Approaches to Antibody-Catalyzed Cationic Cyclizations: Chemical Studies of Leaving Groups and Cyclization Modes

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(Received 15 August 1995 and in revised form 23 October 1995)

Abstract. The solvolysis of a range of esters of 2-methylbenzyl alcohol **10** has been studied. It was found that sulphonate esters are solvolyzed very rapidly in aqueous solution ($t_{1/2} \approx 35$ s), whereas sulphinates react slowly ($t_{1/2} \approx 35$ h) and a phosphonate diester is stable. The fluorescent 5-dimethylaminonaphthalene-1-sulphinate (dansinate) esters (*e.g.* **22**) held the most promise as substrates for antibody-catalyzed cationic cyclizations. It was shown by ^{18}O-labeling studies that ester **13** is hydrolyzed predominantly (but not exclusively) by S_N1 reaction at the benzylic position.

A number of conditions were tried to effect cationic cyclizations of an unsaturated benzylic alcohol **26**, whose ester is intended as the substrate for the antibody-catalyzed reaction. The products obtained were the result of attack of the non-benzylic alcohol on either the benzylic cation, to give **31** and **32**, or on the protonated alkene, to give **29**. No cyclization of the alkene onto the benzylic cation, to give **4**, was observed, suggesting that this is a disfavored reaction in solution.

INTRODUCTION

One of the ways in which Nature makes carbon–carbon bonds is by cationic cyclization reactions. This class of reactions is catalyzed by terpene cyclase enzymes, which operate on a very limited number of substrates. Nevertheless, they are the key steps in the formation of thousands of terpene natural products, ranging from simple monoterpenes, like camphor, to more complex biologically important compounds, like taxol and the steroids.[1] The feature of these cyclization reactions is the ionization of an allylic pyrophosphate to generate a transient allylic cation, which is then attacked by an electron-rich double bond elsewhere in the isoprenoid precursor. This, in turn, generates a further cationic center, which can be attacked by other double bonds or can trigger rearrangement reactions involving alkyl or hydride migrations. The cascade of reactions terminates when the reactive cationic species is quenched, e.g., by loss of a proton or attack of water.

We are interested in generating antibodies designed to catalyze cationic cyclizations. In principle, such antibodies could be used to assemble novel carbon skeletons. A study of these catalytic antibodies may also provide insight into the actual mode of action of terpene cyclases, about which there is very limited structural information.

In our program to generate catalytic antibodies to catalyze cationic cyclizations, a major constraint was to choose a simple reaction for which we could readily make the substrate, product, and hapten. A previous study in our laboratory,[2] aimed at generating antibodies to catalyze the formation of a tetracyclic steroid-like structure, involved long and complex syntheses of both the hapten and the substrate but, at the end of the day, no catalytic antibodies were obtained. In our current study we have chosen a simpler cyclization reaction (Scheme 1). This reaction involves departure of a leaving group from the substrate **1** to generate a benzylic cation **2**, which is then attacked by the double bond to form a six-membered carbocyclic ring. The resulting secondary carbocation **3** will be rapidly quenched by attack of the non-benzylic alcohol to form a tetrahydrofuran ring **4**. Our hapten **5** has a positively-charged nitrogen atom in the correct position to resemble the intermediate secondary carbocation **3** and also a negatively-charged

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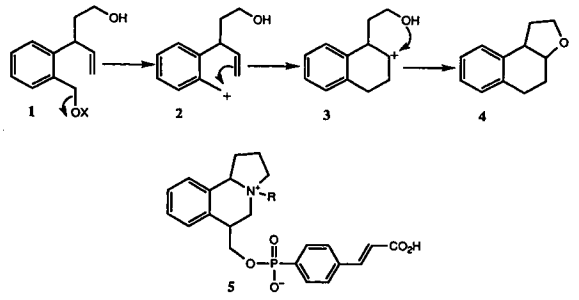


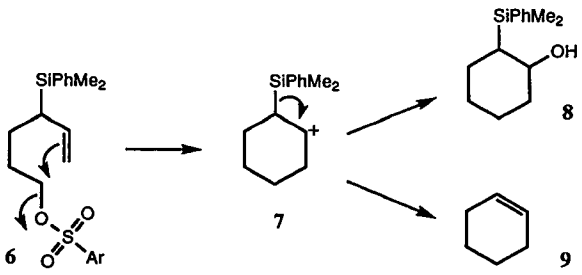
Scheme 1

phosphonate intended to resemble the departing leaving group. The extra carbon atom between the tricyclic skeleton and the phosphonate mimics the lengthened C–OX bond in the transition state of the cyclization reaction.

In this paper we describe in detail two parts of this project. The first part of the paper describes experiments to decide on the leaving group needed to generate the initial cationic species. These studies are of general interest to projects aimed at generating antibodies to catalyze reactions with an S_N1 or E1 component. The second part of the paper describes studies of the acid-catalyzed reactions of the substrate alcohol (**1**, X = H). This study furnished a range of cyclized products displaying the inherent reactivity of the substrate in the absence of conformational binding constraints that should be provided by an antibody catalyst.

Reports of an antibody which catalyzes a somewhat different cationic cyclization have recently appeared.[3] In this reaction the vinyl group of the substrate **6** cyclized onto the developing primary cation generated by departure of the sulphonate leaving group (Scheme 2). The carbon–carbon bond-forming step resulted in the generation of a secondary cation **7**, stabilized by a β-silicon atom. This cation was either trapped by water to give alcohol **8** or quenched by loss of the silyl group to give cyclohexene **9**, depending on the antibody used.

Despite the central importance of carbon–carbon bond-forming reactions in synthetic organic chemistry, there have been few other reports of catalytic antibodies



Scheme 2

which catalyze such reactions. Notable exceptions to this are antibodies which promote Diels–Alder reactions.[4]

EXPERIMENTAL

General Directions

Most general directions are as described in our previous paper.[2] All solvents were distilled and, where indicated, standard procedures were use to dry them. Unless otherwise stated, organic extracts containing the product were combined, dried over sodium sulphate or magnesium sulphate and evaporated to dryness in a rotary evaporator at ca. 15 mmHg before purification. Preparative thin layer chromatography (PLC) was performed on plates (20 × 20 cm) coated with Merck Kieselgel 60 F_{254} (1 mm) and TLC, on commercial plates coated with the same silica (0.25 mm).

*2-Methylbenzyl Toluene-4-Sulphonate **11***

Sodium hydride (65 mg of a 60% dispersion in mineral oil, 1.6 mmol) was washed with dry hexane (1 mL), and a solution of 2-methylbenzyl alcohol **10** (150 mg, 1.23 mmol) in dry diethyl ether (5 mL) was added. The mixture was heated at reflux for 3 h, then stirred at 20 °C for 20 h, then cooled to –35 °C, and a solution of toluene-*p*-sulphonyl chloride (234 mg, 1.23 mmol) in diethyl ether (3 mL) was added dropwise. The mixture was stirred at –20 °C for 2 h, then at 20 °C for 2 h, and then filtered under anhydrous conditions and evaporated. Purification by PLC, eluting with ethyl acetate–petroleum ether (bp 40–60 °C) (3:1), gave the *tosylate* **11** (340 mg, 85%) as an oil; R_f (ethyl acetate–petroleum ether, 1:1) 0.69; ν_{max} ($CHCl_3$) 1375 and 1145 (S=O) cm^{-1}; δ_H (400 MHz, $CDCl_3$) 2.24 and 2.44 (each 3H, s, Me), 5.07 (2H, s, CH_2O), 7.12–7.23 (4H, m) and 7.31 and 7.78 (each 2H, d, *J* 8, Ar).

The tosylate was dissolved in deuteriomethanol at room temperature and its solvolysis was followed by 400 MHz ^1H NMR spectroscopy ($t_{1/2} \approx 18$ min), giving signals for toluene-4-sulphonic acid [δ_H 2.37 (3H, s, Me), 7.24 and 7.69 (each 2H, d, *J* 8, Ar)] and 2-methylbenzyl trideuteriomethyl ether [δ_H 2.32 (3H, s, Me), 4.46 (2H, s, CH_2O), 7.09–7.27 (4H, m, Ar); *m/z* 139 (M^+), 121 ($M^+ - CD_3$), 105 ($M^+ - OCD_3$)].

*2-Methylbenzyl 5-Dimethylaminonaphthalene-1-Sulphonate **15***

The dansylate **15** was made in a similar manner to the tosylate above; δ_H (400 MHz, $CDCl_3$) 2.14 (3H, s, Me), 2.87 (6H, s, NMe_2), 5.05 (2H, s, CH_2O), 7.00–7.17 (5H, m), 7.51 and 7.52 (each 1H, t, *J* 8, 3- and 7-H), 8.24, 8.27, and 8.55 (each 1H, d, *J* 8, 2-, 4- and 8-H).

The dansylate was dissolved in deuteriomethanol at room temperature and its solvolysis was followed by 400 MHz ^1H NMR spectroscopy ($t_{1/2} \approx 7$ min), giving signals for 5-dimethylaminonaphthalene-1-sulphonic acid [δ_H 3.47 (6H, s, NMe_2), 7.70 and 7.73 (each 1H, t, *J* 8, 3- and 7-H), 7.97, 8.19, 8.28, and 9.04 (each 1H, d, *J* 8, 2-, 4-, 6,- and 8-H)] and 2-methylbenzyl trideuteriomethyl ether (as above).

A solution of dansylate **15** in chloroform (2 drops) and methanol (0.5 mL) was rapidly added to 0.1 M sodium phosphate buffer, pH 7.5, containing 0.1 M sodium chloride (3.0 mL)

at room temperature. The absorbance at 345 nm was followed and changed with $t_{1/2} \approx 35$ s.

bis(2-Methylbenzyl) Phenylphosphonate 16

A solution of dry triazole (219 mg, 3.17 mmol) in dry pyridine (2 mL) was stirred with phenylphosphonic dichloride (100 μL, 0.71 mmol) for 15 min and then a solution of 2-methylbenzyl alcohol **10** (172 mg, 1.41 mmol) in pyridine (3 mL) was added dropwise. The mixture was stirred for 3 h, then diluted with water (50 mL) and extracted with dichloromethane (50 mL). Purification by PLC, eluting with light petroleum (bp 60–80 °C)–ethyl acetate (1:1) gave the phosphonate diester **16** (5 mg, 2%); δ_H (400 MHz, $CDCl_3$) 2.28 (6H, s, Me), 5.03 and 5.10 (each 2H, dd, *J* 12, 7, CH_2O), 7.12–7.29 (8H, m), 7.42 (2H, td, *J* 7, 4), 7.51 (1H, td, *J* 7, 1) and 7.78 (2H, ddd, *J* 13, 7, 1, Ar); *m/z* 366 (M^+), 261 ($M^+ - C_8H_9$).

2-Methylbenzyl Trifluoroacetate 12

A solution of 2-methylbenzyl alcohol **10** (302 mg, 2.46 mmol) in trifluoroacetic acid (10 mL) was stirred at 20 °C for 2 h, then at 55 °C for 2 h, then quenched with dilute aqueous sodium carbonate (10 mL) and extracted with dichloromethane (2 × 25 mL). Purification by PLC, eluting with ethyl acetate–petroleum ether (bp 60–80 °C) (1:1; R_f 0.65), gave the *ester* **12** (148 mg, 28%) as an oil [Found: (EI) M^+, 218.0556. $C_{10}H_9F_3O_2$ requires 218.0552]; ν_{max} ($CHCl_3$) 1770 (C=O), 1505 (Ar) cm^{-1}; δ_H (400 MHz, CD_3OD) 2.36 (3H, s, Me), 5.51 (2H, s, CH_2O), 7.22–7.58 (4H, m, Ar); *m/z* (EI) 218 (M^+), 149 ($M^+ - CF_3$), 105 ($M^+ - CF_3CO_2$).

2-Methylbenzyl 4-Nitrophenyl Carbonate 13

A solution of 2-methylbenzyl alcohol **10** (200 mg, 1.64 mmol) in acetonitrile (4 mL) was stirred with triethylamine (340 μL, 2.45 mmol) and *p*-nitrophenyl chloroformate (366 mg, 1.82 mmol) at 20 °C for 1 h and then evaporated. The residue was dissolved in ethyl acetate (5 mL), filtered, and evaporated. Crystallization from ethyl acetate yielded the *carbonate* **13** (307 mg, 65%), mp 85–86 °C (from ethyl acetate); [Found: (EI) M^+, 287.0781. $C_{15}H_{13}NO_5$ requires 287.0789]; R_f (ethyl acetate–hexane, 2:1) 0.78; λ_{max} (CH_3CN) 210, 272 nm; ν_{max} ($CHCl_3$) 1762 (C=O), 1525 and 1349 (N=O) cm^{-1}; δ_H (250 MHz, $CDCl_3$) 2.43 (3H, s, Me), 5.33 (2H, s, CH_2O), 7.20–8.32 (8H, m, Ar); *m/z* (EI) 287 (M^+), 149 ($M^+ - C_6H_4NO_3$), 139 ($C_9H_9O_2$), 105 (C_8H_9).

bis(2-Methylbenzyl) Sulphite 17

To a stirred solution of 2-methylbenzyl alcohol **10** (305 mg, 2.46 mmol) in dry THF (20 mL) was added sodium hydride (140 mg of a 60% dispersion in oil, 2.46 mmol). After the sodium hydride had all reacted, the mixture was cooled to 0 °C, treated with freshly distilled thionyl chloride (0.1 mL, 1.37 mmol), stirred for 4 h, filtered, and evaporated. Purification by PLC, eluting with dichloromethane (R_f 0.6), gave the *sulphite diester* **17** (130 mg, 36%) as an oil [Found: (EI) M^+, 290.0962. $C_{16}H_{18}SO_3$ requires 290.0977]; ν_{max} ($CHCl_3$) 1510 (Ar), 1395 and 1150 (S=O) cm^{-1}; δ_H (250 MHz, $CDCl_3$) 2.33 (6H, s, Me), 4.90 and 5.04 (each 2H, d, *J* 12, $ArCH_AH_BO$), 7.15–7.32 (8H, m, ArH); *m/z* (EI) 290 (M^+), 275 ($M^+ - CH_3$), 260 ($M^+ - C_2H_6$), 169 ($M^+ - C_8H_9O$).

2-Methylbenzyl Toluene-4-Sulphinate 14

2-Chloro-1-methylpyridinium iodide (27 mg, 0.12 mmol) was stirred with a solution of toluene-*p*-sulphinic acid (20 mg, 0.11 mmol) and triethylamine (0.5 mL, 3.6 mmol) in dry dichloromethane (3 mL). The solution was then heated at reflux and 2-methylbenzyl alcohol **10** (13 mg, 0.11 mmol) was added. After 3 h at reflux, diethyl ether (10 mL) was added and the mixture was washed with water (5 mL), 1% aqueous sodium hydrogen carbonate (5 mL) and then water again (2 × 5 mL). Purification by PLC, eluting with ethyl acetate–petroleum ether (bp 60–80 °C) (1:4; R_f 0.33), gave the *sulphinic ester* **14** as an oil (26 mg, 90%) [Found: (EI) M^+, 260.0871. $C_{15}H_{16}O_2S$ requires 260.0871]; λ_{max} (CH_3CN) 198, 222, 247 nm; ν_{max} ($CHCl_3$) 1504 (Ar), 1250 (S=O) cm^{-1}. δ_H (250 MHz, $CDCl_3$) 2.28 and 2.43 (each 3H, s, Me), 4.90 (2H, ABq, *J* 12, CH_2O), 7.1-7.64 (8H, m, Ar); δ_C (62.5 MHz, $CDCl_3$) 18.8 and 21.5 (CH_3), 63.9 (CH_2O), 125.3, 126.0 (2C), 128.9, 129.7, 129.9 (2C), 133.4, 136.5, 137.4, 141.6 and 142.9 (Ar); *m/z* (EI) 260 (M^+), 139 ($M^+ - C_8H_9O$), 105 ($M^+ - C_7H_7SO_2$).

5-Dimethylaminonaphthalene-1-Sulphinic Acid 21[9b]

A stirred solution of sodium metabisulphite (132 mg, 0.69 mmol) in water (2 mL) was heated at reflux with dansyl chloride **20** (150 mg, 0.56 mmol) for 2 h and then evaporated to dryness. The residue was dissolved in methanol (3 mL), filtered and evaporated to give the *sulphinic acid* **21** (118 mg, 90%) as a white solid [Found: M – H^+ (FAB -ve) 234.0572. $C_{12}H_{12}NO_2S$ requires 234.0589]; λ_{max} (CH_3OH) 219, 244, 323 nm; ν_{max} ($CHCl_3$) 1504 (Ar), 1250 (S=O) cm^{-1}; δ_H (200 MHz, DMSO-d_6) 2.50 (6H, s, NMe_2), 7.06–7.16 (2H, m) and 6.84, 7.67, 7.91 and 8.21 (each 1H, dd, *J* 7.5, 1.0, Ar); δ_C (62.5 MHz, d_6 DMSO) 45.2 (NMe_2), 113.9, 122.6, 123.7, 124.4, 125.3, 125.5, 128.9, 130.5, 144.4 and 150.5 (Ar); *m/z* (FAB -ve) 234 (M – H^+), 220 (M – Me).

2-Methylbenzyl 5-Dimethylaminonaphthalene-1-Sulphinate 22

A stirred solution of phenyl dichlorophosphate (9 μL, 60 μmol) in dry pyridine (30 μL, 0.37 mmol) at –15 °C was treated with acid **21** (10 mg, 43 μmol) followed after 4 min by 2-methylbenzyl alcohol **10** (10 mg, 80 mmol). After 1 h at –15 °C the solution was evaporated. Purification by PLC, eluting with ethyl acetate–petroleum ether (bp 60–80 °C) (1:4; R_f 0.40), gave the *sulphinic ester* **22** as an oil (11 mg, 75%) [Found: (EI) M^+, 339.1295. $C_{20}H_{21}NO_2S$ requires 339.1303]; λ_{max} (CH_3CN) 222, 254, 338 nm; ν_{max} ($CHCl_3$) 1504 (Ar), 1250 (S=O) cm^{-1}; δ_H (250 MHz, $CDCl_3$) 2.16 (3H, s, ArMe), 2.90 (6H, s, NMe_2), 4.41 and 5.07 (each 1H, d, *J* 11, $ArCH_2$), 7.02–7.20 (5H, m), 7.41 and 7.61 (each 1H, t, *J* 7.5) and 7.84, 8.20 and 8.45 (each 1H, dd, *J* 7.5, 1.0, Ar); δ_C (62.5 MHz, $CDCl_3$) 18.7 (ArMe), 45.3 (NMe_2), 63.8 (CH_2O), 124.0, 124.2, 125.9 (2C), 127.6, 128.8, 129.1 (2C), 129.2, 130.0 (2C), 130.3, 130.9, 133.2, 137.5 and 139.2 (Ar); *m/z* (EI) 339 (M^+), 218 ($M^+ - C_8H_9O$), 202 ($M^+ - C_8H_9O_2$).

3-Phenylpropyl 5-Dimethylaminonaphthalene-1-Sulphinate 23

To a stirred suspension of 2-chloro-1-methylpyridinium iodide (204 mg, 0.90 mmol) in dry dichloromethane (5 mL) was added sulphinic acid **21** (173 mg, 0.74 mmol), dry triethylamine (0.08 mL, 0.57 mmol), and 3-phenyl-1-propanol

(95 μL, 0.70 mmol). The resultant solution was heated at reflux for 12 h and then evaporated. A solution of the residue in ethyl acetate (10 mL) was washed with water (10 mL) and then dilute aqueous sodium hydrogen carbonate (10 mL). Purification by PLC, eluting with ethyl acetate–petroleum ether (bp 60–80 °C) (1:1; R_f 0.81), gave the *sulphinic ester* **23** as an oil (101 mg, 38%) [Found: (EI) M^+, 353.1452. $C_{21}H_{23}NO_2S$ requires 353.1449]; λ_{max} (CH_3CN) 214, 253, 336 nm; ν_{max} ($CHCl_3$) 1588 and 1551 (Ar), 1120 and 1062 (S=O) cm^{-1}; δ_H (250 MHz, $CDCl_3$) 1.74–1.86 (2H, m, $C\underline{H}_2CH_2O$), 2.57 (2H, m, $ArCH_2$), 2.91 (6H, s, NMe_2), 3.52 and 4.12 (each 1H, dt, *J* 10, 6, CH_2O), 7.08–7.22 (5H, m, Ph), 7.58 and 7.62 (1H, t, *J* 7.5) and 6.98, 7.98, 8.15 and 8.48 (1H, dd, *J* 7.5, 1.0, Ar); δ_C (62.5 MHz, $CDCl_3$) 31.1 ($\underline{C}H_2CH_2O$), 31.6 ($Ar\underline{C}H_2$), 45.2 (NMe_2), 63.0 (CH_2O), 114.8, 116.8, 123.8, 124.0, 125.7, 127.4, 128.1 (2C), 128.2 (2C), 128.9, 130.2, 130.7, 132.1, 139.0 and 140.8 (Ar); *m/z* (EI) 353 (M^+), 337 ($M^+ - CH_3$), 275 ($M^+ - C_6H_4$), 245 ($M^+ - C_8H_{10}$).

*[^{18}O]-2-Methylbenzyl Alcohol **10a***

A solution of the tosylate **11** (136 mg, 0.42 mmol) in dry dioxan (0.5 mL) and $H_2{}^{18}O$ (50 μL, 2.78 mmol) was stirred at 20 °C for 15 h and then extracted with dichloromethane (5 mL). Purification by PLC, eluting with ethyl acetate–petroleum ether (bp 40–60 °C) (3:1), gave the alcohol **10a** (41 mg, 78%) as an oil [Found: (EI) M^+, 124.0764. $C_8H_{10}{}^{18}O$ requires 124.0774]; R_f (ethyl acetate–petroleum ether, 1:4) 0.30; δ_H (250 MHz, $CDCl_3$) 1.75 (1H, s, OH), 2.43 (3H, s, Me), 4.76 (2H, s, CH_2O), 7.24–7.43 (4H, m, Ar), *m/z* (EI) 124 and 122 (M^+; ratio of ^{18}O to ^{16}O, 6.5 : 1), 104 ($M^+ - H_2{}^{18}O$).

*2-Methylbenzyl [^{18}O-alkyl]5-Dimethylaminonaphtha-lene-1-Sulphinate **22a***

The ^{18}O-labeled alcohol **10a** (18 mg, 147 mmol) was converted into its sulphinic ester **22a** (24 mg) as described above for the unlabeled alcohol [Found: (EI) M^+, 341.1343. $C_{20}H_{21}N^{16}O^{18}OS$ requires 341.1335]; *m/z* (EI) 341 and 339 (M^+; ratio of ^{18}O to ^{16}O, 1.9 : 1), 220 ($M^+ - C_8H_9O$), 204 ($M^+ - C_8H_9O_2$).

A solution of [^{18}O]sulphinic ester **23** (3 mg) in acetonitrile (16 mL) and 0.1M sodium phosphate buffer, pH 7.0 (239 mL) were stirred at 20 °C for 14 days (until the absorbance of the ester at 325 nm had disappeared and been replaced by a peak at 305 nm for the sulphinic acid) and then extracted with dichloromethane (3 × 20 mL). The combined extracts were evaporated to give the crude 2-methylbenzyl alcohol **10** [Found: (EI) M^+, 124.0772. $C_8H_{10}{}^{18}O$ requires 124.0774]; *m/z* (EI) 124 and 122 (M^+; ratio of ^{18}O to ^{16}O, 1 : 4.68).

This reaction was repeated using 15% acetonitrile for 28 days; *m/z* (EI) 124 and 122 (M^+; ratio of ^{18}O to ^{16}O, 1: 3.16). As a control, a solution of this last sample of methylbenzyl alcohol in water (1 mL) was stirred at 25 °C for 7 days and then extracted with diethyl ether (3 × 1 mL). The combined extracts were evaporated to give the crude 2-methylbenzyl alcohol **10**; *m/z* (EI) 124 and 122 (M^+; ratio of ^{18}O to ^{16}O, 1 : 3.16).

*3-(2-Hydroxymethylphenyl)pent-4-en-1-ol **26***

A solution of 3-(2-*t*-butyldimethylsilyloxymethylphenyl)pent-4-en-1-ol[2] (451 mg, 1.47 mmol) in dry THF (6 mL) was stirred with tetrabutylammonium fluoride hydrate (575 mg, 2.18 mmol) at 20 °C for 2 h, then diluted with water (10 mL) and extracted with dichloromethane (3 × 20 mL). Purification by flash column chromatography, eluting with dichloromethane–acetonitrile (1:1; R_f 0.44), gave the *diol* **26** (244 mg, 86%) as an oil (Found: (CI +ve) $M+NH_4^+$, 210.1494. $C_{12}H_{20}NO_2$ requires 210.1494); ν_{max} ($CDCl_3$) 3541–3210 (O-H), 1635 (C=C) cm^{-1}; δ_H (400 MHz, $CDCl_3$) 1.76–1.89 and 1.97–2.12 (each 1H, m, $C\underline{H}_2CH_2O$), 3.17 (1H, dt, *J* 11, 3) and 3.50 (1H, dt, *J* 11, 4, $CH_2C\underline{H}_2O$), 3.97 (1H, q, *J* 5, $ArC\underline{H}$), 4.19 and 4.49 (each 1H, br s, OH), 4.39 and 4.79 (each 1H, d, *J* 12, $ArC\underline{H}_2O$), 5.02 (1H, dt, *J* 17, 1) and 5.04 (1H, dt, *J* 10, 1, $CH=C\underline{H}_2$), 5.95 (1H, ddd, *J* 17, 10, 5, $C\underline{H}=CH_2$), 7.13-7.33 (4H, m, Ar); δ_C (62.5 MHz, $CDCl_3$) 38.0 ($\underline{C}H_2CH_2O$), 39.4 ($Ar\underline{C}H$), 59.0 ($CH_2\underline{C}H_2O$), 63.3 ($Ar\underline{C}H_2O$), 113.9 ($CH=\underline{C}H_2$), 126.3, 127.5, 128.6 and 130.1 (aromatic-CH), 138.4 and 141.7 (aromatic-C), 142.3 ($\underline{C}H=CH_2$); *m/z* (CI +ve) 210 ($M+NH_4^+$), 192 ($M+NH_4^+-H_2O$), 175 (M^+-OH).

*3-(2-Acetoxymethylphenyl)pent-4-en-1-yl Acetate **27***

A solution of diol **26** (22 mg, 0.11 mmol) in glacial acetic acid (5 mL) was stirred at reflux for 48 h and then evaporated. A solution of the residue in dichloromethane (5 mL) was washed with dilute aqueous sodium hydrogen carbonate (5 mL). Purification through a short silica plug, eluting with dichloromethane, gave the *diacetate* **27** (31 mg, 98%) as an oil [Found (CI +ve) $M+NH_4^+$, 294.1075. $C_{16}H_{24}NO_4$ requires 294.1075]; R_f (ethyl acetate–petroleum ether, 1:1) 0.55; ν_{max} ($CDCl_3$) 1730 (C=O), 1636 (C=C) cm^{-1}; δ_H (200 MHz, $CDCl_3$) 1.96–2.12 (2H, m, $C\underline{H}_2CH_2O$), 2.01 and 2.07 (each 3H, s, Ac), 3.68 (1H, dt, *J* 11, 3) and 4.01 (1H, dt, *J* 11, 4, $CH_2C\underline{H}_2O$), 4.03 (1H, q, *J* 5, ArCH), 5.00 (1H, dt, *J* 17, 1), and 5.05 (1H, dt, *J* 10, 1, $CH=C\underline{H}_2$), 5.14 (2H, s, $ArC\underline{H}_2O$), 5.92 (1H, ddd, *J* 17, 10, 5, $C\underline{H}=CH_2$), 7.19–7.36 (4H, m, Ar); δ_C (62.5 MHz, $CDCl_3$) 21.0 and 21.1 (CH_3), 34.1 ($\underline{C}H_2CH_2O$), 40.0 ($Ar\underline{C}H$), 62.6 ($CH_2\underline{C}H_2O$), 64.4 ($Ar\underline{C}H_2O$), 115.2 ($CH=\underline{C}H_2$), 126.7, 127.2, 129.3, and 130.5 (aromatic-CH), 133.5 and 142.4 (aromatic-C); 140.8 ($\underline{C}H=CH_2$), 170.9 and 171.1 ($\underline{C}OCH_3$); *m/z* (CI +ve) 294 ($M+NH_4^+$), 277 (MH^+), 252 ($M+NH_4^+-CH_3O$), 234 ($M+NH_4^+-C_2H_3O_2$), 220 (MH^+-CO), 203 (M^+-CO_2H).

*3-(2-Formyloxymethylphenyl)pent-4-en-1-yl Formate **28***

A solution of diol **26** (61 mg, 0.32 mmol) in formic acid (7 mL) was stirred at 20 °C for 15 h and then evaporated. A solution of the residue in dichloromethane (5 mL) was washed with dilute aqueous sodium hydrogen carbonate (5 mL). Purification through a short plug of silica, eluting with dichloromethane, gave the diformate 28 (77 mg, 98%) as an oil [Found; (CI +ve) $M+NH_4^+$, 266.1392. $C_{14}H_{20}NO_4$ requires 266.1392]; R_f (ethyl acetate–petroleum ether, 1:1) 0.50; ν_{max} ($CDCl_3$) 1720 (C=O), 1635 (C=C) cm^{-1}; δ_H (200 MHz, $CDCl_3$) 1.96–2.12 (2H, m, $C\underline{H}_2CH_2O$), 3.69 (1H, dt, *J* 11, 3), and 4.05 (1H, dt, *J* 11, 4, $CH_2C\underline{H}_2O$), 4.07 (1H, q, *J* 5, $ArC\underline{H}$), 5.00 (1H, dt, *J* 17, 1) and 5.06 (1H, dt, *J* 10, 1, $CH=C\underline{H}_2$), 5.22 (2H, s, $ArC\underline{H}_2O$), 5.88 (1H, ddd, *J* 17, 10, 5, $C\underline{H}=CH_2$), 7.15–7.37 (4H, m, Ar), 7.99 and 8.08 (each 1H, s, OCHO); δ_C (50 MHz, $CDCl_3$) 33.7 ($\underline{C}H_2CH_2O$), 40.7 ($Ar\underline{C}H$), 61.8 ($CH_2\underline{C}H_2O$), 63.4 ($Ar\underline{C}H_2O$), 115.4 ($CH=\underline{C}H_2$), 126.6, 127.0, 129.3, and 130.5

(aromatic-CH); 132.7 and 141.5 (aromatic-C); 140.2 (CH=CH_2), 160.5 and 160.8 (OCHO); *m/z* (CI +ve) 266 ($M+NH_4^+$), 238 ($M+NH_4^+$–CO), 220 (M^+–CO), 203 (M^+–CO_2H).

3-(2-Hydroxymethylphenyl)-2-methyltetrahydrofuran 29

Diol **26** (42 mg, 0.22 mmol) was stirred with 50% sulphuric acid (10 mL) at 20 °C for 15 h. The mixture was then extracted with ethyl acetate (3 × 10 mL) and the combined organic fractions were washed with dilute sodium carbonate (15 mL). Purification by PLC, eluting with ethyl acetate–petroleum ether (bp 60–80 °C) (1:1; R_f 0.50), gave the *alcohol* **29** (25 mg, 64%) [Found (CI) M^+–H_2O, 175.1123. $C_{12}H_{15}O$ requires 175.1123]; ν_{max} ($CDCl_3$) 3530–3062 (O-H) cm^{-1}; δ_H (400 MHz, $CDCl_3$) 1.27 (3H, d, *J* 6.5, CHMe), 1.95 and 2.14 (each 1H, dq, *J* 12.5, 7, CH_2CH_2O), 2.67 (1H, q, *J* 6, ArCH), 3.92 (1H, quintet, *J* 7, CHMe), 4.03 and 4.17 (each 1H, dt, *J* 11, 7, CH_2CH_2O), 4.74 (2H, s, $ArCH_2O$), 7.06–7.38 (4H, m, Ar); δ_C (100 MHz, $CDCl_3$) 18.6 (Me), 32.9 (CH_2CH_2O), 40.1 (ArCH), 62.1 (CH_2CH_2O), 65.8 ($ArCH_2O$), 72.7 (CHMe), 124.2, 126.0, 126.7, and 128.5 (aromatic-CH), 134.4 and 135.4 (aromatic-C); *m/z* (CI) 175 (MH^+), 130 (MH^+–CH_2CH_2OH).

Cyclization Reactions of Diol 26

(a) A solution of diol **26** (41 mg, 0.20 mmol) in dry dichloromethane (6 mL) was stirred with stannic chloride (0.2 mL) at 20 °C for 15 h, then quenched with saturated aqueous sodium hydrogen carbonate (5 mL) and extracted with dichloromethane (2 × 8 mL). Purification by PLC, eluting with ethyl acetate–petroleum ether (bp 60–80 °C) (1:1), yielded the *cyclic ether* **31** (12 mg, 30%) (R_f 0.61) and the *dimer* **32** (12 mg, 58%) (R_f 0.46) as oils.

9-Ethenyl-5,7,8,9-tetrahydrobenzo[c]oxepine 31

[Found (EI) M^+, 174.1045. $C_{12}H_{14}O$ requires 174.1045]; ν_{max} ($CDCl_3$) 1634 (C=C) cm^{-1}; δ_H (200 MHz, $CDCl_3$) 1.87–1.97 and 1.98–2.12 (each 1H, m, CH_2CH_2O), 3.55 and 3.65 (each 1H, m, CH_2CH_2O), 3.93 (1H, q, *J* 5, ArCH), 4.68 (2H, s, $ArCH_2O$), 5.02 (1H, dt, *J* 17, 1) and 5.04 (1H, dt, *J* 10, 1, $CH=CH_2$), 5.97 (1H, ddd, *J* 17, 10, 5, $CH=CH_2$), 7.14-7.34 (4H, m, Ar); δ_C (100 MHz, $CDCl_3$) 38.0 (CH_2CH_2O), 40.4 (ArCH), 44.4 (CH_2CH_2O), 60.6 ($ArCH_2O$), 114.7 ($CH=CH_2$), 126.4, 126.6, 129.0, and 130.6 (aromatic-CH), 135.3 and 142.5 (aromatic-C), 141.4 ($CH=CH_2$); *m/z* (CI) 192 ($M+NH_4^+$), 175 (MH^+), 146 (M^+–C_2H_4).

6,17-Diethenyl-3,14-dioxatricyclo[16.4.0.0^{7,12}]docosa-1(22),7,9,11,18,20-hexaene 32

[Found (EI) M^+, 348.2085. $C_{24}H_{28}O_2$ requires 348.2089]; ν_{max} ($CDCl_3$) 1634 (C=C) cm^{-1}; δ_H (400 MHz, $CDCl_3$) 1.91 (2H, ddt, *J* 14, 6, 3) and 2.07 (2H, ddt, *J* 14, 8, 3, CH_2CH_2O), 3.79 (2H, dt, *J* 6.5, 5, ArCH), 3.99 and 4.09 (each 2H, m, CH_2CH_2O), 4.62 and 4.71 (each 2H, d, *J* 13.7, $ArCH_2O$), 4.88 (2H, dt, *J* 17, 1.3) and 5.17 (2H, dt, *J* 10, 1.3, $CH=CH_2$), 6.13 (2H, ddd, *J* 17, 10, 6.5, $CH=CH_2$), 7.13–7.29 (8H, m, Ar); δ_C (100 MHz, $CDCl_3$) 35.1 (CH_2CH_2O), 48.2 (ArCH), 61.4 (CH_2CH_2O), 63.3 ($ArCH_2O$), 115.6 ($CH=CH_2$), 126.4, 127.8, 129.0, and 129.1 (aromatic-CH), 139.5 and 143.0 (aromatic-C), 140.3 ($CH=CH_2$); *m/z* (EI) 348 (M^+), 321 (M^+–C_2H_3), 294 (M^+–C_4H_6).

(b) A solution of diol **26** (31 mg, 0.16 mmol) in dry dichloromethane (6 mL) was stirred with a solution of boron trifluoride in diethyl ether (40 mL, 0.31 mmol) at 20 °C for 15 h, then quenched with dilute aqueous sodium hydrogen carbonate (5 mL) and extracted with dichloromethane (2 × 10 mL). Purification by PLC gave the *dimer* **32** (11 mg, 71%).

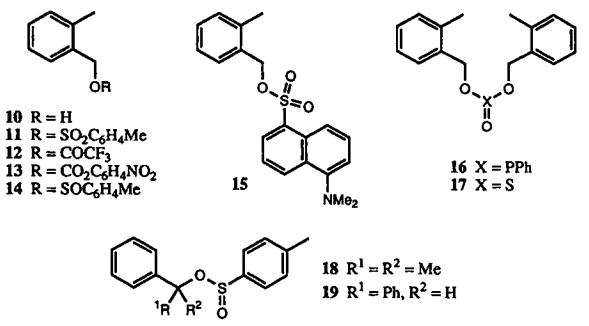
(c) Fluorosulphonic acid (0.2 mL) was carefully added to nitropropane (2.5 mL) at –90 °C and to this solution was added a solution of the diol **26** (42 mg, 0.22 mmol) in nitropropane (2.5 mL). The reaction mixture was stirred at –90 °C for 5 min before being quenched by carefully dripping it into saturated aqueous sodium hydrogen carbonate (5 mL) at 0 °C. The mixture was extracted with diethyl ether (2 × 10 mL) and purification by PLC gave the *dimer* **32** (17mg, 78%).

LEAVING GROUP STUDIES

Nature uses pyrophosphate as a leaving group for cationic cyclizations. This is not a suitable choice for model systems because of problems associated with the synthesis and purification. Also, there is no convenient spectroscopic assay for following the departure of the pyrophosphate group. In choosing a suitable leaving group, we were concerned that the substrate should not be so unreactive that an antibody-catalyzed reaction would be too slow to follow, nor so reactive that it decomposes before having had a chance to bind to the antibodies. Therefore, our intention was to study a range of leaving groups of different leaving ability in order to find one which underwent spontaneous S_N1 substitution in aqueous solution at a slow but measurable rate.

Rather than carry out the leaving group studies on our synthetic diol (**1**, X = H), we decided to use commercially available 2-methylbenzyl alcohol **10**, which should have very similar reactivity as far as the leaving group is concerned. This alcohol was converted into a range of different esters, i.e., tosylate **11**, dansylate **15**, phenylphosphonate diester **16**, trifluoroacetate **12**, and 4-nitrophenyl carbonate **13**. Initially we had some difficulty in preparing the sulphonate esters as it turned out that they were unstable during aqueous workup. However, they could be made satisfactorily by purifying the reaction mixture directly by column chromatography without any workup. The other esters were made by standard procedures.

As expected from the difficulty experienced during their preparation, the sulphonates proved very susceptible to solvolysis in polar solvents. In deuteriomethanol at room temperature tosylate **11** had a half-life of 18 min (by NMR spectroscopy) and the dansylate **15** was even more reactive, half-life 7 min. For the dansylate, the decomposition in aqueous buffer (0.1 M Na_2HPO_4, pH 7.5, containing 0.1 M NaCl) could be followed by UV



Structures **10–19**

spectroscopy and it had a half-life of only 35 s. This acceleration in the more polar solvent is as expected for an S_N1 reaction. The very rapid solvolysis in aqueous solution effectively precludes the use of benzylic sulphonates as substrates for antibody-catalyzed reactions.

Of the other esters, the phosphonate **16** was found to show no solvolysis after one month dissolved in CD_3OD–D_2O (1:1), whereas the trifluoroacetate **12** had a half-life of 40 h in CD_3OD, and the carbonate **13** had a half-life of 15 h in aqueous buffer (0.1 M Na_2HPO_4, pH 7.0, containing 7% MeOH). The pK_a value of a carbonic acid monoester is considerably higher than that of a phosphonate monoester and so a carbonate monoanion would not be expected to be as good a leaving group as a phosphonate monoanion. We suspected, therefore, that the solvolysis of the carbonate **13** was not due to the desired O–alkyl bond cleavage (S_N1) but to an O–acyl cleavage (ester hydrolysis). This was effectively proved by two observations: (a) the hydrolysis was accelerated when the pH of the buffer was lowered from 7.0 to 8.0 (see Table 1); (b) the hydrolysis of a non-benzylic primary alkyl 4-nitrophenyl carbonate ester proceeded at almost exactly the same rate as the benzylic one **13**. Neither observation is consistent with S_N1 reaction, but both are consistent with attack on the carbonyl group by H_2O and/or HO^-. The solvolysis of the trifluoroacetate **12** was not investigated further, but we suspect that it may also occur by attack at the carbonyl group.

As a result of these experiments with esters **11–13**, **15**, and **16**, we wanted to find a class of leaving group that is not as good as sulphonates but better than phosphonates. We therefore investigated esters of sulphinic acids, whose pK_a values are generally intermediate between those of phosphonic and sulphonic acids[5] (e.g., $PhPO_3H_2$, 1.83; $PhSO_2H$, 1.21; $PhSO_3H$, –2.80). There have been previous reports of the solvolysis of benzylic sulphinates **18** in methanol and trifluoroethanol[6] and **19** in dioxan–water.[7] In the latter case both C–O and S–O bond cleavages were observed but C–O cleavage (S_N1) predominated, the more so when the proportion of water in the medium was increased.

The first compound we tested was the sulphite diester **17**, made by treatment of the alcohol **10** with NaH followed by $SOCl_2$, and this had a half-life of 197 h in deuteriomethanol. This result encouraged us to synthesize and study the toluenesulphinate **14**, which, being a monoester (as opposed to the diester **17**), was more suitable for our purposes. This sulphinate was best made (90% yield) by coupling toluene-*p*-sulphinic acid and the alcohol in the presence of 2-chloro-1-methylpyridinium iodide (Mukaiyama's reagent) and triethylamine.[8] NMR spectroscopy showed that solvolysis of the sulphinate occurred in CD_3OD with a half-life of 21 h.

The half-life of the toluenesulphinate **14** in CD_3OD was in the desired range, but it would not be straightforward to measure its half-life in aqueous solution because of its low solubility and the lack of significant

Table 1. Half-lives for the solvolysis of the various 2-methylbenzyl esters

Ester	Solvent[a]	Half-life
Tosylate **11**	CD_3OD	18 min
Dansylate **15**	CD_3OD	7 min
	aq buffer, pH 7.5	35 sec
Phosphonate **16**	CD_3OD–D_2O, 1:1	>1 month
Trifluoroacetate **12**	CD_3OD	40 hours
Carbonate **13**	aq buffer, pH 7.0, 7% MeOH	15 hours
	aq buffer, pH 8.0, 7% MeOH	6 hours
Sulphite **17**	CD_3OD	197 hours
Toluenesulphinate **14**	CD_3OD	21 hours
Dansinate **22**	aq buffer, pH 7.0, 6.7% CH_3CN	35 hours
	aq buffer, pH 7.0, 15% CH_3CN	97 hours
3-Phenylpropyl dansinate **23**	aq buffer, pH 7.0, 6.7% CH_3CN	58 hours
	aq buffer, pH 7.0, 15% CH_3CN	109 hours

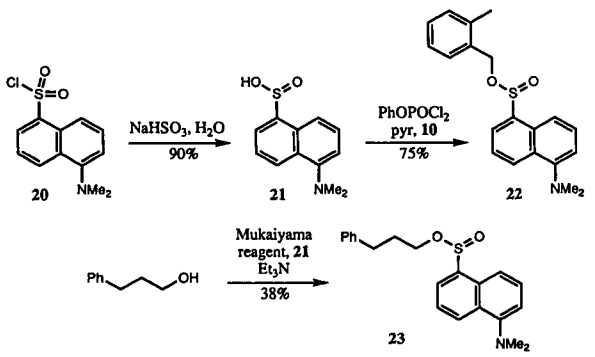
[a]The aqueous buffer used was 0.1M Na_2HPO_4 adjusted to the stated pH.

change in UV absorbance upon its solvolysis. This would be even more of a problem when assaying antibodies for activity and so we chose to synthesise the sulphinate analogue **22** of the dansylate ester; from here on these esters are referred to as dansinates.

Reduction of dansyl chloride **20** to the sulphinic acid **21** (Scheme 3) was attempted by two literature procedures, $Na_2SO_3/NaOH$[9] and Zn/H_2O,[10] but both methods resulted in more hydrolysis (to give the sulphonic acid) than reduction. We reasoned that less basic conditions should result in less hydrolysis and, indeed, treatment of **18** with aqueous $NaHSO_3$ gave the sulphinic acid **21** in 90% yield without any contaminating sulphonic acid. To our surprise, the previous conditions for making a sulphinate ester (Mukaiyama's reagent) gave only a low yield of dansinate **22** (20%) but a much better yield (75%) was obtained when the sulphinic acid was activated by reaction with $PhOPOCl_2$ and pyridine and then treated with the alcohol **10**. Before studying the solvolysis of this sulphinate, an analogous non-benzylic primary dansinate **23** was also made, starting from 3-phenylpropanol. We believe that these are the first reported examples of dansinate esters.

The dansinates **22** and **23** were both highly fluorescent (for **22** λ_{max} excitation 330 nm, emission 455 nm) and their solvolysis was accompanied by a change in fluorescence (λ_{max} emission 478 nm) as well as of absorbance in the UV spectrum around 330 nm. In order to follow the solvolysis in aqueous buffer (0.1 M Na_2HPO_4, pH 7.0), small amounts of an organic cosolvent had to be employed due to the low solubility of the hydrophobic esters in water. Saturated solutions of **22** were prepared in this buffer containing 6.7% and 15% CH_3CN, and their concentrations were determined by UV spectrophotometry to be 6.7 μM and 77 μM, respectively. The decrease of the absorbance at 325 nm (and concomitant increase at 310 nm) followed good first-order kinetics in each case and gave the half-lives shown in Table 1. It can be seen that solvolysis of the benzylic dansinate **22** is ca. 60% faster than the non-benzylic one **23** in 6.7% CH_3CN but only ca. 10% faster in 15% CH_3CN.[11]

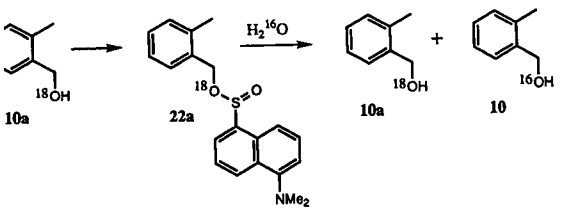
It is most unlikely that the non-benzylic dansinate **23** would react by a S_N1 mechanism, and the solvolysis in this case is probably entirely due to attack of water (or hydroxide ion) at the sulphur atom with subsequent S–O bond cleavage. It seemed likely, however, that the extra reactivity of the benzylic dansinate **22** (especially in the more polar solvent) is due to S_N1 reaction, but this still needed to be proved. We therefore undertook an ^{18}O-labelling experiment, which would give a direct measure of the ratio of C–O to S–O bond cleavage.^{18}O-labeled 2-methylbenzyl alcohol **10a** was synthesized by hydrolysis of the tosylate **11** in dioxan–$H_2{}^{18}O$, and this alcohol was then converted into its dansinate **22a** in the usual way (Scheme 4). Mass spectrometry at this stage showed a ratio of ^{18}O-labeled to unlabeled material of 66:34 (see Table 2). This labeled dansinate was then hydrolyzed under the same two sets of conditions as previously, and the alcohol produced was analyzed by mass spectrometry. The results, shown in Table 2, indicate that only 27% of the original ^{18}O remained when the hydrolysis occurred in aqueous buffer containing 6.7% CH_3CN and 36% of the original ^{18}O remained using 15% CH_3CN. As a control, it was shown that no further exchange occurred when [^{18}O]2-methylbenzyl alcohol was dissolved in water for 7 days at room temperature. Retention of ^{18}O is due to S–O bond cleavage, whereas loss of ^{18}O is due to C–O bond cleavage (presumably S_N1 reaction). Therefore we can conclude that the ratio of C–O to S–O bond cleavage is 73:27 in the more polar medium (6.7% CH_3CN) and 64:36 in the less polar medium (15% CH_3CN). This knowledge of the ratio of the two types of reaction allows calculation of the rate constant for each from the half-life measurement described above. The values for k_{C-O} and k_{S-O} are given in Table 2. The k_{C-O} values are the k_{uncat} values which will be needed for comparison with the k_{cat} values for any catalytic antibodies that are obtained.



Scheme 3

MODEL CYCLIZATION STUDIES

Polyene cyclization studies were pioneered in the work of Johnson.[12] These reactions were generally catalyzed



Scheme 4

Table 2. Percentages of ^{18}O and ^{18}O in the 2-methylbenzyl alcohol **10** resulting from hydrolysis of ^{18}O-labeled **22a** and calculated rate constants

Compound	CH_3CN^a %	^{18}O %	^{16}O %	k_{c-o} /10^{-6} s^{-1}	k_{s-o} /10^{-6} s^{-1}
2-Methylbenzyl dansinate **22a**		66	34		
2-Methylbenzyl alcohol **10**	6.7	18	82	3.97	1.47
2-Methylbenzyl alcohol **10**	15	24	76	1.25	0.73

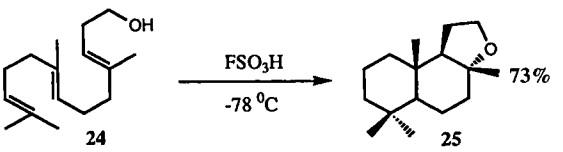
[a]Hydrolysis reactions were carried out in 0.1M aqueous Na_2HPO_4, pH 7.0, containing the indicated amount of CH_3CN as cosolvent.

by protic acids, such as formic acid and trifluoroacetic acid, or Lewis acids, such as tin tetrachloride. More recently, improved yields for some of these type of reactions have been obtained in superacidic media by Vlad and by others.[13–15] The advantages of superacids are reported to be that "being supernonnucleophilic media, they contribute to a greater development of positive charges on the carbocationic centers, and suppress the elimination and isomerisation reactions of intermediates".[13] A reaction which is related to the cyclization reaction we are attempting to promote is the cyclization of homofarnesol **24** to give the tricyclic product **25** shown in Scheme 5.

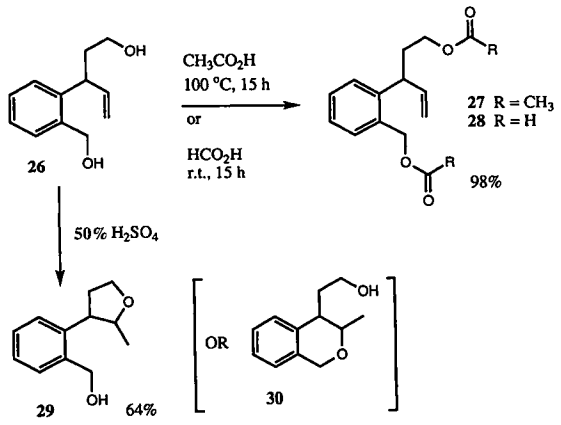
For our cyclization studies we used diol **26**, the alcohol form of the substrate **1** for our catalytic antibodies. The diol was made by deprotection of the corresponding benzylic *t*-butyldimethylsilyl ether (an intermediate that we had made in our earlier synthesis of substrates for cyclization studies[16]) using tetra-*n*-butyl ammonium fluoride in THF in 83% yield.

Attempts to cyclize the diol **26** in acetic acid at room temperature gave no reaction, whereas at 100 °C the diacetate **27** was formed in 98% yield (Scheme 6). Treatment of the diol with formic acid at room temperature gave only the diformate **28** in 98% yield. Using more forcing acidic conditions, 50% sulphuric acid, a bicyclic product was obtained in 64% yield whose ^1H NMR spectrum showed that the alkene functionality had disappeared. The structure of this product is probably **29** (although the alternative structure **30** cannot be ruled out). This compound is formed by Markovnikov-type protonation of the alkene to generate a secondary cation, which is trapped by the non-benzylic alcohol to form the tetrahydrofuran ring.

As catalysis by protic acids had not produced the desired cyclization product, we investigated the use of Lewis acids. Treatment of the diol **26** with tin tetrachloride in dichloromethane gave two new cyclized products, both of which retained their alkene functionality. The minor product (30%) was the seven-membered cyclic ether **31** and the major one (58%) was the dimeric



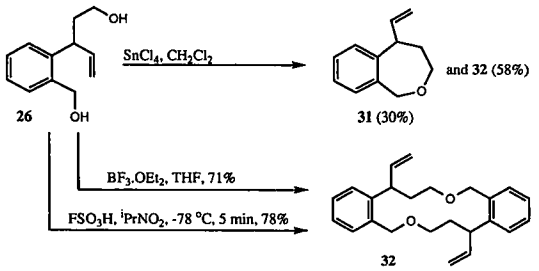
Scheme 5



Scheme 6

cyclic diether **32** (Scheme 7). In forming both products, it appears that the tin tetrachloride has promoted ionization at the benzylic position. However, rather than being attacked by the double bond, the benzylic cation is attacked by the remaining alcohol group, either intramolecularly to form **31**, or intermolecularly to form the dimer **32**. When the cyclization was attempted in the presence of boron trifluoride diethyl etherate, the only product isolated (71%) was the dimer **32**.

Encouraged by the results of polyene cyclization in superacidic media,[13–15] we attempted to cyclize the diol **26** in fluorosulphonic acid.[14] The diol was dissolved in carbon disulphide and added to a solution of fluorosulphonic acid in sulphur dioxide at –78 °C. Even though the expected red color was generated when the diol was added, no identifiable products or starting material was recovered. It was thought that the product (and any unreacted starting material) was being lost during the workup, which involved transfer of the reaction mixture by cannula into a slurry of potassium carbonate in methanol at –78 °C. This prompted a change in the reaction conditions so that we could use a simpler aqueous workup. The diol was dissolved in nitropropane and added to a solution of fluorosulphonic acid in nitropropane at –78 °C.[15] The reaction mixture was kept at –78 °C for 5 min and then carefully quenched with saturated sodium hydrogen carbonate at 0 °C and extracted with diethyl ether. All the starting material had been consumed and a single product, the dimer **32**, was isolated in 78% yield. The reaction was left for longer



Scheme 7

times (20 or 30 min) in the hope of dissociating the dimer to regenerate reactive cationic species, but no identifiable products could be obtained even when care was taken to minimize the loss of any volatile products.

CONCLUSIONS

In this paper we describe the development of the sulphinate group as a suitable leaving group for studies of reactions which involve ionization at a benzylic position at a moderate rate in aqueous media. Esters of 5-dimethylaminonaphthalene-1-sulphinic acid **21** are particularly suitable because of the change in their UV aborbance and fluorescence when the sulphinate acts as a leaving group.

We have also shown that the diol **26** does undergo acid-catalyzed cyclization reactions. However, the two modes of cyclization observed are attack of the non-benzylic alcohol on either the benzylic cation or the protonated alkene. Interestingly, no cyclization of the alkene onto the benzylic cation has been observed. This suggests that in free solution the p-orbital of the benzylic cation may not be in the correct orientation for reaction with the double bond. It is hoped that the catalytic site generated in response to the hapten **5** will hold the substrate in the correct orientation to specifically promote this "disfavored" reaction.

The synthesis of the hapten **5** and generation of antibodies will be reported elsewhere.

Acknowledgments. We are grateful to the SERC for a studentship to C.L., the SERC and CellTech for a studentship to I.M.B., to the European Network for Antibody Catalysis for financial support, and to Dr. D. Whitaker (University of Liverpool) for his advice on the safe use of superacids.

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(11) It can be seen from Table 1 that the solvolysis of the toluenesulphinate **14** in CD_3OD is faster than of dansinate **22** in the aqueous buffers. This may, in part, be due to a greater intrinsic reactivity of **14,** but it should also be borne in mind that the reactions in CD_3OD were not buffered, and autocatalysis by the sulphinic acid produced during the reaction is known to occur (see ref 7). The reactions in aqueous buffer should not show any such acid catalysis, and this is confirmed by the good first-order kinetics observed.
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