Detergents Containing a 1,3-Diene Group in the Hydrophobic Segment. Facile Chemical Modification by a Diels-Alder Reaction with Hydrophilic Dienophiles in Aqueous Solution

John F. W. Keana,* Anthony P. Guzikowski, Claude Morat, and Johannes J. Volwerk

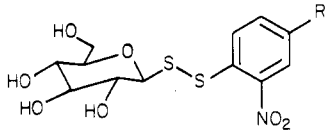
Department of Chemistry, University of Oregon, Eugene, Oregon 97403

Received January 25, 1983

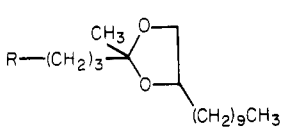
The objective of this investigation is a series of 1,3-diene-containing detergents that may be converted into derivatives of relatively high monomeric aqueous solubility by a Diels-Alder reaction with hydrophilic dienophiles under mild aqueous conditions. Triton X-100 analogue **10**, sodium dodecadienyl sulfate **16**, and dodecadienyl maltoside **18** were synthesized. Detergents **16** and **18** reacted rapidly with the potent hydrophilic triazolinedione dienophile **19** in aqueous solution at 25 °C, forming the adducts **20** and **21** quantitatively. Diels-Alder adducts also formed readily between the three diene detergents and sulfophenyl maleimide **26**, although the reaction rate was somewhat slower than that of **19**. Diene detergent **18** led to a stable $CHCl_3$-water emulsion while its Diels-Alder adduct did not. By monitoring the enzymatic activity of phospholipase A_2 and α-chymotrypsin as a function of added **19** both in the presence and absence of detergents, it was shown that **19** reacts preferentially with the 1,3-diene unit of the detergents rather than with the proteins.

Detergents are amphipathic molecules consisting of a hydrophobic segment and a hydrophilic segment, the latter being either ionic or nonionic in nature. Applications in chemistry include use as phase-transfer catalysts in organic synthesis[1] and as necessary reagents for the isolation, characterization, and reconstitution of membrane proteins.[2] Nearly always, the detergent must be eventually removed from the reaction mixture or biochemical preparation. Removal can be complicated by the formation of emulsions or tight binding to the preparation.

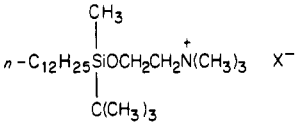
As one approach to this problem, several new detergents have been described recently that can be cleaved by a chemical reaction into nondetergent fragments. Examples include the unsymmetrical aryl glucosyl disulfides **1**,[3] ketals



1, R = *tert*-butyl, *tert*-octyl, *sec*-butyl, or H



2, R =



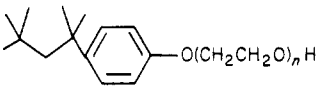
3, X = Cl, NO_3, $B(C_6H_5)_4$

2,[4] and siloxanes **3**.[5] Limitations associated with these novel molecules include low solubility for **1** and the requirement of extremes of pH for the cleavage of detergents **2** and **3** coupled with possible difficulties in the separation of the resulting fragments from the desired product(s).

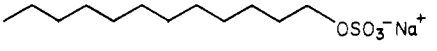
Herein, we describe a conceptually different approach in which a 1,3-diene unit is incorporated in the hydrophobic segment. An aqueous[6,7] Diels-Alder reaction with a powerful hydrophilic dienophile[8] is then used to render this segment largely hydrophilic. The approach in principle is extendable to include, for example, 1,3-diene-containing trialkylsulfonium, tetraalkylphosphonium, and quaternary ammonium salts that are used extensively as phase-transfer catalysts.

Results and Discussion

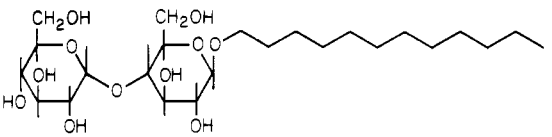
A. Synthesis of the 1,3-Diene-Containing Detergents. The new detergents were patterned after the two widely used, commercially available detergents Triton X-100 (**4**) and sodium dodecyl sulfate (SDS) (**5**) and the



4, *n* = 8-10



5



6

recently reported membrane protein detergent dodecyl maltoside (**6**).[9] Analogues of **4** in which the benzene ring was replaced by a cyclopentadiene ring were the first objective. Cyclopentadiene is among the most reactive 1,3-dienes in the Diels-Alder reaction, and the shape and polarity of the molecule approximates that of benzene.

Lithium *tert*-butylcyclopentadienide (**7**), obtained as a light yellow solid by the reaction of dimethylfulvene with methyllithium in ether,[10] underwent alkylation by the

(1) Starks, C. M.; Liotta, C. "Phase Transfer Catalysis: Principles and Techniques"; Academic Press: New York, 1978. Menger, F. N.; Rhee, J. U.; Rhee, H. K. *J. Org. Chem.* **1975**, *40*, 3803. Weber, W. P.; Gokel, G. W. "Phase Transfer Catalysis in Organic Synthesis", Springer-Verlag: New York, 1977.

(2) Helenius, A.; Simons, K. *Biochim. Biophys. Acta* **1975**, *415*, 29. Tanford, C.; Reynolds, J. A. *Biochim. Biophys. Acta* **1976**, *457*, 133.

(3) Cuomo, J; Merrifield, J. H.; Keana, J. F. W. *J. Org. Chem.* **1980**, *45*, 4216.

(4) Jaeger, D. A.; Frey, M. R. *J. Org. Chem.* **1982**, *47*, 311.

(5) Jaeger, D. A.; Ward, M. D. *J. Org. Chem.* **1982**, *47*, 2221.

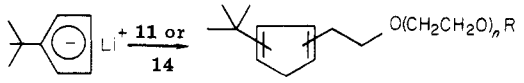
(6) Significant rate enhancements have been observed for certain Diels-Alder reactions run in dilute aqueous solutions. See: Rideout, D. C.; Breslow, R. *J. Am. Chem. Soc.* **1980**, *102*, 7816.

(7) The effect of microemulsions on the Diels-Alder reaction has been investigated, see: Gonzalez, A.; Holt, S. L. *J. Org. Chem.* **1982**, *47*, 3186.

(8) Keana, J. F. W.; Guzikowski, A. P.; Ward, D. D.; Morat, C.; Van Nice, F. L. *J. Org. Chem.*, preceding paper in this issue.

(9) Rosevear, P.; Van Arken, T.; Baxter, J.; Ferguson-Miller, S. *Biochemistry* **1980**, *19*, 4108.

0022-3263/83/1948-2661$01.50/0



7

8, n = 2; R = THP
9, n = 2; R = H
10, n = 8; R = H

$ClCH_2CH_2OCH_2CH_2OCH_2CH_2OTHP$

11

readily available tetrahydropyranyl (THP) ether 11 in THF to give 8 (yellow oil, 35%) as a mixture of double-bond and positional isomers. For the envisaged application as detergents it was not necessary to separate the various isomers. Acid-catalyzed deprotection gave the amphipathic alcohol 9 as a yellow oil in 35% yield.

Initial experiments toward detergent 10, an amphipathic molecule that more closely resembles Triton X-100, utilized benzenesulfonate–THP-derivatized nonaethylene glycol 13,[11] prepared in several steps from 12.[12] In this series,

$RO(CH_2CH_2O)_9R'$

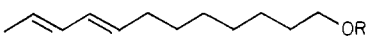
12, R = R′ = H

13, R = $C_6H_5SO_2$; R′ = THP

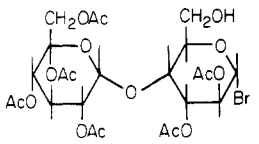
14, R = $C_6H_5SO_2$; R′ = $SiMe_3$

however, the acidic conditions required for cleavage of the THP ether group also caused decomposition of the cyclopentadienyl ring. Therefore, trimethylsilyl benzenesulfonate 14 was used for the alkylation of 7, affording detergent 10 as a dark yellow oil in 35% yield.

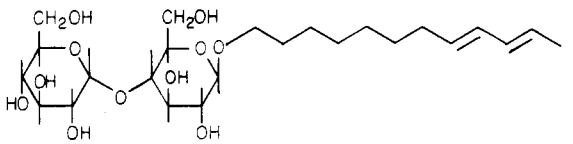
SDS analogue 16 was obtained as a colorless waxy solid in 47% yield by the reaction of 8(*E*),10(*E*)-dodecadienol (15)[13] with 1 equiv of freshly prepared SO_3–pyridine com-



15, R = H
16, R = $SO_3^-Na^+$



17



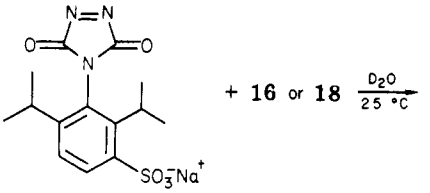
18

plex[14] followed by treatment with aqueous NaOH. Alcohol 15 was also used to prepare the diene analogue 18 of dodecyl maltoside (6).[9] Thus acetobromomaltose (17)[15] was coupled to 15 in the presence of freshly prepared Ag_2CO_3, and the resulting syrup was deprotected with 0.01 N H_2SO_4[16] followed by Et_3N in methanol–water, affording detergent 18 as a colorless powder in 34% yield.

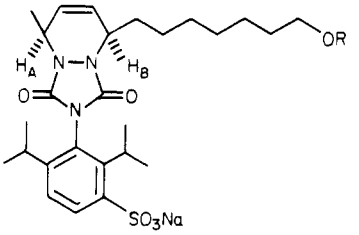
Detergents 9 and 10 were best stored in frozen benzene solution and showed little evidence of dimerization under these conditions after several months.[17] Detergents 16 and 18 could be stored in pure form in the freezer for several months without change. All of these new diene detergents showed evidence (by NMR) of decomposition when stored for days or weeks on the benchtop, however.

B. Chemical Modification of the Diene Detergents by a Diels–Alder Reaction. In the accompanying paper[8] we detailed the synthesis of several new highly hydrophilic triazolinediones (TADs) and demonstrated that the 2,6-diisopropyl-substituted TADs persisted in aqueous solution over several minutes at 25 °C. As TADs are among the most powerful dienophiles known for the Diels–Alder reaction,[18] their reaction in aqueous solution with the new diene detergents was investigated.

Addition of 1 equiv of *deep purple* 19 in small portions to a rapidly stirred D_2O solution of SDS analogue 16 at 25 °C caused an immediate discharge of the color upon dissolution. The solution assumed the purple color of 19 if slightly more than 1 equiv was added. In the absence of 16 the purple color of 19 faded over about 1 min (gas evolution). The ^1H NMR (100 MHz) of the 1:1 reaction mixture indicated that Diels–Alder adduct 20 had formed



19



20, R = SO_3Na
21, R = β-maltose

cleanly. The most striking feature of the spectrum was the replacement of the characteristic four-vinyl-proton multiplet of 16 at δ 5.10–6.02 by a slightly broadened two-proton singlet at δ 5.92 corresponding to the vinyl protons of adduct 20. Also, the terminal methyl doublet of 16 was shifted to δ 1.44 in adduct 20. All other signals were as expected, with H_A and H_B hidden under the HOD peak. When D_2O was replaced by CD_3OD as solvent, H_A and H_B were evident as a three-proton multiplet, which included one of the methine protons of the isopropyl group (vertified by double-resonance experiments), all centered about δ 4.50. Adduct 20 was isolated as a colorless powder that gave a satisfactory microanalysis after precipitation from ether–MeOH. The reaction between TAD 19 and dodecadienyl maltoside 18 in D_2O proceeded in a like

(10) Knox, G. R.; Pauson, P. L. *J. Chem. Soc.* **1961**, 4610.
(11) Roman, R. B.; Keana, J. F. W. *Chem. Phys. Lipids* **1982**, *31*, 161.
(12) Krespan, C. G. *J. Org. Chem.* **1974**, *39*, 2351.
(13) Commercially available from Fluka. Alcohol 15 can be prepared in quantity from sorbic acid and 6-bromohexanol. See: Samarin, D.; Descoins, C. *Synthesis* **1978**, 388.
(14) Gilbert, E. E. *Chem. Rev.* **1962**, *62*, 549. SO_3–pyridine complex prepared by the method of Baumgartner (Baumgartner, P. *Chem. Ber.* *1926*, *59*, 1166) did not perform satisfactorily.
(15) Commercially available acetobromomaltose was unsatisfacotry. Thus, this substance was freshly prepared from maltose monohydrate by the procedure of Rosevear et al.[9]
(16) Omission of this step led to contamination of 18 with an ortho ester side product.[9]
(17) Cyclopentadienes substituted with bulky groups tend to be quite stable toward dimerization (Reimschneider, R.; Nehring, R. *Monatsh. Chem.* **1959**, *90*, 568) while still retaining their extraordinary reactivity toward other dienophiles (Holmes, H. L. *Org. React.* **1946**, *4*, 60).
(18) Burrage, M. E.; Cookson, R. C.; Gupte, S. S.; Stevens, I. D. R. *J. Chem. Soc., Perkin Trans. 2* 1975, 1325.

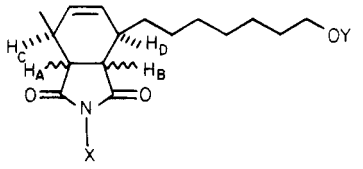
Table I. Effect of 19 and 26 on the Activity of Phospholipase A_2 in the Presence or Absence of Detergents at pH 7 and 25 °C

expt	reagent	molar ratio reagent/protein	detergent	molar ratio detergent/protein	activity remaining,[a] %
1	19	1:1	none		91
2	19	10:1	none		11
3	19	100:1	none		0
4	19	10:1	$C_{16}PN$[c]	1000:1	50
5	19	10:1	5	10:1	13
6	19	10:1	16	10:1	87
7	NEM[b]	10:1	none		95
8	26	10:1	none		81

[a] Estimated error is 10%. [b] *N*-Ethylmaleimide. [c] Hexadecylphosphocholine.

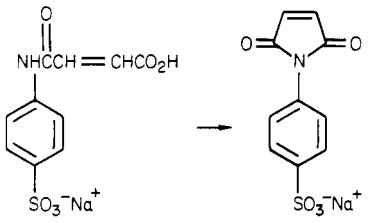
manner, affording adduct **21**.[19]

Less potent dienophiles also reacted with the diene detergents in dilute aqueous solution at 25 °C. Maleic anhydride, for example, and **16** gave the corresponding Diels–Alder adduct (by NMR); however, the hydrolysis of the anhydride to (inert) maleic acid competed effectively with the cycloaddition reaction. The addition of excess maleimide to **16** gave adduct **22** quantitatively (by NMR) in less than 1 h.[20]



22, X = H; Y = $SO_3^-Na^+$
23, X = *p*-$C_6H_4SO_3^-Na^+$; Y = $SO_3^-Na^+$
24, X = *p*-$C_6H_4SO_3^-Na^+$; Y = β-maltose

This latter result portended the use of a more hydrophilic maleimide analogue. Thus, conditions were devised ($NaOAc–Ac_2O$, 25 °C, 5 days) for the cyclization of the known maleamic acid **25**[21] to sulfophenyl maleimide **26**.[22]



25 **26**

A 30-min reaction between **26** and SDS analogue **16** (1:1) in D_2O[23] at 25 °C gave adduct **23** in quantitative yield. The structure of **23** was confirmed both by elemental analysis and by 360 MHz-NMR spectroscopy.[24] The reaction (1:1) between **26** and dodecadienyl maltoside **18** in D_2O at 25° C proceeded somewhat more slowly, requiring about 2 h for the quantative formation of adduct **24** (by NMR). The reaction of dienophile **26** with 1 equiv of Triton X-100 analogue **10** under similar conditions was complete in less than 20 min, as shown by the loss of the characteristic vinyl proton singlet of **26** at δ 6.93. The upfield aromatic proton doublet appeared at δ 7.18 in the adduct(s) (not shown), consistent with that observed in adduct **23**.[24] The remainder of the NMR spectrum was more complex than those described above because of the isomeric mixture constituting **10**. Those absorptions were generally consistent with those observed in $CDCl_3$ for the adduct prepared by treating cyclopentadiene with an excess of maleimide.

The similarity in structure between the new diene-containing detergents and their well-known saturated counterparts virtually assures satisfactory behavior as detergents. This was demonstrated operationally in the case of dodecadienyl maltoside **18** by shaking a 1-mg sample in a mixture of 0.5 mL of water and 0.5 mL of $CHCl_3$. An emulsion was produced that remained visually unchanged for at least 24 h. By contrast, a similar experiment with adduct **21** resulted in a slight emulsion that broke up after a few minutes. Adduct **24** gave a clean two-phase system. The $CHCl_3$ emulsion with **18** was rapidly broken up by the addition of a 3–4-fold excess of TAD **19** to the mixture. Since an excess of **19** was needed, it was likely that the lipophilic diene-containing end of the detergent was protected somewhat from reaction with the hydrophilic dienophile by the surrounding hydrophobic $CHCl_3$ molecules. It is interesting that the less potent hydrophilic dienophile **26** failed to destroy the emulsion over a 24-h period at 25 °C. Thus, for some applications, the added reactivity of TAD **19** would appear to be essential.

C. The Effect of Dienophiles 19 and 26 on the Enzymatic Activity of Phospholipase A_2 and α-Chymotrypsin in the Presence and Absence of Diene Detergents 10, 16, and 18. One envisaged application of the new 1,3-diene-containing detergents was in the isolation and reconstitution of membrane-bound proteins (see above).[2] The in situ chemical modification of the detergent

(19) TAD **19** also reacted rapidly with Triton X-100 analogue **10** in D_2O. While the NMR spectrum of the 1:1 reaction mixture showed new absorptions in the vinyl region and the four-proton multiplet at δ 2.6 present in **10** had disappeared, the overall rather nondescript spectrum was not very helpful in ascertaining the structure of the adduct. Moreover, the reaction mixture darkened with time and the residue upon removal of the solvent became black upon standing.

(20) The rate of reaction of **16** with **19** in D_2O is qualitatively much greater than that of a Diels–Alder reaction between two similar substrates, diene alcohol **15** and *N*-ethylmaleimide (both chosen for solubility reasons) in C_6D_6. Thus, after 20 min at 25 °C there was no observable reaction by NMR. The adduct became observable after 2 h at 35 °C; however, only after 22 h was the reaction nearly complete. These observations are consistent with the rate enhancements observed by Rideout and Breslow[6] for certain Diels–Alder reactions in water.

(21) Merz, H.; Pfleiderer, G.; Wieland, T. *Biochem. Z.* **1965**, *342*, 66.

(22) Use of a shorter reaction time and higher temperature led to the formation of difficultly removable side products.

(23) Maleimide **26** underwent slow hydrolysis back to **25** upon standing in D_2O. Thus, after 8 h at 25 °C, small amounts of **25** could be observed by NMR. After 6 days about 30% of the original **26** remained, producing a mixture of **25**, sulfanilic acid, and maleic acid.

(24) H_A and H_B appeared as one-proton apparent triplets at δ 3.12 and 3.23, respectively, while H_C and H_D appeared as one-proton multiplets at δ 2.35 and 2.17, respectively. These assignments were confirmed by double-resonance experiments. Thus, irradiation at δ 2.35 (H_C) caused the triplet at δ 3.12 (H_A) to collapse to a doublet (J = 8 Hz) and caused a shoulder at δ 1.23 to disappear. The shoulder was due to the mostly buried methyl doublet, which then collapsed to a singlet no longer distinguishable from the other aliphatic proton absorptions. Irradiation at δ 2.17 (H_D) caused the triplet at δ 3.23 (H_B) to collapse to a doublet as the only obvious change. Also, the upfield aromatic doublet of **26** at δ 7.34 was shifted to δ 7.17 in adduct **23**. This shift was a useful diagnostic for Diels–Alder adduct formation with **26**.

Table II. Effect of 19 and 26 on the Activity of α-Chymotrypsin in the Presence or Absence of Detergents at pH 7 and 25 °C

expt	reagent	molar ratio reagent/protein	detergent	molar ratio detergent/protein	activity remaining,[a] %
1	19[b]	10:1	none		40
2	19	10:1	5[c]	10:1	73
3	19	10:1	16	10:1	96
4	19	10:1	6	10:1	32
5	19	10:1	18	10:1	96
6	19	10:1	4	10:1	42
7	19	10:1	10	10:1	81
8	NEM[d]	10:1	none		95
9	26	10:1	none		95

[a] Estimated error is 10%. [b] Addition of 19 either in the absence or in the presence of detergent resulted in some precipitation of chymotrypsin. However, in all cases, the precipitated protein redissolved upon dilution in the assay mixture. Less precipitate was formed in the presence of diene-containing detergent. [c] Among the detergents, only 5 caused some precipitation of chymotrypsin, but 90% of the enzyme activity was recovered upon dilution in the assay medium. [d] *N*-Ethylmaleimide.

molecules via the Diels–Alder reaction into derivatives of high monomeric aqueous solubility ideally should take place without appreciable attack of the hydrophilic dienophiles on the proteins themselves. In order to probe this point, the enzymatic activity of two representative water soluble enzymes, phospholipase A_2 and α-chymotrypsin, was monitored during exposure to various molar ratios of **19** and **26** both in the presence and absence of the diene detergents.[25]

Results with phospholipase A_2 are summarized in Table I. Experiment 6 shows that most of the enzymatic activity is retained upon reaction of the enzyme (7.1 μM) with 10 equiv of TAD **19** in the presence of 10 equiv of diene detergent **16**. Activity is largely lost, however, when saturated detergent analogue **5** is used in place of **16** (expt 5). This demonstrates that **19** indeed reacts preferentially with the diene unit in **16** rather than with the protein. Interestingly, reaction of **19** with the protein could also be partially prevented by an entirely different mechanism, namely, that induced by the presence of the micellar substrate analogue hexadecylphosphocholine ($C_{16}PN$), which is known to form a lipid–protein complex with phospholipase A_2 (expt 4).[26,27] It is likely that in the enzyme–micelle complex, some of the susceptible groups on the surface of the enzyme are no longer accessible to the hydrophilic TAD **19**.

In the *absence* of any detergent, TAD **19** is an effective protein modifier as shown by experiments 1–3. For example, addition of 10 equiv of **19** (final [**19**] is only 71 μM) led to irreversible loss of close to 90% of the enzymatic activity.[28] By contrast, a similar experiment with either *N*-ethylmaleimide (expt 7) or hydrophilic maleimide **26** (expt 8) had only a marginal effect on enzyme activity.[29]

An amino acid analysis profile was obtained for both native and TAD **19** treated phospholipase A_2 (data not shown). Only the tyrosine value was significantly reduced (from 8 residues observed per molecule of native enzyme vs 5.2 residues for the modified enzyme) in the treated enzyme. Quite possibly, inactivation of the enzyme was caused by nucleophilic attack of a side-chain residue on one of the carbonyl groups of the TAD,[30,31] giving rise to a linkage that, except in the case of tyrosine, is cleaved back to the amino acid under the vigorous acidic conditions of the amino acid analysis.

Results with α-chymotrypsin were similar and are collected in Table II. Relatively little enzymatic activity was lost when the enzyme was exposed to TAD **19** in the presence of either diene detergents **16** (expt 3), **18** (expt 5), or **10** (expt 7). Omission of the detergent (expt 1) or substitution of the saturated analogues **6** (expt 4) or **4** (expt 6), however, led to significant inactivation of the enzyme, demonstrating once again that **19** reacts selectively with a 1,3-diene unit rather than with protein.[31,32]

Experimental Section[33]

***tert*-Butyl[(9-tetrahydropyranyl)-3,6,9-trioxanonyl]-cyclopentadiene (8).** To a stirred ice bath-cooled solution of 1.85 g (17.4 mmol) of dimethylfulvene in 10 mL of dry ether was added 27 mL of a 1.3 M MeLi in ether solution. The ice bath was removed, and the mixture was stirred for 1 h and then centrifuged. The solid was washed three times with ether. The resulting light yellow solid was dissolved in 12 mL of dry THF to give a dark orange solution. This was added to a stirred solution of 3.3 g (13 mmol) of 8-chloro-3,6-dioxaoctyl tetrahydropyranyl ether (**11**) dissolved in 5 mL of dry THF over a 2-min period. The mixture was stirred overnight and then added to 20 mL of ice water and extracted with ether. The extract was washed with saturated NaCl solution and dried ($MgSO_4$). Evaporation of the solvent gave 4.1 g of a dark orange oil. This was placed on a 40 × 2.5 cm silica gel column and eluted with 1:3 ether–hexane to yield 1.8 g (35%) of **8** as a light yellow oil: ^1H NMR (benzene-d_6) δ 0.90–1.97 (m, 15 H), 2.57–3.04 (m, 4 H), 3.27–4.09 (m, 12 H), 4.67 (t, J = 3, 1 H), 5.83–6.37 (m, 2 H). The analytical sample

(25) Changes in enzymatic activity are assumed to be indicative of the overall extent of reaction with the protein.

(26) Soares de Araujo, P.; Rosseneu, M. Y.; Kremer, J. M. H.; van Zoelen, E. J. J.; de Haas, G. H. *Biochemistry* **1979**, *18*, 580.

(27) Donné-op den Kelder, G. M.; Hille, J. D. R.; Dijkman, R.; de Haas, G. H.; Egmond, M. R. *Biochemistry* **1981**, *20*, 4074.

(28) Activity was not restored, for example, upon incubation of the treated protein at pH 9 in the presence of 1 N NH_2OH.

(29) That little inactivation of either enzyme occurred by treatment with either NEM or hydrophilic maleimide **26** was not surprising since maleimides are primarily sulfhydryl reagents (Vallee, B. L.; Riordan, J. F. *Annu. Rev. Biochem.* **1969**, *38*, 733) and neither enzyme contains cysteine residues.

(30) Wamhoff, H.; Wald, K. *Chem. Ber.* **1977**, *110*, 1699.

(31) For both enzymes essentially no loss of activity was observed upon addition of a solution containing the aqueous decomposition product(s)[30] of **19**, indicating that **19** itself modifies the enzymes. Also, enzyme inactivation was pH independent over the range 4.5–10. In view of the uniqueness of TAD reagent **19** in comparison to other protein modification reagents and its good reactivity, the exact nature of the protein modification by **19** would appear to merit further investigation.

(32) Apparently, α-chymotrypsin was somewhat more resistant toward inactivation by **19** than was phospholipase-A_2 (cf. expt 1, Table II, and expt 2, Table I). This may be partially ascribed to the precipitation observed when α-chymotrypsin was treated with **19**. Precipitation was also observed when SDS **5** was added, which explains the partial protection observed in the presence of this saturated detergent (expt 2).

(33) Melting points were obtained in a Thomas-Hoover apparatus and are uncorrected. NMR spectra were recorded either on a Varian XL-100 or on a Nicolet 360 MHz spectrometer in $CDCl_3$ unless otherwise indicated. Chemical shifts are expressed in δ with Me_4Si as an internal standard. J values are in hertz. Elemental analyses were determined at the University of Oregon by Dr. R. Wielesek. All reactions were routinely run under a N_2 atmosphere. Solvents were routinely distilled.

was prepared by preparative TLC on silica gel (1:4 ether–hexane); R_f 0.11–0.29). Anal.[34] Calcd for $C_{20}H_{34}O_4 \cdot 0.1H_2O$: C, 70.59; H, 10.13. Found: C, 70.50; H, 10.00.

***tert*-Butyl(8-hydroxy-3,6-dioxaoctyl)cyclopentadiene (9).** A solution of 274 mg (0.81 mmol) of **8**, 20 mL of MeOH, 10 drops of water, and 12 mg of TsOH was stirred at 25 °C for 4.5 h. The solution was concentrated to 5 mL and then taken up in 30 mL of ether. This was washed with ice water, dried ($MgSO_4$), and concentrated to dryness. Preparative TLC over silica gel (1:2 hexane–ether, R_f 0.23–0.43) gave 73 mg (35%) of **9** as a dark yellow oil: ^{1}H NMR (benzene-d_6) δ 0.89–1.30 (m, 9 H), 2.50–2.97 (m, 4 H), 3.10 (br s, 1 H), 3.30–3.74 (m, 10 H), 5.78–6.60 (m, 2 H). Anal.[34] Calcd for $C_{15}H_{26}O_3 \cdot 0.1H_2O$: C, 70.32; H, 10.31. Found: C, 70.17; H, 10.13.

28-(Phenylsulfonyl)-1-(trimethylsilyl)-1,4,7,10,13,16,19,22,25,28-decaoxaoctacosane (14). To a solution of 240 mg (0.58 mmol) of nonaethylene glycol (**12**)[12] in 3 mL of CH_2Cl_2 containing 0.5 mL of dry pyridine was added benzenesulfonyl chloride 102 mg (0.58 mmol) dropwise at 0 °C with stirring. The solution was stirred overnight and then treated with 0.5 mL of water, and the solvent was removed. The residue was taken up in 2 mL of water and extracted twice with 3-mL portions of ether. The ether extract was washed with 1 mL of a saturated NaCl solution, dried ($MgSO_4$), and concentrated to dryness. The residue was dried at 25 °C (0.005 mm), affording 88 mg (22%) of the bis(benzenesulfonated) product as a viscous, colorless oil: ^{1}H NMR (acetone-d_6) δ 3.46–3.76 (m, 32 H), 4.21 (t, J = 4, 4 H), 7.64–8.04 m, 10 H). The ether-extracted aqueous portion was next extracted twice with 3-mL portions of benzene. The extract was washed with 1 mL of a saturated NaCl solution, dried ($MgSO_4$), and concentrated to dryness. The residue was dried at 25 °C (0.005 mm), affording 95 mg (29%) of the monobenzenesulfonated product as a viscous, colorless oil: ^{1}H NMR (acetone-d_6) δ 3.46–3.76 (m, 34 H), 4.21 (t, J = 4, 2 H), 7.64–8.04 (m, 5 H). Anal. Calcd for $C_{24}H_{42}O_{12}S$: S, 5.78. Found: S, 6.22. To a stirred solution of 84 mg (0.15 mmol) of this latter compound, 77 mg of triethylamine, and 1 mL of dry CH_2Cl_2 at 0 °C was added dropwise 82 mg (0.76 mmol) of trimethylsilyl chloride. The solution stirred for 2 h at 25 °C, and then the solvent was removed. The residue was treated with dry ether and then filtered through a fine frit. The filter cake was washed with ether and, the combined filtrate was concentrated to dryness. The residue was dried at 25 °C (0.005 mm), affording 93 mg (98%) of **14** as a colorless viscous liquid: ^{1}H NMR (benzene-d_6) δ 0.14 (s, 9 H), 3.22–3.78 (m, 34 H), 4.00 (t, J = 5, 2 H), 7.00–7.15 (m, 3 H), 7.78–7.92 (m, 2 H). This was used immediately for the next experiment.

***tert*-Butyl(26-hydroxy-3,6,9,12,15,18,21,24-octaoxahexacosyl)cyclopentadiene (10).** To an ice-bath-cooled solution of 1.0 g (9.4 mmol) of dimethylfulvene in 1 mL of dry ether was added 15 mL of a 1.3 M MeLi in ether solution. The ice bath was removed, and the mixture was stirred for 1 h and then centrifuged. The solid was washed three times with ether. The washed solid was dissolved in 10 mL of THF and cooled in an ice bath. A solution of 0.749 g (1.19 mmol) of **14** in 10 mL of dry THF was added with stirring. The ice bath was removed, and the mixture was stirred for 16 h. After 3 mL of water was added, stirring was continued for 1 h, and then the mixture was added to 20 mL of a saturated NH_4Cl solution. The resulting two layers were separated, and the aqueous layer was extracted with 20 mL of ether. The combined organic phase was washed with a saturated NaCl solution, dried ($MgSO_4$), and concentrated to dryness, affording 1.0 g of an orange oil. This was purified by silica gel preparative TLC (1:1 THF-hexane, R_f 0.13–0.30), affording 220 mg (35%) of **10** as a dark yellow oil: ^{1}H NMR (benzene-d_6) δ 1.08–1.28 (m, 9 H), 2.50–3.00 (m, 5 H), 3.28–3.72 (m, 34 H), 5.78–6.34 (m, 2 H). Anal.[34] Calcd for $C_{27}H_{50}O_9 \cdot 0.1H_2O$: C, 62.30; H, 9.72. Found: C, 62.12; H, 9.33.

Sodium 8(*E*),10(*E*)-Dodecadienyl Sulfate (16). To a solution of 113 mg (0.62 mmol) of 8(*E*),10(*E*)-dodecadienol (**15**)[13] in 1 mL of dry pyridine was added 99 mg (0.62 mmol) of freshly prepared sulfur trioxide–pyridine complex.[14] The mixture was stirred for 10 min, resulting in a colorless homogeneous solution. The solution was added dropwise with stirring to 6.2 mL of 0.1 N aqueous NaOH, and then the solvent was removed, leaving a colorless syrup. This was dissolved in 15 mL of absolute EtOH and evaporated to dryness. The residue was dissolved in 20 mL of boiling isopropyl alcohol and filtered, and the filtrate was placed in a freezer to give a gelatinous solid. This was collected on a coarse frit and squeezed free of most of the solvent. The solid was peeled off the frit and reprecipitated a second time from isopropyl alcohol. The resulting solid was dried at 25 °C (0.005 mm) to yield 85 mg (47%) of **16** as a waxy colorless solid: decomposes without melting at 155 °C (preheated oil bath); ^{1}H NMR (D_2O) δ 1.10–2.12 (m, 15 H), 3.94 (t, J = 7, 2 H), 5.16–6.06 (m, 4 H). Anal.[34] Calcd for $C_{12}H_{21}NaO_4S \cdot 0.2H_2O$: C, 50.05; H, 7.49. Found: C, 50.06; H, 7.65.

8(*E*),10(*E*)-Dodecadienyl β-D-Maltopyranoside (18). To a solution of 1.55 g (2.22 mmol) of freshly prepared acetobromomaltose[9,15] in 25 mL of dry CH_2Cl_2 were added (in the order listed) 0.444 g (2.44 mmol) of 8(*E*),10(*E*)-dodecadienol (**15**),[13] 2 g of 4-Å molecular sieves, 0.04 g of iodine, and 0.692 g (2.51 mol) of freshly prepared silver carbonate. The mixture was stirred at 25 °C in the dark for 2 h. The resulting bright yellow reaction mixture was filtered through a fine frit, and the filter cake was washed with CH_2Cl_2. The solvent was removed, and the resulting light yellow syrup was dissolved in 50 mL of a solution of H_2SO_4 (0.01 N) in 10% aqueous acetone. After 30 min at 25 °C, the solution was neutralized with pyridine and stripped of solvent, and the resulting yellow syrup was dissolved in 20 mL of MeOH. A mixture of 5 mL of water and 10 mL of triethylamine was added to this solution, and after 24 h of stirring at 25 °C, the solvent was removed, giving a brown syrup. This was dissolved in the minimum amount of MeOH and placed on a 32 × 2.5 cm column of Dowex-1 strongly basic ion-exchange resin (hydroxide form; 2% cross-linking; 200–400 mesh) that had been equilibrated with MeOH. Elution with MeOH gave the unreacted alcohol as the initial band followed closely by the desired maltopyranoside. After MeOH removal and lyopholization, 387 mg (34%) of **18** was obtained as a colorless powdery solid: TLC on silica gel (4:2 EtOAc–MeOH) R_f 0.60; ^{1}H NMR (CD_3OD) δ 1.21–2.20 (m, 15 H), 3.10–4.04 (m), 4.26 (d, J = 8, 1 *H*), 5.16 (d, J = 4, 1 H), 5.26–6.10 (m, 4 H). An analytical sample was obtained by recrystallization from acetone: mp 108–112 °C (liquid crystal), 152–160 °C (clear melt). Anal.[34] Calcd for $C_{24}H_{42}O_{11} \cdot 0.2H_2O$: C, 56.50; H, 8.38. Found: C, 56.49; H, 8.23.

2-(3-Sodium sulfo-2,6-diisopropylphenyl)-5,8-dihydro-5β-(7-sodium sulfatoheptyl)-8β-methyl-1*H*-[1,2,4]triazolo-[1,2-*a*]pyridazine-1,3(2*H*)-dione (20). To a stirred solution of 20 mg (0.07 mmol) of **16** in 1 mL of water at 25 °C was added in small portions 25.4 mg (0.07 mmol) of solid **19**.[8] The purple color of the dienophile discharged immediately upon going into solution. After addition, the colorless solution was filtered and the solvent was removed, giving 45 mg (99%) of **20** as a colorless solid: mp. 171–173 °C dec (preheated oil bath); ^{1}H NMR (CD_3OD) δ 1.10–2.30 (m, 27 H), 2.38–2.74 (m, 1 H), 4.02 (t, J = 6, 2 H), 4.30–4.66 (m, 3 H), 6.00 (br s, 2 H), 7.40 (d, J = 8, 1 H), 8.12 (d, J = 8, 1 H). An analytical sample was prepared by the dropwise addition of a concentrated MeOH solution into ether, cooling the resulting mixture in a freezer, and drying the resulting colorless powder at 56 °C (0.005 mm). Anal.[34] Calcd for $C_{26}H_{37}N_3Na_2O_9S_2 \cdot 1.5H_2O$: C, 46.42; H, 5.99; N, 6.25. Found: C, 46.53; H, 6.34; N, 6.46.

2-(3-Sodium sulfo-2,6-diisopropylphenyl)-5,8-dihydro-5β-[7-(β-D-maltopyranosyloxy)heptyl]-8β-methyl-1*H*-[1,2,4]triazolo[1,2-*a*]pyridazine-1,3(2*H*)-dione (21). An 11.9-mg (31.3 μmol) quantity of **19**[8] was added as a solid in small portions to a rapidly stirred solution of an equimolar amount of the diene detergent **18** in 0.3 mL of D_2O. The purple color of TAD **19** discharged immediately upon going into solution. The NMR spectrum, taken 20 min after the end of addition, showed that the reaction had proceeded to completion: ^{1}H NMR (D_2O) δ 1.00–2.62 (m, 28 H), 3.05–3.90 (m, 14 H), 4.34 (d, J = 8, 1 H), 5.31 (d, J = 4, 1 H), 5.86 (br s, 2 H), 7.44 (d, J = 8, 1 H), 8.11 (d, J = 8, 1 H). The water was removed under vacuum, and the residue was dried at 56 °C (0.005 mm), giving **21** as a colorless powder: mp 110 °C (phb); ^{1}H NMR (360 MHz, CD_3OD) δ 1.17–2.25 (m, 24 H), 1.56 (d, 3 H), 2.60 (m, 1 H, *H*C(CH_3)$_2$), 3.10–4.04 (m), 4.31 (d, J = 8, 1 H, anomeric proton), 4.55 (m, 3 H, H_A, H_B, and *H*C-

(34) Microanalyses typically indicated the retention of fractional amounts of water by these highly polar oils or waxy solids despite drying under vacuum.

(CH_3)$_2$), 5.20 (d, J = 4, 1 H), 6.00 (m, 2 H), 7.42 (d, J = 8, 1 H), 8.22 (d, J = 8, 11 H). Irradiation at δ 4.55 caused a narrowing of the vinyl proton signal at δ 6.00, the methyl doublet at δ 1.56 to collapse to a singlet, and a three-line pattern at δ 1.31 to become a two-line pattern. Irradiation at δ 2.60 caused a predominantly three-line pattern at δ 1.23 to collapse to a two-line multiplet as the only observed change. Anal.[34] Calcd for $C_{38}H_{58}N_3O_{16}SNa\cdot 3H_2O$: C, 49.48; H, 7.00. Found: C, 49.37; H, 6.79.

1-(4-Sodium sulfophenyl)maleimide (26). A mixture of 0.30 g (1.11 mmol) of **25**,[21] 0.3 g of anhydrous NaOAc, and 15 mL of Ac_2O was stirred at 25 °C for 5 days. The mixture was filtered, and the collected solid was washed with acetone and then dried at 56 °C (0.005 mm). The solid was taken up in 5 mL of water, and the solution was brought to pH 2 with dilute HCl. The solvent was removed, and the resulting solid was dissolved in 25 mL of hot HOAc. The solution was filtered, the solvent was removed, and the residue was dried at 25° C (0.005 mm). The resulting yellow solid was recrystallized from 95% EtOH and dried at 56 °C (0.005 mm) to yield 132 mg (47%) of **26** as light yellow star-shaped needles: mp >300 °C (preheated oil bath); ^{1}H NMR (D_2O) δ 6.93 (s, 2 H), 7.38 (d, J = 9, 2 H), 7.84 (d, J = 9, 2 H). Anal. Calcd for $C_{10}H_6NNaO_5S$: C, 43.64; H, 2.20; N, 5.10. Found: C, 43.59; H, 2.21; N, 5.04.

3a,4,7,7a-Tetrahydro-2-(4-sodium sulfophenyl)-4β-(7-sodium sulfatoheptyl)-7β-methyl-1*H*-isoindole-1,3(2*H*)-dione (23). A solution of 19 mg (0.067 mmol) of **16** in 0.3 mL of D_2O was added to 17 mg (0.067 mmol) of **26** at 25 °C. After 35 min, the NMR spectrum[24] showed the Diels–Alder adduct to be formed quantitatively: ^{1}H NMR (360 MHz, D_2O) δ 1.02–1.84 (m, 15 H), 2.17 (m, 1 H), 2.35 (m, 1 H), 3.12 (apparent t, 1 H), 3.23 (apparent t, 1 H), 3.90 (t, J = 6, 2 H), 5.64 (br s, 2 H), 7.17 (d, J = 8, 2 H), 7.80 (d, J = 8, 2 H). An analytical sample was prepared by removing the solvent and recrystallizing the residue from absolute EtOH–EtOAc. The resulting crystalline solid was dissolved in water, evaporated to dryness, dissolved in absolute EtOH, evaporated to dryness and finally dried at 56° C (0.005 mm) to yield **23** as a colorless solid that analyzed as the disodium salt: mp 225 °C dec (preheated oil bath). Anal.[34] Calcd for $C_{22}H_{27}NNa_2O_9S_2\cdot 2H_2O$: C, 44.37; H, 5.25; N, 2.35. Found: C, 44.32; H, 4.87; N, 2.26.

3a,4,7,7a-Tetrahydro-2-(4-sodium sulfophenyl)-4β-[7-β-D-maltopyranosyloxy)heptyl]-7β-methyl-1*H*-isoindole-1,3-(2*H*)-dione (24). A 17.2 mg (62.6 μmol) quantity of **26** was added as a solid all at once to an equimolar solution of diene detergent **18** in 0.3 mL of D_2O. The NMR spectrum showed that the reaction was complete in 2.5 h: ^{1}H NMR (360 MHz, D_2O) δ 0.98–1.90 (m, 15 H), 2.04 (m, 1 H), 2.22 (m, 1 H), 3.00–3.92 (m, 16 H), 4.27 (d, J = 8, 1 H), 5.26 (d, J = 4, 1 H), 5.65 (br s, 2 H), 7.18 (d, J = 8, 2 H), 7.83 (d, J = 8, 2 H). The solvent was removed, and the residue was taken up in 10 mL absolute EtOH and evaporated. This process was repeated twice, and then the residue was dried at 25 °C (0.005 mm). The resulting solid was recrystallized from absolute EtOH to give a colorless powder: ^{1}H NMR (CD_3OD) δ 1.24–2.10 (m, 15 H), 2.24–2.78 (m, 2 H), 3.04–4.10 (m), 4.28 (d, J = 8, 1 H), 5.23 (d, J = 4, 1 H), 5.86 (br s, 2 H), 7.28 (d, J = 8, 2 H), 7.96 (d, J = 8 , 2 H).

Methods Employed in the Enzyme Studies. A. Enzyme Assays. Phospholipase A_2 (from porcine pancreas)[35] activity was determined by using the egg yolk lipoprotein assay as described by Nieuwenhuizen et al.[36] Chymotrypsin (from bovine pancreas, Sigma) activity was determined by measuring the steady-state rate of hydrolysis of p-nitrophenyl acetate[37] (PNPA). The final assay mixture contained 0.1 M Tris/HCl, pH 8, 2 mM PNPA and 5% isopropyl alcohol.

B. Treatment of Enzymes with 19, 26, and *N*-Ethylmaleimide. Enzymes were treated with **19**, **26**, and *N*-ethylmaleimide in the presence or absence of various detergents in 0.05 or 0.1 M Tris/HCl pH 7 buffer at 25 °C. To determine pH dependence, other buffers used were 0.1 M NaOAc/HOAc pH 4.5 and 0.1 M Tris/NaOH pH 10. Protein concentrations were 0.1 mg/mL (7.1 μM) and 5 mg/mL (0.231 mM) for phospholipase A_2 and chymotrypsin, respectively. To avoid autocatalytic degradation of chymotrypsin, a 10 mg/mL stock solution of this enzyme was prepared in distilled water, acidified with 1 drop of glacial HOAc and kept at 0 °C. An aliquot of this solution was diluted with an equal volume of the appropriate buffer and allowed to warm to 25 °C immediately before addition of detergent and/or modifying reagent. Stock solutions of **19** and *N*-ethylmaleimide were freshly prepared in reagent-grade acetone while those of **26** and the various detergents were freshly prepared in water. Concentrations were chosen so that a 10-fold dilution in the reaction mixture gave the desired molar excess over protein. The final concentration of acetone never exceeded 10% (by volume). Enzyme activities were measured within 4–5 min after the final addition of reagents. Appropriate controls were run to estimate the loss of enzyme activity resulting from addition of detergents and/or acetone. Enzyme activities of the controls were usually within 90% of the value determined for the fresh enzyme stock, except for chymotrypsin at pH 10 where a 20% loss of activity was observed. This is probably due to autocatalytic degradation.

Amino Acid Analysis of Phospholipase A_2 Treated with 19. To study the effect of **19** on the amino acid composition of phospholipase A_2, 0.5 mg of protein was treated with a 100-fold molar excess of **19** at pH 7 as described above except that the protein concentration was 1 mg/mL. The hydrolysis products of the excess **19** were removed by gel filtration on Sephadex G75 fine, elution with a 0.1% NH_4HCO_3 solution followed by lyophylization of the combined protein fractions. Untreated phospholipase A_2 (0.5 mg) was run parallel as a control. The amino acid analysis was performed on a Dionex amino acid analyzer, after hydrolysis of the native and treated protein in 6 N HCl at 110 °C for 22 h in an evacuated sealed tube.[38]

Acknowledgment. This research was supported by PHS Research Grants GM-24951, 27137, and 25698 from the National Istitute of General Medical Sciences. We thank Dr. C. E. Klopfenstein for assistance in obtaining the 360-MHz NMR spectra.

Registry No. **7**, 50356-03-1; **8**, 86217-92-7; **9**, 86217-93-8; **10**, 86217-94-9; **11**, 85539-28-2; **12**, 3386-18-3; **14**, 86217-84-7; **15**, 33956-49-9; **16**, 86217-85-8; **18**, 86217-86-9; **19**, 86217-87-0; **20**, 86217-88-1; **21**, 86217-89-2; **23**, 86217-90-5; **24**, 86217-91-6; **25**, 1886-77-7; **26**, 86238-49-5; phospholipase A_2, 9001-84-7; chymotrypsin, 9004-07-3; *N*-ethylmaleimide, 128-53-0; benzenesulfonyl chloride, 98-09-9; acetobromomaltose, 14257-35-3.

(35) Phospholipase A_2 and n-hexadecylphosphocholine (C_{16}PN) were generously supplied by Prof. G. H. de Haas, Biochemical Laboratory, State University of Utrecht, The Netherlands.

(36) Nieuwenhuizen, W.; Kunze, H.; de Haas, G. H. *Methods Enzymol.* **1974**, *32B*, 147.

(37) Hartley, B. S.; Kilby, B. A. *Biochem. J.* **1952**, *50*, 672.

(38) The amino acid analysis was performed at the University of Oregon by Linda Paulson.