of atoms was updated every 100 integration steps. Initial random velocity setup of the system was taken from a Maxwell distribution at $T = 300$ K. The simulation temperature was kept constant by coupling the system to a simulated heat bath, rescaling the velocities with a temperature relaxing time of $t = 0.4$ ps. Coordinates and energies were stored on disk after every 100 integrations steps (0.1 ps) resulting in 284 distinct conformations. Evolution of the system's potential energy shows equilibrium conditions after about 14 ps. Extracted conformations were additionally energy minimized to remove the kinetic energy of the system.

[6] a) Nevertheless, as shown in a previous modeling study docking a nonapeptide into the binding cleft of HLA-B27 [6b], there is a certain probability to dock residue 3 into pocket D. However, that docking scheme does not agree with our sequencing results for HLA-A2. Residue 3 of the peptide antigen is not conserved and therefore not predestined for docking in the hydrophobic D pocket. Further modeling studies with different docking schemes may reveal the location of antigen residue 6 more precisely. b) After preparation of this manuscript similar interactions were found modeling a nonapeptide into the binding site of HLA-B27: D. R. Madden, J. C. Gorga, J. L. Strominger, D. C. Wiley, *Nature* **1991**, *353*, 321–325.

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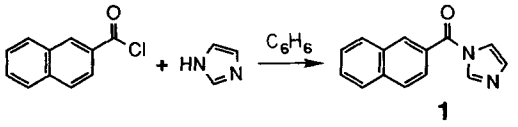
Detection of Subpicomole Levels of Compounds Containing Hydroxyl and Amino Groups with the Fluorogenic Reagent, 2-Naphthoylimidazole**

By *Norihiro Ikemoto, Lee-Chiang Lo,* and *Koji Nakanishi**

Highly sensitive methods for the identification of natural products such as oligosaccharides have become increasingly important with the advances in the biosciences. A commonly employed strategy is HPLC analysis following derivatization with a chromophore. Among the various chromophores used to derivatize amino and/or hydroxyl groups,[1] benzoylation[2, 3] has attracted attention because of the strong UV absorbance of the chromophore, ease of derivatization, and stability of the derivatives. The 2-naphthoate chromophore,[4] however, not only possesses these attributes but also exhibits fluorescence, a feature that dramatically enhances sensitivity. To demonstrate the advantages of using this chromophore, we have investigated its application to sugar analysis.

Most methods for compositional analysis of oligosaccharides involve derivatization of the monosaccharides obtained after acid hydrolysis.[3, 5] In the present microscale method, this derivatization scheme consists of a reduction/naphthoylation sequence (Scheme 1, bottom), which was performed on an artificial mixture of nine monosaccharides most commonly encountered in bioconjugates. The resulting alditol pernaphthoates have UV maxima at 234 nm and possess large ε values (in acetonitrile): 254000 for hexanaphthoates and 220000 for pentanaphthoates, that is, 43000 per naphthoate chromophore, a value three times larger than that of the benzoate. Furthermore, the naphthoates exhibit strong fluorescence at 374 nm upon irradiation at 234 nm, which enhances the sensitivity of detection.



aldoses $\xrightarrow{NaBH_4}$ alditols $\xrightarrow[CH_3CN]{1,\ DBU}$ alditol pernaphthoates $\Rightarrow$ HPLC

Scheme 1. Preparation of naphthoyl imidazole (**1**) and the derivatization/analysis scheme. DBU = 1,8-diazabicyclo[5.4.0]undec-7-ene.

The microscale naphthoylation reaction was performed with 2-naphthoylimidazole (**1**, Scheme 1 top) prepared according to a procedure given for cinnamoylimidazole;[6] the latter reagent has proven to be an excellent reagent for the microscale acylation of acyclic polyols.[7] The pernaphthoylation of alditols and other natural products[8] on a 10 nmol scale occurred rapidly and completely, and only minimal amounts of reagent-derived impurities were formed; in contrast, reactions with 2-naphthoyl chloride, pyridine, and dimethylaminopyridine (DMAP) were slower, required heating, and yielded more extraneous impurity peaks. Prepurification of samples of biological origin prior to derivatization and HPLC analysis minimizes the contaminants of biological origin that may have similar spectroscopic characteristics. Figure 1 shows the chromatogram with about 0.4 pmol of sample injected and monitored with a fluorescence detector (see Experimental). The nine alditol naphthoate derivatives are well resolved on a silica gel column. The absence of incomplete derivatization products in the chromatogram indicates high conversion at the 10 nmol scale.[9] The detection limit is about 0.1 pmole (signal-to-noise ratio S/N $\approx$ 5). Because of the high absorption coefficient of 2-naphthoates and multiple derivatization on each

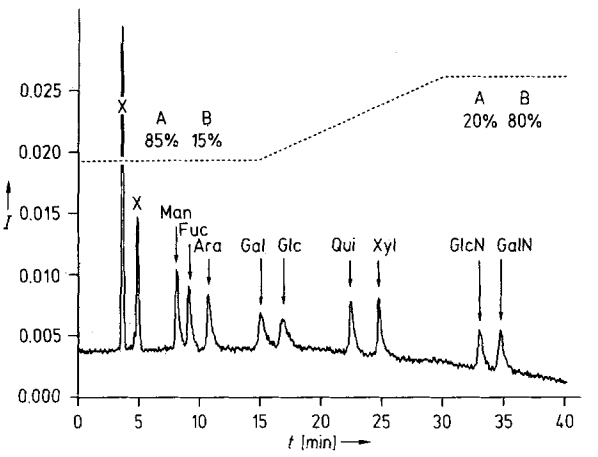


Fig. 1. Chromatogram of ca. 0.4 pmole of pernaphthoate derivatives of mannitol, fucitol, arabitol, dulcitol, sorbitol, quinovitol, xylitol, glucosaminitol, and galactosaminitol (X represents reagent-derived impurities). Solvent A: 0.025% 2-propanol/dichloromethane. Solvent B: 4% acetonitrile/0.025% 2-propanol/dichloromethane. Flow rate: 1 mL min^{-1}. Column: YMC silica gel (4.6 × 250 mm^2, 5 μm). Absorption at 234 nm, Emission intensity I at 374 nm.

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sugar, high sensitivity can still be achieved with UV detection (down approximately to the 1 pmole level, S/N ≈ 2), although it is an order of magnitude less sensitive than fluorescence detection.

Although alditol benzoates have been reported to show poor separation with reverse phase HPLC,[3] the alditol naphthoates could be well separated by normal phase HPLC. Diastereomers differing by one stereocenter, for example, sorbitol/mannitol/dulcitol, xylitol/arabitol, fucitol/quinovitol, and glucosaminitol/galactosaminitol are all well resolved. The amino alditols galactosaminitol and glucosaminitol are retained more strongly by the column than the oxygen sugars and are eluted last. In addition to their higher sensitivity of detection, the good HPLC separation of these 2-naphthoate derivatives offers an important advantage over the use of the benzoate chromophore.

When two or more 2-naphthoate chromophores are present in chiral compounds, exciton coupling interactions are often observed by circular dichroic spectroscopy (CD).[10] The resulting CD curve not only reflects the stereochemistry of the molecule but can be used as an additional handle for structural identification. With the use of a CD beam condenser and a microcell (100 μL, 1 cm), compounds bearing 2-naphthoate chromophores have yielded distinct CD spectra with 50 pmole of sample; ten times more sample is required with a regular CD cell (1 mL, 1 cm).

In conclusion, the advantages of the 2-naphthoate chromophore are evident in the ease of derivatization, the high sensitivity of detection, the good chromatographic behavior, and the possibility of further CD characterization.

Experimental Procedure

Preparation of **1**: Imidazole (0.71 g) was suspended in benzene (40 mL) and cooled to 8 °C. A solution of 2-naphthoyl chloride (1.0 g) in benzene (10 mL) was added dropwise over 10 min, and the mixture stirred for 1 h at room temperature. The reaction mixture was filtered through Celite and lyophilized to afford the product (1.2 g, quantitative).

Mannose, fucose, arabinose, galactose, glucose, quinovose, xylose, glucosamine hydrochloride, and galactosamine hydrochloride (5 μmol each) were stirred for 2 h at room temperature with $NaBH_4$ (10 mg) in water (1 mL). The reaction was quenched with aqueous HCl, and the resulting mixture evaporated six times with methanol. An aliquot (10 nmol of each sugar) was stirred in acetonitrile (0.2 mL) with **1** (10 μmol) and DBU (10 μmol) for 2 h at room temperature. After quenching with water, an aliquot (2 nmol) was evaporated in a silanized test tube. The residue was dissolved in dichloromethane, and aliquots were injected for HPLC analysis [11] (Perkin-Elmer Series 4 Liquid Chromatograph and 7500 Data Station, Shimadzu RF-551 Fluorescence HPLC Monitor).

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1, 141903-34-6; imidazol, 288-32-4; mannose, 3458-28-4; fucose, 2438-80-4; arabinose, 147-81-9; galactose, 59-23-4; glucose, 50-99-7; quinovose, 7658-08-4; xylose, 58-86-6; glucosamine hydrochloride, 66-84-2; galactosamine hydrochloride, 1772-03-8; 2-naphthoyl chloride, 2243-83-6.

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[8] Smaller scale reactions (< 0.5 nmol), HPLC separation and CD measurements have been successfully carried out with different series of compounds containing hydroxyl groups of biological origin.
[9] The isolated yield was ca. 32% when naphthoylation was performed at the smaller 0.6 nmol scale.
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[11] If desired, a prepurification step with C18 cartridges may be added to remove the very polar reagent-derived impurities. See [3].

Tetramethyl-*ortho*-quinodimethane, First Member of a Family of Custom-Tailored Cheletropic Spin Traps for Nitric Oxide**

By *Hans-Gert Korth**, *Keith U. Ingold**, *Reiner Sustmann*, *Herbert de Groot*, and *Helmut Sies*

Nitric oxide, •NO, has been shown to be an intra- and intercellular signal molecule; it regulates vascular tone, is a messenger molecule in the central nervous system, and mediates cytotoxicity of macrophages (see reviews[1–4]). Even if •NO is not itself the "Endothelium Derived Relaxing Factor" (EDRF),[5] it certainly appears to be the active agent released by the EDRF. Nitric oxide also plays an important role in air pollution arising from photochemical and/or combustion processes. Because of the well-recognized importance of •NO, some rather sensitive methods have been developed for its identification and quantitation,[4] for example, the spectrophotometrically monitored •NO-induced conversion of oxyhemoglobin (HbO_2) to methemoglobin (MetHb) [Eq. (a)], monitoring its vasorelaxant activity on arterial segments, and in the gas phase, its chemiluminescent reaction with ozone [Eq. (b)].

$$HbO_2 + {}^{\bullet}NO \longrightarrow MetHb + NO_3^- \qquad (a)$$

$$O_3 + {}^{\bullet}NO \longrightarrow O_2 + {}^{\bullet}NO_2^* \longrightarrow {}^{\bullet}NO_2 + h\nu \qquad (b)$$

Nevertheless, despite all this effort there is as yet no method that, even in principle, would be suitable for monitoring •NO production continuously, both *in time and in space*, in cell cultures, perfused organs, and living organisms. The most attractive route to this objective appeared to be a chemical reaction by which •NO (itself a persistent radical) could be converted into a persistent nitroxide radical. The

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