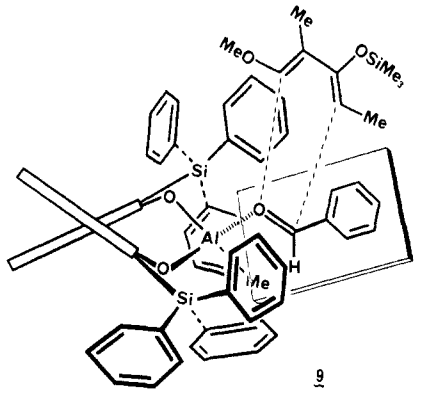

9

residue and **3** in order to minimize the steric repulsion between the incoming diene and the front triarylsilyl moiety, thereby yielding the cis adduct **4** predominantly in accord with the experimental findings. The observed higher cis as well as enantioselectivity by the use of the sterically more hindered triarylsilyl moiety in **1** would be accommodated in this explanation. It should be noted that the hetero-Diels–Alder adduct, once it formed, split off readily from the aluminum center in view of the steric release between the adduct and the aluminum reagent, resulting in regeneration of the catalyst **1** for further use in the catalytic cycle of the reaction. In marked contrast, the chiral organoaluminum reagent derived from Me_3Al and (*R*)-(+)-3,3′-dialkylbinaphthol (alkyl = H, Me, and Ph) was employable only as a stoichiometric reagent and gave fewer satisfactory results in reactivity and enantioselectivity in the hetero-Diels–Alder reaction.

Acknowledgment. We thank Dr. T. Ibuka (Kyoto University) for a generous supply of 1,3-bis(trimethylsiloxy)-1,3-butadiene. Financial support of this work was provided by the Kurata Foundation.

A Potent New Class of Active-Site-Directed Glycosidase Inactivators

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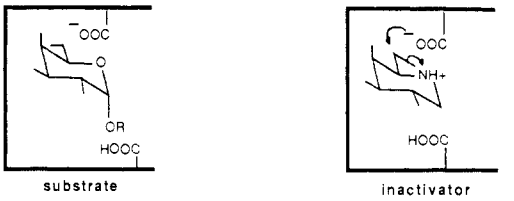
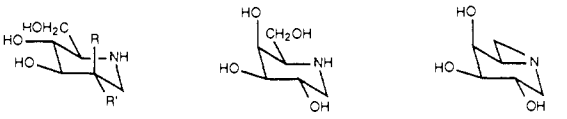
In the past several years, sugar- and anomer-specific inhibitors of glycosylhydrolases have helped unravel the catalytic mechanisms of these important enzymes.[1,2] The latest generation of such compounds manifests promising therapeutic applications as antiviral agents[3] and in the regulation of carbohydrate metabolic disorders.[1b] We wish to report a new class of potent active-site-directed glycosidase inactivators[4] capable of alkylating the key catalytic carboxylate group invoked in most currently accepted mono- or bilateral mechanisms of enzyme-assisted glycoside hydrolysis (Scheme I).[5]

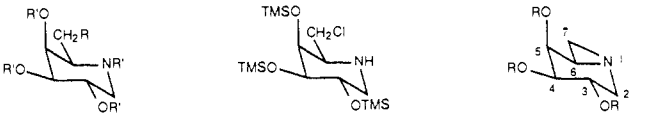
Scheme I





1 R=H, R'= OH
2 R=OH, R'=H
3
4

Scheme II



5 R=OH, R'=Bn
6 R=Cl, R'=Bn
7 R=Cl, R'=H
8
9 R=TMS
4 R=H

In the lysozyme model (Scheme I),[6] competitive inhibition of gluco-, manno-, and galactosidases by azasugars like **1**,[7] **2**,[8] and **3**,[9] respectively, arises from H bonding and electrostatic interactions with a nearby carboxylate (Scheme I).[1b] We speculated that protonated aziridine[10] analogues like **4** might preferentially interfere with *α-glycoside* hydrolysis by S_N2 esterification of the enzyme's *β*-face carboxylate anion. There were several reasons for choosing galactosidases to test this hypothesis: (a) compared to **1** or **2**, azasugar **3** was 100-fold more active[9c] against its target, *α*-galactosidase, (b) reactive aziridine electrophiles were expected to be more stable at the near-neutral pH optima of most galactosidases, and (c) despite its metabolic significance, relatively little has been learned about the active site of *α*-galactosidase.[11]

The synthesis of aziridinyltriol **4**[12] from the known[9c] piperidine **5** is outlined in Scheme II. Mesylation and displacement of **5** afforded chloride **6** (76%) which could be hydrogenolyzed to triol **7** (100%). When direct cyclization of **7** to **4** proved impossible, the triol was exhaustively silylated [$(TMS)_2NH$, TMSCl, pyr, then H_2O] to afford **8** (88%). Exposure of **8** to *n*-butyllithium (1 equiv, THF, −78 °C) produced aziridine **9** (36–40%).[13]

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(10) The pK_A of an aziridine like **4** is expected to be ca. 8.0–9.5: *Small Ring Heterocycles*; Hassner, A., Ed.; Wiley: New York, 1983; Part 1, pp 9–10.

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(12) (3*S*,4*R*,5*S*,6*R*)-1-Aza-3,4,5-trihydroxybicyclo[4.1.0]heptane.

(13) For **9**: ^1H NMR (300 MHz, acetone-d_6) δ 4.42 (dd, H-5, *J* = 3.6, 5.5 Hz), 3.82 (ddd, H-3, *J* = 3.4, 4.3, 5.9 Hz), 3.71 (dd, H-4, *J* = 3.6, 5.9 Hz), 3.08 (dd, H-2*β*, *J* = 3.4, 13.4 Hz), 2.87 (dd, H-2*α*, *J* = 4.3, 13.4 Hz), 2.06 (ddd, H-6, *J* = 2.7, 5.5, 5.5 Hz), 1.85 (d, H-7*β*, *J* = 2.7 Hz), 1.65 (d, H-7*α*, *J* = 5.5 Hz); CMR (100 MHz, C_6D_6) 78.11, 76.89, 66.19, 61.28, 55.18, 43.59; CIMS (methane) *m/e* 362 (M + 1, 13%), 73 (100%).

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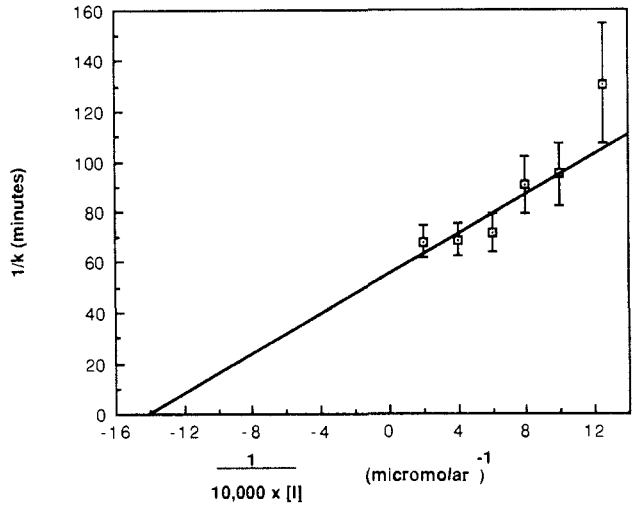

Figure 1. Lineweaver–Burk analysis of time-dependent kinetics with **4**.

Deprotection (K_2CO_3–CH_3OH room temperature) afforded **4**.[14]

At 1 mM concentration, aziridinyltriol **4** showed potent inhibition of green coffee bean α-galactosidase (Sigma; either pH 5 or pH 6.6), but had little or no effect on yeast α-glucosidase (pH 6.6), jackbean α-mannosidase (pH 5), or bovine β-galactosidase (pH 7). Detailed kinetic studies of *p*-nitrophenyl α-D-galactopyranoside hydrolysis at different inhibitor concentrations (pH 6.6) revealed time-dependent first-order inactivation of α-galactosidase.[15] A Lineweaver–Burk plot of $1/k$ vs $1/I$ (Figure 1) gave the dissociation constant of the noncovalent enz-**4** complex [$K_M = 7.1 \pm 2$ μM] as well as the first-order rate constant with which the complex was converted into inactivated enzyme [$k_{inact} = 1.8 \pm 0.51 \times 10^{-2}$ min^{-1}]. In the presence of competitive inhibitor **3**, the enzyme was protected against irreversible inactivation by **4**. Moreover, inactivated enzyme, when treated with 1 M NH_2OH[17] and then $FeCl_3$, gave rise to a strong absorbance at 510 nm characteristic of an enzyme–hydroxamic acid–iron(III) chelate. Controls using fresh enzyme with and without **3** showed no such absorbance, strongly suggesting that inactivation by **4** led to a new ester linkage. Judging from the apparent second-order rate constant for the association of free enzyme and inhibitor [$k_{inact}/K_M = 2540$ min^{-1} M^{-1}], aziridine **4** is, to our knowledge, the most potent and specific α-galactosidase inactivator yet reported.[18] These findings support the proposed orientation of proton-donating and nucleophilic groups at the α-galactosidase active site and may find application in the study of other carbohydrate-processing enzymes.

Acknowledgment. We thank the National Institutes of Health for grant support (GM 35712) and a predoctoral traineeship to M.K.T. (GM97273) and Prof. T. Begley for helpful discussions. Support of the Cornell Nuclear Magnetic Resonance Facility by NSF (CHE 7904825, PCM 8018643) and NIH (RR02002) is gratefully acknowledged.

(14) For **4**: ^{1}H NMR (300 MHz, D_2O) δ 4.53 (br t, H-5, $J = 5.2, 6.6$ Hz), 3.88 (dd, H-4, $J = 5.6, 6.6$ Hz), 3.77 (m, H-3), 3.09 (m, H-2α, 2β), 2.37 (q, H-6, $J = 5.7, 10.9$ Hz), 1.96 (d, H-7β, $J = 4.1$ Hz), 1.91 (d, H-7α, $J = 6.1$ Hz).

(15) All enzyme assays were conducted in triplicate at 37 °C in citrate–phosphate buffer with added KCl to a constant ionic strength of 0.5 M (ref 16). After interim exposure of enzyme to inhibitor at various concentrations, residual activity was measured by incubating the enzyme with substrate (5 mM) for 15 min in a final volume of 200 μL, the basifying to pH 10.4 and monitoring absorbance at 400 nm.

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(18) For conduritol C epoxide (ref 4a) $k_{inact}/K_M = 6.6$ min^{-1} M^{-1}; for α-D-galactopyranosylmethyl(*p*-nitrophenyl)triazine (ref 4b) $k_{inact}/K_M = 0.96$ min^{-1} M^{-1}.

Preparation of the First Stable Formylsilane, $(Me_3Si)_3SiCHO$, from a Zirconium η^2-Silaacyl Complex

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Despite intent interest in the chemistry and properties of acylsilane derivatives ($RCOSiR'_3$),[1] little has been reported regarding formylsilanes (R_3SiCHO). Early attempts to prepare formylsilanes led to the conclusion that they were unstable under a variety of reaction conditions.[1a,2] Hydrolysis of the ozonide adduct of vinyltrimethylsilane with zinc dust gave trimethylsilanol and formaldehyde, possibly via Me_3SiCHO.[2a] Speier attempted unsuccessfully to prepare Me_3SiCHO by treatment of $Me_3SiCHCl_2$ with potassium acetate and sodium ethoxide and by catalytic dehydrogenation of Me_3SiCH_2OH over copper metal at 260 °C. The latter reaction produced trimethylsilane and carbon monoxide, possible decomposition products of Me_3SiCHO.[2b] Reaction of triphenylsilyllithium with ethyl formate also failed to produce an isolable formylsilane, but Ph_3SiCHO was postulated as an intermediate.[1a] More recently, Ireland and Norbeck have obtained evidence for Me_3SiCHO, generated at low temperature by Swern oxidation of Me_3SiCH_2OH and trapped by reaction with a Wittig reagent.[1h]

A possible route to formylsilanes is suggested by the reported acidification of zirconium acyl derivatives $Cp_2Zr(\eta^2\text{-COR})Cl$ (Cp = η^5-C_5H_5) to produce aldehydes.[3] We have prepared a number of early transition-metal η^2-silaacyl complexes that are potential starting materials for such a synthesis.[4] Indeed, reaction of $Cp_2Zr(\eta^2\text{-COSiMe}_3)Cl$ with 1 equiv of HCl at low temperature generated a product that was identified by NMR spectroscopy as Me_3SiCHO. This species was not thermally stable, however, and decomposed to a number of products above −25 °C.[4d] To obtain a more stable formylsilane derivative, we sought a route to the more sterically hindered $(Me_3Si)_3SiCHO$. Unfortunately, the obvious precursor to this compound, $Cp_2Zr[\eta^2\text{-COSi}(SiMe_3)_3]Cl$, is not available via carbonylation of $Cp_2Zr[Si(SiMe_3)_3]Cl$.[4d] Here we report a successful preparation of an $\eta^2\text{-COSi}(SiMe_3)_3$ derivative of zirconium and its conversion to the first stable, isolable formylsilane $(Me_3Si)_3SiCHO$ (**3**).

The zirconium silyl $CpCp^*Zr[Si(SiMe_3)_3]Cl$ (**1**, $Cp^* = \eta^5$-C_5Me_5) is prepared from $CpCp^*ZrCl_2$[5] and $(THF)_3LiSi(SiMe_3)_3$[6] in benzene.[7] The silaacyl $CpCp^*Zr[\eta^2\text{-COSi}(SiMe_3)_3]Cl$ (**2**)[8] is obtained as pink crystals in 71% yield by reaction of **1** with

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(8) For **2**: IR (Nujol) ν(CO) = 1440 cm^{-1}; ^{1}H NMR (benzene-d_6, 22 °C, 300 MHz) δ 0.29 (s, 27 H, $SiMe_3$), 1.73 (s, 15 H, C_5Me_5), 5.70 (s, 5 H, C_5H_5); $^{13}C\{^1H\}$ NMR (benzene-d_6, 22 °C, 75.5 MHz) δ 1.98 ($SiMe_3$), 12.19 (C_5Me_5), 110.58 (C_5H_5), 117.26 (C_5Me_5), 382.79 (ZrCOSi). Anal. ($C_{25}H_{47}ClOSi_4Zr$) C, H.

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