PREPARATION OF 2,2,2($^2H_3$)-DIAZOETHANE

Claus O.Meese
Fischer-Bosch-Institute of Clinical Pharmacology
Auerbachstraße 112, D-7000 Stuttgart 50 (FRG)

SUMMARY

2,2,2($^2H_3$) Ethyl-N-nitroso-p-toluenesulfonamide 6a, prepared in three steps from trideuteroacetonitrile 2a (overall yield 71%), has been subjected to alkaline decomposition to give after co-distillation with ether/n-hexane solutions of 2,2,2($^2H_3$)-diazoethane 1a. As shown by quantitative studies and from NMR and mass spectra, respectively, carboxylic acids were completely converted by 1a under mild reaction conditions into highly labelled (>96% d_3, <0.3% d_0) 2,2,2($^2H_3$)-ethyl esters. The ready preparation of 1($^2H_{1,2}$)-ethyl 4-nitrobenzoate 15 from 9 and unlabelled 1b under H/D exchange conditions illustrates the possibility of further introduction of ^2H or ^3H into an ethyl group. The molar extinction coefficient of 1a, b has been determined (ε(470 nm)=8.4).

Key Words: deuterated diazoethane, ethyl esters (d_x), H/D exchange

INTRODUCTION

The most efficient reagent for the smooth and quantitative conversion of a carboxylic acid into its methyl ester, even at low temperature, is diazomethane (1-4). However, the analogous preparation of trideuteromethyl esters, useful as stable isotope labelled internal standards in the GC/MS trace analysis of organic acids, from dideuterodiazomethane under H/D exchange conditions is less convenient since the appropriate labelled precursor is not readily available, a great excess of highly labelled deuterated solvents has to be applied for its preparation, the d_3-methyl esters obtained this way show considerable scrambling, and no further introduction of deuterium or tritium into the alcoholic part of the labelled ester is possible (5-8). To obtain a general and ready access to a variety of deuterated esters, a new synthetic route to hitherto unknown 2,2,2($^2H_3$)-diazoethane (1a) was elaborated.

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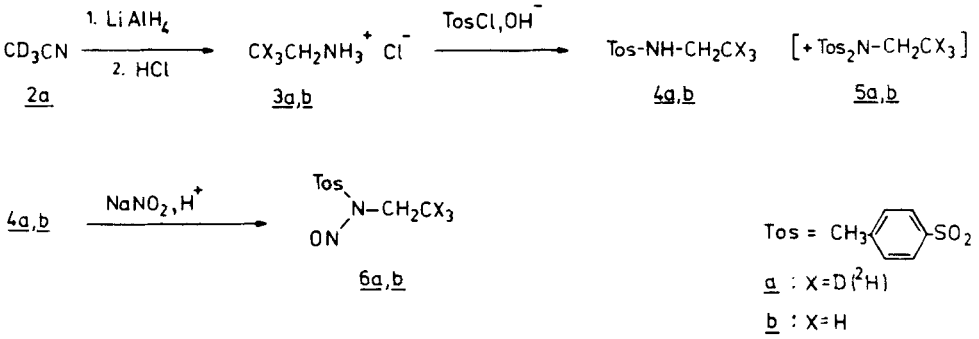
RESULTS AND DISCUSSION

Solutions of unlabelled diazoethane (1b) have been prepared in satisfactory yields from N-ethyl-N-nitrosourethane (9), N-ethyl-N-nitrosourea(10,11), N-ethyl-N-nitroso-N'-nitroguanidine (12), and N-nitroso-N-ethylaminoisobutyl methyl ketone (13). All these precursors are prepared in fair to moderate yields, but are thermically instable, in part skin irritating, potentially carcinogenic, and are therefore inconvenient substrates for preparations of d_3-diazoethane 1a.

Since good results have been reported for a number of different diazoalkanes which have been generated from more stable and less toxic N-alkyl-N-nitroso-p-toluenesulfonamides (14-18), it seemed promising to utilize the N-ethyl analogues 6a and 6b (19) for the synthesis of 1a and 1b (Scheme 1).

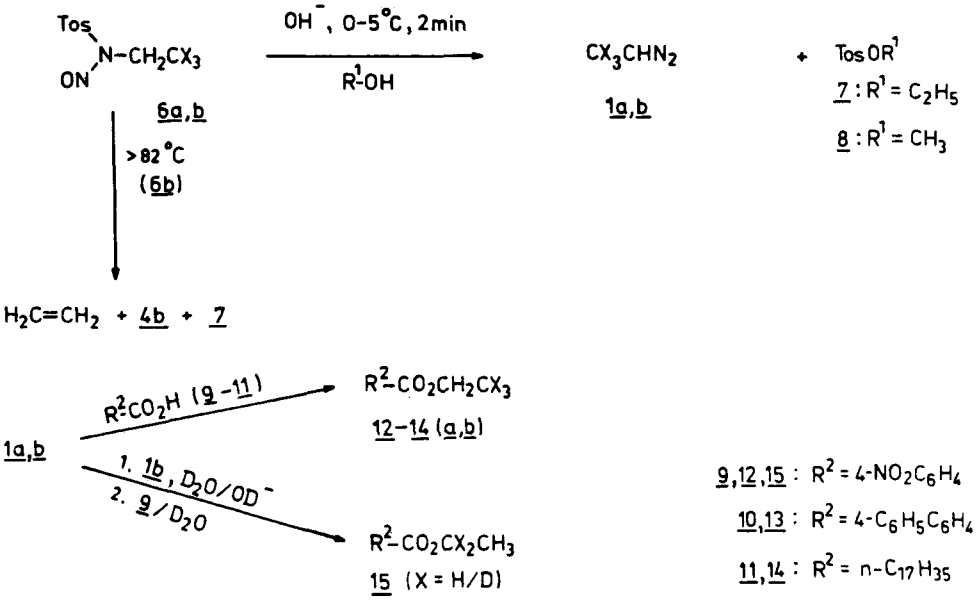


Scheme 1: Synthesis of diazoethane precursors 6a and 6b.

The desired precursor 6a was conveniently prepared in excellent yields by reduction of trideuteroacetonitrile 2a with $LiAlH_4$ ((20),82%), tosylation of the labelled amine (92%), and nitrosation of the sulfonamide 4a (94%). The formation of the bis-tosylate 5 may be completely suppressed if optimum reaction conditions are applied.

Although 6 represents a close homologue of N-methyl-N-nitroso-p-toluenesulfonamide, the most widely used substrate for the generation of diazomethane (14,15), surprisingly

low yields of 1 were reached (1-5%) when analogous reaction conditions (50-70°C, alkaline alcoholic solution, distillation) were employed. A closer investigation of the stability of 6b revealed that rapid denitrosation takes place at elevated temperatures (> 44°C). If neat 6b is heated at 82-85°C a violent decomposition occurs, traces of ethylene may be trapped, and the residue mainly consists of ethyl p-toluenesulfonate 7 and 4b (Scheme 2).



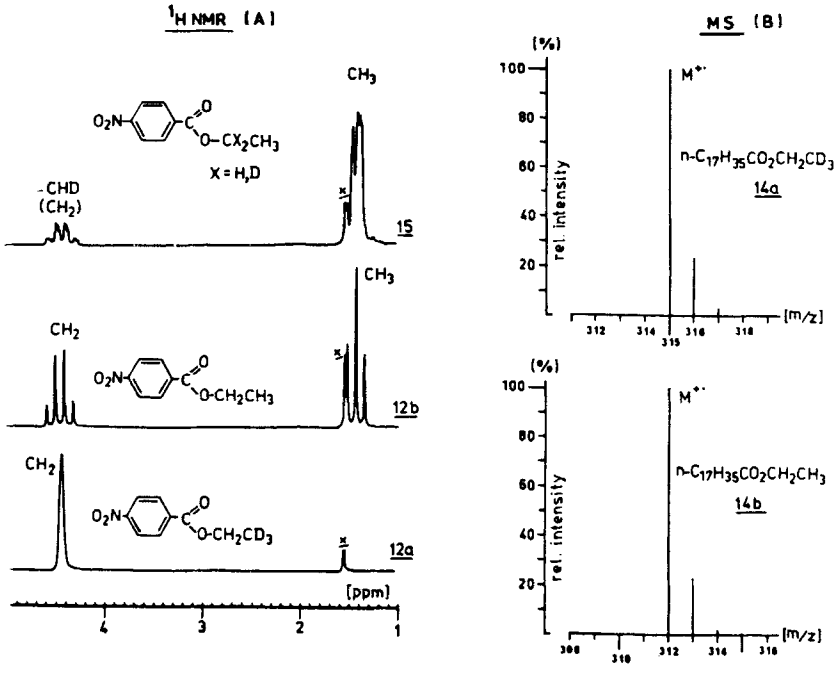
Scheme 2: Reactions of 6 and 1 (a: X=D, b: X=H).

These findings are in accordance with previous reports on the reactivity of N-alkyl-N-nitroso-p-toluenesulfonamides (19, 21-23) and explain the low yield in this reaction. On the contrary, satisfactory yields of 1 may be reached if the alkaline decomposition of 6 is carried out within several minutes at low temperature and in the presence of alcohols (R^1-OH, Scheme 2). The organic extracts of these reaction mixtures contain up to 60% of desired 1. Although these solutions may be suitable for the esterification of organic acids, considerable amounts of unwanted tosylates 7 or 8 were co-extracted. Since these esters may interfere in a subsequent analytical derivatization step (e.g. pyridine rapidly converts 7, 8 into the corresponding N-alkyl-pyridinium tosylates), the ethereal co-distillation of the crude n-hexane extracts which proceeds without substantial loss of diazoethane 1a or 1b, is recommended.

The determination of the content of (d_3) diazoethane in these solutions was performed in three ways: 1) direct acidimetric titration (24,25), 2) esterification of excess organic acids and quantitative isolation of the ethyl esters formed, and 3) esterification of organic acids and ^{1}H NMR quantification in the presence of known amounts of an internal standard. The results of these three methods were in good agreement and allow the calculation of the molar extinction coefficient of 1a and 1b (identical values of ε=8.4 ($\pm$ 0.5) at λ_{max}470nm) (26)). Thus, the spectrophotometric method presents the most rapid and convenient way of measuring the concentration of diazoethane in these preparations.

Ethereal solutons of diazoethane 1 are stable for several weeks if stored at -80°C, and no insoluble polymeric methyl-methylenes beeing detected. However, at ambient temperature these samples show a steady decrease in concentration (5-10% in 5 hr) which is enhanced in the presence of alcohols. Pure solutions of 1 convert organic carboxylic acids (e.g. 9-11) in quantitative yield into the corresponding chemically and isotopically pure ethyl esters (Scheme 2, Fig.1). Thin layer chromatography, ^{1}H , ^{13}C NMR spectroscopy, and mass spectrometry failed to reveal the presence of significant amount of by-products.

Figure 1: (A) Partial ^{1}H NMR spectra ($CDCl_3$,x:trace of H_2O) and (B) partial mass spectra (70 eV,$M^{+\cdot}$ region) of labelled and unlabelled ethyl esters 12a, b, 15, and 14a, b.



The complete retention of deuterium throughout all synthetic steps may be judged from the ^1H NMR spectra and mass spectra in the significant ranges and in comparison with the unlabelled ethyl esters (Fig.1).

To examine the possibility of the introduction of tritium or further deuterium in the course of the preparation of (deuterium labelled) ethyl esters, 1b was subjected to H/D exchange (D_2O,OD^-)in a model experiment and then allowed to react with 4-nitrobenzoic acid-d_1 (from 9 preequilibrated with D_2O) (Scheme 2). The ester 15 obtained this way showed conversion to 47% CD_2, 49% CHD, and 4% CH_2 by mass spectrometry.

In conclusion, 6a, readily prepared from trideuteroacetronitrile 2a, provides a convenient source of highly labelled d_3-diazoethane 1a. This reagent 1a allows the simple and effectice preparation of deuterated ethyl esters and permits the further introduction of ^2H or ^3H into the methylene group of the ester.

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EXPERIMENTAL SECTION

Materials: Trideuteroacetonitrile (Fluka, > 99 atom% D), toluene-4-sulfochloride (Fluka, >99%), dioxane (E.Merck, < 0.01% H_2O), lithium aluminium hydride (E.Merck, powdered, 95%), deuterium oxide (E.Merck, 99.75%), sodium deuteroxide (Aldrich, 40% soln. in D_2O,99 atom% D).

TLC: SiO_2 60-F_{254} (E.Merck, "Fertigplatten"); solvent system (I): EtOAc/n-hexane (2:3), solvent system (II): EtOAc/n-hexane (1:5). -Melting points, mp, uncorrected (Electrothermal). - IR (KBr/film, cm^{-1}): Perkin-Elmer 283, only intense or characteristic absorptions are reported. - NMR (80 MHz/^1H,20 MHz/^{13}C-proton decoupled): Bruker WP 80, 39°C, int. stand. TMS($CDCl_3$) or 3-(trimethylsilyl) propionic acid-d_4 (D_2O), shifts in ppm (δ-scale). - UV/VIS: Zeiss PMQ 3 and Perkin-Elmer 330, λ in nm (ε), d=1 cm. - MS (70 eV,EI): Hewlett-Packard 5985, fragments reported as m/z (rel. abund., %).

2,2,2($^2H_3$) -Ethylamine hydrochloride, 3a

A suspension of lithium aluminium hydride (5.9g, 0.15 mol) in 200 ml of dry diethyl ether (freshly distilled from $LiAlH_4$) was stirred for 20 min under argon atmosphere. Then the

soln. was cooled to 0-5°C and 2a (CD_3CN, 5.2 ml, 4.4g, 0.1 mol, dried over $CaCl_2$) was added over a period of 2 hr. The reaction mixture was stirred for a further 15 min and then successively quenched at the same temp. during 3 hr with the dropwise addition of H_2O (6 ml), 20% aq. NaOH (4.5 ml), and H_2O (21 ml). Volatile amine and most of the ether were distilled into a receiving flask charged with 200 ml of rapidly stirred 1n HCl (0°C). 200 ml of Et_2O were added to the white, granular inorganic residue and distillation was continued until the distilling Et_2O was neutral (ca. 3 x 200 ml). The ethereal layer from the receiver was discarded, the aqueous phase was evaporated to dryness on a rotary evaporator, redissolved in dry EtOH (200 ml), concentrated under vacuum, and recrystallized from hot dry EtOH under the addition of ethyl acetate. Filtration and drying (P_2O_5) afforded pure 3a (6.9g, 82%), mp 99-107°C (fused capillary), as colourless, highly hygroscopic plates.

Anal. Calc. for $C_2H_5D_3ClN$ (84.5): C 28.41, (H+D) 13.10, Cl 41.93, N 16.56.
Found: C 28.21, (H+D) 13.09, Cl 41.82, N 16.81. - ^{1}H NMR (D_2O): 3.08 (broad s, CH_2), 4.8 (HOD); $d_o+d_1+d_2$ <1.5% by integration at 1.27 ppm (CH_3 resonance of 3b). - ^{13}C NMR (D_2O): 37.7(CH_2), 3b: 14.6(CH_3), 37.7(CH_2). - IR: 3050 (broad), 1606, 1483, 1469, 1163, 914.

N-2,2,2($^2H_3$) Ethyl-p-toluenesulfonamide, 4a

A cold soln. of NaOH (136.4g, 3.4 mol) in water (300 ml) was added slowly to a stirred soln. of 3a (59.4 g, 0.7 mol) in the same solvent (150 ml, 0-5°C). During 6.5 hr a soln. of p-tolylsulfonylchloride (143 g, 0.75 mol) in 200 ml of dry 1,4-dioxane was added. The reaction mixture was stirred for additional 17 hr (~4°C) and then heated at 50-60°C in a water bath (30 min). Removal of the organic solvent under reduced pressure and addition of water (1000 ml) gave a clear soln. from which after acidification to pH 1-2 (20% HCl) cryst. sulfonamide 4a precipitated. The solid product was filtered off, thoroughly washed with water, and then dried (P_2O_5), yield 131.2 g (92.5%), mp 64°C (4b : mp 62.5-63.5 (19)). Three recrystallizations from boiling n-hexane did not change the mp. As judged from TLC (R_f 0.54(I), 0.15(II)) and its analytical and spectral properties, this procedure gave pure 4a free of di-tosylate 5a (mp 114-6°C, TLC: R_f 0.73(I)).

Anal. Calc. for $C_9H_{10}D_3NO_2S$ (202.3): C 53.44, (H+D) 7.97, N 6.92, S 15.85.
Found: C 53.42, (H+D) 7.80, N 6.92, S 16.03. - ^{1}H-NMR ($CDCl_3$): 2.43 (s,3H,aryl-CH_3), 3.00 (d,J=5.8 Hz, 2H, CH_2), 4.28 (t, J=5.8 Hz, 1H, NH), 7.32 and 7.77 (d each, J=8.5 Hz, 4H, aryl-H), $d_o+d_1+d_2$ < 1% by integration at 1.10 ppm (CH_3 CH_2-resonance of 4b). ^{13}C-NMR ($CDCl_3$): 21.5 (aryl - CH_3), 38.2 (CH_2); 127.0, 130.0, 137.5, 143.7 (aryl). No resonance signal was observed at 15.0 ppm (CH_3CH_2 of 4b). -
IR: 3280, 2234, 1602, 1428, 1323, 1312, 1164, 1090, 900, 812.-
MS: 202 (49,$M^{+\cdot}$), 184(78,$M^{+\cdot}$-CD_3), 155(80,$M^{+\cdot}$-$NHCH_2CD_3$), 91(100,C_7H_7), d_3 > 96%, d_o < 0.2% (by multiple ion detection, MID).

N-2,2,2($^2H_3$) Ethyl-N-nitroso-p-toluenesulfonamide, 6a

To a stirred soln. of 4a (8.2 g, 40.6 mmol) in 96% acetic acid (80 ml) and water (8 ml) was added at 0°C, over 30 min, a soln. of $NaNO_2$ (5 g) in water (10 ml). Stirring was continued for 1 hr and then the reaction mixture was slowly diluted (30 min) with 450 ml of cold water. The resulting yellow precipitate was filtered off, repeatedly washed with water, and dried over P_2O_5 in the dark. This afforded 8.9 g (94%) of pure 6a, mp 42-44°C. A sample recryst. from EtOAc/n-hexane had the same mp(6b: mp 42-43.5°C(19)).
TLC: R_f 0.68(I), 0.39 (II)- Anal., Calc. for $C_9H_9D_3N_2O_3S$ (231.3): C 46.74, (H + D) 6.54, N 12.11, S 13.86. Found: C 46.78, (H + D) 6.67, N 12.04, S 13.97. -
^{1}H-NMR ($CDCl_3$): 2.46(s, 3H, aryl-CH_3), 3.76 (broad s, 2H, CH_2), 7.38 and 7.91 (d each, J=8.3 Hz, 4H, aryl-H), $d_o+d_1+d_2$ < 0.6% by integration at 1.0 ppm (CH_3CH_3-resonance of 6b).- ^{13}C-NMR ($CDCl_3$): 12.2 (very weak quint., J=19.5 Hz, CD_3), 21.7 (aryl-CH_3), 38.4 (NCH_2); 128.3, 130.6, 135.5, 146.3 (aryl). No signal occured at 12.8 ppm (CH_3CH_2 of 6b).-
IR: 2242, 1601, 1510, 1383, 1198, 1180, 1156, 1086, 848, 663.-
UV/VIS ($5.57\cdot10^{-3}$ m/n-hexane): 412 (793), 395 (883), 380 (591), ~366, shoulder (~1.8); superimposable with 6b.-

When stored in the dark and under refrigeration (4°C), pure cryst. 6a (as well as 6b) showed no decomposition after six months. Solutions (EtOAc, $CDCl_3$) of 6 are stable at ambient temp. for at least 48 hr if kept in the dark but reveal the slow formation of 4

when exposed to light. When neat 6b is heated above its melting point, a rapid denitrosation takes place. Above 82°C the decomposition becomes violent and ethylene may be identified by ^{1}H-NMR when the escaping gases are trapped in cold $CDCl_3$ (singlett at 5.4 ppm which disappeared if argon gas is bubbled through this soln. for a few minutes). Besides some insoluble material the residue mainly consists of a 1:1 mixture of 4b and 7 (ethyl p-toluenesulfonate 7 is isolated as an oil by preparative layer chromatography (SiO_2-60, R_f 0.32 (II), identical with an authentic sample).

2,2,2($^2H_3$) - Diazoethane, 1a

CAUTION: Prior to synthetic work reviews on the chemistry of diazoalkanes should be consulted, e.g. (1).

The reaction and extraction was carried out in a well-ventilated refrigeration chamber (+4°C). Completely identical results may be obtained for 1a and 1b.

To a magnetically stirred soln. of KOH(12.5g, 0.22 mol) in methanol (100 ml) was added solid 6a (2.3g 10mmol) in one portion. After 1.5 - 2 min the mixture was rapidly extracted with cold n-hexane (6x50 ml) and the combined layers were then dired immediately over KOH (pellets). The procedure should be terminated after about 20 min. The crude extract (30) was diluted with ether (100-200 ml), placed in a flask connected with an efficient condenser cooled with circulating ice water (all glassware with smooth surfaces, "Diazald Kit", Aldrich) and water bath at 65°C. Distillation into a pre-cooled receiver (-78°C) was continued during ~30 min until the distillate was colourless (ca. 150-250 ml). Depending on the volume of ether used and distilled, concentrations of 1a (or 1b) are in the range of 0.02-0.06 mmol/ml, yield 45-55%. As judged from ^{1}H-NMR these yellow ethereal solutions contained 44 to 52 vol% of n-hexane but no detectable amounts of methanol (< 1 vol%). The varying ratios of the solvents do not affect the spectrophotometric determination of 1, as dilution with equal volumes of either n-hexane or ether results in identical rel. absorbances (E at 470 nm).

Stability of 1

At 21°C (protected from light) co-distillates of 1 showed 5-10% decomposition after 5 hr. In the presence of 33 vol% of ethanol or methanol the concentration of 1 decreased rapidly (40% decomp. after 5 hr, and 40% decomp. after 30 min, respectively). If stored for five weeks at -80°C, samples of 1 contained ca. 75-80% of the initial concentration. Prolonged drying over KOH should be avoided because an insoluble yellow precipitate is formed slowly. If stored in a stoppered flask, any pressure caused by liberated nitrogen gas should be released weekly.

Determination of diazoethane

1. Titration

To an accurately known excess of pure benzoic acid in acetone or ethanol is added at 4°C a known volume of cold diazoethane solution. After rapid decolourization ice water is added and excess acid is immediately titrated with 0.1 n NaOH (phenolphthalein). Irreproducible results were obtained with mineral acids, acetic acid, propionic acid, methanol as solvent, and when the mixtures were titrated slowly at ambient temperature. A soln. of 1b (E(470 nm)=0.451) gave a conc. of 1b of 0.055 mmol/ml (ε (470 nm)=8.2), a sample of 1a (E(470 nm)=0.207) showed a content of 0.025 mmol/ml (ε (470 nm)= 8.3).

2. Esterification (excess acid)

An ethereal co-distillate of 1a (25.0 ml, E(470 nm)=0.201) was added at 4°C to a soln. of 4-nitrobenzoic acid 9 (331.2 mg, 2 mmol, freshly sublimed) in EtOAc (100 ml). After evaporation the cryst. residue was suspended in EtOAc/n-hexane (3ml, 1:5, sov. (II)). Excess acid was filtered off and the filtrate was subjected to a short column (2.5 x 10 cm) charged with equal amounts of alumina (lower layer, Woelm neutral TSC) and silica (upper layer). Elution with solv. (II), evaporation and drying in vacuo (10 min, 0.01 mbar) left 116.1 mg(0.586 mmol) of pure cryst. 12a. Assuming a quantitative reaction (see below) a concentration of 1a of 0.023 mmol/ml was calculated, i.e. ε (470 nm)=8.7.

3. ^{1}H-NMR determination

To three samples of pure 9 (0.1 mmol/50 ml Et_2O) were added at 4°C (a) 2 ml, (b) 3 ml, and (c) 4 ml, respectively, of an ethereal soln. of d_3-diazoethane 1a (E(470 nm)=0.176). After the addition of 0.1 mmol/ml Et_2O of internal standard (methyl 4-phenylbenzoate (31)) the mixture was evaporated, redissolved (c) or suspended and filtrated (a,b), respectively, in 2 ml of $CDCl_3$. Integration of the OCH_3 signals (3.95 ppm) and OCH_2 singlets (4.43 ppm) in the ^{1}H-NMR spectra gave an initial conc. of 1a of 0.021 (± 0.001) mmol/ml, i.e. ε (470 nm)=8.4. A similar procedure using 10 and a soln. of 1b (E(470 nm)=0.451) afforded a conc. of 1b of 0.053 mmol/ml (ε (470 nm)=8.5).

As a result, from numerous determinations a mean molar extinction coefficient (at 470 nm) of ε =8.4(± 0.5) was obtained.

Esterfication (excess 1) - general procedure

To a soln. of pure 4-nitrobenzoic acid 9 (169.3 mg, 1.01 mmol) in dioxane (50 ml) were added at 4°C 60 ml of a soln. of 1a (0.025 mmol/ml). After removal of excess reagent with a gentle stream of inert gas the soln. was evaporated and dried (30 min, 0.01 mbar) to give pure (TLC, ^{1}H-NMR) cryst. 12a (196.4 mg, 97.8%, mp 57-58°C). Since 12a is slightly volatile in vacuo the esterification with excess 1 may be regarded as quantitative. Instead of dioxane any other acid-free solvent may be used, in the presence of methanol ethyl esters are formed exclusively. In the case of acids which contain other functional groups (e.g. aliphatic, allylic, or phenolic hydroxyl) it is essential to remove excess 1 immediately after esterification.

2,2,2($^2H_3$)-Ethyl 4-nitrobenzoate, 12a,

prepared as described above (97.8%), mp 57-8°C (unchanged if recryst. from n-hexane), TLC: R_f 0.52 (II).- Anal. Calc. for $C_9H_6D_3NO_4$ (198.2): C 54.54, (H+D) 6.10, N 7.07. Found: C 54.58, (H+D) 6.26, N 6.91. - ^{1}H-NMR ($CDCl_3$): 4.43 (broad s, 2H, OCH_2), 8.14-8.39 (symm.m, 4H, aryl-H). - ^{13}C-NMR ($CDCl_3$): 61.9 (CH_2), 165.0 (C=0), 123.8, 131.0, 136.3, 150.9 (aryl-C). - IR: 3125, 2978, 1724, 1613, 1529, 1354, 1327, 1304, 1283, 1105,

714. -MS: 198 (16,$M^{+\cdot}$), 181 (56, $M^{+\cdot}$-OH), 168(42, $M^{+\cdot}$-$C_2H_2D_2$), 150(100,$M^{+\cdot}$-OCH_2CD_3).

2,2,2($^2H_3$)-Ethyl 4-phenylbenzoate, 13a,

prepared as described above from 1a and 4-phenylbenzoic acid 10 (99.7 mg, 0.5 mmol), 114.0 mg(98.9%), mp 49.5-50.5°C (n-hexane), TLC: R_f 0.57 (II).- Anal. Calc. for $C_{15}H_{11}D_3O_2$ (229.3): C 78.57, (H+D): 7.47. Found: C 78.37, (H+D) 7.59. - ^{1}H-NMR ($CDCl_3$): 4.40 (broad s, 2H, OCH_2), 7.4 - 8.2 (m, 9H, aryl-H).- ^{13}C-NMR ($CDCl_3$): 60.9 (CH_2), 166.9 (C=O), 127.3, 127.6, 128.4, 129.2, 129.7, 130.4, 140.5, 145.9 (aryl-C). - IR: 1714, 1613, 1298, 1285, 1121, 753. - MS: 229 (83, $M^{+\cdot}$), 199 (19, $M^{+\cdot}$-$C_2H_2D_2$), 181 (100, $M^{+\cdot}$-OCH_2CD_3); d_0 < 0.6% (MID).

2,2,2($^2H_3$)-Ethyl and ethyl octadecanoate, 14a and 14b,

analytical samples of stearic acid 11 (0.5 mg) in 0.5 ml of EtOAc were esterified with excess 1a and 1b, respectively. GC analysis showed a single peak with identical retention times. MS (14a): 315 (41, $M^{+\cdot}$), 286 (4, $M^{+\cdot}$-C_2H_5), 272 (22, $M^{+\cdot}$-C_3H_7), 267 (9, $M^{+\cdot}$-OCH_2CD_3), 160 (20, $M^{+\cdot}$-$C_{11}H_{23}$), 104 (75, $C_5H_6D_3O_2^+$), 91 (100, $C_4H_5D_3O_2^{+\cdot}$); d_0 < 0.3%, determined by MID.

MS (14b): 312 (50, $M^{+\cdot}$), 283 (5, $M^{+\cdot}$-C_2H_5), 269 (26, $M^{+\cdot}$-C_3H_7), 267 (15, $M^{+\cdot}$-OCH_2CH_3), 157 (17, $M^{+\cdot}$-$C_{11}H_{23}$), 101 (54, $C_5H_9O_2^+$), 88 (100, $C_4H_8O_2^{+\cdot}$).

The fragments of 14a, b around the molecular ion (normalized intensities) are shown in Fig. 1.

Synthesis of 15 by H/D exchange

8 ml of a soln. of diazoethane 1b (0.038 mmol/ml) were shaken vigorously for 1 min at 4°C with 2 ml D_2O and 2 ml of 40% sodium deuteroxide. The upper layer was separated and then added to a stirred mixture of 4-nitrobenzoic acid 9 (38 mg, 0.227 mmol) in Et_2O (50 ml) and D_2O (10ml). The organic phase was dried (Na_2SO_4) and evaporated to give a solid residue which was recryst. from hot n-hexane (ca. 0.2 ml). TLC (R_f 0.52(II)) showed a single spot for 15 (40.0 mg, 89%). - ^{1}H-NMR ($CDCl_3$, see Fig. 1): 1.3-1.5 (m, 3H, CH_3), 4.43 (complex q., ^3J (H,H) = 7.3 Hz, ^2J (H,D) = 1.5 Hz, ca. 0.6H, OCHD/OCH_2), 8.15-8.40 (symm. m, 4H, aryl-H). Irradiation at 4.43 ppm transformed the multiplett of CH_3 into a singlet. From integration an isotope ratio of 50% d_1 (1 (^{2}H)-ethyl 4-nitrobenzoate) and 50% d_2 (1,1 ($^2H_2$)-ethyl 4-nitrobenzoate) was estimated. - ^{13}C-NMR ($CDCl_3$): 14.1 (CH_3),

61.8 (weak m, J=23 Hz, OCHD/OCD_2), 165.1 (C=O); 123.8, 131.0, 136.3, 150.9 (aryl-C).- IR : 3124, 2979, 1723, 1608, 1529, 1348, 1325, 1304, 1296, 1278, 1103, 870, 712.- MS : 197 (3, $M^{+\cdot}/d_2$), 196 (4, $M^{+\cdot}/d_1$), 180 (21, $M^{+\cdot}/d_2$-OH), 179 (20,$M^{+\cdot}/d_1$-OH), 167 (38, $M^{+\cdot}$- $C_2H_3D/C_2H_2D_2$), 150 (100, $M^{+\cdot}$- $OCHDCH_3/OCD_2CH_3$). In comparison with 12b the isotopic composition was established in the range of m/z 176-182 ($M^{+\cdot}$- 17): 47% d_2, 49% d_1, ca. 4% d_o. - Anal. Calc. for $C_9H_{7.5}D_{1.5}NO_4$ (196.7): C 54.96, (H+D) 5.38, N 7.12. Found: C 54.89, (H+D) 7.08, N 5.34. -

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30. Evaporation to dryness left a yellow oil which consisted of almost pure methyl p-toluene sulfonate 8 (TLC: R_f 0.28 (II)), identical with an authentic sample (IR, NMR).

31. Prepared from 4-phenylbenzoic acid 10 and etheral diazomethane, mp 117°C (from n-hexane), ^{1}H-NMR (CDCl): 3.95 ppm (s, 3H, OCH_3). TLC: R_f0.58 (II).