Communications to the Editor

[Chem. Pharm. Bull. 34(2) 931—934 (1986)]

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

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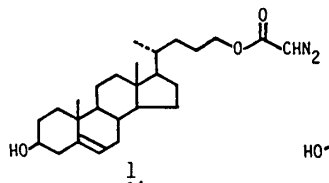
As photoaffinity-labelled analogs of cholesterol, the diazoacetate 1, the aryldiazirines 2 and 3, and the fluorodiazirine 4 were prepared.

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 3β-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 observations[7] that relatively large modification of the side chain structure of cholesterol causes little or no



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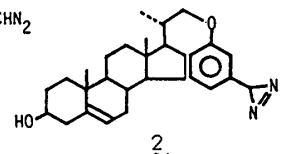

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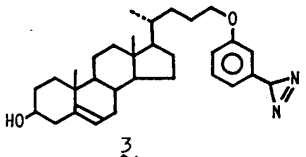

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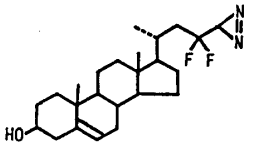

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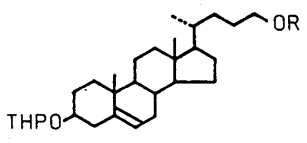
perturbation in the properties of liposomes, suggest that compounds 1-4 would be promising alternatives as membrane probes.

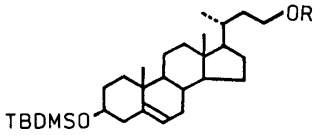
We first attempted to prepare diazoacetate 1 from 24-hydroxy-3-tetrahydropyranyl(THP)- or 3-t-butyldimethylsilyl(TBDMS)-ether 5a[8] or 6a.[9] When 5a or 6a were treated with glyoxylic acid chloride p-toluenesulfonyl hydrazone and triethylamine according to the published method,[3,10] the sulfinate 5b 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 CH_2Cl_2-dimethylformamide (10 : 1) at O°C, followed by treatment with tiethylamine(5 eq). Flash chromatography[13] 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) λ_{max}247nm(ε, 1.0 x 10^4); IR($CHCl_3$) 2105(diazo), 1680cm^{-1}(carbonyl); ^{1}H-NMR($CDCl_3$, 270 MHz): 4.13(2H, td, J=6.8 and 3Hz, 24-H_2), 4.68ppm(1H, s, -CHN_2)], 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 8a[15] and sodium m-(3H-diazirino)phenoxide[14] 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) λ_{max}363nm(ε, 340); IR($CHCl_3$) 1585cm^{-1} (diazo); ^{1}H-NMR($CDCl_3$): 1.99(1H, s, $CH\langle^{N}_{N}$), 3.64(1H, dd, J=9 and 7Hz, 22-H_a), 3.87 ppm(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) λ_{max}363 nm(ε, 340); IR($CHCl_3$) 1585cm^{-1}(diazo); ^{1}H-NMR: 1.99(1H, s, $CH\langle^{N}_{N}$), 3.89ppm(2H, t, J=7Hz, 24-H_2)].

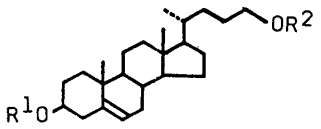
For synthesis of the fluorodiazirine 4, the 22-aldehyde 10a[16] was subjected to the Horner-Emmons reaction with $(MeO)_2P(O)CH(OTHP)CO_2Me$/lithium diisopropylamide[17] in tetrahydrofuran to give the enol ether 10b (1 : 1 mixture of E and Z isomers) in 85% yield, which on refluxing with zinc acetate in acetic acetic acid afforded the oxoester 11a[18] in 72% yield. Reaction of 11a with diethylaminosulfur trifluoride in CH_2Cl_2[19] gave the difluoride 11b[18] (60%). This was converted into the aldehyde hydrate 12a in 57% overall yield by the sequences: 1) hydrolysis (K_2CO_3/methanol); 2) esterification(CH_2N_2/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



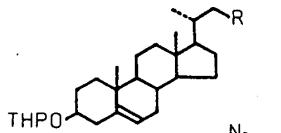
5a, R=H
5b, R=SO-⟨⟩-Me
5c, R=COCHN$_2$



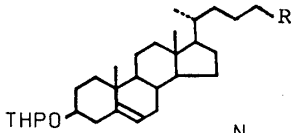
6a, R=H
6b, R=SO-⟨⟩-Me
6c, R=COCHN$_2$



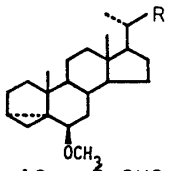
7a, R^1=R^2=H
7b, R^1=H;R^2=SO-⟨⟩-Me
7c, R^1=COCHN$_2$;R^2=H



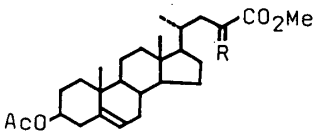
8a, R=I
8b, R=O-⟨⟩



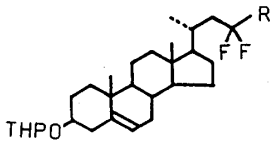
9a, R=I
9b, R=O-⟨⟩



10a, R=CHO
10b, R=CH=C(CO$_2$Me)(OTHP)



11a, R=O
11b, R=F$_2$



12a, R=CH(OH)$_2$
12b, R=CH(NH)(N^{t}Bu)
12c, R=CH(N=N)

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 O°C to yield the N-tert-butyldiazirine 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 O°C to yield the diazirine 12c in 90% yield. Acid treatment (d.HCl/tetrahydrofuran-methanol) furnished the fluorodiazirine 4 [amorph. UV(EtOH) λ_{max}^{306}, 314 and 319nm($\varepsilon_{306-318}^{160}$); IR ($CHCl_3$) 1600cm^{-1}(diazo); ^{1}H-NMR($CDCl_3$): 0.72(3H, s, 18-H$_3$), 1.00(3H, s, 19-H$_3$), 3.5(1H, m, 3α-H), 5.3ppm(1H, m, 6-H)].

ACKNOWLEDGEMENT This research was supported by grant(No. 58570867) from the Ministry of Education, Science and Culture.

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(Received November 28, 1985)