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SYNTHESES OF CHOLESTEROL ANALOGS WITH A CARBENE-GENERATING
SUBSTITUENT ON THE SIDE CHAIN

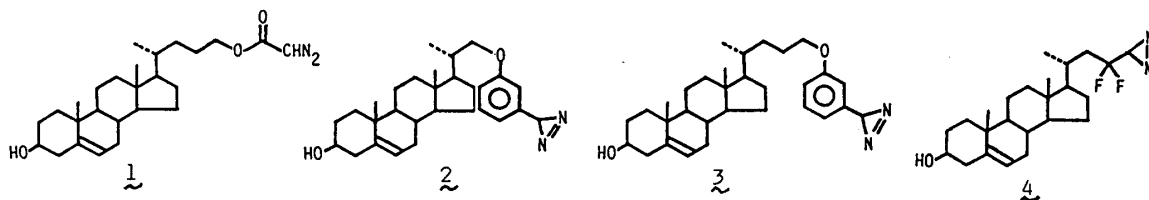
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As photoaffinity-labelled analogs of cholesterol, the diazoacetate **1**, the aryldiazirines **2** and **3**, and the fluoro-diazirine **4** were prepared.

KEYWORDS-cholesterol analog; photoaffinity label;
diazoacetate; aryldiazirine; fluorodiazirine; phospholipid-
cholesterol interaction

Since cholesterol is a major lipid constituent of biological membrane, knowledge of its interaction with phospholipid is essential in understanding the architectural and functional roles of cholesterol.¹⁾ Although the molecular interaction of cholesterol with phospholipids has been studied extensively by physicochemical techniques such as differential scanning calorimetry, X-ray diffraction, NMR and ESR, there has been no definite proof of direct interaction between the two components.²⁾ One approach to this problem is photoaffinity labelling, and cholesteryl diazoacetate has recently been reported as a useful membrane photolabelling reagent.^{3,4)} However, in view of the importance of the 3 β -hydroxyl group of cholesterol for interaction with phospholipids,²⁾ cholesteryl diazoacetate does not appear to be ideal as a membrane probe. Here we describe the syntheses of cholesterol analogs **1**, **2**, **3** and **4** having a carbene-generating substituent on the side chain,⁵⁾ and in the accompanying paper, the photochemical behavior of these compounds is reported.⁶⁾ The hydrophobic nature of these substituents and also our previous observations⁷⁾ that relatively large modification of the side chain structure of cholesterol causes little or no

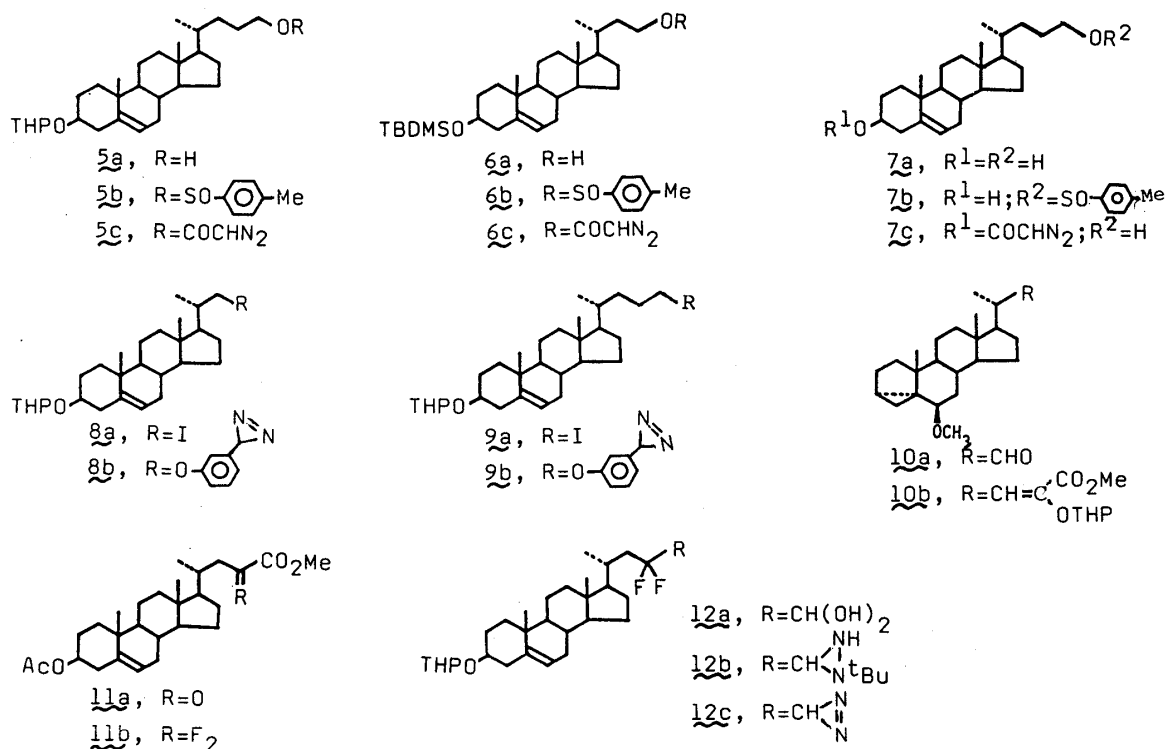


perturbation in the properties of liposomes, suggest that compounds 1-4 would be promising alternatives as membrane probes.

We first attempted to prepare diazoacetate 1 from 24-hydroxy-3-tetrahydropyranyl(THP)- or 3-*t*-butyldimethylsilyl(TBDMS)-ether 5a⁸⁾ or 6a.⁹⁾ When 5a or 6a were treated with glyoxylic acid chloride *p*-toluenesulfonyl hydrazone and triethylamine according to the published method,^{3,10)} the sulfinate 5b and 6b, instead of the desired diazoacetate 5c and 6c, were the major products (64% and 51% yield, respectively). Substitution of triethylamine for the weaker base dimethylaniline,¹¹⁾ gave 5c and 6c in 40% and 49% yield, respectively without formation of 5b and 6b. However, various attempts to deprotect the 3-THP or 3-TBDMS groups of 5c and 6c, induced a concomitant decomposition of the diazoacetyl group. Accordingly, we turned our attention to selective diazoacetylation of the 3,24-diol 7a. Treatment of 7a with glyoxylic acid *p*-toluenesulfonyl hydrazone in the presence of dicyclohexylcarbodiimide and 4-dimethylaminopyridine,¹²⁾ again afforded the sulfinate 7b as a major product accompanied with a trace of the expected diazoacetate 1. A modest success finally came when the 3,24-diol was subjected to esterification with glyoxylic acid chloride *p*-toluenesulfonyl hydrazone (1 eq) in the presence of dimethylaniline in CH₂Cl₂-dimethylformamide (10 : 1) at 0°C, followed by treatment with triethylamine (5 eq). Flash chromatography¹³⁾ of the crude product gave the recovered diol 7a (40%), the less polar 3-diazoacetate 7c (4%), the more polar 24-diazoacetate 1 [13%, mp 83-85°C, UV(EtOH) $\lambda_{\max}$ 247nm(ϵ , 1.0 $\times 10^4$); IR(CHCl₃) 2105(diazo), 1680cm⁻¹(carbonyl); ¹H-NMR(CDCl₃, 270 MHz): 4.13(2H, td, J=6.8 and 3Hz, 24-H₂), 4.68ppm(1H, s, -CHN₂)], and a mixture of 1 and 7c (25%).

Syntheses of the aryldiazirines 2 and 3 were patterned after the published preparation of the diazirinophenoxy derivatives of fatty acids.¹⁴⁾ Thus, 22-iodide 8a¹⁵⁾ and sodium *m*-(3H-diazirino)phenoxide¹⁴⁾ were coupled in hexamethylphosphoramide-tetrahydrofuran (3 : 2) to give the ether 8b (40%), which on acid treatment yielded 2 [mp 133-134°C, UV(EtOH) $\lambda_{\max}$ 363nm(ϵ , 340); IR(CHCl₃) 1585cm⁻¹(diazo); ¹H-NMR(CDCl₃): 1.99(1H, s, CH₂^N), 3.64(1H, dd, J=9 and 7Hz, 22-H_a), 3.87ppm(1H, dd, J=9 and 3Hz, 22-H_b)]. Similarly, 24-iodide 9a, which was prepared by the iodide substitution reaction on the corresponding 24-tosylate,⁸⁾ was converted into the ether 9b (78%), and then into 3 [mp 65-67°C, UV(EtOH) $\lambda_{\max}$ 363nm(ϵ , 340); IR(CHCl₃) 1585cm⁻¹(diazo); ¹H-NMR: 1.99(1H, s, CH₂^N), 3.89ppm(2H, t, J=7Hz, 24-H₂)].

For synthesis of the fluorodiazirine 4, the 22-aldehyde 10a¹⁶⁾ was subjected to the Horner-Emmons reaction with (MeO)₂P(O)CH(OTHP)CO₂Me/lithium diisopropylamide¹⁷⁾ in tetrahydrofuran to give the enol ether 10b (1 : 1 mixture of E and Z isomers) in 85% yield, which on refluxing with zinc acetate in acetic acid afforded the oxoester 11a¹⁸⁾ in 72% yield. Reaction of 11a with diethylamino-sulfur trifluoride in CH₂Cl₂¹⁹⁾ gave the difluoride 11b¹⁸⁾ (60%). This was converted into the aldehyde hydrate 12a in 57% overall yield by the sequences: 1) hydrolysis (K₂CO₃/methanol); 2) esterification(CH₂N₂/ether); 3) THP ether formation (dihydropyran/*p*-toluenesulfonic acid); 4) reduction (diisobutyl aluminum hydride/ether). Transformation of the aldehyde hydrate 12a into the diazirine 12c was patterned after the method of Khorana.²⁰⁾ The compound 12a was dehydrated by azeotropic refluxing with benzene using a Soxhlet apparatus filled



with 4A molecular sieves. The resulting free aldehyde was refluxed with excess tert-butylamine to give the tert-butylimine derivative. This was unstable and so, it was directly treated with hydroxylamine O-sulfonic acid in ethanol-benzene-triethylamine (10 : 2 : 5) at 0°C to yield the N-tert-butyl diazirine 12b (12% yield from 12a), together with the recovered aldehyde hydrate 12a (60%). The diazirine 12b was oxidized with tert-butyl hypochlorite in tert-butanol-ethanol-tetrahydrofuran in the presence of triethylamine at 0°C to yield the diazirine 12c in 90% yield. Acid treatment (d.HCl/tetrahydrofuran-methanol) furnished the fluorodiazirine 4 [amorph. UV(EtOH) $\lambda_{\max}$ 306, 314 and 319nm ($\epsilon_{306-318}$ 160); IR (CHCl₃) 1600cm⁻¹ (diazo); ¹H-NMR(CDCl₃): 0.72(3H, s, 18-H₃), 1.00(3H, s, 19-H₃), 3.5(1H, m, 3 α -H), 5.3ppm(1H, m, 6-H)].

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