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# A convergent synthesis of (17*R*,5*Z*,8*Z*,11*Z*,14*Z*)-17-hydroxyeicosa-5,8,11,14-tetraenoic acid analogues and their tritiated derivatives

Igor V. Ivanov,[a,b,*] Stepan G. Romanov,[a] Valery P. Shevchenko,[c] Elena A. Rozhkova,[d] Mikhail A. Maslov,[a] Nataliya V. Groza,[a] Nikolai F. Myasoedov,[c] Hartmut Kuhn[b] and Galina I. Myagkova[a]

[a]*M.V. Lomonosov State Academy of Fine Chemical Technology, Chemistry and Technology of Fine Organic Compounds, Vernadskogo Pr. 86, Moscow 119571, Russian Federation*
[b]*Institute of Biochemistry, University Clinics Charité, Humboldt University, Berlin D-10117, Germany*
[c]*Institute of Molecular Genetics, Kurchatov Square 2, Moscow 123182, Russian Federation*
[d]*Department of Chemistry, Princeton University, Princeton, NJ 08544, USA*

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**Abstract**—17(*R*)-OH-AA and 14,15-dehydro-17(*R*)-OH-AA were synthesized from a common tetraacetylenic precursor and their azide derivatives were obtained in moderate yields via the corresponding *p*-toluenesulfonates. Since the azido group remained stable during tritiation procedure on Lindlar's catalyst in benzene, both 14,15-dehydro-17(*S*)-$N_3$-AA and 14,15-dehydro-17(*R*)-OH-AA constitute useful intermediates in the synthesis of radio-labelled 17(*S*)-$N_3$-AA and 17(*R*)-OH-AA. In contrast, reduction of azide in methanol afforded 17(*S*)-$NH_2$-AA with 95% yield.


## 1. Introduction

Lipoxygenases (LOXs) constitute a heterogeneous family of lipid peroxydizing enzymes, which catalyze oxygenation of polyunsaturated fatty acids to their corresponding hydroperoxy derivatives.[1] LOXs were isolated from various sources and some isoforms have been characterized comprehensively with respect to their enzymatic and molecular biological properties.[2–4] Although the X-ray structures of some LOX-isoforms have recently been solved[5,6] there are a number of open questions on the structural biology of these enzymes. Studying the structural basis for the specificity of the LOX we recently observed that hydroxylated derivatives of arachidonic acid (ω/ω-4-OH-AA) may constitute valuable tools to investigate the mechanism of the oxygenation process. Preliminary experiments suggested that a hydroxy group in close proximity to the methyl terminus of the fatty acid chain may impact the oxygenation characteristics of these substrates[7] and we observed a pronounced oxygen dependence of the reaction rate even at normoxic oxygen tensions. The presence of an OH-group in a hydrocarbon chain allows simple derivatization leading to both, photosensitive azido and primary amino derivatives.[8] Azido fatty acid can be used as active site affinity labels for fatty acid oxygenases, such as lipoxygenase- and/or cyclooxygenase isoforms.

An enantiospecific procedure has already been reported for synthesis of 17-OH-AA.[9] However, this method may not be applicable for the preparation of tritiated compounds, unless an inefficient isotope exchange procedure is employed. To obtain preparative amounts of radio-labelled 17(*R*)-OH-AA with high specific radioactivity a convergent and stereoselective synthesis is still required. This paper reports an efficient method for the synthesis of (17*R*,5*Z*,8*Z*,11*Z*,14*Z*)-17-hydroxyeicosa-5,8,11,14-tetraenoic acid (17(*R*)-OH-AA), (17*S*,5*Z*,8*Z*,11*Z*,14*Z*)-17-azidoeicosa-5,8,11,14-tetraenoic (17(*S*)-$N_3$-AA), (17*S*,5*Z*,8*Z*,11*Z*,14*Z*)-17-aminoeicosa-5,8,11,14-tetraenoic acids (17(*S*)-$NH_2$-AA) and their tritiated analogues. The procedure is illustrated for 17(*R*)-OH-AA (Scheme 1), but it works equally well for the *S*-enantiomer.

## 2. Results and discussion

The most crucial point of our synthetic strategy was to minimize number of manipulations with radioactive material. Thus, radioactive labeling should be performed

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* Corresponding author. Tel.: +7-95-434-8544; fax: +7-95-434-86-78; e-mail: ivanov.mitht@mail.ru

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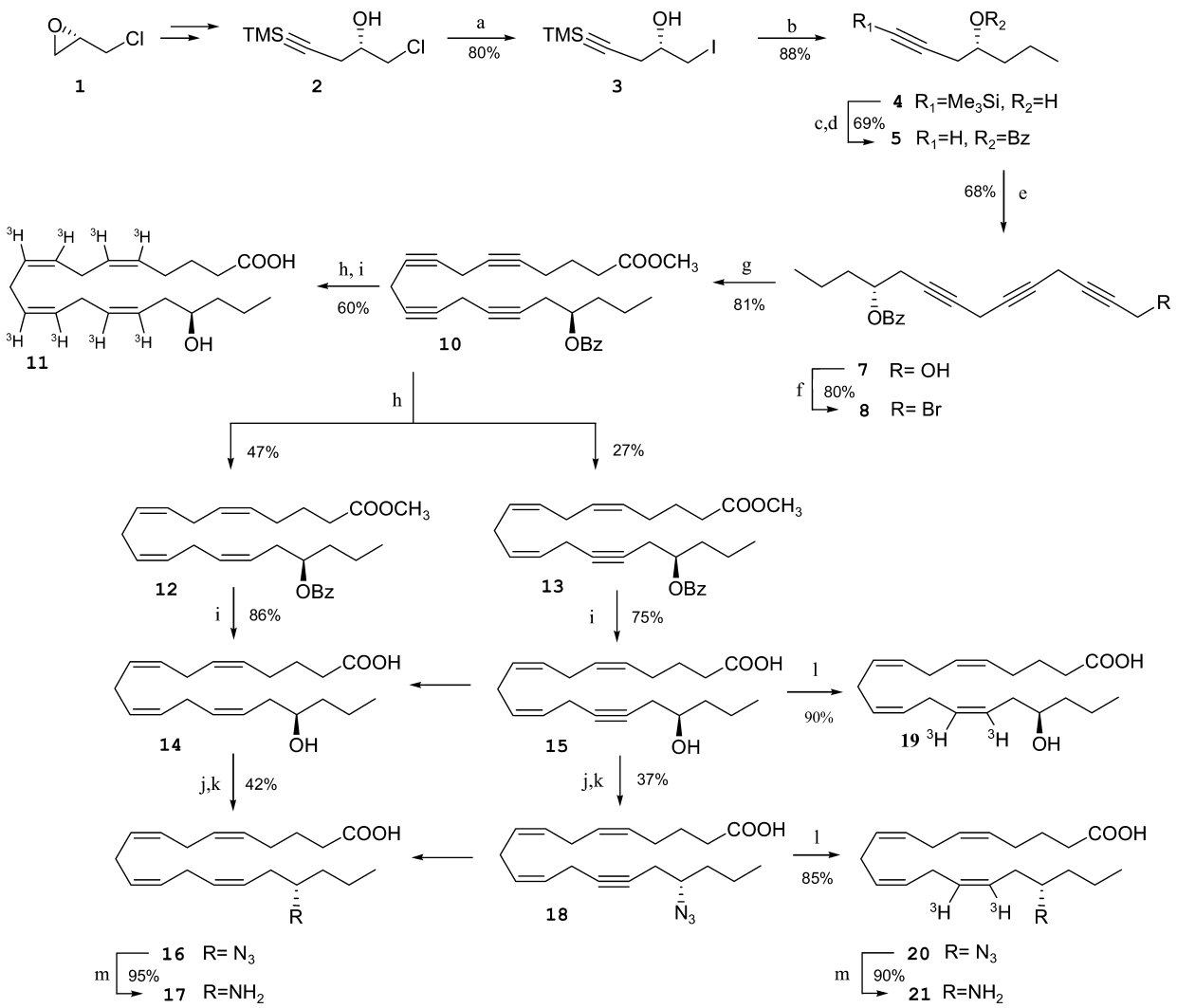


**Scheme 1.** (a) NaI, acetone, 65°C, 78 h; (b) CuI, EtMgBr, THF-HMPA, −45°C, 65 h; (c) BzCl, Py, benzene, rt, 10 h; (d) *n*-$Bu_4NF$, THF, rt, 2 h; (e) **6**, CuI, NaI, $K_2CO_3$, DMF, rt; (f) $CBr_4$, $PPh_3$, $CH_2Cl_2$, rt, 1 h; (g) **9**, CuI, NaI, $K_2CO_3$, DMF, rt then HPLC; (h) $H_2$ or tritium/Lindlar's catalyst, quinoline, benzene, 10°C; (i) NaOH, MeOH–$H_2O$, rt; (j)TsCl, $CH_2Cl_2$, Py, rt, 24 h; (k) $NaN_3$, DMF, 75–80°C, 2.5 h; (l) $T_2$/Lindlar's catalyst, benzene; (m) $H_2$/Lindlar's catalyst, MeOH.

in the synthesis as late as possible. Since acetylenic functions permit the incorporation of tritium (partial tritiation) to yield labeled compounds with high specific activity, the acetylenic approach was selected as method of choice. To construct the tetraacetylenic precursor **10** (Scheme 1) we elected three building blocks: (*R*)-4-(benzoyloxy)hept-1-yn (**5**), 7-bromo-2,5-heptadiyne-1-ol (**6**)[7] and methyl 5-hexynoate (**9**). Heptyne **5** was obtained as indicated in Scheme 1 starting from (*S*)-epichlorohydrin (**1**). Oxirane ring opening was achieved by the Yamaguchi method as described previously for the *R*-enantiomer[10] affording (*S*)-1-chloro-5-(trimethylsilyl)pent-4-yne-2-ol (**2**). Conversion of the chloride **2** to corresponding iodide **3** and chain elongation using $Et_2CuMgBr$ resulted in (*R*)-1-(trimethylsilyl)hept-1-yne-4-ol (**3**). Protection of OH-group followed by desilylation (TBAF, THF) afforded **5** in an overall yield of 42% for four-step procedure. Copper(I) mediated cross-coupling[11] of methyl 5-hexynoate (**9**) and propargyl bromide **8** obtained from acetylene **5** and bromo alcohol **6** resulted in methyl (*R*)-17-(benzoyloxy)eicosa-5,8,11,14-tetraynoate (**10**) in 81% yield. Stereospecific hydrogenation of the skipped triple bonds of **10** using Lindlar's catalyst (catalyst/substrate ratio, 1.2:1) afforded both methyl (*R*,5*Z*,8*Z*,11*Z*,14*Z*)-17-(benzoyloxy)eicosa-5,8,11,14-tetraenoate (**12**) and its 14,15-dehydro analogue **13**. Recently, we have shown that the close neighborhood of a benzoate residue to an acetylene moiety may impact its hydrogenation efficiency,[7] which may be due to a steric hindrance of the accessibility for the Pd-catalyst. For the tetraacetylene **10** this also seems to be the case. Increase in the catalyst concentration (catalyst/substrate ratio, 2:1) diminished the formation of **13** to trace amounts. Compounds **12** and **13** were separated by preparative reverse phase HPLC (MeOH/$H_2O$, 95:5). The structural identification of **13** as methyl (*R*,5*Z*,8*Z*,11*Z*)-17-(benzoyloxy)-eicosa-5,8,11-trien-14-ynoate was based on both mass spectral data and NMR spectroscopy. Two dimensional $^1H/^{13}C$ heteronuclear multiple-bond correlation (HMBC) spectra were used to confirm the position of the triple bond (Fig. 1). We observed correlation between neighboring atoms (C-15/$H_2$-16) and remote ones (between others C-15/H-17, C-15/$H_2$-13). Moreover, three separate cross peaks indicated the additional correlations: C-14/$H_2$-13, C-14/H-12 and C-14/$H_2$-16. Simultaneous deprotection of both,

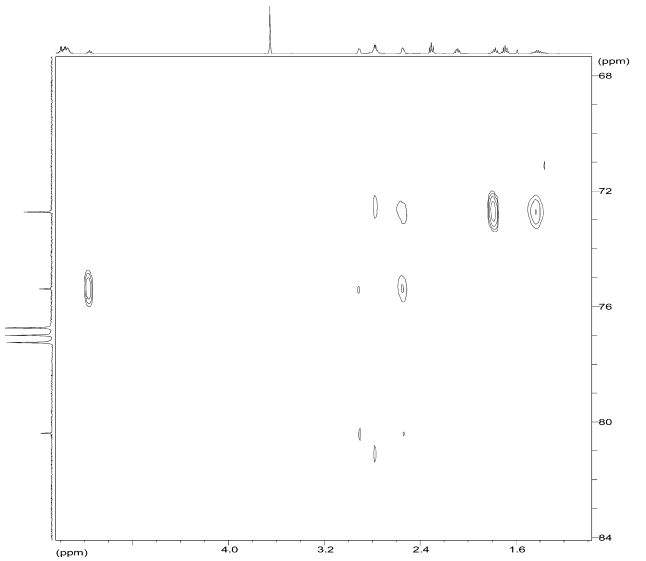


**Figure 1.** Fragment of 2D $^{1}H/^{13}C$ heteronuclear multiple-bond correlation (HMBC) spectra for compound **13**.

carboxy and hydroxy groups of **12** and **13**, using NaOH in MeOH–$H_2O$ solution proceeded well to give the free acids **14** and **15** in 86 and 75% yields, respectively. Tritiation of **10** was performed in benzene with a method used before[12] with slight modifications. Subsequent deprotection afforded **11** with high specific activity (175–180 Ci/mmol) and a radiochemical purity of more than 98%.

Conversion of the hydroxy compounds **14** and **15** to the corresponding *p*-toluenesulfonates followed by tosylate substitution with azide in DMF[13] afforded the (*S*)-azido acids **16** and **18** in moderate yields, 42 and 37%, respectively. In contrast to the tosylate pathway, the synthesis via bromides retained the *R*-configuration of the final product. However, substitution of bromide during the reaction with $NaN_3$ was incomplete and we recovered about 30% of the starting material. The corresponding (*R*)-azides were prepared from 17(*S*)-OH-AA, itself obtained by exactly the same procedure starting with commercially available (*R*)-epichlorohydrin. Mitsunobu inversion of 17(*R*)-OH-AA[9] may also serve as alternative way to obtain the *S*-isomer.

The high specific activity of [5,6,8,9,11,12,14,15-$^{3}H_8$]-17(*R*)-OH-AA (**11**), which is required for biological applications, complicates its extensive derivatization in the preparative scale. Hence, the monoacetylenes **15** and **18** were used as key intermediates for partial tritiation of the acetylene moiety at the final step. To optimize the tritiation procedure we first performed model experiments on hydrogenation of **15** and **18** and the analytical data of hydrogenated compound **15** appeared to be identical with that of obtained previously for 17(*R*)-OH-AA (**14**). Importantly, hydrogenation of azide **18** using Lindlar's catalyst in dry benzene (catalyst/substrate ratio, 2:1) proceeded without sizeable azide reduction. Tritiation was carried out by the same procedure as hydrogenation to yield [14,15-$^{3}H$]-17(*R*)-OH-AA (**19**) and [14,15-$^{3}H$]-17(*S*)-$N_3$-AA (**20**) with a high specific activities of 45 Ci/mmol and 40 Ci/mmol, respectively. In contrast, catalytic hydrogenation of **16** and **20** in methanol[14,15] afforded corresponding amino acids **17** and **21** with good yields.

## 3. Conclusion

In conclusion, a novel convergent synthetic procedure is reported for enantio-selective synthesis of 17-OH-AA, 17-$NH_2$-AA and 17-$N_3$-AA. This method can be used to prepare the corresponding tritiated derivatives, which constitute valuable tools for mechanistic studies of eicosanoid biosynthesis.

## 4. Experimental

### 4.1. General

$^{1}H$ and $^{13}C$ NMR spectra were recorded either on a Brucker MSL 200, Brucker MSL 300 or Brucker MSL 500 spectrophotometers in $CDCl_3$ as solvent. Chemical shifts are referenced to tetramethylsilane as an internal standard for $^{1}H$ NMR or to the deuterium lock signal of $CDCl_3$ ($\delta$ $^{13}C$=77.19 ppm). IR spectra were recorded on Shimadzu IR-435. HPLC analysis was carried out on a Shimadzu LC-10Avp liquid chromatograph connected to SPD-10Advp UV detector. RP-HPLC analysis was performed on a Nucleosil C18-column; 250×4 mm, 5 μm particle size (Machery-Nagel, Düren, Germany) with different solvent systems: MeOH/$H_2O$ (95:5, by vol.) and a flow rate of 1 mL/min were used for analysis of compound **12**, **13**. MeOH/$H_2O$/Ac (85:15:0.1, by vol.) and a flow rate of 1 mL/min were used for other compounds. Preparative HPLC was carried out on a Lichrospher 100 RP18 column; 250×22.5 mm, 10 μm particle size (Knauer, Berlin, Germany) with MeOH/$H_2O$/AcOH (95:5, by vol.) and a flow rate of 10 mL/min. For EIMS analysis a Shimadzu QP-2000 system was used. High resolution mass spectra were recorded either on a MAT 711 mass spectrometer (Finigan MAT) or VG Autospec instrument. Column chromatography was carried out on Silica Gel 60 (Merck, Darmstadt, Germany particle size ranging from 70–230 mesh). For thin-layer chromatography we employed precasted Silica Gel 60 $F_{254}$ sheets (Merck, Darmstadt, Germany). THF was freshly distilled from sodium/benzophenone ketyl, and HMPA was dried over $CaH_2$. All solvents and reagents used were of extra pure grade and purchased from Merck, Aldrich or Across (Germany). Prior to use all glassware and syringes were dried at 140°C overnight and all reactions were carried out under atmosphere of dry argon.

**4.1.1. (*S*)-1-Iodo-5-(trimethylsilyl)pent-4-yne-2-ol (3).** A mixture of **2** (2.54 g, 13.31 mmol) and NaI (5.99 g, 39.95 mmol) in dry acetone (15 mL) was stirred at 65°C for 78 h. After acetone was evaporated, $Et_2O$ (60 mL) was added to the residue. The resulting mixture was washed with $H_2O$ (2×50 mL) and the organic layer was dried over $Na_2SO_4$. After evaporation under reduced pressure, the crude residue was purified on silica gel (hexane/$Et_2O$, 3:1) to yield **3** as a colorless oil, yield: 3.02 g (80%). $[\alpha]_D^{20}$=+18.3 (*c* 0.7, acetone). TLC: $R_f$=0.42 (hexane/$Et_2O$, 1:1). IR (neat)/cm$^{-1}$: 3600–3200 (OH), 2170 (C≡C),

1273 (C–O), 1245, 839 ($Si(CH_3)_3$), 590 (C–I). $^1H$ NMR (300 MHz, $CDCl_3$): $\delta$ 3.71 (m, 1H, 2-CH), 3.43 (dd, 1H, $J$=3.7, 10.2 Hz) and 3.32 (dd, 1H, $J$=6.1, 10.2 Hz, 1-$CH_2$), 2.61 (dd, 1H, $J$=5.8, 16.9 Hz) and 3.52 (dd, 1H, $J$=6.2, 16.9 Hz, 3-$CH_2$), 2.24 (s,1H, OH), 0.09 (m, 9H, $(CH_3)_3Si$). $^{13}C$ NMR (50 MHz, $CDCl_3$): $\delta$ 101.61, 88.49, 69.89, 48.36, 12.67, 0.31 (3C). Anal. calcd for $C_8H_{15}IOSi$: C, 34.05; H, 5.36. Found: C, 34.14; H, 5.49.

**4.1.2. (*R*)-1-(Trimethylsilyl)hept-1-yne-4-ol (4).** To a suspension of CuI (3.39 g, 17.82 mmol) in THF (80 mL) and HMPA (10 mL), which was cooled to −10°C, a solution of EtMgBr (33.30 mL, 35.64 mmol, 1.07 M) in THF was added with a syringe. The mixture was stirred for 45 min at −5–0°C till homogenous dark solution formed. After the mixture was called to −50°C, a solution of iodide **3** (2.01 g, 7.13 mmol) in THF (10 mL) was added. The resulting mixture was kept with a stirring at −45°C for 1.5 h, then was quenched with satd aq. $NH_4Cl$, acidified with HCl (1 M) to pH 5.0 and the organic products were extracted with $Et_2O$ (3×60 mL). The combined ethereal extracts were washed with satd aq. NaCl, dried over $Na_2SO_4$ and then concentrated under vacuum. Purification by silica gel chromatography (hexane/$Et_2O$ 2:1) gave 1.15 g (88%) of pure **4**. $[\alpha]_D^{20}$=+21.8 (*c* 0.9, acetone). TLC: $R_f$=0.39 (hexane/$Et_2O$, 1:1). IR (neat)/$cm^{-1}$: 3600–3200 (OH), 2240 (C≡C), 1245, 840 ($Si(CH_3)_3$). $^1H$ NMR (300 MHz, $CDCl_3$): $\delta$ 3.74 (m, 1H, 4-CH), 2.45 (dd, 1H, $J$=4.7, 16.9 Hz) and 2.30 (dd, 1H, $J$=7.1, 16.9 Hz, 3-$CH_2$), 1.92 (s, 1H, OH), 1.30–1.49 (m, 4H, 5- and 6-$CH_2$), 0.92 (t, 3H, $J$=6.8 Hz, $CH_3$), 0.1 (m, 9H, $(CH_3)_3Si$). $^{13}C$ NMR (50 MHz, $CDCl_3$) $\delta$: 103.66, 87.78, 69.92, 38.72, 29.20, 19.02 14.17, 0.31 (3C). Anal. calcd for $C_{10}H_{20}OSi$ C, 65.15; H, 10.94. Found: C, 65.34; H, 11.15.

**4.1.3. (*R*)-4-(Benzoyloxy)hept-1-yn (5).** A solution of BzCl (0.762 mL, 6.56 mmol) in benzene (15 mL) was added to a solution of **4** (0.805 g, 4.37 mmol) in benzene (30 mL) and pyridine (20 mL). The mixture was stirred overnight at rt, then acidified with $H_2SO_4$ (1 M, 50 mL). Reaction products were extracted with $Et_2O$ (2×50 mL) and the extracts were concentrated under reduced pressure. The residue was filtrated through a silica gel column (hexane/$Et_2O$, 4:1). The product which showed an $R_f$=0.60 in silica gel thin layer chromatography (hexane/$Et_2O$, 1:1) was dissolved in THF (50 mL) and the silyl group was removed by addition of $n$-$Bu_4NF$ (2.37 g, 7.53 mmol) within 2 h at rt. The mixture was quenched with $H_2O$ (100 mL), organic layer was separated and lipophilic products were extracted from the water phase with $Et_2O$ (2×40 mL). The combined organic layers were washed with satd aq. NaCl (70 mL), dried over $Na_2SO_4$ and concentrated under vacuum. Silica gel chromatography using gradient elution with hexane/$Et_2O$ (from 1 to 10% $Et_2O$) gave pure **5** as a colourless oil in a yield 0.745 g (79%). $[\alpha]_D^{20}$=+29.2 (*c* 0.8, acetone). TLC: $R_f$=0.55 (hexane/$Et_2O$, 1:1). IR (neat)/$cm^{-1}$: 3272 (C≡C), 1725 (C=O), 1273 (C–O), 710 (Ph). $^1H$ NMR (200 MHz, $CDCl_3$): $\delta$ 8.09 (m, 2H, *o*-Bz), 7.54 (m, 1H, *p*-Bz), 7.40 (m, 2H, *m*-Bz), 5.19 (m, 1H, 4-CH), 2.59 (m, 2H, 3-$CH_2$), 1.89 (t, 1H, $J$=2.7 Hz, 1-CH), 1.75–1.82 (m, 2H, 5-$CH_2$), 1.35–1.45 (m, 2H, 6-$CH_2$), 0.89 (t, 3H, $J$=6.8 Hz, $CH_3$). $^{13}C$ NMR (50 MHz, $CDCl_3$): $\delta$ 166.26, 133.07, 132.06, 129.88 (2C), 128.55, 127.30, 79.96, 72.39, 70.63, 35.51, 24.36, 18.77, 14.77. Anal. calcd for $C_{14}H_{16}O_2$: C, 77.75; H, 7.46. Found: C, 77.99; H, 7.34.

**4.1.4. (*R*)-11-(Benzoyloxy)tetradeca-2,5,8-triyne-1-ol (7).** To a suspension of previously dried salts CuI (1.12 g, 5.90 mmol), NaI (0.885 g, 5.90 mmol) and $K_2CO_3$ (0.610 g, 4.42 mmol) in DMF (25 mL) were added bromide **6** (0.548 g, 2.93 mmol) and acetylene **5** (0.637 g, 2.95 mmol) under argon atmosphere. After stirring overnight at rt, the mixture was quenched with satd aq. $NH_4Cl$ (100 mL) and the products were extracted with $Et_2O$ (4×100 mL). The combined organic extracts were washed with satd aq. NaCl (2×50 mL), dried over $Na_2SO_4$ and the solvent was evaporated under reduced pressure. The residue was purified by silica gel flash chromatography (hexane/$Et_2O$, 1:2) to afford 0.680 g (72%) of pure acetylene alcohol **7**. TLC: $R_f$=0.30 (hexane/$Et_2O$, 1:2). IR (neat)/$cm^{-1}$: 3200–3600 (OH), 2240, 2170 (C≡C), 1725 (C=O), 1115 (C–O), 709 (Ph). $^1H$ NMR (200 MHz, $CDCl_3$): $\delta$ 8.05 (m, 2H, *o*-Bz), 7.49 (m, 3H, (*m*+*p*)-Bz), 5.15 (m, 1H, 11-CH), 4.17 (m, 2H, 1-$CH_2$), 3.09 (m, 4H, 4- and 7-$CH_2$), 2.52 (m, 2H, 10-$CH_2$), 1.75 (m, 2H, 12-$CH_2$), 1.37 (m, 2H, 13-$CH_2$), 0.88 (t, 3H, $J$=6.8 Hz, $CH_3$). $^{13}C$ NMR (50 MHz, $CDCl_3$): $\delta$ 166.29, 132.91, 130.82, 129.79 (2C), 128.43 (2C), 80.06, 79.07, 76.39, 76.28, 75.25, 74.11, 72.78, 51.21, 35.63, 24.56, 18.65, 13.98, 9.90 (2C). Anal. calcd for $C_{21}H_{22}O_3$: C, 78.23; H, 6.88. Found: C, 78.01; H, 6.76.

**4.1.5. (*R*)-4-(Benzoyloxy)-14-bromotetradeca-6,9,12-triyne (8).** To a solution of alcohol **7** (600 mg, 1.86 mmol) and $CBr_4$ (925 mg, 2.79 mmol) in $CH_2Cl_2$ (30 mL) was added a solution of $PPh_3$ (732 mg, 2.79 mmol) in $CH_2Cl_2$ (15 mL) at 10°C. The reaction mixture was stirred for 1 h at rt, then quenched with MeOH (2 mL) and volatile components were removed under vacuum. Column chromatography on silica gel (hexane/$Et_2O$, 1:1) afforded pure **8**. Yield: 616 mg (86%). TLC: $R_f$=0.69 (hexane/$Et_2O$, 1:2). IR (neat)/$cm^{-1}$: 2240, 2170 (C≡C), 1725 (C=O), 1115 (C–O), 710 (Ph), 598 (C-Br). $^1H$ NMR (200 MHz, $CDCl_3$): $\delta$ 8.05 (m, 2H, *o*-Bz), 7.49 (m, 3H, (*m*+*p*)-Bz), 5.12 (m, 1H, 4-CH), 3.89 (m, 2H, 14-$CH_2$), 3.18 (m, 2H, 8-$CH_2$), 3.10 (m, 2H, 11-$CH_2$), 2.54 (m, 2H, 5-$CH_2$), 1.75 (m, 2H, 3-$CH_2$), 1.41 (m, 2H, 2-$CH_2$), 0.92 (t, 3H, $J$=6.8 Hz, $CH_3$). $^{13}C$ NMR (50 MHz, $CDCl_3$): $\delta$ 166.29, 132.91, 130.85, 129.78 (2C), 128.43 (2C), 81.49, 79.11, 76.13 (2C), 75.76, 75.54, 72.71, 35.63, 24.56, 18.68, 14.56, 14.01, 9.89 (2C). Anal. calcd for $C_{21}H_{21}O_2Br$: C, 65.46; H, 5.49. Found: C, 65.26; H, 5.35.

**4.1.6. Methyl (*R*)-17-(benzoyloxy)eicosa-5,8,11,14-tetraynoate (10).** In an Ar filled previously dried round-bottomed flask equipped with magnetic stirrer anhydrous $K_2CO_3$ (261 mg, 1.89 mmol), NaI (378 mg, 2.52 mmol) and CuI (480 mg, 2.52 mmol) were suspended in DMF (15 mL). Methyl 5-hexynoate (**9**) (159 mg, 1.26 mmol) was added at once to the suspension followed by bromide **8** (490 mg, 1.27 mmol). The reaction mixture was vigorously stirred overnight at rt, then quenched with satd aq. $NH_4Cl$ (200 mL). The lipophilic products were extracted with $Et_2O$ (4×100 mL). The combined organic extracts were washed with satd aq. NaCl (2×150 mL). After drying over $Na_2SO_4$ the ethereal solution was concentrated in vacuum. The crude residue was purified by silica gel flash

chromatography (hexane/$Et_2O$, 3:1) under Ar to give pure **10** as yellow oil. Yield of **10**: 439 mg (81%). TLC: $R_f$=0.42 (hexane/$Et_2O$, 1:1). IR (neat)/$cm^{-1}$: 2240 (C≡C), 1740, 1725 (C=O), 1115 (C–O), 710 (Ph). $^1H$ NMR (200 MHz, $CDCl_3$): $\delta$ 8.01 (m, 2H, *o*-Bz), 7.48 (m, 3H, (*m*+*p*)-Bz), 5.15 (m, 1H, 17-CH), 3.65 (s, 3H, $OCH_3$), 3.19 (m, 6H, 7-, 10- and 13-$CH_2$), 2.65 (dt, 2H, *J*=6.0, 2.1 Hz, 16-$CH_2$), 2.40 (t, 2H, *J*=7.2 Hz, 2-$CH_2$), 2.21 (tt, 2H, *J*=6.8, 1.7 Hz, 4-$CH_2$), 1.75 (m, 4H, 3- and 18-$CH_2$), 1.41 (m, 2H, 19-$CH_2$), 0.90 (t, 3H, *J*=6.8 Hz, $CH_3$). $^{13}C$ NMR (50 MHz, $CDCl_3$): $\delta$ 173.42, 166.17, 132.77, 130.75, 129.95 (2C), 128.25 (2C), 81.28, 79.18 (2C), 77.47, 76.01 (2C), 75.51, 73.97, 72.36, 51.48, 35.04, 32.95, 24.02, 23.91, 18.26, 14.07, 13.95, 9.83 (3C).

**4.1.7. Methyl (17*R*,5*Z*,8*Z*,11*Z*,14*Z*)-17-(benzoyloxy)-eicosa-5,8,11,14-tetraenoate (12) and methyl (17*R*,5*Z*,8*Z*,11*Z*)-17-(benzoyloxy)eicosa-5,8,11-trien-14-ynoate (13).** The suspension of Lindlar's catalyst (500 mg) in dry benzene (15 mL) was saturated with $H_2$ in a 100-mL Erlenmeyer flask at rt and cooled to 10°C. Then a solution of **10** (400 mg, 0.93 mmol) in benzene (25 mL) and quinoline (0.40 mL) were added to the catalyst under a stream of Ar. The Ar was exchanged with $H_2$ and the mixture was stirred for 1 h at 10°C. After the absorption of hydrogen was completed the solution was filtered, washed with HCl (2 M, 2×30 mL) and the solvent was evaporated. The crude residue was filtered over silica gel (hexan/$Et_2O$, 2:1) and the products formed were separated by preparative RP-HPLC (solvent system MeOH/$H_2O$, 95:5) to yield 192 mg (47%) of pure tetraenoate **12** and 113 mg (27%) of 14,15-dehydroanalogue **13**. Data for **12**: $[\alpha]_D^{20}$=+17.2 (*c* 0.5, acetone). TLC: $R_f$=0.53 (hexane/$Et_2O$, 1:1). Analytical RP-HPLC: $R_t$=6.58 min. $^1H$ NMR (300 MHz, $CDCl_3$): $\delta$ 8.01 (m, 2H, *o*-Bz), 7.52 (m, 1H, *p*-Bz), 7.40 (m, 2H, *m*-Bz), 5.42–5.46 (m, 2H, CH=CH), 5.29–5.39 (m, 6H, CH=CH), 5.15 (m, 1H, 17-CH), 3.61 (s, 3H, $OCH_3$), 2.79 (m, 6H, 7-, 10- and 13-$CH_2$), 2.46 (m, 2H, 16-$CH_2$), 2.30 (t, 2H, *J*=7.21 Hz, 2-$CH_2$), 2.07 (m, 2H, 4-$CH_2$), 1.62–1.70 (m, 4H, 3- and 18-$CH_2$), 1.35 (m, 2H, 19-$CH_2$), 0.89 (t, 3H, *J*=6.81 Hz, $CH_3$). $^{13}C$ NMR (50 MHz, $CDCl_3$): $\delta$ 173.22, 166.41, 132.92, 131.07 (2C), 130.71, 129.73 (2C), 129.13, 128.99, 128.46 (2C), 128.40, 128.24, 128.17, 124.95 (2C), 74.40, 51.65, 36.08, 32.27, 26.71, 25.92, 25.78 (2C), 24.94, 18.89, 14.16. EIMS *m*/*z* (%): 316 (2.74) [M-BzCOO]$^+$. Anal. calcd for $C_{28}H_{38}O_4$: C, 76.68; H, 8.73. Found: C, 76.44; H, 8.91. Data for **13**: $[\alpha]_D^{20}$=+26.0 (*c* 0.5, acetone). TLC: $R_f$=0.53 (hexane/$Et_2O$, 1:1). Analytical RP-HPLC: $R_t$=5.91 min. $^1H$ NMR (500 MHz, $CDCl_3$): $\delta$ 8.02 (m, 2H, *o*-Bz), 7.52 (m, 1H, *p*-Bz), 7.41 (m, 2H, *m*-Bz), 5.30–5.45 (m, 6H, CH=CH), 5.14 (m, 1H, 17-CH), 3.64 (s, 3H, $OCH_3$), 2.89 (m, 2H, 13-$CH_2$), 2.78 (m, 6H, 7-and 10-$CH_2$), 2.53 (m, 2H, 16-$CH_2$), 2.29 (t, 2H, *J*=7.21 Hz, 2-$CH_2$), 2.07 (m, 2H, 4-$CH_2$), 1.76 (m, 2H, 18-$CH_2$), 1.69 (m, 2H, 3-$CH_2$), 1.41 (m, 2H, 19-$CH_2$), 0.93 (t, 3H, *J*=6.81 Hz, $CH_3$). $^{13}C$ NMR (125 MHz, $CDCl_3$): $\delta$ 174.18, 166.27, 132.97, 130.77, 129.81 (2C), 129.43, 129.18, 128.92, 128.70, 128.46 (2C), 127.73, 125.30, 80.58, 75.59, 72.92, 51.62, 35.55, 33.61, 26.73, 25.77, 25.66, 24.95, 24.65, 18.72, 17.37, 14.10. EIMS *m*/*z* (%): 314 (1.35) [M−BzCOO]$^+$. Anal. calcd for $C_{28}H_{36}O_4$: C, 77.03; H, 8.31. Found: C, 76.84; H, 8.43.

**4.1.8. (17*R*,5*Z*,8*Z*,11*Z*,14*Z*)-17-Hydroxyeicosa-5,8,11,14-tetraenoic acid (14).** An aqueous solution (15 mL) of NaOH (164 mg, 4.10 mmol) was added to a solution of **12** (180 mg, 0.41 mmol) in methanol (40 mL) under argon atmosphere. The resulting mixture was stirred for 30 h at rt. After the reaction was completed, methanol was removed by evaporation under reduced pressure and the residue was carefully acidified to pH 4.0 using HCl (1 M). The lipophilic products were extracted with $Et_2O$ (3×40 mL), combined organic extracts were dried over $Na_2SO_4$ and concentrated in vacuum. The crude residue was purified by silica gel column chromatography (hexane/$Et_2O$, 1:3) to give **14** in 113 mg (86%). $[\alpha]_D^{20}$=+4.3 (*c* 0.6, acetone). TLC: $R_f$=0.31 (hexane/$Et_2O$, 1:3). Analytical RP-HPLC: $R_t$=6.07 min. $^1H$ NMR (300 MHz, $CDCl_3$): $\delta$ 5.50–5.60 (m, 2H, CH=CH), 5.29–5.45 (m, 6H, CH=CH),3.65 (m, 1H, 17-CH), 2.82 (m, 6H, 7-, 10- and 13-$CH_2$), 2.33 (m, 2H, *J*=7.11 Hz, 2-$CH_2$), 2.24 (m, 2H, 16-$CH_2$), 2.10 (m, 2H, 4-$CH_2$), 1.68 (m, 2H, 3-$CH_2$), 1.41 (m, 4H, 18- and 19-$CH_2$), 0.89 (t, 3H, *J*=6.8 Hz, $CH_3$). $^{13}C$ NMR (75 MHz, $CDCl_3$): $\delta$ 178.03, 131.55, 129.16, 128.99, 128.48, 128.39, 128.11, 128.05, 125.70, 71.60, 38.94, 35.40, 33.30, 26.58, 25.97, 25.87, 25.84, 24.65, 19.08, 14.23. EIMS *m*/*z* (%): 320 (6.95) [M]$^+$, 302 (15.94) [M−$H_2O$]$^+$, 259 (8.08) [M−$H_2O$−$C_3H_9$]$^+$. FABMS *m*/*z*: 319 [M−H]$^-$. EIHRMS calcd for $C_{20}H_{32}O_3$ [M]$^+$: 320.2351. Found: 320.2364.

**4.1.9. (17*R*,5*Z*,8*Z*,11*Z*)-17-Hydroxyeicosa-5,8,11-trien-14-ynoic acid (15).** Free acid **15** was prepared by analogues procedure as described for **14** from methyl (*R*,5*Z*,8*Z*,11*Z*)-17-(benzoyloxy)eicosa-5,8,11-trien-14-ynoate (**13**) (209 mg, 0.25 mmol) and LiOH (105 mg, 2.50 mmol). Yield of pure **15**: 59.6 mg (75%). $[\alpha]_D^{20}$=+5.0 (*c* 0.6, acetone). TLC: $R_f$=0.31 (hexane/$Et_2O$, 1:3). Analytical RP-HPLC: $R_t$=4.98 min. $^1H$ NMR (300 MHz, $CDCl_3$): $\delta$ 5.30–5.45 (m, 6H, CH=CH), 3.70 (m, 1H, 17-CH), 2.94 (m, 2H, 10-$CH_2$), 2.81 (m, 4H, 7- and 13-$CH_2$), 2.34 (m, 2H, *J*=7.11 Hz, 2-$CH_2$), 2.22–2.45 (m, 2H, 16-$CH_2$), 2.10 (m, 2H, 4-$CH_2$), 1.68 (m, 2H, 3-$CH_2$), 1.41 (m, 4H, 18- and 19-$CH_2$), 0.89 (t, 3H, *J*=6.8 Hz, $CH_3$). $^{13}C$ NMR (75 MHz, $CDCl_3$): $\delta$ 177.94, 129.77, 129.03 (2C), 128.72, 127.63, 125.07, 81.35, 76.34, 70.21, 38.51, 33.22, 27.86, 26.63, 25.82, 25.72, 24.67, 19.02, 17.36, 14.18. ESIHRMS calcd for $C_{20}H_{30}O_3Na$ 341.2093 [M+Na]$^+$. Found 341.2079.

**4.1.10. [5,6,8,9,11,12,14,15-$^3$H]-(17*R*,5*Z*,8*Z*,11*Z*,14*Z*)-17-Hydroxyeicosa-5,8,11,14-tetraenoic acid (11).** A solution of **10** (5 mg, 0.012 mmol) in benzene (1 mL), Lindlar's catalyst (10 mg) and quinoline (0.10 mL) were placed in the reaction ampule and the mixture was frozen with liquid nitrogen. After evacuation the ampule was filled with gaseous tritium up to a pressure 400 hPa. The reaction mixture was stirred for 1 h at rt and then frozen again. Unreacted tritium gas was removed by applying vacuum. The catalyst was removed by filtration over silica gel and washed with benzene (3×1 mL). The combined filtrates were concentrated under reduced pressure and the residue was evaporated two times with methanol (2×2 mL) to remove labile tritium. The crude product was purified by preparative HPLC followed by deprotection according to procedure described for preparation of compound **14**. Yield of **11**: 1.16 Ci (57%) with specific radioactivity 175 Ci/mmol. The radiochemical purity of [$^3$H-8]-**11** was found to be 95%. Analytical RP-HPLC: $R_t$=6.07 min.

**4.1.11. (17*S*,5*Z*,8*Z*,11*Z*,14*Z*)-17-Azidoeicosa-5,8,11,14-tetraenoic acid (16).** A mixture of 17-HETE (**14**) (42 mg, 0.13 mmol), *p*-toluenesulphonyl chloride (95 mg, 0.5 mmol) in $CH_2Cl_2$ (2 mL) and anhydrous pyridine (3 mL) was stirred for 24 h at rt and the reaction was monitored by HPLC using MeOH/$H_2O$/AcOH (85:15:0.1, by vol.) as solvent system. After the reaction was completed the mixture was poured into diluted HCl (1 M, 50 mL), and extracted with $Et_2O$ (3×40 mL). Combined organic extracts were additionally washed with HCl (1 M, 50 mL), water (50 mL) and dried over $Na_2SO_4$. The crude residue was purified by silical gel column chromatography (hexane/$Et_2O$, 1:1) to give 37 mg of the product with $R_t$=7.98 min, as it was shown by RP-HPLC. The latter was then stirred with sodium azide (20 mg, 0.31 mmol) in DMF (3 mL) for 2.5 h at 75–80°C. The reaction mixture was quenched with satd aq. $NH_4Cl$ (20 mL) and the products were extracted with $Et_2O$ (3×30 mL). The combined organic extracts were washed with satd aq. NaCl (20 mL), dried over $Na_2SO_4$ and the solvent was evaporated under reduced pressure. The residue was purified by silica gel flash chromatography (hexane/$Et_2O$, 1:1) to afford pure **16** with overall yield 19 mg (42%). $[\alpha]_D^{20}=-43.6$ (*c* 0.5, acetone). Analytical RP-HPLC: $R_t$=13.5 min. $^1$H NMR (300 MHz, $CDCl_3$): $\delta$ 5.30–5.50 (m, 8H, CH=CH), 3.30 (m, 1H, 17-CH), 2.81 (m, 6H, 7-, 10- and 13-$CH_2$), 2.30 (m, 4H, 2- and 16-$CH_2$), 2.09 (m, 2H, 4-$CH_2$), 1.65 (m, 2H, 3-$CH_2$), 1.41 (m, 4H, 18- and 19-$CH_2$), 0.89 (t, 3H, $J$=6.8 Hz, $CH_3$). $^{13}$C NMR (75 MHz, $CDCl_3$): $\delta$ 178.90, 131.06, 129.22, 128.92, 128.59, 128.49, 128.20, 127.98, 125.32, 62.73, 36.30, 32.50, 26.78, 25.97, 25.81 (3C), 25.02, 19.57, 14.03. ESIHRMS calcd for $C_{20}H_{31}N_3O_2Na$ $[M+Na]^+$: 368.2314. Found: 368.2316.

**4.1.12. (17*S*,5*Z*,8*Z*,11*Z*)-17-Azidoeicosa-5,8,11-trien-14-ynoic acid (18).** Azido acid **18** was prepared from acid **15** (40 mg, 0.126 mmol) by an analogous procedure as described for tetraene **16**. Yield of pure **18**: 16 mg (37%). $[\alpha]_D^{20}=-28.5$ (*c* 0.4, acetone). Analytical RP-HPLC: $R_t$=9.64 min. $^1$H NMR (300 MHz, $CDCl_3$): $\delta$ 5.30–5.50 (m, 6H, CH=CH), 3.38 (m, 1H, 17-CH), 2.95 (m, 2H) and 2.79 (m, 4H, 7-, 10- and 13-$CH_2$), 2.35 (m, 4H, 2- and 16-$CH_2$), 2.09 (m, 2H, 4-$CH_2$), 1.70 (m, 2H, 3-$CH_2$), 1.41 (m, 4H, 18- and 19-$CH_2$), 0.92 (t, 3H, $J$=6.8 Hz, $CH_3$). $^{13}$C NMR (75 MHz, $CDCl_3$): $\delta$ 178.87, 129.64, 129.10, 129.00, 128.69, 127.71, 124.99, 81.18, 75.84, 61.31, 35.83, 33.25, 26.62, 25.79, 25.69, 25.34, 24.67, 19.41, 17.39, 13.97. ESIHRMS calcd for $C_{20}H_{29}N_3O_2Na$ $[M+Na]^+$: 366.2157. Found: 366.2160.

### 4.2. General procedure for tritiation of (17*R*,5*Z*,8*Z*,11*Z*)-17-hydroxyeicosa-5,8,11-trien-14-ynoic acid (15) and (17*R*,5*Z*,8*Z*,11*Z*)-17-azidoeicosa-5,8,11-trien-14-ynoic acid (18)

In a 10 mL reaction ampule was placed a solution of either **15** (5 mg, 0.016 mmol) or **18** (5 mg, 0.014 mmol) in dry benzene (2 mL) and Lindlar's catalyst (5 mg) was added to the solution. The mixture was frozen with liquid nitrogen. After evacuation the ampule was filled with gaseous tritium up to a pressure 400 hPa. The reaction mixture was stirred for 1.5 h at rt and then frozen again. Tritium was removed by vacuum, more catalyst (5 mg) was added and the mixture was stirred additionally for 1 h in tritium atmosphere. Unreacted tritium gas was removed by applying vacuum. The catalyst was filtered off over silica gel and washed with benzene (3×1 mL). The combined filtrates were concentrated under reduced pressure and the residue was evaporated two times with methanol (2×2 mL) to remove labile tritium. The crude products were purified by HPLC to give either 630 mCi (90%) of **19** (specific activity 45 Ci/mmol) or 490 mCi (85%) of **20** (specific activity 40 Ci/mmol). Analytical RP-HPLC for **19**: $R_t$=6.07 min. Analytical RP-HPLC for **20**: $R_t$=13.5 min.

**4.2.1. (17*S*,5*Z*,8*Z*,11*Z*,14*Z*)-17-Aminoeicosa-5,8,11,14-tetraenoic acid (17).** In a three-neck flask was placed a solution of **16** (5.0 mg, 0.014 mmol) in methanol (3 mL). The flask was purged with argon and Lindlar catalyst (10 mg) was added to the solution. The argon was exchanged with hydrogen and the mixture was stirred for 1 h at rt. After the reaction was completed the catalyst was removed by filration over Celite and washed with methanol (3 mL). Combined filtrates were concentrated in vacuum. Yield of **17**: 4.4 mg (95%). $[\alpha]_D^{20}=-45.7$ (*c* 0.7, acetone). TLC: $R_f$=0.17 ($CHCl_3$/MeOH, 4:1 with 0.1% $NH_3$). $^1$H NMR (300 MHz, $CD_3OD$): $\delta$ 5.30–5.50 (m, 8H, CH=CH), 3.30 (m, 1H, 17-CH), 2.81 (m, 6H, 7-, 10- and 13-$CH_2$), 2.30 (m, 4H, 2- and 16-$CH_2$), 2.09 (m, 2H, 4-$CH_2$), 1.65 (m, 2H, 3-$CH_2$), 1.41 (m, 4H, 18- and 19-$CH_2$), 0.89 (t, 3H, $J$=6.8 Hz, $CH_3$). $^{13}$C NMR (75 MHz, $CDCl_3$): $\delta$ 132.20, 129.61, 128.78, 128.61, 128.54, 127.78, 127.46, 124.51, 51.12, 35.85, 35.73, 27.30, 26.05 (4C), 25.83, 19.00, 14.17. ESIMS *m*/*z* (%): 319 (100) $[M+H]^+$, 341 (20) $[M+Na]^+$.

**4.2.2. [14,15-$^3$H]-(17*S*,5*Z*,8*Z*,11*Z*,14*Z*)-17-Aminoeicosa-5,8,11,14-tetraenoic acid (21).** Radio-labelled acid **21** was prepared by entyrely analogous procedure as described for **17** from 90 mCi (specific radioactivity 40 Ci/mmol). Yield of **21** was 90%, while specific radioactivity remained unchanged.

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