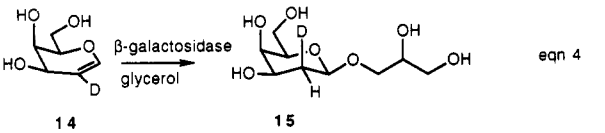
bonding. One can interpret the observation of a β-glycoside in the allal series simply as the result of the steric repulsion of the 3-axial substituent overriding the KAE. Our results are consistent with the Ad_E2 mechanism with a kinetically (sterically?) controlled protonation followed by glycosyl transfer directed by the KAE. They are only surprising to the extent that the intuition of many practicing organic chemists assumes that trans diaxial addition of electrophilic reagents to alkenes is the common behavior. Our results confirm a related experiment with similar mild conditions where Michalska et al. added deuterated thiophosphoric acid to glucal to afford cleanly a 2-deoxypyranose thiophosphate product resulting from cis addition from the α-face.[7] In a control experiment for enzyme studies (vide infra) using rather unusual conditions, Lehmann treated the triacetate of galactal-2-*d* (**14**) with a melt of phenol and p-TosH at 60 °C to afford largely α-glycoside with no stereoselectivity in the proton delivery.[8]

It is interesting to compare our acid-catalyzed results with those of enzyme-catalyzed additions. Lehmann[8] and Hehre,[9] in a pioneering series of experiments that anticipated recent work applying enzymology to preparative glycoside chemistry,[10,11] demonstrated that glycals could be stereospecifically converted to 2-deoxyglycosides using glycosidase enzymes. For example, the galactal-2-*d* (**14**) was cleanly transferred to glycerol using a β-galactosidase, affording glyceryl 2-deoxy-β-galactoside (**15**) (eq 4). De-



eqn 4

14 **15**

livery of the proton to C-2 of the galactal took place specifically from the bottom face to afford apparent trans addition. Although it would be simple to explain the observation as a direct trans addition, the accepted interpretation is more complex. Thus, it has been shown that the enzyme delivers the glycerol to the β-face of the galactosyl anomeric carbon in a step subsequent to the initial bonding of an enzymic nucleophile on the α face,[12] ostensibly the microscopic reversal of the enzyme's natural and stereospecific cleavage mechanism.[13] The attachment of a proton at C-2 and the presumed enzymic nucleophile at the intermediate stage must have been via cis addition. We are now able to speculate that the β-galactosidase-promoted addition with an overall trans outcome was really a more stereoselective version of a simple acid-catalyzed cis addition, subject to stereoelectronic control, with the β-enzyme affording below-plane protonation corresponding to our observation. In contrast, the above-plane protonation with the α-glucosidase enzyme is not consistent with the simple acid-catalyzed chemistry of glycals followed by glycosyl transfer. The unanswered question concerning our chemical results and also the larger field of electrophilic addition to glycals is what is the basis for "below-plane" or "equatorial-developing" attack at C-2? One popular explanation, namely pyramidalization of the alkene carbon,[14] is belied by the reported crystal structures for glycals.[15] A rationalization, commonly known as the Cieplak effect,[16] where the more electron-rich bond vicinal and anti-parallel to the forming bond, directs the attack, can explain the outcome only in the 3-deoxyglucal case. Some as yet undefined steric effect of the 3-equatorial substituent and the 4-axial proton may be a part of the explanation in the glucal series while in the galactal and allal cases, a steric repulsion of 4-axial and 3-axial groups, respectively, are probably determinant. More work remains to be done to discover the balance of forces that controls the face-selectivity of electrophilic attack in glycals.[17,18]

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Substitution Reactions of a Nucleofugal Group in Heptamethine Cyanine Dyes. Synthesis of an Isothiocyanato Derivative for Labeling of Proteins with a Near-Infrared Chromophore

Lucjan Strekowski,* Malgorzata Lipowska,[1] and Gabor Patonay

Department of Chemistry, Georgia State University, Atlanta, Georgia 30303-3083

Received April 22, 1992 (Revised Manuscript Received June 29, 1992)

Summary: The reactions of dye **1** with MeONa, $MeNH_2$, PhONa, PhSNa, PhSH, and 4-H_2NPhSH to yield the corresponding derivatives **2a–e**, hydrodechlorination of **1** to **3** in the presence of EtSNa or PhSNa/Ph_2PH, and synthesis of the SCN-substituted dye **4**, a new reagent for ultratrace detection of proteins, are described.

Currently there is immense interest in the chemistry of polymethinamidinium salts (cyanine dyes) that absorb in the near-infrared (NIR) region. This class of compounds is important as sensitizers for photography and xerography,

(1) On leave of absence from the Department of Chemistry, Jagiellonian University, 30-060 Krakow, Poland.

0022-3263/92/1957-4578$03.00/0

Table I. Reactions of 1 with Various Reagents

reagent[a]	temp (°C)	time[b] (min)	product[c] (% yield)
MeONa	23	600	**2a** (90)
$MeNH_2$	23	360	**2b** (83)
PhONa	23	<1	**2c** (87)
PhSNa	23	<1	**2d** (86)
PhSH	23	5	**2d** (85)
4-H_2NPhSH	23	5	**2e** (81)
EtSNa	100	60	**3** (89)
PhSNa/Ph_2PH	60	10	**3** (91)

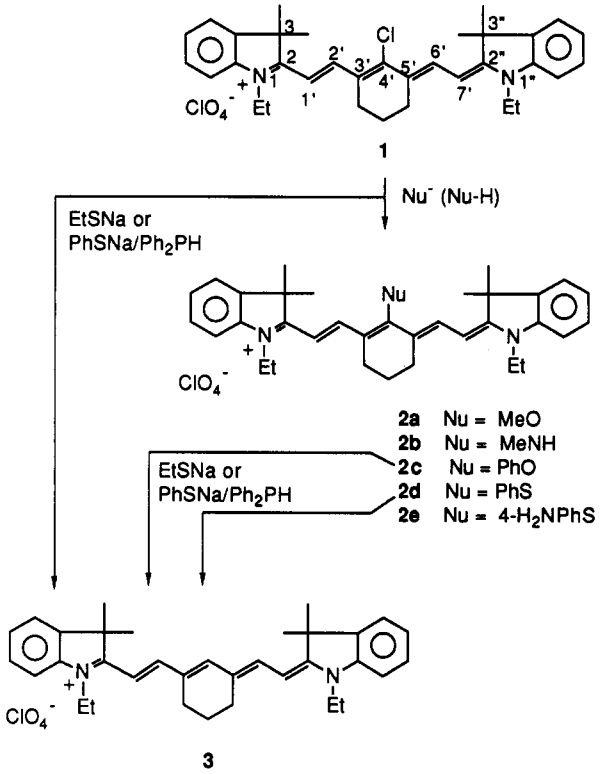
[a] All reactions were conducted with 20 mg (0.033 mmol) of 1 and 0.66 mmol of the reagent under a nitrogen atmosphere in 10 mL of solvent: MeOH for MeONa and DMF for the remaining reagents. A molar ratio of 1:1 for PhSNa/Ph_2PH was used in the last entry. [b] The reaction progress was monitored by VIS-NIR absorption changes for solutions diluted with MeOH, and the given reaction times correspond to disappearance of the absorption band for 1 at 778 nm. [c] The mixtures were treated with CO_2, concentrated, and then purified by silica gel flash chromatography ($CHCl_3$/MeOH, up to 50% of MeOH). Workup of the chromatography fractions included concentration followed by treatment of the residues with $CHCl_3$ to precipitate silica gel eluted with MeOH. Products were crystallized from MeOH/Et_2O in the presence of $HClO_4$ (0.2 equiv), mp >200 °C (dec at >150 °C). VIS-NIR [compound, λ_{max}^{MeOH} (ϵ)]: **2a**, 753 (182 000); **2b**, 615 (84 300); **2c**, 764 (206 000); **2d**, 790 (168 000); **2e**, 791 (168 000); and **3**, 747 nm (262 000 M^{-1} cm^{-1}). Fluorescence for **2a–e**, **3**: approximately at λ_{max} +30 nm (excitation at λ_{max}) with quantum yields in the range of 0.1–0.5.

laser dyes, pleochroic dyes for LC displays, dyes for polymers, cosmetic ingredients,[2] analytical indicators,[3] and fluorescent markers for biological macromolecules[4] (vide infra).

In this paper we report a novel approach to derivatization of cyanine dyes in reactions of compounds containing a nucleofugal group at the central position, such as 1,[5] with nucleophiles. As can be seen from Scheme I and Table I, dye 1 undergoes efficient substitution reactions in the presence of various reagents to give the corresponding derivatives **2a–e**, albeit with different facilities.

In contrast to the fast reaction of 1 with PhSNa at 23 °C, dye 1 was stable in the presence of EtSNa under otherwise identical conditions but underwent reaction at an elevated temperature to furnish a dehalogenated dye **3** as the sole NIR-absorbing product. The treatment of the phenoxy and phenylthio derivatives, **2c** and **2d**, with EtSNa under similar conditions also gave **3** in a high yield.

Scheme I



A sharply accelerated reduction of **2d** to **3** was observed with a system consisting of PhSNa, a putative electron donor, and Ph_2PH, a good hydrogen donor[6,7] (analogous data as for **3** in Table I).

The unusually rapid substitution reactions of 1 with PhONa and PhSNa are remarkable. When 1 was allowed to react with a mixture of PhONa (10 equiv) and PhSNa (1.5 equiv), the phenylthio derivative **2d** was formed exclusively. Treatment of 1 with PhONa to preform **2c** and followed by addition of PhSNa under these molar ratios of the reagents also furnished **2d**. In a similar way, the reaction of **2a** with PhSNa gave **2d** rapidly. Finally, the treatment of 1 (1 equiv) in DMF or DMSO at 23 °C for 5 min with a mixture consisting of PhSNa (1.5 equiv) and PhONa, MeONa, NaOH,[8] EtSNa, NaI, $MeNH_2$, and $PhNH_2$ (10 equiv each) furnished the phenylthio derivative **2d** (83%) as the sole NIR-absorbing product.[9] The yield of **2d** did not decrease after the mixture was allowed to stand for 3 h before workup. Dye **2d**, thus, is formed rapidly and is stable at 23 °C in the presence of other common nucleophiles including aliphatic mercaptides.

The high stability of a phenylthio derivative prompted us to synthesize isothiocyanato-substituted dye **4** by functionalization of the amino group in **2e** (eq 1). The rationale was to obtain a reagent for NIR labeling of proteins at amino groups.[4b] It was hoped that such labeling would permit ultratrace determination of proteins from clinical isolates by HPLC taking advantage of the high absorbance and fluorescence of the NIR dye system and inherently low interference in the NIR region. The pre-

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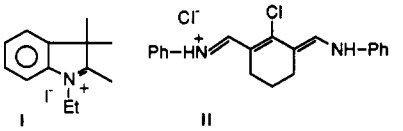
(5) Condensation (EtOH, AcONa, 80 °C, 1 h) of i and ii was followed by chromatography (silica gel, $CHCl_3$/MeOH (19:1)).



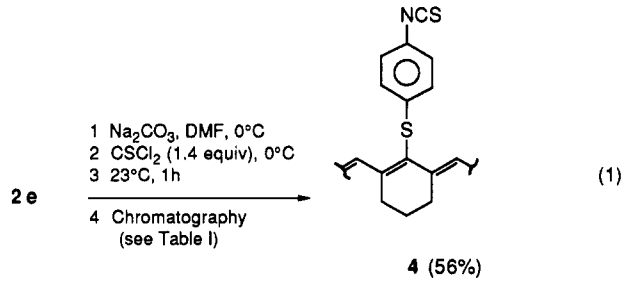
2-[4′-Chloro-7′-(1″-ethyl-3″,3″-dimethylindolin-2″-ylidene)-3′,5′-(propane-1‴,3‴-diyl)-1′,3′,5′-heptatrien-1′-yl]-1-ethyl-3,3-dimethylindolinium perchlorate (1) crystallized from EtOH upon treatment with 0.1 M $HClO_4$: yield 81%; mp >200 °C (dec > 150 °C); UV-NIR λ_{max}^{MeOH} 778 nm (219 000). For preparation of ii, its synthetic equivalents, and analogs, see: (a) Makin, S. M.; Boiko, L. I.; Shavrygina, O. A. *Zh. Org. Khim.* **1977**, *13*, 1189. (b) Slominski, Yu. L.; Radchenko, I. D.; Popov, S. V.; Tolmachev, A. I. *Zh. Org. Khim.* **1983**, *19*, 2134. (c) Sosnovskii, G. M.; Lugovskii, A. P.; Tishchenko, I. G. *Zh. Org. Khim.* **1983**, *19*, 2143. (d) Reynolds, G. A.; Drexhage, K. H. *J. Org. Chem.* **1977**, *42*, 885. (e) Arnold, Z. *Collect. Czech. Chem. Commun.* **1965**, *30*, 2783.

(6) Ashby, E. C.; Park, W. S.; Goel, A. B.; Su, W.-Y. *J. Org. Chem.* **1985**, *50*, 5184.

(7) The use of Bu_3SnH caused disappearance of VIS-NIR absorption.

(8) Although all dyes **1–3** are relatively stable at 23 °C in DMF or DMSO in the presence of NaOH under concentration conditions of Table I, upon heating they undergo decomposition to unidentified products that do not absorb in the NIR region. Treatment of 1 with NaOH in MeOH (65 °C, 30 min) gave **2a**.

(9) Chromatography gave an iodide which was treated with $HClO_4$ to furnish the perchlorate **2d**.



(1)

liminary results showed that for detection of proteins[10,11] **4** is vastly superior to fluoresceinisothiocyanate, a classical reagent for protein labeling with UV–vis chromophore.[12]

Finally, we wish to comment on possible mechanisms for the discussed reactions. The yields of **2b–e** were reduced in the presence of molecular oxygen, a free-radical scavenger, and nitrobenzene, an electron scavenger,[6] with a concomitant formation of a number of unidentified products that lacked NIR chromophore. When dry oxygen was bubbled through the mixtures in DMF/$PhNO_2$ (1:1), the yields of **2c** and **2d** from the fast reactions of **1** with PhONa and PhSNa, respectively, were decreased by 5%, the yields of **2d** and **2e** from the reactions with PhSH and 4-H_2NPhSH, respectively, were decreased by 15%, and product **2b** was not formed at all in an attempted reaction of **1** with $MeNH_2$ although **1** was consumed.[13] Under anaerobic conditions in the presence of $PhNO_2$ the yield of **2b** was only 60%, and the apparent rates for disappearance of **1** and formation of **2b** were both smaller by about 30% in comparison to that for the reaction conducted in the absence of $PhNO_2$. By contrast, molecular oxygen and $PhNO_2$ had no effect[13] on the slow reaction of **1** with MeONa.

These results are consistent with a unified mechanism (eqs 2–5) in which an $S_{RN}1$ pathway[14] leads to **2b–e**, and **3** is produced from an intermediate cation radical[14c] (eq 3). The suggested presence of radical intermediates was

$$(R\text{–}X)^+ + Y^- \rightarrow (R\text{–}X)^\bullet + Y^\bullet \quad (2)$$

$$(R\text{–}X)^\bullet \rightarrow R^{\bullet+} + X^- \quad (3)$$

$$R^{\bullet+} + Y^- \rightarrow (R\text{–}Y)^\bullet \quad (4)$$

$$(R\text{–}Y)^\bullet + (R\text{–}X)^+ \rightarrow (R\text{–}Y)^+ + (R\text{–}X)^\bullet \quad (5)$$

supported by observation of an EPR absorption for a mixture of **1** and PhSH in DMF.[15] Semiempirical calculations were also consistent with a high electron affinity of the cation **1**.[16] On the other hand, the reaction of **1** with MeONa to give **2a** may involve a direct addition of the nucleophile followed by elimination of chloride.[17]

We have shown straightforward and highly efficient chemistry for modification of the optical chromophore of **1**. This method should be applicable to the preparation of a broad spectrum of compounds[18,19] starting with similarly substituted analogs of **1**. A vast number of such dyes is readily available through classical syntheses.[2–5]

Acknowledgment. Support of this research by NIH (Grant AI28903) and NSF (Grants CHEM-8409599 and CHEM-890456) is gratefully acknowledged. We thank Dr. W. H. Nelson for EPR analyses and Dr. A. L. Baumstark for critical reading of the manuscript.

Supplementary Material Available: Analytical data, VIS-NIR, and ^1H NMR (400 MHz) spectra for **1–4** and analogs and a procedure for the preparation of **4** (16 pages). This material is contained in many libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

(10) Human angiotensin I (10^{-4} M in MeOH/H_2O (1:1)) was allowed to react with a 10-molar excess of fluoresceinisothiocyanate (FL-NCS) or **4** for 15 min at 23 °C followed by removal of the unbound dye on a short C18 cartridge (MeOH/H_2O (1:1)). Fluorescence of the labeled oligopeptide samples was observed on an SLM 8000 fluorometer (λ_{ex} = 499 nm, λ_{em} = 530 nm) for the labeling with FL-NCS and by using a 30-mW laser diode (λ_{ex} = 785 nm, λ_{em} = 820 nm) for the labeling with **4** to give the detection limits of 2×10^{-10} M and 4×10^{-14} M, respectively. A laser diode excitation source for fluorescein is not available.

(11) An attempted use of reactive dyes **1**, **2a**, or **2c** for direct labeling of proteins gave negative results at low concentration levels.

(12) *Luminescence Techniques in Chemical and Biochemical Analysis*; Baeyens, W. R. G., De Keukeleire, D., Korkidis, K., Eds.; Marcel Dekker: New York, 1991.

(13) Dye **1** itself is stable in the presence of oxygen under similar conditions of solvent, temperature and time.

(14) (a) Rossi, R. A.; de Rossi, R. H. *Aromatic Substitution by the $S_{RN}1$ Mechanism*; ACS Monograph 178; American Chemical Society: Washington, D.C., 1983. (b) Bunnett, J. F. *Acc. Chem. Res.* **1978**, *11*, 413. (c) Bard, A. J.; Ledwith, A.; Shine, H. J. In *Advances in Physical Organic Chemistry*; Gold, V., Bethell, D., Eds.; Academic: London, 1976; Vol. 13, p 155.

(15) A poorly resolved signal was observed, as expected for several radical intermediates. Neither DMF with **1** nor DMF with PhSH showed this absorption. This result does not rule out partial involvement of alternative mechanistic pathways in which (i) **2d** is formed by recombination of a radical pair (eq 2) followed by elimination of chloride, and (ii) hydrogen abstraction by the radical (R-SPh)• (eq 2) followed by elimination of benzenethiolate anion leads to **3**.

(16) AMPAC and MOPAC computations gave lower energy for the free radical derived from **1** over the cation **1** by 130 and 126 kcal/mol, respectively.

(17) Methoxide ion appears to be inert toward $S_{RN}1$ reactions (ref 14b). The slow substitution reaction of **1** with MeONa is not due to deprotonation of **1** at the methylene of N-Et to form an ylide, although reversible addition reactions at positions other than 4′ cannot be ruled out (see Scheme I for numbering). After the reaction was conducted in MeOD at 23 °C, the resultant product **2a** was devoid of deuterium. Heating of **2a** with MeOD/MeONa at 65 °C for 2 h (see Table I for concentrations) gave regioselective and complete deuteration at positions 1′ and 7′. With **1** the rate for formation of nondeuterated **2a** in the MeOD/MeONa system was four times greater at 65 °C than the apparent rate for hydrogen–deuterium exchange at this temperature. This unusual regioselective deuterium labeling can be rationalized in terms of two consecutive additions of methoxide anion at positions 2 and 2″ of the chromophore to form the most thermodynamically stable anion.

(18) Substitution of a 1,2-ethanediyl bridge for the 1,3-propanediyl bridge in the chromophore of **1** does not affect these efficient transformations.

(19) The same chemistries, albeit with lower yields (by ca. 5%), were observed for cation **1** with iodide counterion. The use of the iodide salt may be preferred for larger scale preparations due to a more facile chromatographic purification of the less polar iodides than perchlorates. Of interest is the reported lack of reactivity of iodide ion as a nucleophile with cation radicals (ref 14c).

Enantioselective Cation Binding with a Functionalized Podand Ionophore

Alan Armstrong and W. Clark Still*

Department of Chemistry, Columbia University, New York, New York 10027

Received May 28, 1992

Summary: By adding an acetamido group to podand **1**, a new ionophore **2** is created which binds peptidic ammonium ions with high enantioselectivity.

The methylated podand ionophore **1** has been shown to bind simple chiral ammonium ions with modest enantioselectivity (up to 40% ee).[1] One likely reason for this

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