectra Identified | % of Total Spectra |
|---|---|---|---|---|---|
| >99 (2.0) | 0 | 0 | 0 | 0 | 0.0 |
| >95 (1.3) | 1 | 1 | 2 | 2 | 0.1 |
| >66 (0.47) | 5 | 10 | 7 | 12 | 0.6 |
| As shown: >95 (1.30) | 1 | 1 | 2 | 2 | 0.1 |

**1.1 Data File: f:\project\wiff file\2007\03\ICAT_A28B28_070316.wiff**
**Interrogator DB: Test_Inter_Sear_DB_0919; ID Range: 1-1340; Quant Range: 1-1340; Available Range: 1-1340**
**Comments:**
**Date of Run: 3/16/2007 22:45:32**
**MS Tolerance: 0.3; MSMS Tolerance: 0.15; Modification Tolerance: 0**
**Max #IDs: 20; Confidence Threshold: 0**
**Custom amino acids: O -> M, Z -> C**
**Result DB: C:\Program Files\Pro ICAT\Pro ICAT tutorial.mdb; Result #: 15**

| Group Report | | | | | | Protein Summary | | | |
|---|---|---|---|---|---|---|---|---|---|
| - | N | Unused ProtSc | Total ProtSc | Accession | Protein Name | Species | Conf | Sequence | Mod | Zone |
| - | 1 | 2.00 | 2.00 | gi\|125142 | Ig kappa chain C region, A allele | | 99 | HNLYTCEVVHK | (heavy) | |
| | | | | | | | 0 | HNLYTCEVVHK | (heavy) | |

図 10　ラット尿中タンパク質の２次元電気泳動解析




（MALDI-TOF マス解析による同定結果）

| spot No. | MS | MS/MS |
|---|---|---|
| 1 | nd | nd |
| 2 | nd | nd |
| 3 | nd | AY327506 |
| 4 | nd | prostatic steroid-binding protein chain C3 precursor |
| 5 | nd | nd |
| 6 | nd | urinary protein 2 precursor |
| 7 | nd | nd |
| 8 | RATVPSP | RATVPSP |
| 9 | alpha-2u-globulin, cain A | alpha-2u-globulin, cain A (precursor) |
| 10 | alpha-2u-globulin, cain A | alpha-2u-globulin, cain A (precursor) |
| 11 | alpha-2u-globulin, cain A | alpha-2u-globulin, cain A (precursor) |
| 12 | alpha-2u-globulin, cain A | alpha-2u-globulin, cain A (precursor) |
| 13 | nd | urinary protein 2 precursor |
| 14 | alpha-2u-globulin (precursor) | alpha-2u-globulin (precursor) |
| 15 | alpha-2u-globulin (precursor) | alpha-2u-globulin (precursor) |
| 16 | nd | alpha-2u-globulin, cain A (precursor) |
| 17 | nd | alpha-2u-globulin, cain A (precursor) |
| 18 | Alpha-amylase | Alpha-amylase |
| 19 | Albumin | nd |
| 20 | Albumin | serum albumin precursor |
| 21 | Albumin | nd |
| 22 | Albumin | nd |
| 23 | nd | nd |
| 24 | alpha-2u-globulin, cain A | nd |

図 11 血清アルブミン等除去キットによる効果

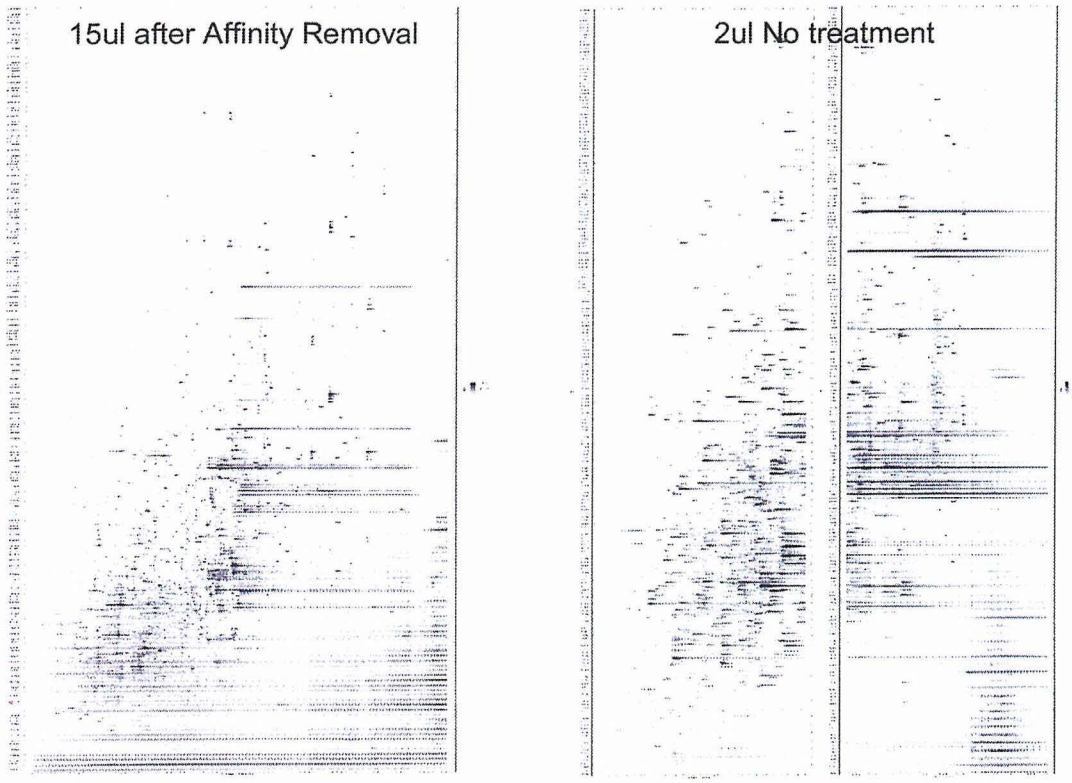



図 12 分子量分画フィルターを用いた前処理効果

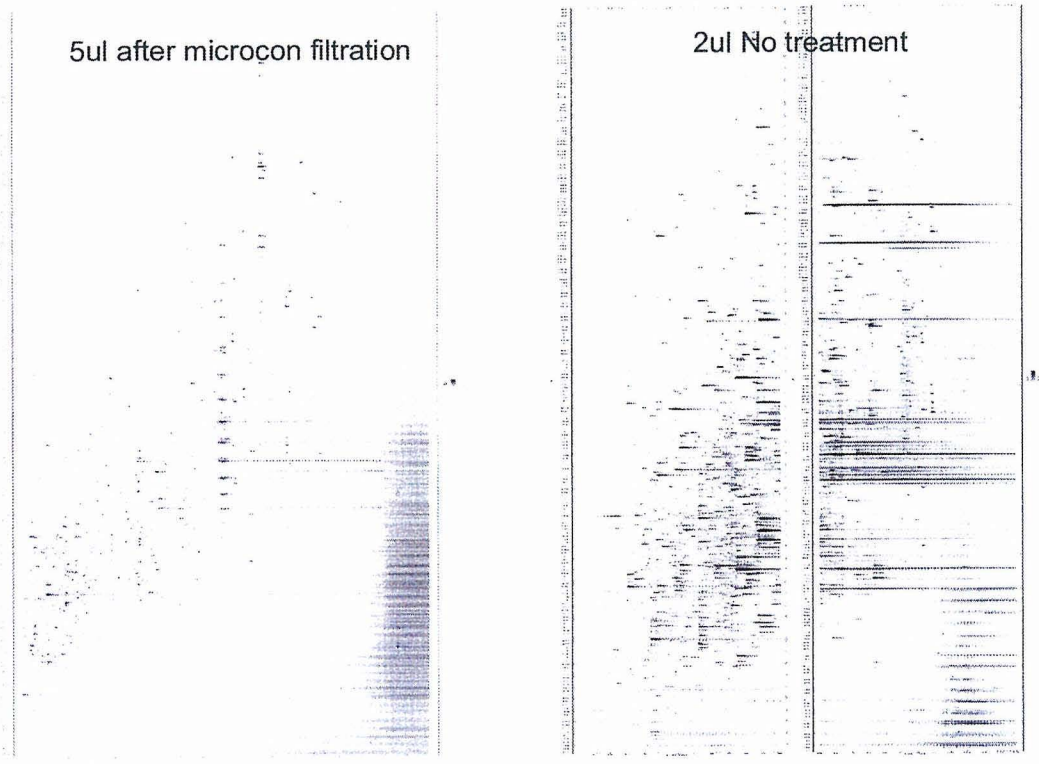
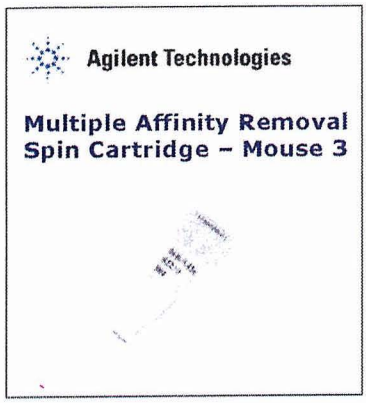
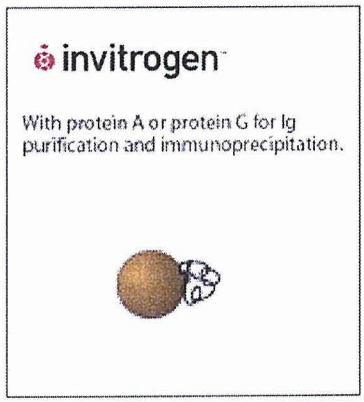


図 13　前処理用に用いたキット等

血中主要タンパク除去カラム




Dynabeads® IgG




Tharma-Max LPA

厚生労働科学研究費補助金（トキシコゲノミクス研究事業）
分担研究報告書

# 分担研究課題：細胞レベルでのメタボロミクス技術の開発

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## 研究要旨

細胞レベルでの薬剤による毒性の予測を行うため、細胞試料を用いたNMRによるメタボノミクス技術の開発を目的とした。本年度は細胞（各種臓器由来）のメタボローム解析に必要なサンプル調製法（特にアルブミン除去などの前処理法）の検討を行い、超音波破砕試料を用いたNMRによるメタボローム解析を実施した。

## A. 研究目的

ヒトに投与して初めて起こる副作用を予測するため網羅的な遺伝子発現解析が注目されトキシコゲノミクスと呼ばれる研究が実施されてきた。しかし毒性の予測までには至っていないのが現状である。本研究では非侵襲試料を用いたメタボノミクス解析手法を確立し、トキシコゲノミクスで得られた網羅的な遺伝子発現情報を補完し、毒性予測につながる評価系開発を行うことを目的としている。そこで我々はよりスクリーニング研究に適していると考えられる培養細胞を用いたメタボノミクス解析技術の確立を目指す。本年度は予備検討として医薬基盤研究所・実験動物開発室で保有している疾患モデルマウスの尿を用いてNMR解析を行い、感度の評価並び解析手法の選択を行った。今後技術開発により、細胞レベルでのメタボロミクス研究を推進する。ヒト肝･腎細胞およびヒト由来培養細胞を用いて、毒性を有する薬剤の網羅的遺伝子発現

## B. 研究方法

＜細胞に関して＞

LI90（肝臓）

肝臓のイトウ細胞由来の正常細胞であり、脂肪蓄積能がある細胞。

培地：Dulbecco's modified Eagle's medium with 10% fetal bovine serum.

継代方法：Cells are harvested after 0.25% trypsin and 0.02% EDTA treatment.

HepG2（肝臓）

毒性の分野で広く用いられているヒト肝癌由来細胞株であり、ダイオキシンに対する受容体発現が確認されている。

培地：Dulbecco's modified Eagle's medium with 10% fetal bovine serum.

継代方法：Cells are harvested after 0.25% trypsin and 0.02% EDTA treatment.

KMRC-3（腎臓）

腎明細胞がん由来細胞株。

培地：Dulbecco's modified Eagle's medium with 10% fetal bovine serum.
継代方法：Cells are harvested after 0.25% trypsin and 0.02% EDTA treatment.

HUV-EC-C（血管内皮）

ヒト正常血管内皮細胞であり、in vitroにおける血管内皮の研究に多用されている。
培地：MCDB 107 with 10% FBS, 100 μg/ml heparin and 50 μg/ml endothelial cell growth supplement
継代方法：Cells are harvested after 0.25% trypsin and 0.02% EDTA treatment.

NCR-G1（精巣）

AFP産生能のほか、Type IV collagen, fibronectin, lamininなど細胞外基質の産生能を保持した細胞株
培地：G031101
継代方法：The cell is peeled off by pipetting. (handle it like embryonic stem cell. )

HeLaS3（子宮頸部）

浮遊培養可能としたHeLa細胞亜株。子宮頸部由来のがん細胞として非常に有名。
培地：Eagle's minimal essential medium with 10% calf serum.
継代方法：Cells are treated with 0.05 % trypsin.

HL60（急性前骨髄球性白血病由来細胞）

がん遺伝子による分化誘導やDMSOによる分化誘導による研究に良く用いられる細胞株。
培地：RPMI1640 medium with 20% fetal calf serum.
継代方法：Simple dilution.

＜超音波破砕＞

細胞を酵素安定化バッファー中で氷冷し、マイクロソン微量超音波細胞破砕機XL2000にて細胞を超音波破砕した。

＜試料の前処理＞

Albumin Segregation Kitを用いて、培養液中の血清成分（特にアルブミン）を除去し、サンプルとした。

＜NMR測定に関して＞

ブルカーバイオスピン社の協力を得て、UltraShield Plus 500MHz NMRにより$^{1}$H-NMRを測定した。

## C. 結果

本分担研究では、細胞レベルでのメタボロミクス技術の開発を目的としており、NMRを用いた高感度解析技術を採用する予定である。しかし、導入予定である800MHzNMR設備整備が遅れており、NMR解析はブルカーバイオスピン社にご協力頂き実施した。本年度は細胞レベルでのメタボノミクス解析の前段階として、細胞の超音波破砕における試料の安定性評価，試料の前処理法の検討ならびに基礎解析を行った。

今回用いた細胞の倍加時間はそれぞれ、LI90（肝臓）35時間，HUV-EC-C（血管内皮）38時間，NCR-G1（精巣）3.5日，HeLaS3（子宮頸部）24時間，HL60（急性前骨髄球性白血病由来細胞）18時間，HepG2（肝臓）32時間，KMRC-3（腎臓）14日であ

った。細胞培養に際しては形態学的特徴を細胞の情報として記録するために動画撮影を行い、細胞増殖の過程を撮影した。

細胞試料の作成法として超音波による細胞破砕を用い、氷冷酵素安定化バッファー中で細胞を超音波破砕し、酵素活性 (G6PD (Glucose-6-phosphate dehydrogenase), LD (Lactate dehydrogenase), NP (Nucleoside phosphorylase)) を有した状態で細胞破砕を行った。

NMR 解析の前処理としてヒトアルブミンを除去するキット Albumin Segregation Kit を用い、多量に含まれるアルブミンの影響を除去し、より微量な成分まで検出できるように前処理を行った。

ブルカーバイオスピン社の協力を得て、UltraShield Plus 500MHz NMR により $^{1}$H-NMR を測定した。

## D. E 考察及び結論

細胞レベルでのメタボロミクス技術の開発を目的として、高感度かつ簡便解析が実施できる NMR を採用することとした。医薬基盤研究所では来年度 800MHz 高感度 LC-MS-NMR を設備整備する予定であったが、その整備が遅れている。本年度はブルカーバイオスピン社の協力を得て、NMR 解析を実施し、細胞超音波破砕試料を用いた NMR 解析を実施した。今回の解析に用いた細胞は、LI90 (肝臓), HUV-EC-C (血管内皮), NCR-G1 (精巣), HeLaS3 (子宮頸部), HL60 (急性前骨髄球性白血病由来細胞), HepG2 (肝臓), KMRC-3 であり、いずれも研究によく利用されている細胞を使用した。特に HepG2 は肝癌由来細胞であり、肝臓を標的とする毒性試験に汎用されている。また、もう一つの毒性標的臓器としてよく取り上げられるのが腎臓であり、今回 KMRC-3 を用いてそのモデルとして可能性を検討した。用いた細胞の内、KMRC-3 は非常に増殖が遅く、倍加時間が 14 日であったことから考えると、スクリーニングのような大量に細胞を使用する研究には適していないことがわかった。

細胞の形態観察のため行った動画撮影においては前述の増殖速度の低さが問題となったが、実際に細胞分裂はしないものの、細胞が培養容器表面を非常によく運動している状態が記録され、分裂には至らないが、細胞活動は活発であるということが確認できた。細胞は生命の最小単位として最も基本的な生物とみなされており、生命科学においては『構造』と『機能』が重要な研究対象である。分子生物学が大きく発展した現代においても、重要な分野であることは間違い無く、細胞の分化の指標になっている。この点から考えても、化学物質作用時の経時的形態観察は他の網羅的解析と同時に行うことによってより多くの情報をもたらすと考えられる。今後他の解析情報とどのように結び付けられるかが重要となるだろう。

また、細胞レベルでのメタボノミクス解析においては、NMR が非常に高感度であり、煩雑な前処理を必要としないことから、細胞を薬剤処理し、一定時間後超音波破砕した細胞試料をアルブミン除去後解析する方法を検討しており、実際に NMR 解析したところ、ノイズ等を気にすることなく解析が可能であるという結果を得ている。試料として用いる細胞破砕液に関してはいろいろな方法によって調製が可能であるが、簡

便な方法であり、酵素活性も維持できるように、酵素安定化バッファー中での超音波破砕を実施した。本方法は発熱による酵素活性への影響が考えられるため、氷冷したバッファー中にて実施することによりウ、酵素活性を維持した状態での細胞破砕液調製を行うことができた。今後は毒性のある薬剤を選択して細胞を処理し、その毒性のタイプを詳細解析できる手法の確立を目指す。

## F. 健康危険情報

なし

## G. 学会発表

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Chromosomal Instability in Human Mesenchymal Stem Cells Immortalized with Human Papilloma Virus E6, E7 and hTERT Genes. In Vitro. 2007 in press

## H. 知的所有権の取得状況

なし

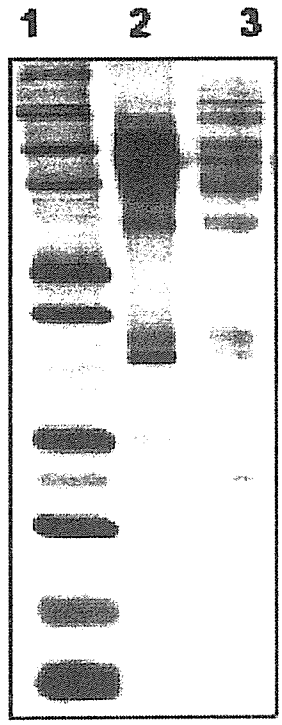


図１　アルブミン除去したサンプルの電気泳動像
（1：分子量マーカー，２：未処理細胞破砕液，３：アルブミン除去細胞破砕液）

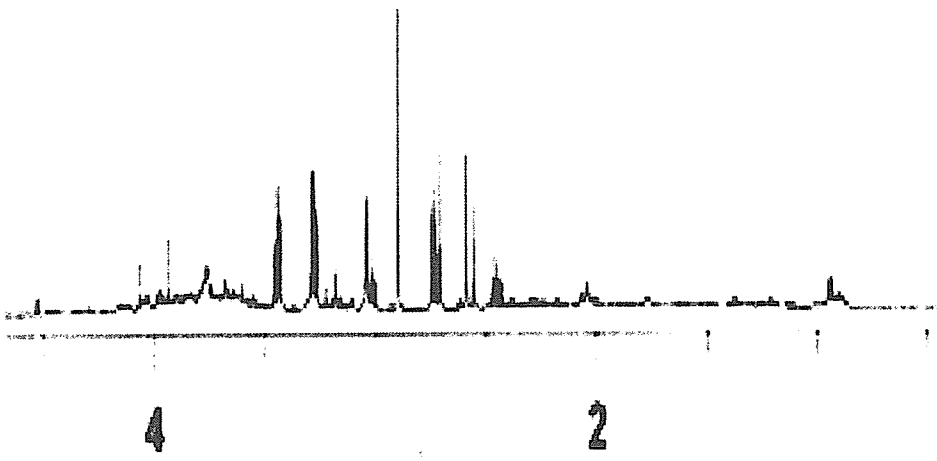


図２　アルブミン除去細胞破砕液の NMR

# 研究成果の刊行に関する一覧表

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# 9-Nitroanthracene derivative as a precursor of anthraquinone for photodynamic therapy

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**Abstract**—Anthraquinones are typical photosensitizers used in photodynamic therapy (PDT). However, systemic toxicity is a major problem for anthraquinones due to their ability not only to bind DNA but also to cause oxidative stress even without photoirradiation. To avoid such disadvantages in cancer therapy, we designed and synthesized a novel 9-nitroanthracene derivative (**1**) as a precursor of anthraquinone. Under photoirradiation, **1** is converted into anthraquinone via generation of nitric oxide as confirmed by ESR. Strong DNA cleavage specifically at guanine under photoirradiation was also observed, characteristic of DNA-cleaving reactions by photoirradiated anthraquinones. We propose development of **1** as an alternative approach toward PDT that reduces the systemic toxicity of anthraquinone.


## 1. Introduction

Photodynamic therapy (PDT) is an attractive approach to selectively localize toxicity using photosensitizers activated by light to induce cell death.[1,2] A large number of porphyrins have been tested for their efficacy in PDT, photofrin and photosan are currently in clinical use for PDT of lung cancer.[3,4] There is also considerable interest in understanding the factors contributing to the photodynamic activity of anthraquinones.[5–7] The ability of some anthraquinone derivatives to form cytotoxic reactive oxygen species (ROS) after illumination can result in effective ablation of targeted tissue. However, anthraquinones often carry complications of systemic toxicity as they tend to both bind DNA[8,9] and also cause oxidative stress even without photoirradiation.[10] Therefore, a prodrug of anthraquinone, which can be converted into anthraquinone only under photoirradiation, is required to overcome these disadvantages in cancer therapy. We have focused on the mechanism of photochemical degradation of 9-nitroanthracene to form anthraquinone via generation of nitric oxide (NO). A similar reaction is observed in 6-nitrobenzo[a]pyrene where the orientation of the nitro group is perpendicular to the aromatic ring due to the two protons located at the peri position, a similar situation for the nitro group in 9-nitroanthracene.[11] For 6-nitrobenzo[a]pyrene, three isomers of benzo[a]pyrenequinone are formed as a result of photodecomposition. If 9-nitroanthracene is used as a precursor of anthraquinone for PDT, complications of systemic toxicity can be avoided thereby benefiting its use in therapy. In this communication, we highlight a 9-nitroanthracene derivative (**1**) as a precursor of anthraquinone, an effective photosensitizer agent, and demonstrate its DNA cleaving activities under photoirradiation.

*Keywords*: Anthraquinone; 9-Nitroanthracene; Photodynamic therapy; DNA cleavage.
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## 2. Results and discussion

For the purpose of PDT, **1** was designed to have a dimethyl aminoalkyl group at the 2-position of 9-nitroanthracene. This group should increase solubility and DNA-binding affinity compared to 9-nitroanthracene itself. The synthesis of **1** and its isomer (**2**) with a nitro group at the 1-position is described in Scheme 1. 2-Aminoanthracene was condensed with 4-dimethylaminobutylic acid to give the dimethylaminoalkyl derivative at 87% yield. Nitration of the derivative with $HNO_3$ in acetic anhydride proceeded at 1- and 9-positions to give compounds **2** and **1** at 25% and 58% yields, respectively.

Comparing the stabilities of **1** and **2** under photoirradiation, **1** was much more susceptible to decomposition than **2**, consistent with the fact that **1** is almost completely decomposed 3 hours later, while photodegradation of **2** is not observed under the same conditions as shown in Figure 1a. Product analysis after photolysis of **1** showed that anthraquinone derivative (AQ) was formed at 77% yield, the structure of which was confirmed by $^1H$ NMR and mass spectrometry. It is likely that photolabile **1** releases NO in the course of anthraquinone formation. To assess the generation of NO upon photoirradiation, an ESR experiment with (MGD)-$Fe^{2+}$ as the spin trap[12] was performed. As shown in Figure 1b, a three-line spectrum consistent of $a^N = 1.25$ mT and $g^{iso} = 2.04$, characteristic of the [$(MGD)_2$-$Fe^{2+}$-NO] complex, was observed from photoirradiated **1**, indicating that degradation of photolabile **1** is accompanied by generation of NO. In contrast, we could barely observe a similar peak in the case of photostable **2**. These results show that **1** is a promising photosensitizer that is converted into an AQ structure under photoirradiation via a NO-releasing mechanism.

Photosensitizers are activated by light to induce cell death or modulation of immunological cascades, presumably via formation of ROS. Therefore, the abilities of **1** and **2** to cleave DNA were examined by agarose gel electrophoresis of pBR322DNA. As shown in Figure 2a, no effect of **1** and **2** on DNA was observed in the dark condition. When irradiation was performed for 30 min, **1** induced efficient strand cleavage as shown in Figure 2b, and in the presence of 200 μM **1**, almost all

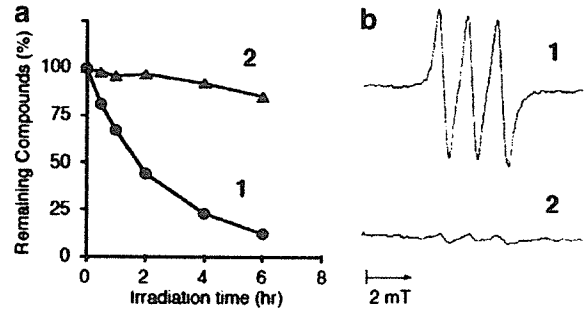



**Figure 1.** Photodegradation of **1** and **2**, and generation of NO. (a) Remaining **1** and **2** after photodegradation. (b) ESR spectra of (MGD)-$Fe^{2+}$ in the presence of **1** and **2** after photoirradiation for 6 min.

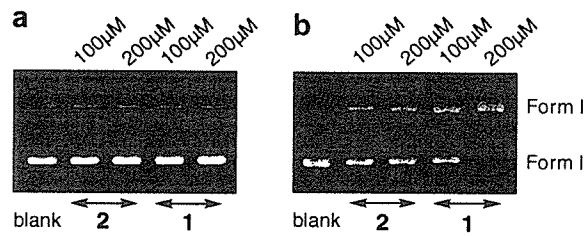
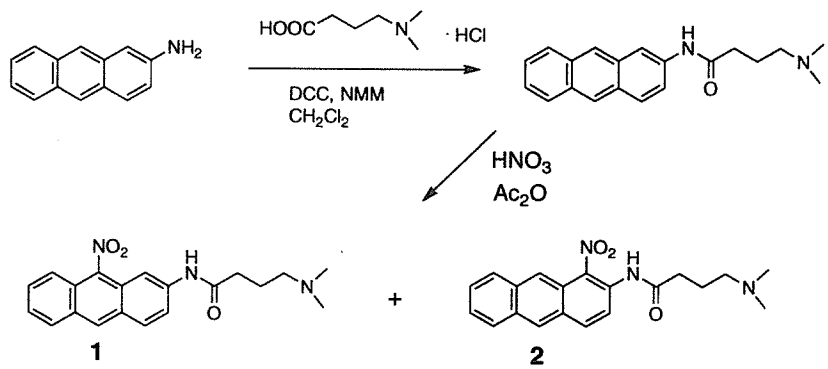



**Figure 2.** Effects of **1** and **2** on supercoiled pBR322DNA in the dark (a) or under photoirradiation (b).

of Form I (supercoiled) DNA was converted to Form II (closed circular) DNA. In contrast, although strand scission by **2** was obviously observed, the DNA-cleaving ability was not so strong compared with that of **1**. The strong DNA cleavage induced by **1** might be triggered by a structural change in AQ under photoirradiation. To confirm the generation of $^1O_2$ under photoirradiation, ESR spectra were observed in the presence of 2,2,6,6-tetramethyl-4-piperidone (4-oxo-TEMP), a spin trap for $^1O_2$. As shown in Figure 3, a three-line spectrum consistent of $a^N = 1.60$ mT, which is characteristic of 4-oxo-TEMPO,[13] was observed from both **1** and **2**, indicating generation of $^1O_2$. Importantly, the ability of **1** to generate $^1O_2$ as reflected in the peak height of 4-oxo-TEMPO was stronger than that for **2**, demonstrat-




**Scheme 1.** Structures of **1** and **2**, and their synthesis.

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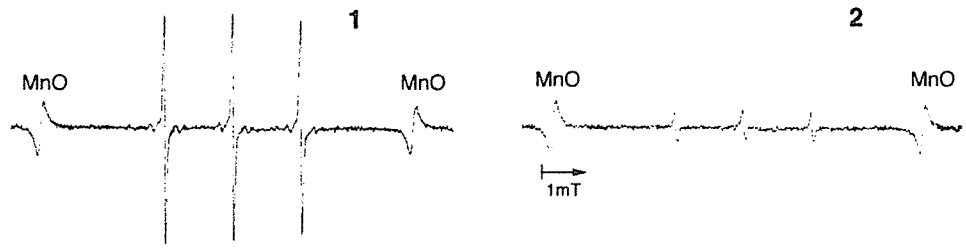



**Figure 3.** ESR spectra of 4-oxo-TEMP in the presence of **1** and **2** after photoirradiation for 10 min.

ing the advantage of **1** in cleaving DNA after conversion into AQ.

Photoexcited AQ ($AQ^{3*}$) is responsible for DNA damage through electron transfer from the DNA base (Type I mechanism)[14] or by generation of ROS such as $^1O_2$ (Type II mechanism).[15] Both mechanisms would result in DNA strand scission primarily at guanines. Therefore, photocleavage experiments induced by **1** were also performed with 5′-$^{32}$P-end labeled oligonucleotides to test targeting of specific cleaving sites. As expected, irradiation of DNA in the presence of **1** for 30 min and subsequent treatment with piperidine revealed that strand cleavage occurs predominantly at guanine, as shown in Figure 4.

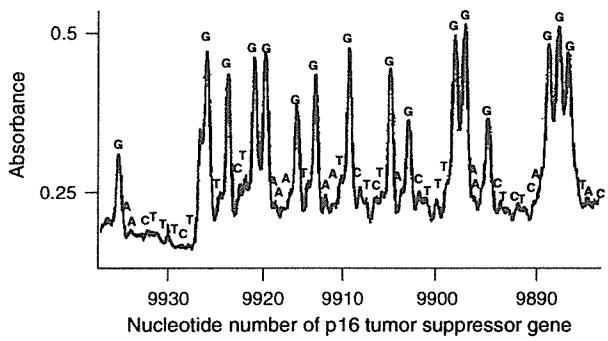
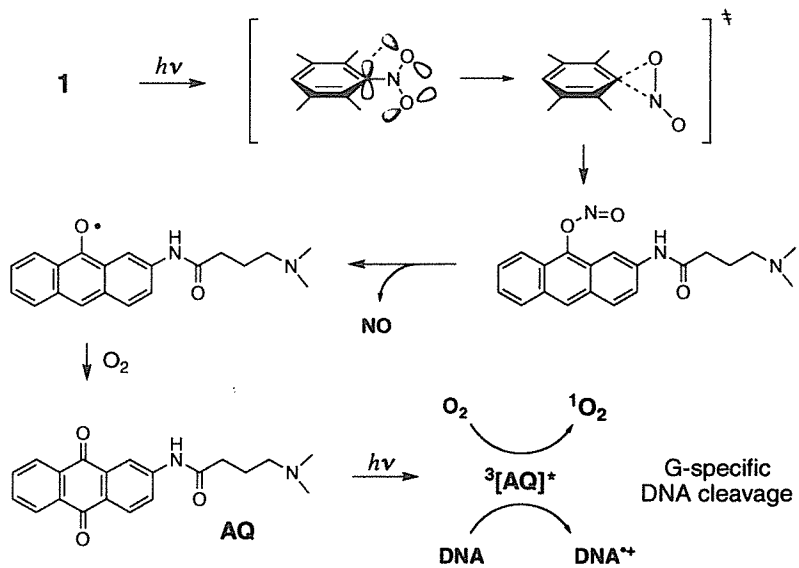



**Figure 4.** Sequence specificity of DNA damage in the p16 tumor suppressor gene, induced by **1** upon photoirradiation.

In summary, we have described the design and synthesis of **1** as a precursor of anthraquinone, a typical photosensitizer. Photochemical conversion of **1** into anthraquinone via generation of NO and specific DNA cleavage at guanine were demonstrated, characteristic of DNA-cleaving reactions of anthraquinones. A possible mechanism of oxidative DNA cleavage induced by photoirradiated **1** is shown in Scheme 2. The perpendicular conformation of the nitro group to the anthracene is characteristic for **1** and is responsible for the ease of NO generation which is followed by structural conversion into anthraquinone.[16] The great difference of NO-generating activities between **1** and **2** is attributed to the conformation of nitro group. That is, the perpendicular conformation of nitro group in **1** suggests that the overlap of half-vacant nonbonding orbital of the nitro group with the adjacent orbital of the aromatic ring increases susceptibility to intramolecular rearrangement from nitro to nitrite. In comparison, such rearrangement does not occur in the case of **2** where the conformation of nitro group is not perpendicular (data not shown). There are a number of nitro polycyclic aromatic hydro-




**Scheme 2.** Possible mechanism of oxidative DNA damage induced by photoirradiated **1**.

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carbons (nitroPAHs) showing mutagenicity and/or carcinogenicity. However, no such toxicity has been shown by 9-nitroanthracene because the nitro group at 9-position is insensitive to enzymatic reduction, essential for the toxicity of nitroPAH.[17] Interestingly, two peri protons at the 1- and 8-positions, which lead to a perpendicular conformation of the nitro group, impede access of reductase to 9-nitroanthracene.[17,18] Finally, **1** might be favorable for PDT as an approach to reducing the systemic toxicity of anthraquinone. Further investigation to understand the mechanism of DNA cleaving activity and apoptotic cell death induced by **1** is underway and will be reported in due course.

## 3. Experimental

### 3.1. General methods

The reagents and solvents used were of commercial origin (Wako chemicals, Sigma, Aldrich) and were employed without further purification. The progress of all reactions was monitored by thin-layer chromatography on silica gel 60 $F_{254}$ (0.25 mm, Merck). Column chromatography was performed on silica gel 60 (0.063–0.200 mm, Merck). The $^1$H NMR spectra were recorded with a Varian AS 400 Mercury spectrometer. Chemical shifts were expressed in ppm downfield shift from $Me_4Si$. High resolution mass spectra were obtained on a JEOL MS700 mass spectrometer. ESR spectra were obtained with a JEOL X-band spectrometer (JES-FA100) under nonsaturating microwave power conditions.

### 3.2. Synthesis of *N*-(anthracen-2-yl)-4-(dimethylamino)butanamide (3)

To a solution of 2-aminoanthracene (580 mg, 3 mmol) and *N*-methylmorpholine (303 mg, 3 mmol) in DMF (10 ml) were added 4-(dimethylamino)butyric acid hydrochloride (503 mg, 3 mmol) and *N*-methylmorpholine (303 mg, 3 mmol) in DMF (10 ml) at 0 °C and the mixture was stirred for 18 h. After removal of the solvent in vacuo, the residue was dissolved in $CH_2Cl_2$ and filtered. The filtrate was washed with saturated NaCl, dried over anhydrous $Na_2SO_4$, and concentrated in vacuo. The residue was purified by silica gel column chromatography ($CH_2Cl_2$/MeOH/Satd $NH_3$ in MeOH, 100:20:1) to afford the title compound (796 mg, 87%) as a white solid. $^1$HMR ($CDCl_3$, 400 MHz) $\delta$ 1.95 (2H, m), 2.40 (6H, s), 2.54 (2H, m), 2.61 (2H, m), 7.38 (1H, d, $J$ = 9.2 Hz), 7.43 (2H, m), 7.94 (1H, d, $J$ = 8.8 Hz), 7.95 (1H, d, $J$ = 7.6 Hz), 7.96 (1H, d, $J$ = 9.2 Hz), 8.34 (1H, s), 8.35 (1H, s), 8.47 (1H, s), 10.11(1H, br); HR-MS (+EI) $(M)^+$ found 306.1731; $(M)^+$ calcd for $C_{20}H_{22}N_2O$ 306.1734.

### 3.3. Synthesis of 4-(dimethylamino)-*N*-(9-nitroanthracen-2-yl)butanamide (1) and 4-(dimethylamino)-*N*-(1-nitroanthracen-2-yl)butanamide (2)

To a suspension of **1** (238 mg, 0.78 mmol) in acetic anhydride (35 mL) was added $HNO_3$ (0.17 mL) in acetic acid (1.8 mL) dropwise over 10 min at −10 °C. After stirring at 0 °C for 20 min, the mixture was poured onto crushed ice and stirred for 2 h, extracted with $CH_2Cl_2$, and the extracts were washed with $H_2O$ and brine, dried over anhydrous $Na_2SO_4$, and concentrated in vacuo. The residue was purified by silica gel column chromatography (benzene/ethyl acetate/MeOH/Satd $NH_3$ in MeOH, 40:40:15:5) to afford **2** and **1** in this order as a light orange solid.

**1.** 159 mg, 58% yield, UV $\lambda_{max}$ (MeOH) nm: 224, 260, 364; $^1$HMR ($CDCl_3$, 400 MHz) $\delta$ 1.93 (2H, m), 2.43 (6H, s), 2.57 (2H, m), 2.62 (2H, m), 7.51 (1H, m), 7.63 (1H, m), 7.89 (1H, s), 7.93 (1H, d, $J$ = 8.8 Hz), 8.02 (1H, d, $J$ = 9.1 Hz), 8.03 (1H, d, $J$ = 8.4 Hz), 8.09 (1H, d, $J$ = 9.1 Hz), 8.54 (1H, s), 11.22 (1H, br); HR-MS (+EI) $(M)^+$ found 351.1585; $(M)^+$ calcd for $C_{20}H_{21}N_3O_3$ 351.1584.

**2.** 68 mg, 25% yield, UV $\lambda_{max}$ (MeOH) nm: 241, 269, 379; $^1$HMR ($CDCl_3$, 400 MHz) $\delta$ 1.96 (2H, m), 2.32 (6H, s), 2.49 (2H, m), 2.61 (2H, m), 7.55 (2H, m), 8.01 (2H, m), 8.15 (1H, d, $J$ = 9.4 Hz), 8.29 (1H, d, $J$ = 9.4 Hz), 8.45 (1H, s), 8.62 (1H, s), 10.20 (1H, br); HR-MS (+EI) $(M)^+$ found 351.1581; $(M)^+$ calcd for $C_{20}H_{21}N_3O_3$ 351.1584.

### 3.4. Photolysis of 1

A 3.5 mg (0.01 mmol) of **1** was dissolved in 4 ml of benzene/acetone (1:1) and irradiation was performed through a Pyrex filter with a 300W photoreflector lamp for 3 h. The reaction mixture was concentrated in vacuo and purified by silica gel column chromatography (benzene/ethyl acetate/MeOH/Satd $NH_3$ in MeOH, 40:45:10:5) to afford 4-(dimethylamino)-*N*-(9,10-dioxo-9,10-dihydroanthracen-2-yl)butanamide (AQ) as a pale yellow solid (2.6 mg, 77% yield). UV $\lambda_{max}$ (MeOH) nm: 219, 272, 356; $^1$HMR ($CDCl_3$, 400 MHz) $\delta$ 2.29 (2H, m), 2.89 (6H, s), 2.94 (2H, m), 3.18 (2H, m), 7.97 (2H, m), 8.10 (1H, d, $J$ = 8.8 Hz), 8.21 (1H, d, $J$ = .8 Hz), 8.26 (1H, d, $J$ = 8.0 Hz), 8.29 (1H, d, $J$ = 8.0 Hz), 8.61 (1H, s), 10.33 (1H, br); HR-MS (+EI) $(M)^+$ found 336.1476; $(M)^+$ calcd for $C_{20}H_{20}N_2O_3$ 336.1475.

### 3.5. Detection of nitric oxide and $^1O_2$ by ESR

The $Fe^{2+}$ complex of MGD [$Fe^{2+}$-$MGD_2$, (Fe-MGD)] was used to trap NO. Fresh stock solutions of Fe-MGD (1:5) were prepared by adding ferrous ammonium sulfate to an aqueous solution of MGD. A sample containing 200 μM of **1** or **2** and 15 mM MGD-Fe in phosphate buffer, pH 7.6 (5% DMF) was introduced into a quartz flat cell. ESR spectra were recorded after light irradiation (5 J/cm$^2$ UVA) at 30 cm distance with a JES-FE 2XG spectrometer (JEOL Co. Ltd., Tokyo, Japan). The spectrometer settings used were: modulation frequency, 100 kHz; amplitude, 100–1000; scan time, 4 min; microwave power, 16 mW; microwave frequency, 9.394 GHz. Detection of $^1O_2$ was performed by ESR using 2,2,6,6-tetramethyl-4-piperidone (4-oxo-TEMP) as a spin trap. A sample containing 100 μM of **1** or **2**

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and 100 mM of TEMP in phosphate buffer, pH 7.4 (5% DMF) was introduced into a quartz flat cell. ESR spectra were recorded in a similar manner as above.

### 3.6. Assays for DNA strand breaks

DNA strand breakage was measured by the conversion of supercoiled pBR322 plasmid DNA to the open circular form. Reactions were carried out in 20 μL (total volume) of 50 mM Na cacodylate buffer (5% DMF), pH 7.2, containing 45 μM of pBR322 DNA and 100 and 200 μM of **1** and **2**. The mixture was exposed to 5 J/cm$^2$ UVA light on ice using a 10 W UV lamp (365 nm, UVP, Inc., CA, USA) placed at a distance of 20 cm. After 30 min, the reaction mixtures were treated with 5 μL of loading buffer (100 mM TBE buffer, pH 8.3, containing 30% glycerol, 0.1% bromophenol blue) and applied to a 1% agarose gel. Horizontal gel electrophoresis was carried out in 50 mM TBE buffer, pH 8.3, and gels were stained with ethidium bromide (1 μg/ml) for 30 min, followed by destaining in water for 30 min and photography with UV translumination.

### 3.7. Site specificity of DNA damage induced by 1

**3.7.1. Preparation of $^{32}$P-5′-end-labeled DNA fragments.** DNA fragments were obtained from the human p16 tumor suppressor genes. The $^{32}$P-3′-end-labeled 460-base pair fragment (*Eco*RI*9481–*Eco*RI*9940) containing exon 2 of the human p16 tumor suppressor gene was obtained as previously described.[19] The 460-bp fragment was further digested with *Bss*HII to obtain the singly labeled 309-base pair (*Bss*HII 9789–*Eco*RI*9481) and 147-base pair (BssHII*9794–*Eco*RI*9940) DNA fragments.[20] An asterisk indicates $^{32}$P labeling.

**3.7.2. Detection of DNA damage induced by UVA in the presence of 1.** The standard reaction mixture in a microtube (1.5-ml Eppendorf) contained a $^{32}$P-labeled DNA fragment, 5 μM calf thymus DNA, and **1** in 10 mM sodium phosphate buffer (pH 7.8) containing 5 μM DTPA. The mixture was exposed to 5 J/cm$^2$ UVA light on ice using a 10 W UV lamp (365 nm, UVP, Inc., CA, USA) placed at a distance of 10 cm. After irradiation, the DNA fragments were treated for 20 min at 90 °C in 1 M piperidine and then electrophoresed on an 8% polyacrylamide/8 M urea gel. The autoradiogram was obtained by exposing an X-ray film to the gel. The preferred cleavage sites were determined by direct comparison of the position of the oligonucleotides with those produced by the chemical reactions of Maxam–Gilbert procedure using a DNA sequencing system (LKB2010 Macrophor). The relative amounts of oligonucleotides from treated DNA fragments were measured with a laser densitometer (LKB 2222 UltroScan XL).


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**Regular Paper**

# Design and Synthesis of α-Glucosidase Inhibitor Having DNA Cleaving Activity

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**Abstract: Apoptosis, or programmed cell death, is a mechanism by which cells undergo death to control cell proliferation or in response to DNA damage. The present study was designed to explore small molecule apoptosis inducers for antitumor agents. The synthesis of 4-sulfonylphenyl α-D-glucopyranoside derivatives 1–6 and 4-(sulfonylamino)phenyl α-D-glucopyranoside derivatives 7–12, endoplasmic reticulum (ER)-targeted small molecules that were designed to induce apoptosis from ER stress by ER glucosidase inhibition and DNA damage is described. Compounds 6 and 12, with a terminal 2-naphthyl group, indicated inhibitions of α-glucosidases from *S. cerevisiae* ($IC_{50}$=51.7 μM and $IC_{50}$=74.1 μM) and *B. stearothermophilus* ($IC_{50}$=60.1 μM and $IC_{50}$=89.1 μM). Moreover, compound 12 strongly induced the DNA strand breakage condition. When compounds 1–12 were assayed for their ability to inhibit processing by glucosidases at the cellular level, no effects on glycoprotein processing were observed.**

**Key words: α-glucosidase, inhibitor, DNA cleavage, apoptosis, ER stress**

The cell is perturbed by environmental stress conditions. In order to avoid cell death from the stress, cells must sense and respond to stress, including viral infection, genetic mutation, chemical insult, and nutrient depletion.[1)] In the ER, stress is a condition that accumulates misfolded or unfolded proteins by disturbing these ER circumstances. Specific response programs are activated to circumvent each type of stress. The ER stress induces a coordinated adaptive program called the unfolded protein response (UPR).[2)] The UPR is activated upon disruption of the ER environment by such events as the inhibition of *N*-linked oligosaccharide processing, which results in the accumulation of unfolded or misfolded proteins in the ER.[3)] *N*-Linked oligosaccharide processing is carried out by ER glucosidases I and II. Both enzymes are key enzymes in the biosynthesis of *N*-linked oligosaccharides that catalyze the first processing event after the transfer of $Glc_3Man_9GlcNAc_2$ to proteins.[4)] The inhibition of ER glucosidases induces the accumulation of unfolded proteins in the ER, and increases ER stress. The UPR caused by ER stress is insulted due to DNA damage, and the cell is led to apoptosis. Apoptosis targets are currently being explored for antitumor agent discovery, such as the tumor-necrosis factor (TNF)-related apoptosis-inducing ligand (TRAIL) receptors, the BCL2 family of anti-apoptotic proteins, and inhibitor of apoptosis (IAP) proteins.[1,5)]

We think that the inhibition of ER glucosidases can be used to trigger ER stress, and that the ER stress may trigger the UPR. Further, following interruption of the UPR by DNA damage, the cell is led to apoptosis. We think that compounds that have α-glucosidase inhibitory activity and DNA breakage activity may be developed into an ER-targeted small molecule apoptosis inducer for use as an antitumor agent. We have already elucidated the molecular recognition properties[6–13)] and the inhibition[13,14)] of α-glycosidases necessary for the molecular design of glycosidase inhibitors using synthetic probes. Based on our knowledge, we designed compounds **1–12** to have α-glucosidase inhibitory activity and DNA breakage activity (Fig. 1). The enzymatic liberation of the aglycon from compounds **1–12** might be followed by the ejection of a $R^2SO_2H$ with the concomitant formation of *p*-benzoquinone or *p*-benzoquinone imine,[15)] which would then generate reactive oxygen species (ROS), leading to DNA breakage,[16)] shown in Fig. 2. The group of Taylar *et al.* has developed a series of 4-(sulfonylamino)phenyl α-D-glucopyranosides.[15)] These compounds have been reported to act as competitive yeast α-glucosidase inhibitors. We suspect that these compounds may also be enhanced in their inhibitory activity by changing the sulfonamide of 4-(sulfonylamino)phenyl α-D-glucopyranoside to sulfonate, since the liberation of *p*-benzoquinone is easier than that of *p*-benzoquinone imine.

In this report, we first describe the design and synthesis series of 4-sulfonylphenyl α-D-glucopyranoside derivatives **1–6** and 4-(sulfonylamino)phenyl α-D-glucopyranoside derivatives **7–12**. These compounds **1–12** were evaluated with regard to their ability to inhibit three kinds of α-glucosidases, and the effects of α-glucosidase triggered

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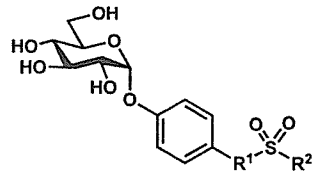

1: $R^1$=O, $R^2$=4-$NO_2$-$C_6H_4$
2: $R^1$=O, $R^2$=4-Cl-$C_6H_4$
3: $R^1$=O, $R^2$=4-$CF_3$-$C_6H_4$
4: $R^1$=O, $R^2$=4-$CH_3$-$C_6H_4$
5: $R^1$=O, $R^2$=4-$C(CH_3\text{-}C_6H_4)_3$
6: $R^1$=O, $R^2$=2-naphthyl
7: $R^1$=NH, $R^2$=4-$NO_2$-$C_6H_4$
8: $R^1$=NH, $R^2$=4-Cl-$C_6H_4$
9: $R^1$=NH, $R^2$=4-$CF_3$-$C_6H_4$
10: $R^1$=NH, $R^2$=4-$CH_3$-$C_6H_4$
11: $R^1$=NH, $R^2$=4-$C(CH_3)_3$-$C_6H_4$
12: $R^1$=NH, $R^2$=2-naphthyl

**Fig. 1.** Chemical structure of target compounds **1**–**12**.

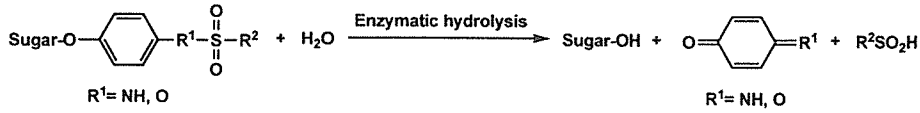


**Fig. 2.** Schematic diagram of enzymatic liberation of quinone derivatives.

ROS-mediated DNA breakage. Finally, these compounds were also tested in a cell culture system.

## MATERIALS AND METHODS

***General methods.*** Optical rotations were measured with a JASCO DIP-370 digital polarimeter at 25°C. The NMR spectra were recorded with a Varian Mercury 400 spectrometer (400 MHz for $^1$H). Chemical shifts were expressed in ppm downfield relative to $Me_4Si$. Low resolution mass spectra were obtained with a Waters MicroMass ZQ instrument under positive and negative ion ESI conditions. Column chromatography was performed on silica gel 60 (0.063–0.200 mm, Merck). The progress of all reactions was monitored by thin-layer chromatography on silica gel 60 $F_{254}$ (0.25 mm, Merck).

***Method A.*** To the solution of α-arbutin (**1**, 0.5 g, 1.8 mmol) in 100 mL of dry acetone was added triethylamine ($NEt_3$, 10 mL) and the sulfonyl chloride derivative (2.8 mmol). After the mixture was stirred for 15 min, the resulting salt was removed by filtration through a cotton filter, and the solvent was concentrated.

***Method B.*** To the solution of α-arbutin (**1**, 1.0 g, 3.7 mmol), in 50 mL of dry acetone was added dry potassium carbonate ($K_2CO_3$, 1.52 g, 11 mmol) and the sulfonyl chloride derivative (5.5 mmol). After the mixture was stirred overnight, the $K_2CO_3$ was removed by filtration through Celite, and the solvent was concentrated.

***Method C.*** To the solution of *p*-nitrophenyl α-D-glucopyranoside (**14**, 1.0 g, 3.3 mmol), in pyridine (50 mL) at room temperature was added acetic anhydride (10 mL). The mixture was stirred overnight and poured into water. The product was extracted with AcOEt (3 × 50 mL) and washed with water, 1 M HClaq, satd. $NaHCO_3$aq and brine, and then dried over $Na_2SO_4$. The product was purified by column chromatography on silica gel (1:1 hexane-AcOEt) to afford a quantitative yield of **15**. A mixture of compound **15** (1.6 g, 3.3 mmol) in ethanol (EtOH, 100 mL) was hydrogenated under $H_2$ with 20% palladium hydroxide on carbon (150 mg). After the mixture was stirred for 2 h, the palladium charcoal was removed by filtration through Celite and the solvent was concentrated. The product was purified by column chromatography on silica gel (1:1 hexane-AcOEt) to afford 1.5 g (93.9%) of **16**.

***Method D.*** To the solution of compound **16** (0.5 g, 1.2 mmol), in pyridine (20 mL) at room temperature was added sulfonyl chloride derivative (1.36 mmol). The mixture was stirred for 10 min and poured into water. The product was extracted with AcOEt (3 × 50 mL) and washed with water, 1 M HClaq, satd. $NaHCO_3$aq and brine, and then dried over $Na_2SO_4$, and the solvent was concentrated.

***Method E.*** A mixture of methanol (MeOH) : $NEt_3$ : $H_2O$ (5:1:1, 60 mL) was added to a stirred solution of the sulfonyl derivative (0.85 mmol). After the mixture was stirred for 6 h at room temperature, the solvent was evaporated.

***Synthesis of compounds 1–12.***

**4-(4-Nitrobenzenesulfonyl)phenyl α-D-glucopyranoside (1).** According to method A, compound **1** was prepared from **13** (0.5 g, 1.8 mmol). The product was purified by column chromatography on silica gel (5:1 dichloromethane ($CH_2Cl_2$)-MeOH) to afford 0.46 g (54.8%) of **2**: $[\alpha]_D$ +14.9° (*c* 0.93, MeOH); $^1$H NMR ($CD_3OD$) δ 3.38 (dd, 1H, $J_{3-4}$=8.8 Hz, $J_{4-5}$=10.0 Hz, H-4), 3.55 (dd, 1H, $J_{1-2}$= 3.8 Hz, $J_{2-3}$=9.8 Hz, H-2), 3.58 (ddd, 1H, $J_{4-5}$=10.0 Hz, $J_{5-6a}$=5.2 Hz, $J_{5-6b}$=2.4 Hz, H-5), 3.67 (dd, 1H, $J_{5-6a}$=5.0 Hz, $J_{6a-6b}$=11.8 Hz, H-6a), 3.73 (dd, 1H, $J_{5-6b}$=2.4 Hz, $J_{6a-6b}$=12.0 Hz, H-6b), 3.80 (dd, 1H, $J_{2-3}$=$J_{3-4}$=9.2 Hz, H-3), 5.42 (d, 1H, *J*=3.6 Hz, H-1), 6.95 (d, 2H, *J*=8.8 Hz, -$OC_6H_4O$-), 7.12 (d, 2H, *J*=9.2 Hz, -$OC_6H_4O$-), 8.07 (d, 2H, *J*=9.2 Hz, -$SO_2C_6H_4NO_2$-), 8.44 (d, 2H, *J*=9.2 Hz, -$SO_2C_6H_4NO_2$), MS: 480 $(M+Na)^+$.

**4-(4-Chlorobenzenesulfonyl)phenyl α-D-glucopyranoside (2).** According to method A, compound **2** was prepared from **13** (0.5 g, 1.8 mmol). The product was purified by column chromatography on silica gel (5:1 $CH_2Cl_2$-MeOH) to afford 0.59 g (72.5%) of **3**: $[\alpha]_D$ +13.8° (*c* 1.51, MeOH); $^1$H NMR ($CD_3OD$) δ 3.40 (dd, 1H, $J_{3-4}$=9.0 Hz, $J_{4-5}$=9.8 Hz, H-4), 3.55 (dd, 1H, $J_{1-2}$=3.6 Hz, $J_{2-3}$=9.6 Hz, H-2), 3.59 (ddd, 1H, $J_{4-5}$=10.0 Hz, $J_{5-6a}$=5.4 Hz, $J_{5-6b}$ =2.2 Hz, H-5), 3.67 (dd, 1H, $J_{5-6a}$=5.4 Hz, $J_{6a-6b}$=12 Hz, H-6a), 3.73 (dd, 1H, $J_{5-6b}$=2.4 Hz, $J_{6a-6b}$=12.0 Hz, H-6b), 3.81 (dd, 1H, $J_{2-3}$=9.6 Hz, $J_{3-4}$=9.2 Hz, H-3), 5.43 (d, 1H, *J*=3.6 Hz, H-1), 6.92 (d, 2H, *J*=9.6 Hz, -$OC_6H_4O$-), 7.12 (d, 2H, *J*=9.2 Hz, -$OC_6H_4O$-), 7.62 (d, 2H, *J*=8.8 Hz, -$SO_2C_6H_4Cl$), 7.78 (d, 2H, *J*=8.8 Hz, -$SO_2C_6H_4Cl$), MS: 469 $(M+Na)^+$.

**4-(4-Trifluorobenzenesulfonyl)phenyl α-D-glucopyranoside (3).** According to method A, compound **3** was prepared from **13** (0.5 g, 1.8 mmol). The product was purified by column chromatography on silica gel (5:1 $CH_2Cl_2$-MeOH) to afford 0.59 g (88.4%) of **3**: $[\alpha]_D$ +11.7° (*c* 1.25, MeOH); $^1$H NMR ($CD_3OD$) δ 3.40 (dd, 1H, $J_{3-4}$=9.0 Hz, $J_{4-5}$=9.8 Hz, H-4), 3.55 (dd, 1H, $J_{1-2}$=3.6 Hz, $J_{2-3}$= 10.0 Hz, H-2), 3.58 (m, 1H, H-5), 3.66 (dd, 1H, $J_{5-6a}$=5.0

Hz, $J_{6a-6b}$=11.8 Hz, H-6a), 3.73 (dd, 1H, $J_{5-6b}$=2.4 Hz, $J_{6a-6b}$=12.0 Hz, H-6b), 3.81 (dd, 1H, $J_{2-3}$=$J_{3-4}$=9.4 Hz, H-3), 5.43 (d, 1H, $J$=3.6 Hz, H-1), 6.93 (d, 2H, $J$=9.2 Hz, -$OC_6H_4O$-), 7.12 (d, 2H, $J$=9.2 Hz, -$OC_6H_4O$-), 7.94 (d, 2H, $J$=8.0 Hz, -$O_2C_6H_4O$-), 8.02 (d, 2H, $J$=8.4 Hz, -$SO_2C_6H_4CF_3$), MS: 503 $(M+Na)^+$.

**4-(4-Methylbenzenesulfonyl)phenyl α-D-glucopyranoside (4)**. According to method B, compound **4** was prepared from **13** (1.1 g, 5.5 mmol). The product was purified by column chromatography on silica gel (5:1 $CH_2Cl_2$-MeOH) to afford 1.03 g (63.4%) of **4**: $[\alpha]_D$ +18.4° (*c* 0.97, MeOH); $^1H$ NMR ($CD_3OD$) δ 2.44 (s, 3H, -$CH_3$), 3.39 (dd, 1H, $J_{3-4}$=8.8 Hz, $J_{4-5}$=10.0 Hz, H-4), 3.54 (dd, 1H, $J_{1-2}$=3.8 Hz, $J_{2-3}$=9.8 Hz, H-2), 3.58 (ddd, 1H, $J_{4-5}$= 10.0 Hz, $J_{5-6a}$=5.2 Hz, $J_{5-6b}$=2.4 Hz, H-5), 3.66 (dd, 1H, $J_{5-6a}$=5.2 Hz, $J_{6a-6b}$=12.0 Hz, H-6a), 3.73 (dd, 1H, $J_{5-6b}$= 2.6 Hz, $J_{6a-6b}$=11.8 Hz, H-6b), 3.80 (dd, 1H, $J_{2-3}$=9.8, $J_{3-4}$ =9.0 Hz, H-3), 5.41 (d, 1H, $J$=3.6 Hz, H-1), 6.88 (d, 2H, $J$=9.6 Hz, -$OC_6H_4O$-), 7.09 (d, 2H, $J$=9.2 Hz, -$OC_6H_4O$-), 7.40 (d, 2H, $J$=8.0 Hz, -$SO_2C_6H_4CH_3$), 7.66 (d, 2H, $J$=8.4 Hz, -$SO_2C_6H_4CH_3$), MS: 449 $(M+Na)^+$.

**4-(4-*tert*-Butylbenzenesulfonyl)phenyl α-D-glucopyranoside (5)**. According to method B, compound **3** was prepared from **13** (0.5 g, 1.8 mmol). The product was purified by column chromatography on silica gel (5:1 $CH_2Cl_2$-MeOH) to afford 0.39 g (43.9%) of **5**: $[\alpha]_D$ +13.6° (*c* 1.40, MeOH); $^1H$ NMR ($CD_3OD$) δ 1.36 (s, 9H, -$C(CH_3)_3$), 3.39 (dd, 1H, $J_{3-4}$=8.8 Hz, $J_{4-5}$=10.0 Hz, H-4), 3.54 (dd, 1H, $J_{1-2}$=3.6 Hz, $J_{2-3}$=9.6 Hz, H-2), 3.59 (ddd, 1H, $J_{4-5}$=10.0 Hz, $J_{5-6a}$=5.0 Hz, $J_{5-6b}$=2.5 Hz, H-5), 3.66 (dd, 1H, $J_{5-6a}$=5.0 Hz, $J_{6a-6b}$=11.8 Hz, H-6a), 3.73 (dd, 1H, $J_{5-6b}$=2.4 Hz, $J_{6a-6b}$=12.0 Hz, H-6b), 3.80 (dd, 1H, $J_{2-3}$ =$J_{3-4}$=9.4 Hz, H-3), 5.41 (d, 1H, $J$=3.6 Hz, H-1), 6.89 (d, 2H, $J$=9.2 Hz, -$OC_6H_4O$-), 7.10 (d, 2H, $J$=9.2 Hz, -$OC_6H_4O$-), 7.64 (d, 2H, $J$=8.8 Hz, -$SO_2C_6H_4C(CH_3)_3$), 7.72 (d, 2H, $J$=8.8 Hz, -$SO_2C_6H_4C(CH_3)_3$), MS: 491 $(M+Na)^+$.

**4-(2-Naphthalenesulfonyl)phenyl α-D-glucopyranoside (6)**. According to method B, compound **6** was prepared from **13** (0.5 g, 1.8 mmol). The product was purified by column chromatography on silica gel (5:1 $CH_2Cl_2$-MeOH) to afford 0.63 g (74.6%) of **6**: $[\alpha]_D$ +12.3° (*c* 1.32, MeOH); $^1H$ NMR ($CD_3OD$) δ 3.38 (dd, 1H, $J_{3-4}$=8.8 Hz, $J_{4-5}$=10.0 Hz, H-4), 3.52 (dd, 1H, $J_{1-2}$=3.6 Hz, $J_{2-3}$=9.6 Hz, H-2), 3.55 (ddd, 1H, $J_{4-5}$=10.0 Hz, $J_{5-6a}$=4.8 Hz, $J_{5-6b}$ =2.8 Hz, H-5), 3.64 (dd, 1H, $J_{5-6a}$=4.8 Hz, $J_{6a-6b}$=12.0 Hz, H-6a), 3.68 (dd, 1H, $J_{5-6b}$=2.8 Hz, $J_{6a-6b}$=12.0 Hz, H-6b), 3.78 (dd, 1H, $J_{2-3}$=$J_{3-4}$=9.0 Hz, H-3), 5.38 (d, 1H, $J$=3.6 Hz, H-1), 6.89, 7.05 (d, 2H × 2, $J$=9.6 Hz, -$OC_6H_4O$-), 7.64–7.83, 8.00–8.11, 8.35 (m, 7H, -$SO_2C_{10}H_7$), MS: 485 $(M+Na)^+$.

**4-(4-Nitrophenylsulfonylamino)phenyl 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranoside (17)**. According to methods C and D, compound **17** was prepared from **16** (0.5 g, 1.1 mmol). The product was purified by column chromatography on silica gel (1:1 hexane-AcOEt) to afford 0.67 g (94.6%) of **17**.

**4-(4-Chlorophenylsulfonylamino)phenyl 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranoside (18)**. According to methods C and D, compound **18** was prepared from **16** (0.4 g, 1.0 mmol). The product was purified by column chromatography on silica gel (1:1 hexane-AcOEt) to afford 0.54 g (93.1%) of **17**.

**4-(4-Trifluoromethylphenylsulfonylamino)phenyl 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranoside (19)**. According to methods C and D, compound **19** was prepared from **16** (0.4 g, 1.0 mmol). The product was purified by column chromatography on silica gel (1:1 hexane-AcOEt) to afford 0.65 g (99.9%) of **19**.

**4-(4-Methlphenylsulfonylamino)phenyl 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranoside (20)**. According to methods C and D, compound **20** was prepared from **16** (0.5 g, 1.1 mmol). The product was purified by column chromatography on silica gel (1:1 hexane-AcOEt) to afford 0.64 g (93.1%) of **20**.

**4-(4-tert-Butylphenylsulfonylamino)phenyl 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranoside (21)**. According to methods C and D, compound **20** was prepared from **16** (0.4 g, 1.0 mmol). The product was purified by column chromatography on silica gel (1:1 hexane-AcOEt) to afford 0.55 g (88.5%) of **21**.

**4-(2-Naphthalenephenylsulfonylamino)phenyl 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranoside (22)**. According to methods C and D, compound **22** was prepared from **16** (0.4 g, 1.0 mmol). The product was purified by column chromatography on silica gel (1:1 hexane-AcOEt) to afford 0.53 g (92.5%) of **22**.

**4-(4-Nitrophenylsulfonylamino)phenyl α-D-glucopyranoside (7)**. According to method E, compound **7** was prepared from **17** (0.7 g, 1.1 mmol). The product was purified by column chromatography on silica gel (5:1 $CH_2Cl_2$-MeOH) to afford 0.41 g (83.3%) of **7**: $[\alpha]_D$ +12.1° (*c* 1.23, MeOH); $^1H$ NMR ($CD_3OD$) δ 3.38 (dd, 1H, $J_{3-4}$=8.8 Hz, $J_{4-5}$=10.0 Hz, H-4), 3.53 (dd, 1H, $J_{1-2}$=3.6 Hz, $J_{2-3}$= 9.6 Hz, H-2), 3.59 (ddd, 1H, $J_{4-5}$=10.0 Hz, $J_{5-6a}$=5.0 Hz, $J_{5-6b}$=2.4 Hz, H-5), 3.65 (dd, 1H, $J_{5-6a}$=5.0 Hz, $J_{6a-6b}$=11.8 Hz, H-6a), 3.72 (dd, 1H, $J_{5-6b}$=2.4 Hz, $J_{6a-6b}$=12.0 Hz, H-6b), 3.80 (dd, 1H, $J_{2-3}$=$J_{3-4}$=9.2 Hz, H-3), 5.38 (d, 1H, $J$= 3.6 Hz, H-1), 7.0 (d, 2H, $J$=8.8 Hz, -$OC_6H_4NH$-), 7.05 (d, 2H, $J$=9.2 Hz, -$OC_6H_4NH$-), 7.91 (d, 2H, $J$=8.8 Hz, -$SO_2C_6H_4NO_2$), 8.31 (d, 2H, $J$=8.8 Hz, -$SO_2C_6H_4NO_2$), MS: 455 $(M-H)^-$.

**4-(4-Chlorophenylsulfonylamino)phenyl α-D-glucopyranoside (8)**. According to method E, compound **8** was prepared from **18** (0.54 g, 0.9 mmol). The product was purified by column chromatography on silica gel (5:1 $CH_2Cl_2$-MeOH) to afford 0.38 g (95.2%) of **8**: $[\alpha]_D$ +13.4° (*c* 1.42, MeOH); $^1H$ NMR ($CD_3OD$) δ 3.39 (dd, 1H, $J_{3-4}$ =9.2Hz, $J_{4-5}$=10.0 Hz, H-4), 3.53 (dd, 1H, $J_{1-2}$=3.8 Hz, $J_{2-3}$=9.8 Hz, H-2), 3.60 (ddd, 1H, $J_{4-5}$=10.0 Hz, $J_{5-6a}$=4.8 Hz, $J_{5-6b}$=2.6 Hz, H-5), 3.66 (dd, 1H, $J_{5-6a}$=4.8 Hz, $J_{6a-6b}$ =11.9 Hz, H-6a), 3.72 (dd, 1H, $J_{5-6b}$=2.6 Hz, $J_{6a-6b}$=11.9 Hz, H-6b), 3.80 (dd, 1H, $J_{2-3}$=$J_{3-4}$=9.2 Hz, H-3), 5.38 (d, 1H, $J$=4.0 Hz, H-1), 6.98 (d, 2H, $J$=9.2 Hz, -$OC_6H_4NH$-), 7.05 (d, 2H, $J$=9.6 Hz, -$OC_6H_4NH$-), 7.48 (d, 2H, $J$=8.8 Hz, -$SO_2C_6H_4Cl$), 7.65 (d, 2H, $J$=8.8 Hz, -$SO_2C_6H_4Cl$), MS: 444 $(M-H)^-$.

**4-(4-Trifluoromethylphenylsulfonylamino)phenyl α-D-glucopyranoside (9)**. According to method E, compound **9** was prepared from **19** (0.7 g, 1.0 mmol). The product was purified by column chromatography on silica gel (5:1 $CH_2Cl_2$-MeOH) to afford 0.44 g (90.9%) of **7**: $[\alpha]_D$ +13.4°