Chemistry and Physics of Lipids 195 (2016) 58–62

Contents lists available at ScienceDirect

Chemistry and Physics of Lipids

journal homepage: www.elsevier.com/locate/chemphyslip

Optimised conditions for the synthesis of ^{17}O and ^{18}O labelled cholesterol

Celia de la Calle Arregui[a], Jonathan A. Purdie[a], Catherine A. Haslam[a], Robert V. Law[b], John M. Sanderson[a,*]

[a] Chemistry Department, Durham University, Durham, DH1 3LE, UK
[b] Institute of Chemical Biology, Imperial College, Exhibition Road, London, SW7 2AZ, UK

ARTICLE INFO

Article history:
Received 28 October 2015
Received in revised form 30 November 2015
Accepted 17 December 2015
Available online 24 December 2015

Keywords:
Cholesterol
Synthesis
Oxygen isotope

ABSTRACT

Conditions are described for the preparation of cholesterol with ^{17}O and ^{18}O labels from *i*-cholesteryl methyl ether using minimal amounts of isotopically enriched water. Optimum yields employed trifluoromethanesulfonic acid as catalyst in 1,4-dioxane at room temperature with 5 equivalents of water. An isotopic enrichment >90% of that of the water used for the reaction could be attained. Tetrafluoroboric acid could also be used as catalyst, at the expense of a lower overall reaction yield. Byproducts from the reaction included dicholesteryl ether, methyl cholesteryl ether, compounds formed by ether hydrolysis, and olefins arising from elimination reactions. Reactions in tetrahydrofuran yielded significant amounts of cholesteryl ethers formed by reaction with alcohols arising from hydrolysis of the solvent.

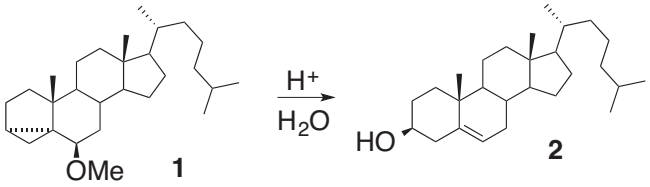
1. Introduction

Cholesterol is one of the most important natural sterols because of its role in modifying the properties of cell membranes (Róg and Vattulainen, 2014) and the link between *in vivo* transport of cholesterol in plasma lipoprotein fractions and disease states related to dietary intake of cholesterol (Birner-Gruenberger et al., 2014). Its presence modifies membrane fluidity (Fraenza et al., 2014; Sanderson, 2012) and rigidity (Dimova, 2014), and in mixtures of high and low melting lipids it is capable of inducing phase separation to form cholesterol-rich liquid ordered phases in the presence of more fluid disordered phases (Róg and Vattulainen, 2014; Simons and Vaz, 2004). The distribution of cholesterol within plasma membranes has consequently been subject to a great deal of investigation using a range of spectroscopic and analytical techniques. The membrane activity of cholesterol is intrinsically linked to its hydrocarbon structure. Minor perturbations to the structure of cholesterol, such as the addition of a chromophore to facilitate luminescence studies, frequently modify the behaviour of the sterol in an unpredictable manner. For example, the addition of an 7-nitro-2,1,3-benzoxadiazol-4-yl (NBD) group in the aliphatic hydrocarbon region produces a fluorescent analogue that preferentially partitions into the liquid disordered phase rather than the liquid ordered phase (Loura et al., 2001). It is therefore desirable to be able to label cholesterol with isotopic labels that are less intrusive than bulky chromophores and permit study by NMR or mass spectrometry methodologies. Isotopes of oxygen (^{17}O, ^{18}O) are potentially valuable for this purpose. The ^{17}O isotope, with spin 5/2, is suitable for NMR studies, particularly as it exhibits a wide chemical shift range of ~2000 ppm (Lemaître et al., 2004; Klemperer, 1978). However, with a natural abundance of 0.038% and low receptivity, isotopic enrichment is essential. The ^{18}O isotope is not directly observable by NMR, but does induce isotopic shifts in ^{13}C NMR spectra (Risley and Van Etten, 1979) that can be used analytically (Risley and Van Etten, 1989). Both isotopes also have utility as isotopic markers for mass spectrometry studies (Ye et al., 2009; Zyakun, 2011). A convenient route to cholesterol analogues has been described in the literature, involving acid-catalyzed reaction of the commercially available *i*-cholesteryl methyl ether (**1**) with water (Scheme 1).

This reaction has been known for some time and generally gives good yields of cholesterol (**2**) when water is present in large excess (Chu and Li, 2000; Riegel et al., 1946; Steele and Mosettig, 1963; Smith et al., 1993; Nicotra et al., 1981). It has been used for the preparation of ^{18}O-cholesterol (Wong et al., 1995). However, for the preparation of isotopically enriched cholesterol, particularly with a ^{17}O isotope, the use of a large excess of water is undesirable given the high cost of water enriched with oxygen isotopes (*e.g.* ^{17}O enriched water typically costs >$1000 per ml). Furthermore, in order to maximize isotopic enrichment, it is desirable to either use an acid catalyst without exchangeable oxygen, or if the

* Corresponding author. Fax: +44 1913844737.
E-mail address: j.m.sanderson@durham.ac.uk (J.M. Sanderson).

http://dx.doi.org/10.1016/j.chemphyslip.2015.12.003



Scheme 1. Acid-catalyzed reaction of *i*-cholesteryl methyl ether (**1**) with water to form cholesterol (**2**).

use of such a catalyst is unavoidable, minimize the amount of catalyst required. In this communication, we investigate the ability of different acids to catalyse the reaction, and establish conditions that use minimal amounts of water and catalyst.

2. Results and discussion

2.1. Choice of acid catalyst

Initial studies were conducted using **1** and non-enriched water in 1,4-dioxane using a selection of acid catalysts (Table 1). This solvent was chosen because of its miscibility with water and its good solvation properties for cholesterol. In addition, as an aprotic solvent it was not expected to participate in the reaction. All acid catalysts were investigated at a ratio of 5 mol% with respect to **1**. The acids investigated covered a range of pK_a values in organic solvents, including the superacids trifluoromethanesulfonic acid (pK_a = −11.4 in dichloroethane) and tetrafluoroboric acid (pK_a = −10.3 in dichloroethane) (Kütt et al., 2011). From the data it is apparent that trifluoromethanesulfonic acid produced by some margin the greatest yield of cholesterol, at the expense of having exchangeable oxygen. Tetrafluoroboric acid, on the other hand, gave a poor yield of cholesterol but, being a hydracid, did offer the potential for preparing cholesterol with an isotopic enrichment matching that of the water used for its preparation. It should be noted though, that in the reaction utilizing this acid, a significant quantity of **1** remained unreacted after 5 h, raising the possibility that a longer reaction time would increase the yield of cholesterol. In all cases, unreacted **1** was present, alongside a number of byproducts, the structures of which are given in Fig. 1.

Some of these byproducts, including dicholesteryl ether **3** and cholesteryl methyl ether **4** could be accounted for by reaction of **1** with other nucleophiles formed during the progress of the reaction. Alcohol **5** was formed by a competing nucleophilic displacement of methanol from **1**. The other products, **6** and **7**, could be accounted for by elimination reactions of either cholesterol, **3** or **4** (for **6**) and **1** or **5** (for **7**).

2.2. Optimization of reaction conditions

On the basis of this preliminary work, further experiments were conducted to investigate the effects on cholesterol yield of

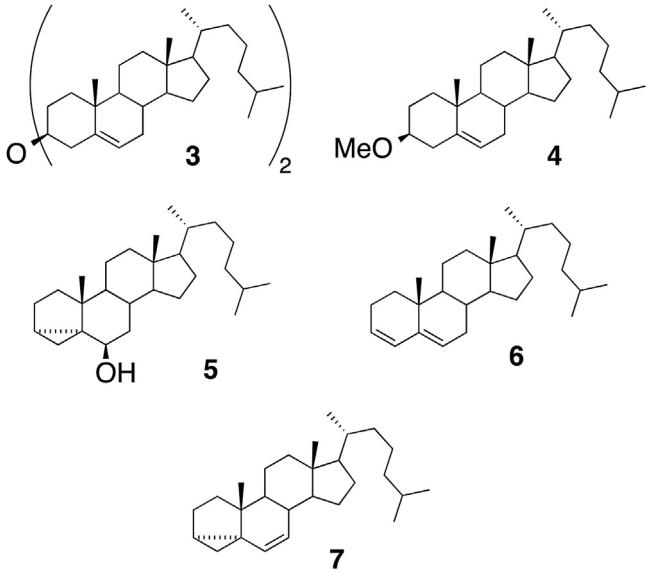


Fig. 1. Byproducts from hydrolysis reactions of **1** in 1,4-dioxane.

temperature and the ratios of trifluoromethanesulfonic acid catalyst and water to ether **1**. These outcomes of these experiments are summarised in Table 2 and Fig. 2. The best yield of cholesterol was found with 0.05 equivalents of acid and 5 equivalents of water at 20 °C (entry 8). Increasing the number of equivalents of water beyond 5 did not lead to significant benefits, either in overall yield (entry 11) or in the ratio of cholesterol to other products (entry 9). Although in the latter case the reaction had not gone to completion within 5 h, the major product after this time was alcohol **5** (full details are in the ESI). Increasing the number of equivalents of acid catalyst led to a significant increase in the formation of byproducts, particularly methyl ether **4**, which formed >20% of the products when 1 equivalent of acid was used. Decreasing the amount of acid below 0.05 equivalents gave incomplete reactions after 5 h at 20 °C, but in all cases cholesterol was not the major product after this time and would not have given cholesterol yields greater than entry 8 had they been allowed to go to completion.

When the mole fraction of cholesterol in the products is analysed in relation to the ratio of acid to water (Fig. 2) in the reaction, the conditions corresponding to entries 7 and 8 in Table 2, with an acid/water ratio of 0.01, are found to be optimal for increasing the proportion of cholesterol in the mixture. At lower acid to water ratios, the predominant byproduct is the alcohol **3** resulting from hydrolysis of ether **1**. At higher ratios, the major byproduct becomes methyl ether **4**.

On the basis of the optimal conditions, and assuming complete exchange between the ^{16}O isotope of the catalyst and the ^{18}O isotope of the enriched water, the maximum isotopic enrichment of cholesterol theoretically attainable (not allowing

Table 1
The influence of acid catalyst on the formation of cholesterol from **1** in the presence of water.[a]

Acid	Yield cholesterol (%)[b]	Byproducts[c]
CF_3SO_3H	73 (61)	**3** (9%), **4** (9%), **5** (trace), **6** (trace), other (9%) + 1 (trace)
p-TsOH	trace	**3**,**4**, **5** (all trace) + **1** (>99%)
CH_3SO_3H	1	**3** (trace), **4** (2%), **5** (2%), **7** (1%), other (1%) + **1** (93%)
HBF_4	18 (16)	**3** (1%), **4** (3%), **5** (12%), **6** (1%), **7** (trace), other (10%) + **1** (55%)
HCl	7 (6)	**3** (1%), **4** (2%), **5** (9%), **6** (trace), **7** (1%), other (1%) + **1** (79%)

[a] Conditions in all cases: 5 eq. water, 0.05 eq. acid, 1,4-dioxane, 80 °C, 5 h.
[b] Yields are calculated from analysis of crude NMR spectra. Figures in parentheses indicate isolated yields.
[c] Yields are calculated from analysis of crude NMR spectra. Trace products were detectable by thin layer chromatography but not ^1H NMR. The relative yields of 'other' products were determined on the basis of 3-H signals or pairs of olefinic signals that could not be attributed to any of **1**–**7**.

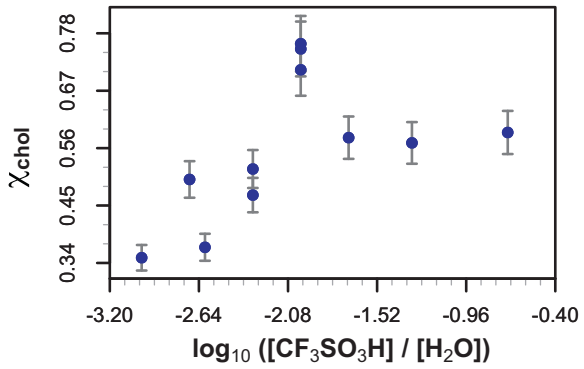


Fig. 2. Mole fraction of cholesterol in the steroid products of reactions in 1,4-dioxane with CF_3SO_3H as catalyst in response to changes in the ratio of acid to water.

for residual water in the catalyst and solvent) would be 97.1% of that of the original water, which is acceptable.

Tetrahydrofuran (THF) was investigated as an alternative solvent to 1,4-dioxane and good yields of cholesterol could be obtained in this solvent. However, in all cases, products arising *via* lysis of the solvent were significant byproducts, with compounds **8**–**10** (Fig. 3) typically forming 20–50% of the reaction products.

Further experiments were not conducted in THF and we would not recommend using this solvent as first choice for reactions that are not conducted in the presence of a large excess of water. For reactions in 1,4-dioxane, products equivalent to those in Fig. 3 were present in some cases (as judged by NMR), but were not isolated in sufficient quantity to characterise and always represented less than 3% of the reaction products.

2.3. Synthesis of labelled cholesterol

Having determined suitable reaction conditions for the preparation of cholesterol labelled with oxygen isotopes, the final step was to verify that yields and isotopic enrichment were as expected. Reactions were therefore performed with **1** and $^{18}OH_2$ (95.7 atom% ^{18}O) in two sets of conditions, with either trifluoromethanesulfonic acid or tetrafluoroboric acid as catalyst. The latter was included to examine the maximum achievable isotopic enrichment achievable:

1. **1** + 0.05 eq. CF_3SO_3H + 5 eq. $^{18}OH_2$ at 20 °C for 5 h; and

Table 2
Formation of cholesterol from **1** using trifluoromethanesulfonic acid as catalyst in 1,4-dioxane.

Entry	T (°C)	Equiv. CF_3SO_3H[a]	Equiv. water[b]	Yield cholesterol (%)[c]	χ_{chol}[d]
1	20	0.005	2	9	0.37
2	20	0.005	5	4	0.35
3	20	0.01	2	30	0.47
4	80	0.01	2	52	0.52
5	20	0.01	5	21	0.50
6	20	0.05	2.5	19	0.58
7	80	0.05	5	73	0.73
8	20	0.05	5	76	0.76
9	20	0.05	20	7	0.27
10	20	1	5	59	0.59
11	20	1	20	57	0.57

[a] Equivalents relative to **1**.
[b] Yields are calculated from analysis of NMR spectra of the crude reaction products.
[c] Cholesterol mole fraction in the steroid products (i.e. excluding **1**). Errors are ±7%.

2. **1** + 0.05 eq. HBF_4 + 5 eq. $^{18}OH_2$ at 80 °C for 40 h.

These gave isolated yields of 62% and 40% respectively. Following purification, the isotopic enrichment of the cholesterol was determined by ^{13}C NMR spectroscopy (Fig. 4).

The inclusion of the ^{18}O induced a readily detectable isotopic shift of the 3-C ^{13}C resonance of 17 Hz. Isotopic enrichment was determined by the relative peak areas for the ^{13}C signals of the ^{16}O- and ^{18}O-isotopomers to be 90.1 ± 1.9 atom % with trifluoromethanesulfonic acid as catalyst, and 90.9 ± 1.9 atom % with tetrafluoroboric acid as catalyst. These isotopic enrichments are lower than the theoretical maximum achievable of 92.9 ± 0.04 atom % when consideration is given to the exchangeable atoms of the catalysts and the isotopic enrichment of the water. The differences are accounted for by residual water in the solvent and acid catalysts. Using the same procedure, ^{17}O-cholesterol was also prepared using $^{17}OH_2$.

3. Conclusions

From the data presented above, we conclude that 1,4-dioxane is the best solvent for the preparation of isotopically enriched cholesterol. For maximum recovery of cholesterol where a small dilution of the ^{17}O or ^{18}O isotope is acceptable, trifluoromethanesulfonic acid is the optimum choice of acid catalyst. In order to obtain an acceptable yield of product, it should not be necessary to use more than 5 equivalents of enriched water relative to *i*-cholesteryl methyl ether.

4. Experimental procedures

4.1. General methods

Dry solvents were sourced from Fisher Scientific (Loughborough, UK; THF) and Sigma–Aldrich (Dorset, UK; 1,4-dioxane; ≤0.003% H_2O manufacturers specification, measured as 0.007% by a Karl–Fischer titration at the time of use). Cholesterol, *i*-cholesteryl methyl ether (95%), 4 M HCl in dioxane, tetrafluoroboric acid diethyl ether complex and the rest of the acids were obtained from Sigma–Aldrich (Dorset, UK). Isotopically enriched water was obtained from Cortecnet (Voisins-Le-Bretonneux, France). Isotopic enrichments were 95.7 atom % for $^{18}OH_2$ (our measurement, lot number 139808A-P) and 41.1% for $^{17}OH_2$ (manufacturers specification, lot number 1040171A-P). Stock solutions of acids in THF or 1,4-dioxane were prepared immediately before use. Purification was performed by flash column chromatography using a silica gel support (230–400 mesh, 40–60 μm) from Sigma–Aldrich (UK). NMR data were collected on a Bruker Avance-400 (at 400 MHz for ^1H; 100.6 MHz for ^{13}C) or Varian VNMRS (at 700 MHz for ^1H; 176 MHz for ^{13}C). NMR spectra were obtained in $CDCl_3$ and are reported in ppm using residual $CHCl_3$ at 7.26 ppm as the internal reference. ^{13}C NMR spectra were referenced to solvent as internal reference (77.23 ppm for $CDCl_3$). Atmospheric solids analysis probe (ASAP) mass spectrometry was performed on a Xevo QToF mass spectrometer (Waters Ltd, Wilmslow, UK) equipped with an Agilent 7890 GC gas

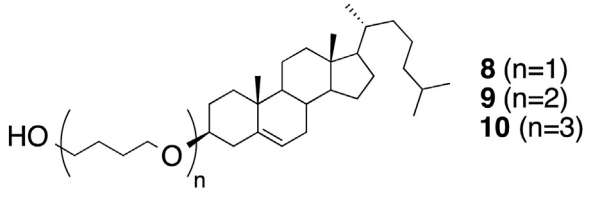


Fig. 3. Major products from reactions of **1** in THF.

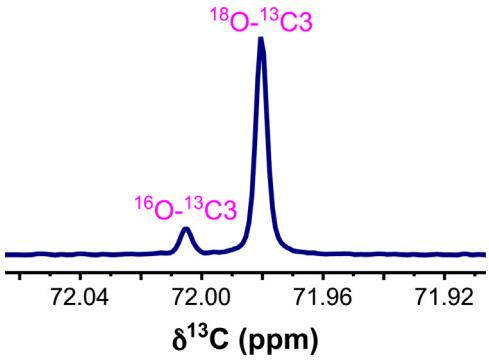


Fig. 4. The region of the ^{13}C NMR spectrum (176 MHz, $CDCl_3$) corresponding to the 3-carbon of ^{18}O-cholesterol prepared by Method 1.

chromatography apparatus (Agilent Technologies UK Ltd, Stockport, UK) at 450 °C. Full details are presented in the supporting information.

4.2. Optimised experimental procedure in 1,4-dioxane

A 12.5 mM solution of the acid in dry 1,4-dioxane (1 ml, 12 μmol) was added to a solution of **1** (0.10 g, 0.25 mmol), and water (22 μl, 1.2 mmol) in 1,4-dioxane (4 ml) and the solution stirred for 5 h at 20 °C. After concentrating to ~1 ml *in vacuo*, CH_2Cl_2 (20 ml) was added and the organic solution washed with water (2 × 10 ml). The organic solution was dried ($MgSO_4$), filtered and concentrated *in vacuo*. Purification was by flash column chromatography on silica using a gradient of CH_2Cl_2/hexane, 1:1 to CH_2Cl_2.

The procedure when using HBF_4 as acid catalyst followed the same procedure, except that the solution was heated at 80 °C for 40 h.

4.3. Synthesis of ^{17}O-cholesterol

Using the optimised procedure with CF_3SO_3H as the acid yielded the title compound (0.069 g, 67%) as a white solid.

^1H NMR (400 MHz, $CDCl_3$) δ (ppm): 0.68 (s, 3H, 18-H), 0.86 (dd, J = 6.6 and 1.8 Hz, 6H, 26-H/27-H), 0.91 (d, J = 6.5 Hz, 3H, 21-H), 0.94–1.68 (m, 24H), 1.77–1.88 (m, 4H), 1.92–2.04 (m, 2H), 2.17–2.34 (m, 2H), 3.46–3.58 (m, 1H, 3α-H), 5.33–5.37 (m, 1H, 6-H).

^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm):

MS (ASAP) m/z (%): 388.4 (8) [^{17}O–M + H]$^+$, 387.4 (8.5) [^{17}O–M$^{+\bullet}$] and [^{16}O–M + H]$^+$, 386.4 (6) [M$^{+\bullet}$, ^{16}O], 385.4 (2), 369.4 (100).

4.4. Synthesis of ^{18}O-cholesterol

Using the optimised procedure with CF_3SO_3H as the acid and **1** (0.075 g) yielded the title compound (0.047 g, 62%) as a white solid.

^1H NMR (400 MHz, $CDCl_3$) δ (ppm): 0.68 (s, 3H, 18-H), 0.86 (dd, J = 6.6 and 1.8 Hz, 6H, 26-H/27-H), 0.91 (d, J = 6.5 Hz, 3H, 21-H), 0.93–1.63 (m, 24H), 1.77–1.88 (m, 4H), 1.92–2.04 (m, 2H), 2.18–2.33 (m, 2H), 3.48–3.57 (m, 1H, 3α-H), 5.32–5.38 (m, 1H, 6-H).

^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 12.08, 18.94, 19.61, 21.31, 22.78, 23.04, 24.05, 24.51, 28.23, 28.45, 31.88, 32.12, 32.13, 36.00, 36.41, 36.72, 37.48, 39.74, 40.00, 42.52, 42.54, 50.35, 56.38, 56.98, 71.98 (3-C; ^{18}O), 72.00 (3-C; ^{16}O), 121.91, 140.97.

MS (ASAP) m/z (%): 389.4 (5.5) [^{18}O-M + H]$^+$, 388.4 (10) [^{18}O-M$^{+\bullet}$], 387.4 (6) [^{16}O–M + H]$^+$, 386.4 (4) [M$^{+\bullet}$, ^{16}O], 385.4 (10), 383.4 (8.5), 369.4 (100).

Conflicts of interest

All third-party financial support for the work in the submitted manuscript: nothing to disclose.

All financial relationships with any entities that could be viewed as relevant to the general area of the submitted manuscript: nothing to disclose.

All sources of revenue with relevance to the submitted work who made payments to you, or to your institution on your behalf, in the 36 months prior to submission: nothing to disclose.

Any other interactions with the sponsor of outside of the submitted work should also be reported: nothing to disclose.

Any relevant patents or copyrights (planned, pending, or issued): nothing to disclose.

Any other relationships or affiliations that may be perceived by readers to have influenced, or give the appearance of potentially influencing, what you wrote in the submitted work: nothing to disclose.

Acknowledgements

We thank the EPSRC (EP/J017566/1) for support and Dr. Darren R. Gröcke for assistance with water isotopic enrichment analyses.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.chemphyslip.2015.12.003.

References

Birner-Gruenberger, R., Schittmayer, M., Holzer, M., Marsche, G., 2014. Understanding high-density lipoprotein function in disease: recent advances in proteomics unravel the complexity of its composition and biology. Prog. Lipid Res. 56, 36–46. doi:http://dx.doi.org/10.1016/j.plipres.2014.07.003.

Chu, G.H., Li, P.K., 2000. Synthesis of [25,26, 26, 26, 27, 27, 27-d_7]-Cholesterol. J. Labelled Cpd. Radiopharm. 43, 773–779. doi:http://dx.doi.org/10.1002/1099-1344(200007) 43:8<773:aid-jlcr361>3.0. co;2-g.

Dimova, R., 2014. Recent developments in the field of bending rigidity measurements on membranes. Adv. Colloid Interface Sci. 208, 225–234. doi:http://dx.doi.org/10.1016/j.cis.2014.03.003.

Fraenza, C.C., Meledandri, C.J., Anoardo, E., Brougham, D.F., 2014. The effect of cholesterol on membrane dynamics on different timescales in lipid bilayers from fast field-cycling NMR relaxometry studies of unilamellar vesicles. ChemPhysChem 15, 425–435. doi:http://dx.doi.org/10.1002/cphc.201301051.

Klemperer, W.G., 1978. ^{17}O-NMR spectroscopy as a structural probe. Angew. Chem. Int. Ed. Engl. 17, 246–254. doi:http://dx.doi.org/10.1002/anie.197802461.

Kütt, A., Rodima, T., Saame, J., Raamat, E., Mäemets, V., Kaljurand, I., Koppel, I.A., Garlyauskayte, R.Y., Yagupolskii, Y.L., Yagupolskii, L.M., Bernhardt, E., Willner, H., Leito, I., 2011. Equilibrium acidities of superacids. J. Org. Chem. 76, 391–395. doi:http://dx.doi.org/10.1021/jo101409p.

Lemaître, V., Smith, M.E., Watts, A., 2004. A review of oxygen-17 solid-state NMR of organic materials-towards biological applications. Solid State Nucl. Magn. Reson. 26, 215–235. doi:http://dx.doi.org/10.1016/j.ssnmr.2004.04.004.

Loura, L.M., Fedorov, A., Prieto, M., 2001. Exclusion of a cholesterol analog from the cholesterol-rich phase in model membranes. Biochim. Biophys. Acta 1511, 236–243. doi:http://dx.doi.org/10.1016/s0005-2736(01) 00269-3.

Nicotra, F., Ronchetti, F., Russo, G., Lugaro, G., Casellato, M., 1981. Stereochemical fate of the isopropylidene methyl groups of lanosterol during the biosynthesis of isofucosterol in *Pinus pinea*. J. Chem. Soc. Perkin Trans. 1 498. doi:http://dx.doi.org/10.1039/p19810000498.

Riegel, B., Hager, G.P., Zenitz, B.L., 1946. Δ^6-*i*-Cholestadiene. J. Am. Chem. Soc. 68, 2562–2564. doi:http://dx.doi.org/10.1021/ja01216a041.

Risley, J.M., Van Etten, R.L., 1979. An oxygen-18 isotope shift upon carbon-13 NMR spectra and its application to the study of oxygen exchange kinetics. J. Am. Chem. Soc. 101, 252–253. doi:http://dx.doi.org/10.1021/ja00495a059.

Risley, J.M., Van Etten, R.L., 1989. Mechanistic studies utilizing oxygen-18 analyzed by carbon-13 and nitrogen-15 nuclear magnetic resonance spectroscopy. Methods Enzymol. 177, 376–389. doi:http://dx.doi.org/10.1016/0076-6879(89) 77021-x.

Róg, T., Vattulainen, I., 2014. Cholesterol, sphingolipids, and glycolipids: what do we know about their role in raft-like membranes? Chem. Phys. Lipids 184, 82–104. doi:http://dx.doi.org/10.1016/j.chemphyslip.2014.10.004.

Sanderson, J.M., 2012. Resolving the kinetics of lipid, protein and peptide diffusion in membranes. Mol. Membr. Biol. 29, 118–143. doi:http://dx.doi.org/10.3109/09687688.2012.678018.

Simons, K., Vaz, W.L.C., 2004. Model systems, lipid rafts, and cell membranes. Annu. Rev. Biophys. Biomol. Struct. 33, 269–295. doi:http://dx.doi.org/10.1146/annurev.biophys.32.110601.141803.

Smith, L.L., Herz, J.E., Ezell, E.L., 1993. ^{17}O nuclear magnetic resonance spectra of steroids. Steroids 58, 260–267.

Steele, J.A., Mosettig, E., 1963. The solvolysis of stigmasteryl tosylate. J. Org. Chem. 28, 571–572. doi:http://dx.doi.org/10.1021/jo01037a505.

Wong, W.W., Hachey, D.L., Clarke, L.L., Zhang, S., 1995. Cholesterol synthesis and absorption by $^{2}H_2O$ and ^{18}O-cholesterol and hypocholesterolemic effect of soy protein. J. Nutr. 125, S612–S618.

Ye, X., Luke, B., Andresson, T., Blonder, J., 2009. ^{18}O stable isotope labeling in MS-based proteomics. Brief. Funct. Genomic Proteomic 8, 136–144. doi:http://dx.doi.org/10.1093/bfgp/eln055.

Zyakun, A.M., 2011. Isotopologs and isotopomers: new analytical aspects of isotopic mass spectrometry in biology. J. Anal. Chem. 66, 1243–1252. doi:http://dx.doi.org/10.1134/s1061934811130119.