Total Synthesis of Leukotriene E_4 Metabolites and Precursors to Radiolabeled Forms of Those Metabolites

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Four novel β-oxidation products of peptido leukotrienes, the 18-carboxy-19,20-dinor-LTE_4 (8), the 16-carboxy-17,18,19,20-tetranor-14,15-dihydro-LTE_4 (17), and their corresponding N-acetylated derivatives 9 and 18 were prepared by total synthesis and used to identify unknown polar metabolites derived from LTC_4 in rats and primates. Their syntheses were accomplished by forming the C-11–C-12 cis double bond using the Wittig reaction on the chiral diene aldehyde epoxide 6. Also the direct precursors of radioactive labeled material, the 18-carbomethoxy-19,20-dinor-14,15-didehydro-LTE_4 triester 12 and 16-carbomethoxy-17,18,19,20-tetranor-14,15-dihydro-11,12-didehydro-LTE_4 triester 26 were synthesized and allowed the incorporation of tritium. In the case of 26 the fragment C-8–C-16 was formed using a Pd^0-catalyzed coupling reaction between the terminal acetylene 20 and the vinyl bromide 22. A subsequent Wittig–Horner reaction furnished the *trans,trans*-diene geometry.

Introduction

Since the discovery of the leukotrienes[1] there has been an enormous ongoing effort to synthesize these products and analogues.[2] The peptido leukotrienes C_4, D_4, and E_4 are sequential metabolites of leukotriene A_4, itself produced by the action of 5-lipoxygenase on arachidonic acid.[3] Very recently a major focus of leukotriene research has been the elucidation of the metabolism of the carbon backbone of these compounds with the goal of accurately measuring leukotriene levels in animal models and human disease states.[4-11] It is known that leukotriene C_4 (LTC_4) is rapidly metabolized in vivo to LTD_4 and then to LTE_4 in the systemic circulation. Further in vivo metabolism of LTE_4 was expected, and recently we have identified ω-oxidation products of LTE_4 as the 20-carboxy-*N*-acetyl-LTE_4 metabolites in rat bile by using chemical syntheses and correlation with biological material.[4,5] However, additional unknown polar metabolites were also present. The correlation of synthetic and biological material reveals that 18-carboxy-19,20-dinor-*N*-acetyl-LTE_4 (9) and 16-carboxy-17,18,19,20-tetranor-14,15-dihydro-*N*-acetyl-LTE_4 (18) were present in rat bile.[12] Moreover, the corresponding free amines 8 and 17 of these two metabolites were present in the bile and urine of primates.[13] It is to be noted that the 16-carboxy-17,18,19,20-tetranor-14,15-dihydro-*N*-acetyl-LTE_4 (18) and its corresponding free amine 17 are double β-oxidation products saturated at the 14,15-position. The saturation of β-oxidation products has been previously observed in the case of linoleic acid[14,15] as well as in prostanoid products.[16,17] Another part of the project was to establish a method to detect the picomole amounts found in vivo and to study further metabolism of these compounds. Radioimmunological assay (RIA) is the method of choice to achieve that goal, and the availability of radioactive labeled material becomes of primary importance.

We wish to report the strategy and the synthesis of 18-carboxy-19,20-dinor-*N*-acetyl-LTE_4 (9), 16-carboxy-17,18,19,20-tetranor-14,15-dihydro-*N*-acetyl-LTE_4 (18), and their corresponding free amines 8 and 17. Also the immediate precursors of radioactive labeled material, 18-carbomethoxy-19,20-dinor-14,15-didehydro-LTE_4 triester 12 and 16-carbomethoxy-17,18,19,20-tetranor-14,15-dihydro-11,12-didehydro-LTE_4 triester 26, were synthesized.

Results and Discussion

A simple method to prepare the 18-carboxy-19,20-dinor-*N*-acetyl-LTE_4 (9) and the corresponding 14,15-didehydro triester derivative 12 is to use a Wittig reaction to construct the C-11–C-12 cis double bond using the appropriate phosphorane derived from 5 or 10 (Scheme I) with the previously reported chiral diene aldehyde epoxide 6.[18] Phosphonium salts 5 and 10 were readily obtained from the same acetylenic precursor 3 either via semihydrogenation, giving the homallylic alcohol 4, which was used for further elaboration to the dinor metabolites 8 and 9, or by keeping the acetylene function in 3 and transforming it to the precursor of radioactive material 12.

Protection of 3-butyn-1-ol[19] (Scheme I) and homologation by two carbons using ethylene oxide[20] gave the mon-

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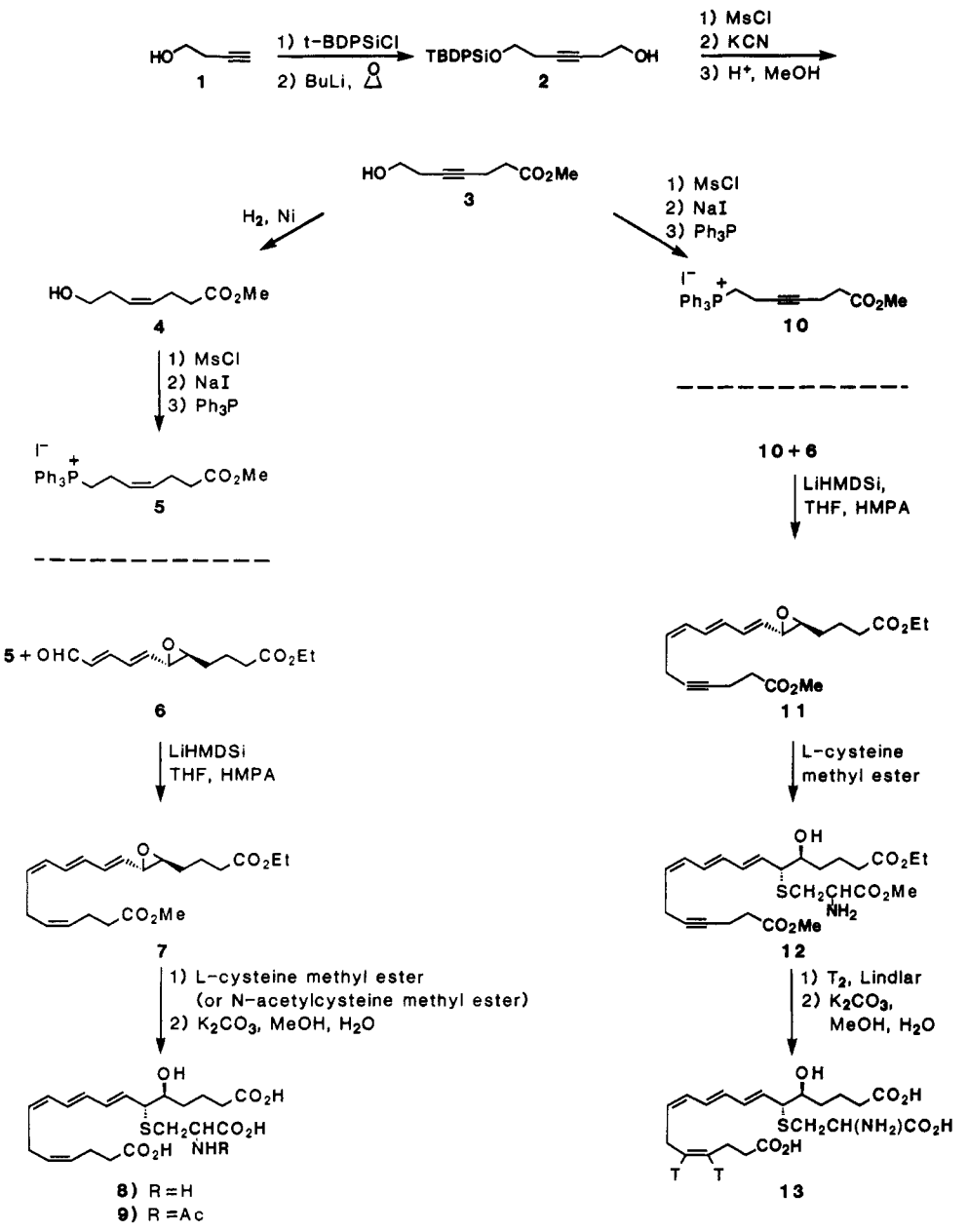
Scheme I



oprotected diol **2** in 66% yield. The hydroxyl group was then mesylated and displaced with potassium cyanide. Treatment with gaseous HCl in ether followed by aqueous acid treatment provided the hydroxy ester **3** in 44% overall yield. An efficient semihydrogenation of **3** using a nickel(0) catalyst[21] gave the homoallylic hydroxy ester **4** in 90% yield. The alcohol **4** was converted to the primary iodide via the corresponding mesylate, and treatment of the iodide with triphenylphosphine afforded the phosphonium salt **5**. Condensation of the phosphorane, generated from the phosphonium salt **5** (LiHMDSi, THF, HMPA, −70 °C), with the aldehyde **6**[18] furnished the tetraene epoxide **7** in 56% yield. Regiospecific epoxide opening with either L-cysteine methyl ester or *N*-acetylcysteine methyl ester in a mixture of methanol and triethylamine in the presence of 4-hydroxy-TEMPO (4-hydroxy-2,2,6,6-tetramethylpiperidinyloxy free radical) gave, after basic hydrolysis (0.25 M K_2CO_3 in methanol), the 18-carboxy-19,20-dinor-LTE_4 (**8**) or the corresponding *N*-acetyl derivative **9**.

The acetylenic alcohol **3** was also used to prepare **12**, the precursor of radiolabeled 18-carboxy-19,20-dinor-LTE_4 **13**. A similar sequence to that previously described for the formation of phosphonium salt **5** was applied to **3** to afford phosphonium salt **10** (Scheme I). Condensation of the phosphorane with the aldehyde **6** using conditions similar to those described above, followed by regiospecific epoxide opening with L-cysteine methyl ester, afforded the 14,15-acetylenic precursor **12**. Introduction of the tritium by semihydrogenation[22] followed by basic hydrolysis gave the

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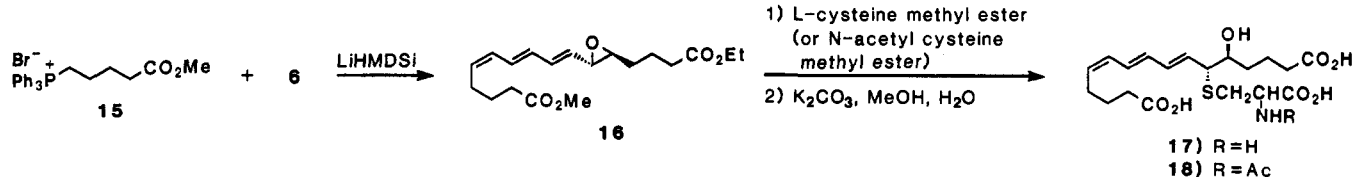
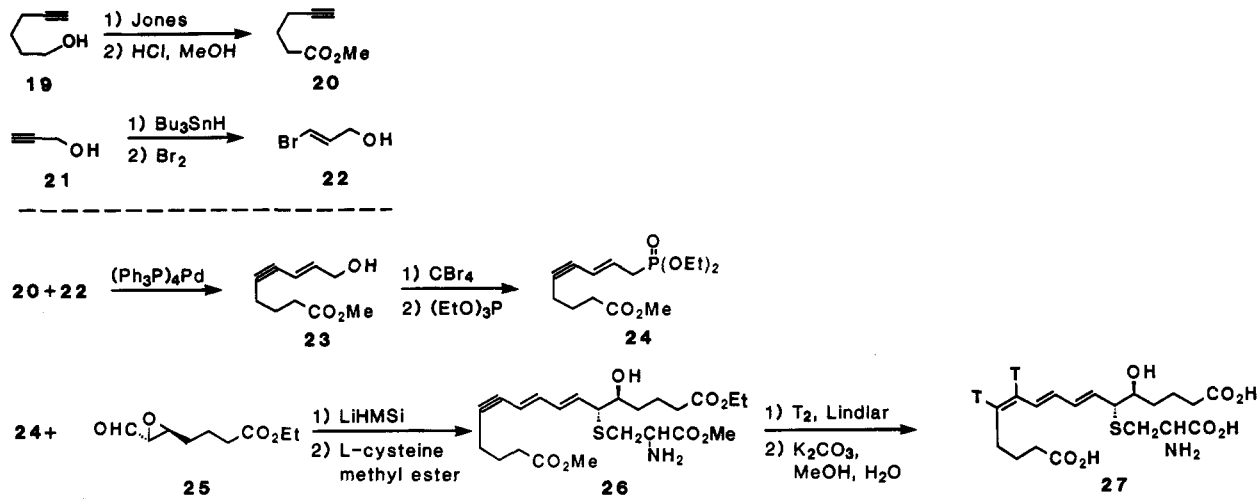
(22) The semihydrogenation with tritium were performed by E. Do and G. Iles of the lipid labs of New England Nuclear. General semihydrogenation procedure: the acetylene derivative and Lindlar catalyst in methanol containing 2% of 4-hydroxy-TEMPO (4-hydroxy-2,2,6,6-tetramethylpiperidinyloxy free radical) were stirred under an atmosphere of tritium (1 atm) for 1 h. At this time a mixture of methanol and 1% triethylamine was added, and the residue was purified by preparative HPLC. This yielded material with a specific activity of approximately 33 Ci/mmol. Subsequent basic hydrolysis using 0.25 M potassium carbonate in methanol for 18 h furnished the corresponding triacid, which was purified by reverse-phase HPLC.

Scheme II



Scheme III



radioactive triacid **13**, which was used in radioimmunological assays.

The second β-oxidation metabolites, 16-carboxy-17,18,19,20-tetranor-14,15-dihydro-LTE_4 (**17**) and it corresponding *N*-acetyl derivative **18** were obtained by using a similar strategy (Scheme II). The readily available phosphonium salt **15** was transformed to the ylide (LiHMDSi, THF, HMPA, −70 °C) and condensed with the diene aldehyde epoxide **6** to furnish the triene epoxide **16**, which was followed without delay by a regiospecific epoxide opening with L-cysteine methyl ester or *N*-acetylcysteine methyl ester to give, after basic hydrolysis, the corresponding triacid **17** or the *N*-acetyl derivative **18**. In both cases (**9** and **18**), the 11-trans isomer, which is produced in 5–10% during the Wittig reaction, elute before their corresponding 11-cis isomer on reverse-phase HPLC in contrast to corresponding isomers of the peptido leukotrienes C_4, D_4, E_4.

Since the tetranor derivative **17** has no double bond at the 14,15-position we had to consider another position in the molecule in order to introduce the radioactive label. Considering the synthetic aspects as well as the choice of the method of introducing the label, the appropriate precursor that we chose was the 11,12-acetylenic derivative. The synthesis of the 16-carbomethoxy-17,18,19,20-tetranor-14,15-dihydro-11,12-didehydro-LTE_4 was to be based on coupling the chiral epoxy aldehyde **25**[18] with the phosphonate **24** to obtain the *trans,trans*-diene geometry. Construction of the C-8–C-16 synthon **24** started with 5-hexyn-1-ol, which gave, after Jones oxidation and esterification, the acetylene ester **20** (Scheme III). Propargyl alcohol **21** was treated with excess tributyltin hydride[23] at 100 °C followed by treatment in situ with bromine at −20 °C to give the vinylic bromide **22**. Coupling of **20** and **22** in the presence of tetrakis(triphenylphosphine)palladium as catalyst in benzene containing *n*-propylamine[24] gave the acetylenic olefin derivative **23** in 51% yield. The allylic alcohol in **23** was transformed to the phosphonate **24** by treatment with carbon tetrabromide and 1,2-bis(diphenylphosphino)ethane (DiPHOS) followed by an Arbusov[25] reaction with triethylphosphite in refluxing benzene. The phosphonate **24** was then treated with LiHMDSi in dry THF at −70 °C for 15 min and then reacted with chiral epoxy aldehyde **25**[18] to give the corresponding diene epoxide, which after a rapid purification by flash chromatography was allowed to react with L-cysteine methyl ester as previously described. Product analysis by normal-phase HPLC revealed that the *trans,trans*-diene compound **26** was the major isomer. Treatment of **26** with Lindlar catalyst and T_2[22] followed by basic hydrolysis (K_2CO_3, MeOH, H_2O) gave the corresponding radioactive triacid **27**.

Comparison of UV spectra and HPLC coelution experiments revealed that compounds **9** and **18** correspond to the polar rat biliary metabolites after administration of [^{3}H]LTC_4.[12] This was further confirmed by coelution of the corresponding methyl esters derivatives. Product analysis of oxidative ozonolysis of the biological material corresponding to 16-carboxy-17,18,19,20-tetranor-14,15-dihydro-*N*-acetyl-LTE_4 (**18**) confirmed the absence of the 14,15-unsaturation.[12] In case of **8** and **17** the comparison with natural source was effected with compounds derived from biliary and urinary [^{3}H]LTC_4 metabolites in the cynomologus monkey.[13] The confirmation of identity was done by using UV spectra and coelution in several HPLC solvent systems as well as by a correlative shift in RPHPLC retention time on N-acetylation. Production of [^{3}H]glutaric acid without concomitant [^{3}H]H_2O on oxidative ozonolysis of the biological material corresponding to 16-carboxy-17,18,19,20-tetranor-14,15-dihydro-LTE_4 indicated saturation at the 14,15-position.[13]

In summary the above sequence for the preparation of metabolites **8**, **9**, **17**, and **18** is simple and efficient, and the strategy of making the corresponding acetylenic precursors

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12 and 26 allows access to the isotopically labeled forms of these important metabolites in substantial quantities.

Experimental Section

Tetrahydrofuran (THF) was distilled from sodium/benzophenone immediately prior to use. Hexamethylphosphoramide (HMPA) and methylene chloride were distilled from CaH_2 and stored under a nitrogen atmosphere. Flash chromatography refers to the procedure described by Still et al.[26] NMR spectra were recorded on a Bruker AM 250 (250 MHz) spectrometer. The purity of all title compounds was judged to be ≥90% by ^1H NMR spectral determinations. High-resolution mass spectra were obtained at the McGill University Mass spectrometry Unit on a ZAB-HS Spectrometer. TLC was performed with E. Merck 60F-254 precoated silica (0.2 cm) on glass.

Methyl 7-Hydroxy-4-heptynoate (3). To a solution of 3-butyn-1-ol (1) (10.0 g, 0.14 mol) in DMF (100 mL) at 0 °C was added imidazole (19.5 g, 0.28 mol) followed by *tert*-butyldiphenylchlorosilane (37.1 g, 0.14 mol). The mixture was stirred at room temperature for 30 min, water (400 mL) was added, and the resulting mixture was extracted with hexanes. The organic phase was washed twice with brine (100 mL), dried over $MgSO_4$, and evaporated to give 40 g (91%) of the silyl ether. ^1H NMR (250 MHz, $CDCl_3$): δ 1.06 (s, 9 H, $(CH_3)_3$), 1.95 (d, 1 H, J = 2 Hz), 2.45 (dt, 2 H, J = 2 Hz, 7 Hz), 3.78 (t, 2 H, J = 7 Hz), 7.4 (m, 6 H), 7.7 (m, 4 H). IR (neat): 3300, 3070, 1580 cm^{-1}. Anal. Calcd for $C_{20}H_{24}OSi$: C, 77.87; H, 7.84; Si, 9.10. Found: C, 78.18; H, 7.76; Si, 9.44.

To the silyl ether (3.0 g, 9.7 mmol) in a mixture of THF (5 mL), ether (14 mL), and HMPA (0.5 mL) was added triphenylmethane (1–2 mg). The solution was cooled to −40 °C, and a solution of *n*-BuLi (1.3 M in hexanes) was added until the color changed from yellow to orange. The solution was stirred for 15 min, and then neat ethylene oxide was added (1 mL, 19.5 mmol). The reaction mixture was stirred at room temperature for 30 h. Ether was added, and the organic phase was washed twice with brine/water (4:1), dried over $MgSO_4$, and evaporated to give after flash chromatography (20% ethyl acetate in hexanes) 2.5 g (73%) of primary alcohol, **2**. ^1H NMR (250 MHz, $CDCl_3$): δ 1.08 (s 9 H), 2.0 (br s, 1 H), 2.45 (m, 4 H), 3.66 (q, 2 H, J = 7 Hz), 3.77 (t, 2 H, J = 7 Hz), 7.4 (m, 6 H), 7.7 (m, 4 H). IR (neat): 3350 (br), 3070, 1580 cm^{-1}. Anal. Calcd for $C_{22}H_{28}O_2Si$: C, 74.95; H, 8.01; Si, 7.97. Found: C, 75.0; H, 8.17; Si, 8.22.

To a cold (−40 °C) solution of **2** (1.0 g, 2.8 mmol) in CH_2Cl_2 (25 mL) was added triethylamine (0.63 mL, 3.7 mmol) followed by dropwise addition of methanesulfonyl chloride (0.3 mL, 3.7 mmol). The resulting mixture was stirred at −20 °C for 30 min. Water was added, and the mixture was extracted with CH_2Cl_2. The combined organic extracts were washed successively with an aqueous 1 N HCl solution, saturated aqueous sodium bicarbonate, and brine and dried over anhydrous $MgSO_4$. The mesylate was obtained in 90% yield (1.1 g). ^1H NMR (250 MHz, $CDCl_3$): δ 1.09 (s, 9 H), 2.4 (m, 2 H), 2.6 (m, 2 H), 3.0 (s, 3 H), 3.75 (m, 2 H), 4.20 (t, 2 H, J = 7 Hz), 7.4 (m, 6 H), 7.7 (m, 4 H).

The mesylate (888 mg, 2.1 mmol) was treated with potassium cyanide (402 mg, 6.2 mmol) in a mixture of ethanol/water (4:1, 2 mL) at 60 °C overnight. The reaction mixture was extracted with CH_2Cl_2, and the combined organic extracts were washed successively with an aqueous 1 N HCl solution, saturated aqueous sodium bicarbonate, and brine and dried over anhydrous $MgSO_4$. The primary cyanide (700 mg, 93%) was obtained. ^1H NMR (250 MHz, $CDCl_3$): δ 1.09 (s, 9 H), 2.45 (t, 2 H, J = 7 Hz, 2 H), 2.50 (br s, 4 H), 3.75 (t, 2 H, J = 7 Hz), 7.4 (m, 6 H), 7.7 (m, 4 H). IR (neat): 3050, 2920, 2840, 2230, 1580 cm^{-1}.

The primary cyanide (320 mg, 0.89 mmol) was stirred in a mixture of methanol and ether (1:1, 10 mL) at room temperature, and HCl gas was bubbled through the solution for 45 min. Then 10% aqueuous HCl was added, and the resulting mixture was stirred overnight. After extraction with ethyl acetate and purification by flash chromatography (35% ethyl acetate in hexane), 73 mg (53%) of compound **3** was obtained. ^1H NMR (250 MHz, $CDCl_3$): δ 2.0 (br s, 1 H, OH), 2.38–2.58 (m, 6 H, 3 CH_2), 3.68 (t, 2 H, J = 7 Hz, CH_2O), 3.73 (s, 3 H, CO_2CH_3). IR (neat): 3450, 2950, 1740 cm^{-1}. High-resolution mass spectrum, m/e calcd for $C_8H_{13}O_3$ (M + H) 157.0865, found 157.0864.

(6-Carbomethoxy-3(*Z*)-hexen-1-yl)triphenylphosphonium Iodide (5). Sodium borohydride (24 mg, 0.64 mmol) was added to a suspension of $Ni(OAc)_2 \cdot 4H_2O$ (159 mg, 0.64 mmol) in 95% ethanol (4 mL) at room temperature. After 5 min ethylenediamine (43 μL, 0.64 mmol) was added followed by a solution of the acetylene **3** (100 mg, 0.64 mmol) in 95% ethanol (2 mL). The mixture was then hydrogenated. After 30 min the mixture was filtered through a pad of Celite to give after evaporation flash chromatograrphy (40% ethyl acetate in hexanes) 97 mg (90%) of the corresponding cis olefin **4**. ^1H NMR (250 MHz, $CDCl_3$): δ 2.35–2.42 (m, 7 H, OH, 3 CH_2), 3.68 (t, 2 H, J = 7 Hz, CH_2O), 3.69 (s, 3 H, CO_2CH_3), 5.40–5.57 (m, 2 H, J = 10.2 Hz, CH=CH).

Compound **4** (97 mg, 0.61 mmol) was mesylated as previously described for compound **2** to give 143 mg (99%) of the corresponding mesylate. The mesylate (143 mg, 0.6 mmol) was dissolved in acetone (3 mL), and sodium iodide (276 mg, 1.84 mmol) was added. The resulting solution was heated at 55 °C overnight and then worked up with CH_2Cl_2 in the usual manner. Flash chromatography (10% ethyl acetate in hexanes) gave 156 mg (93%) of the primary iodide. The iodide was treated with triphenylphosphine (183 mg, 0.7 mmol) in acetonitrile at 80 °C overnight. Evaporation of solvent followed by purification by flash chromatography (5% methanol in dichloromethane) gave a quantitative yield of **5** (310 mg). ^1H NMR (250 MHz, $CDCl_3$): δ 2.11–2.19 (m, 2 H, CH_2CH=), 2.29–2.34 (m, 2 H, CH_2CH=), 2.39–2.53 (m, 2 H, CH_2CO_2Me), 3.62 (s, 3 H, CO_2Me), 3.72–3.83 (m, 2 H, CH_2P), 5.29–5.40 (m, 1 H, CH=), 5.65–5.75 (m, 1 H, CH=), 7.67–7.90 (m, 15 H, 3Ph). High-resolution FAB mass spectrum (glycerol) calcd for $C_{26}H_{28}O_2P$ (M − I) 403.1827, found 403.1825.

(5*S*,6*R*)-5-Hydroxy-6-(*N*-acetylcystein-*S*-yl)-7,9-(*E*),11,14(*Z*)-octadecatetraenedioic Acid (18-Carboxy-19,20-dinor-*N*-acetyl-LTE$_4$, 9). To a stirred solution of the ylide generated by treatment of **5** (117 mg, 0.22 mmol) with a solution of LiHMDSi (0.34 M in THF, 0.72 mL, 0.24 mmol) in THF (2 mL) containing HMPA (0.3 mL) at −78 °C for 20–30 min under argon was added a solution of the aldehyde **6** (50 mg, 0.21 mmol) in THF (0.3 mL). After complete addition the reaction mixture was allowed to warm to 0 °C. After 2 h the reaction was quenched with 25% aqueous solution of ammonium acetate (0.5 mL) diluted with CH_2Cl_2, washed successively with a saturated aqueous sodium bicarbonate solution and brine, and dried over anhydrous Na_2SO_4. Flash chromatography (15% ethyl acetate in hexanes containing 1% triethylamine) gave 43 mg of the tetraene epoxide **7** (56%). The epoxide (6 mg, 0.017 mmol) was allowed to react with *N*-acetylcysteine methyl ester (6 mg, 0.33 mmol) in a mixture of MeOH/Et_3N (3:1, 0.5 mL) containing 4-hydroxy-TEMPO (4-hydroxy-2,2,6,6-tetramethylpiperidinyloxy free radical) (1 mg) at room temperature. After 3 h the solvent was evaporated, and the resulting product was purified by flash chromatography (hexanes–AcOEt–MeOH, 8:5:1) to give 13 mg (75%) of the corresponding ester. UV (MeOH) λ_{max}: 280 nm. ^1H NMR (250 MHz, acetone-d_6): δ 1.28 (t, 3 H, J = 7.5 Hz, CH_2CH_3), 1.44–1.91 (m, 4 H), 2.05 (s, 3 H, $COCH_3$), 2.37 (t, 2 H, J = 7.5 Hz, CH_2CO_2), 2.46 (br s, 2 H), 2.83 (dd, 1 H, J = 7.5, 13 Hz, SCH_2), 2.91 and 2.94 (2 s, 2 H), 3.0 (dd, 1 H, J ≈ 4.5, 13 Hz, SCH_2), 3.08 (t, 2 H, H-13,13′), 3.49 (dd, 1 H, J = 3.7, 10.5 Hz, H-6), 3.68 (s, 3 H, CO_2CH_3), 3.75 (s, 3 H, CO_2CH_3), 3.79 (m, 1 H, H-5), 4.06 (d, 1 H, OH), 4.12 (q, 2 H, J = 7.5 Hz, OCH_2), 4.71 (m, 1 H, CHN), 5.46 (m, 3 H, H-12,14,15), 5.74 (br t, 1 H, J = 11 Hz, H-7), 6.12 (t, 1 H, J = 11 Hz, H-11), 6.32 (m, 2 H, H-8, 9), 6.70 (br t, 1 H, J = 11 Hz, H-10), 7.54 (br d, 1 H, J = 7.5 Hz, NH).

The triester (2 mg) was dissolved in methanol (0.8 mL), cooled to 0 °C, and treated with 1 M potassium carbonate solution (0.2 mL). After the solution was stirred at room temperature for overnight, acetic acid (20 μL) was added, and the product was purified from its 11-trans isomer by reverse-phase HPLC (column: μ-Bondapak C_{18} 7.8 mm × 30 cm) using as solvent a mixture of MeOH/H_2O/AcOH (45:55:0.1) buffered to pH 5.5 with NH_4OH, 4.0 mL min^{-1}, retention time 9.3 min. The title compound **9** was obtained (1 mg, 56%). UV (MeOH) λ_{max}: 280 nm. ^1H NMR (300 MHz, CD_3OD): δ 1.3–1.8 (m), 1.99 (s, 3 H, NAc), 2.28 (m), 2.37 (m), 2.77 (dd, 1 H, J = 7.7, 13.5 Hz, SCH), 2.98 (m, 3 H, H-13,13′,SCH), 3.67 (m, 1 H, H-5), 4.42 (m, 1 H), 5.39 (m, 3 H,

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H-12,14,15), 5.63 (dd, 1 H, J = 9.8, 14 Hz, H-7), 6.01 (t, 1 H, J = 11 Hz, H-11), 6.23 (m, 2 H, H-8,9), 6.58 (t, 1 H, J = 12 Hz, H-10).

(5*S*,6*R*)-5-Hydroxy-6-cystein-*S*-yl-7,9(*E*),11,14(*Z*)-octadecatetraenedioic Acid (18-Carboxy-19,20-dinor-LTE$_4$, 8). The same procedure as for the preparation of compound **9** was used to produce compound **8** except L-cysteine methyl ester was used to open epoxide **7**. Flash chromatography (hex–AcOEt–MeOH, 8:5:1) gave the corresponding triester in 75% yield (11 mg from 11 mg of the epoxide **7**). UV (MeOH) λ_{max}: 280 nm. ^{1}H NMR (250 MHz, acetone-d_6) δ 1.20 (t, 3 H, J = 7.5 Hz, CH_2CH_3), 1.27–1.86 (m, 4 H), 2.28 (t, 2 H, J = 7.5 Hz, CH_2CO_2), 2.39 (br s, 2 H), 2.67 (dd, 1 H, J = 7.5, 13 Hz, SCH_2), 2.80 (dd, 1 H, J = 5.6 Hz, 13 Hz, SCH_2), 2.85 (br s, 4 H), 3.01 (t, 2 H, H-13,13′), 3.45 (dd, 1 H, J = 3.7, 9.4 Hz, H-6), 3.62 (s, 3 H, CO_2CH_3), 3.66 (s, 3 H, CO_2CH_3), 3.67 (m, 1 H), 4.06 (q, 2 H, J = 7.5 Hz, OCH_2), 5.40 (m, 3 H, H-12,14,15), 5.70 (br t, 1 H, J = 11 Hz, H-7), 6.06 (t, 1 H, J = 11 Hz, H-11), 6.26 (m, 2 H, H-8, 9), 6.62 (br t, 1 H, J = 11 Hz, H-10).

The triester was hydrolyzed as previously described to give **8** (56%, 1 mg from 2 mg of the corresponding triester) after purification by reverse-phase HPLC (column: μ-Bondapak C_{18}, 7.8 mm × 30 cm) MeOH/H_2O/ACOH (45:55:0.1) pH 5.5, 4.0 mL min^{-1}, retention time 9.5 min. UV (MeOH) λ_{max}: 280 nm. ^{1}H NMR (300 MHz, CD_3OD): δ 3.75 (m, 1 H), 5.4 (m, 3 H, H-12,14,15), 5.67 (t, 1 H, J = 14 Hz, H-7), 6.0 (t, 1 H, J = 11 Hz, H-11), 6.25 (m, 2 H, H-8,9), 6.6 (t, 1 H, J = 12.5 Hz, H-10).

Ethyl (5*S*,6*R*)-5-Hydroxy-6-[(cysteine methyl ester)-*S*-yl]-17-carbomethoxyheptadeca-7,9(*E*),11(*Z*)-trien-14-ynoate (18-Carbomethoxy-19,20-dinor-14,15-didehydro-LTE$_4$ triester, 12). Same procedure as for the preparation of compound **9** was applied to produce **12** except that the phosphorane derived from **10** was used in the Wittig reaction (48% yield, 73 mg from 100 mg of aldehyde **6**). Opening epoxide **11** with L-cysteine methyl ester gave the triester **12** in 80% yield (80 mg from 73 mg of epoxide **11**) after purification by flash chromatography (80% ethyl acetate in hexanes). UV (MeOH) λ_{max}: 278 nm. ^{1}H NMR (250 MHz, acetone-d_6): δ 1.20 (t, 3 H, J = 7.5 Hz, CH_2CH_3), 1.40–1.81 (m, 4 H), 2.26 (t, 2 H, J = 7.5 Hz, CH_2CO_2) 2.39–2.48 (m, 4 H), 2.66 (dd, 1 H, J = 7.5, 13 Hz, SCH_2), 2.80 (dd, 1 H, J = 5.6 Hz, 13 Hz, SCH_2), 3.05 (AB, 2 H, J = 7.5 Hz, H-13,13′), 3.44 (dd, 1 H, J = 4.5, 9.4 Hz, H-6), 3.61 (s, 3 H, CO_2CH_3), 3.64 (s, 3 H, CO_2CH_3), 3.66 (m, 1 H), 4.04 (q, 2 H, J = 7.5, CH_2O), 5.40 (dd, 1 H, J = 7.5, 11 Hz, H-12), 5.71 (dd, 1 H, J = 9.4, 11 Hz, H-7), 6.06 (br t, 1 H, J = 11 Hz, H-11), 6.26 (m, 2 H, H-8, 9), 6.51 (br t, 1 H, J = 11 Hz, H-10). Mass spectrum FAB (glycerol) 496 (M + H).

(5*S*,6*R*)-5-Hydroxy-6-(*N*-acetylcystein-*S*-yl)-7,9(*E*),11-(*Z*)-hexadecatrienedioic Acid (16-Carboxy-17,18,19,20-tetranor-14,15-dihydro-*N*-acetyl-LTE$_4$, 18). The same procedure as for the preparation of compound **9** was applied for **18** except that the phosphorane derived from **15** was used in the Wittig reaction (61%, 43 mg from 50 mg of aldehyde **6**). The epoxide **16** was treated with *N*-acetylcysteine methyl ester to give after purification by flash chromatography (80% ethyl acetate in hexanes) the corresponding triester (75%, 8 mg from 7 mg of epoxide **16**). UV (MeOH) λ_{max}: 278 nm. ^{1}H NMR (250 MHz, acetone-d_6): δ 1.14 (t, 3 H, J = 7.5 Hz, CH_2CH_3), 1.41–1.80 (m, 4 H), 1.92 (s, 3 H, $COCH_3$), 2.21–2.32 (m, 4 H), 2.72 (dd, 1 H, J = 7.5, 13 Hz, SCH_2), 2.82 (br s, 4 H), 2.86 (dd, 1 H, J = 5.6, 13 Hz, SCH_2), 3.41 (dd, 1 H, J = 3.7, 9.4 Hz, H-6), 3.61 (s, 3 H, CO_2CH_3), 3.66 (s, 3 H, CO_2CH_3), 3.67 (m, 1 H, H-5), 4.05 (q, 2 H, J = 7.5 Hz, CH_2O), 4.64 (m, 1 H, CHN), 5.42 (dd, 1 H, J = 10.5 Hz, 7.5 Hz, H-12), 5.67 (dd, 1 H, J = 9.4, 13 Hz, H-7), 6.07 (br t, 1 H, J = 11 Hz, H-11), 6.25 (m, 2 H, H-8, 9), 6.53 (br t, 1 H, J = 11 Hz, H-10), 7.47 (d, 1 H, J = 7.5 Hz, NH).

The triester was hydrolyzed as previously described to give **18** after purification by reverse-phase HPLC (column μ-Bondapak C_{18}; 7.8 mm × 30 cm) MeOH/H_2O/AcOH (45:55:0.1) pH 5.4, 4.0 mL min^{-1}, retention time 5.5 min. UV (MeOH) λ_{max}: 279 nm. ^{1}H NMR (300 MHz, CD_3OD): δ 2.11 (s, 3 H, $COCH_3$), 2.76 (dd, 1 H, J = 7.9, 13.5 Hz, SCH_2), 2.93 (dd, 1 H, J = 4.2, 13.7 Hz, SCH_2), 3.68 (m, 1 H), 4.38 (m, 1 H), 5.44 (m, 1 H, H-12), 5.62 (dd, 1 H, J = 9.2, 14 Hz, H-7), 6.01 (t, 1 H, J = 11 Hz, H-11), 6.2 (m, 2 H, H-8,9), 6.52 (t, 1 H, J = 12.3 Hz, H-10). Mass spectrum: FAB (neg, glycerol) m/e 456 (M − H$^+$).

(5*S*,6*R*)-5-Hydroxy-6-cystein-*S*-yl-7,9(*E*),11(*Z*)-hexadecatrienedioic Acid (16-Carboxy-17,18,19,20-tetranor-14,15-dihydro-LTE$_4$, 17). The same procedure as for the preparation of compound **9** was applied for **17** except that the phosphorane derived from **15** was used in the Wittig reaction (61% yield 43 mg from 50 mg of aldehyde **6**) and L-cysteine methyl ester was used to open epoxide **16**. Purification by flash chromatography (hexanes–AcoEt–MeOH, 8:5:1) gave the corresponding triester in 71% yield (7 mg from 7 mg of epoxide **16**). UV (MeOH) λ_{max}: 278 nm. ^{1}H NMR (250 MHz, acetone-d_6): δ 0.96 (t, 3 H, J = 7.5 Hz, CH_2CH_3), 1.21 and 1.44 (m, 4 H), 1.99–2.08 (m, 6 H), 2.60 (dd, 1 H, J = 7.5, 13 Hz, SCH_2), 2.61 (br s, 4 H), 2.67 (dd, 1 H, J = 5.6, 13 Hz, SCH_2), 3.22 (dd, 1 H, J = 3.7, 9.4 Hz, H-6), 3.38 (s, 3 H, CO_2CH_3), 3.40 (s, 3 H, CO_2CH_3), 3.42 (m, 2 H), 3.81 (q, 2 H, J = 7.5 Hz, OCH_2), 4.10 (t, 1 H, J = 6.8 Hz), 5.21 (br q, 1 H, J = 11.7 Hz, H-12), 5.50 (dd, 1 H, J = 9.4, 13 Hz, H-7), 5.84 (t, 1 H, J = 11 Hz, H-11), 6.02 (m, 2 H, H-8, 9), 6.28 (br t, 1 H, J = 13 Hz, H-10). Mass spectrum: FAB (glycerol) 472 (M + H).

The triester was hydrolyzed as previously described to give **17** after purification by reverse-phase HPLC (column: μ-Bondapak C_{18}; 7.8 mm × 30 cm) MeOH/H_2O/AcOH (45:55:0.1) pH 5.5, 4.0 mL min^{-1}, retention time 5.5 min. UV (MeOH) λ_{max}: 279 nm. ^{1}H NMR (300 MHz, CD_3OD): δ 1.2–1.8 (m), 2.24 (m), 2.77 (dd, 1 H, J = 10, 14 Hz), 3.08 (d, 1 H, J = 9.7 Hz), 3.43 (dd, 1 H, J = 3.9, 9.8 Hz, SCH_2), 3.75 (dd, 1 H, J = 4.4, 9.2 Hz, H-6), 5.45 (m, 1 H, H-12), 5.65 (dd, 1 H, J = 9.5, 14 Hz, H-7), 6.05 (t, 1 H, J = 11 Hz, H-11), 6.26 (m, 2 H, H-8,9), 6.56 (dd, 1 H, J = 11.7, 14 Hz, H-10).

Methyl 5-Hexynoate (20). To a solution of 5-hexyn-1-ol (1.5 g, 15.3 mmol) in acetone (25 mL) at 0 °C was added dropwise a solution of 2 N Jones reagent (30 mL), and the mixture was allowed to warm to room temperature. Water was added, and the resulting mixture was extracted with ether, washed successively with water and brine, and dried over anhydrous $MgSO_4$. The crude product (1.7 g, 88%) was dissolved in MeOH (10 mL) and added to a solution of anhydrous 5% HCl (prepared by the addition of acetyl chloride (4 mL) to MeOH (80 mL) at 0 °C). The resulting reaction mixture was allowed to warm to room temperature over 45 min. Solid sodium carbonate was added carefully until the reaction was neutral. After filtration the MeOH was distilled slowly to give a concentrated solution of the title compound, which was purified by flash chromatography (5% ether in pentanes). The solvent was distilled slowly to give the volatile title compound **20**. ^{1}H NMR (250 MHz, $CDCl_3$): δ 1.84 (quint, 2 H, J = 7 Hz, $CH_2CH_2CH_2$), 1.93 (t, 1 H, J = 2 Hz, C≡CH), 2.24 (dt, 2 H, J = 7 Hz, 2 Hz, $C{\equiv}CCH_2$), 2.45 (t, 2 H, J = 7 Hz, CH_2CO), 3.66 (s, 3 H, CO_2CH_3).

Methyl 9-Hydroxy-7(*E*)-nonen-5-ynoate (23). A solution of vinylic bromide **22** (642 mg, 4.7 mmol) in benzene (13 mL) was degassed by bubbling argon for 10 min. Distilled *n*-propylamine (577 μL, 7 mmol) was then added followed by tetrakis(triphenylphosphine)palladium (543 mg, 0.47 mmol), and the mixture was stirred under argon and protected from light for 45 min. The acetylene **20** (590 mg, 4.7 mmol) was then added in benzene (10 mL) followed by copper(I) iodide (142 mg, 0.75 mmol). The reaction mixture was stirred for 3 h. Ether was added and washed with saturated ammonium chloride solution. The organic phase was dried over Na_2SO_4 and concentrated, and the crude product was purified by flash chromatography (30% ethyl acetate in hexanes) to give 439 mg (51%) of the title compound **23**. ^{1}H NMR (250 MHz, $CDCl_3$): δ 1.80–1.90 (m, 2 H, $CH_2CH_2CH_2$), 2.33–2.46 (m, 4 H, $CH_2C{\equiv}$, CH_2CO_2), 3.67 (s, 3 H, CO_2CH_3), 4.20 (d, 2 H, J = 6 Hz, CH_2O), 5.68 (dd, 1 H, J = 3.7, 15 Hz, $CHC{\equiv}C$), 6.18 (dt, 1 H, J = 5.6, 15.6 Hz, $CH{=}CHCH_2$).

Methyl 9-(Diethylphosphono)-7(*E*)-nonen-5-ynoate (24). The alcohol **23** (100 mg, 0.55 mmol) was dissolved in CH_2Cl_2 (10 mL), and then carbon tetrabromide (272 mg, 0.82 mmol) was added followed by a solution of 1,2-bis(diphenylphosphino)ethane (328 mg, 0.82 mmol) in CH_2Cl_2 (5 mL) at −10 °C. After 10 min a mixture of 20% ethyl acetate in hexanes was added to precipitate the corresponding phosphine oxide. After silica gel filtration and solvent evaporation, purification by flash chromatography (10% ethyl acetate in hexanes) gave 134 mg (100%) of the primary allylic bromide.

The allylic bromide (41 mg, 0.17 mmol) was dissolved in benzene (1 mL), triethylphosphite (172 μL, 1.0 mmol) was added, and the reaction mixture was heated at 80 °C overnight. Di-

chloromethane was added, and the organic phase was worked up in the usual manner. Purification by flash chromatography (70% ethyl acetate in hexanes) gave 50 mg (99%) of the title compound **24**. ^{1}H NMR (250 MHz, $CDCl_3$): δ 1.31 (t, 6 H, J = 7.5 Hz, 2 CH_2CH_3), 1.84 (quint, 2 H, J = 7.5 Hz, $CH_2CH_2CH_2$), 2.32–2.47 (m, 4 H, $CH_2C{\equiv}C$, CH_2CO_2), 2.62 (dd, 2 H, J = 7.5 Hz, 22.5 Hz, $CH_2P{=}O$), 3.67 (s, 3 H, CO_2CH_3), 4.10 (quint, 4 H, J = 7.5 Hz, 2 CH_2O), 5.60 (br d, 1 H, $CHC{\equiv}C$), 5.93 (hept, 1 H, J = 7.5 Hz, $CHCH_2PO$). IR (neat): 2980, 1740 cm^{-1}. High-resolution mass spectrum: m/e calcd for $C_{14}H_{24}O_5P$ (M + H) 303.1362, found 303.1360.

Ethyl (5*S*,6*R*)-5-Hydroxy-6-[(cysteine methyl ester)-*S*-yl]-15-carbomethoxypentadeca-7,9(*E*)-dien-11-ynoate (16-Carbomethoxy-17,18,19,20-tetranor-14,15-dihydro-11,12-didehydro-LTE$_4$ Triester, 26). To a stirred solution of the phosphonate **24** (50 mg, 0.17 mmol) in THF (1 mL) was added at −70 °C a solution of LiHMDSi (0.34 M in THF, 0.54 mL, 0.18 mmol), and the resulting solution was stirred for 15 min under argon. Then a solution of the aldehyde **25** (34 mg, 0.18 mmol) in THF was added, and the temperature was raised slowly to room temperature. After 2.5 h the reaction was quenched with saturated ammonium chloride solution (0.5 mL) diluted with CH_2Cl_2, washed successively with a saturated solution of sodium bicarbonate and brine, and dried over anhydrous Na_2SO_4. Flash chromatography of the residue (20% ethyl acetate in hexanes containing 1% triethylamine) gave 12 mg (22%) of the corresponding epoxide. The epoxide (0.036 mmol) was dissolved in a mixture of methanol and triethylamine (3:1, 1 mL) containing L-cysteine methyl ester (15 mg, 0.089 mmol) and 4-hydroxy-TEMPO (4-hydroxy-2,2,6,6-tetramethylpiperidinyloxy free radical) (1 mg) at room temperature. After being stirred overnight evaporation of the solvent and flash chromatography (hexanes–AcoEt–MeOH, 8:5:1) gave 11 mg (65%) of the title compound (**26**). UV (MeOH) λ_{max}: 273 nm. ^{1}H NMR (250 MHz, $CDCl_3$): δ 1..25 (t, 3 H, J = 7.3 Hz, CH_2CH_3), 1.44–1.53 (m, 2 H), 1.60–1.91 (m, 6 H), 2.32 (t, 2 H, J = 7.2 Hz, CH_2CO_2), 2.39–2.48 (m, 2 H), 2.75 (dd, 1 H, J = 7.5, 13.7 Hz, SCH_2), 2.86 (dd, 1 H, J = 4.5, 13.8 Hz, SCH_2), 3.42 (dd, 1 H, J = 3.6, 9.7 Hz, H-6), 3.66 (m, 1 H), 3.68 (s, 3 H, CO_2CH_3), 3.74 (s, 3 H, CO_2CH_3), 4.12 (q, 2 H, J = 7.2 Hz, OCH_2), 5.59 (d, 1 H, $J_{9,10}$ = 15.7 Hz, H-10), 5.69 (dd, 1 H, $J_{6,7}$ = 10 Hz, $J_{7,8}$ = 15.6 Hz, H-7), 6.19 (dd, 1 H, $J_{7,8}$ = 15.1 Hz, $J_{8,9}$ = 10.7 Hz, H-8), 6.50 (dd, 1 H, $J_{8,9}$ = 10.7 Hz, $J_{9,10}$ = 15.4 Hz, H-9). High-resolution mass spectrum (glycerol) calcd for $C_{23}H_{36}O_7NS$ (M + H), 470.2212, found 470.2213.

Boc-L-Dmt-OH as a Fully N,S-Blocked Cysteine Derivative for Peptide Synthesis by Prior Thiol Capture. Facile Conversion of N-Terminal Boc-L-Dmt-Peptides to H-Cys(Scm)-Peptides

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An efficient, convenient preparation of *N*-L-(*tert*-butyloxycarbonyl)-2,2-dimethylthiazolidine-4-carboxylic acid (Boc-Dmt-OH) is reported. The following rate constants assess the coupling efficiency and racemization risk of Boc-L-Dmt-OC_6F_5 in THF at 22 °C. With H-Val-OMe, k_{couple} = 3.2 × 10^{-2} M^{-1} s^{-1}, and with Et_3N, k_{rac} < 10^{-8} M^{-1} s^{-1}. Conversions of derivatives of the sequence Cys-Gly-Gly-Ala to the corresponding Scm-functionalized (Scm = methoxycarbonylsulfenyl, MeOCOS) cysteine peptides illustrated transformation of –Dmt– to –Cys(Scm)– with or without prior Boc cleavage. A palladium black catalyzed hydrogenolysis of a benzyl ester in the presence of the thiazolidine is reported in the solution-phase synthesis of the tripeptide Boc-Dmt-Leu-Ala-OBzl. The functionalized octapeptide 58–51 of basic pancreatic trypsin inhibitor, H-Cys(Scm)-Met-Arg-Thr-Cys(Dnp)-Gly-Gly-Ala-OH, was prepared by a 4 + 4 thiol capture and acyl-transfer strategy to demonstrate the applicability of the thiazolidine to this method.

In this paper we report recent findings on the particular utility of cysteine N,S-blocking through thiazolidine formation for amide ligation by means of thiol capture. Previous reports[1] from this laboratory have described in detail the strategy of peptide synthesis by prior thiol capture and its application to the synthesis of a 29 amino acid fragment of Basic pancreatic trypsin inhibitor, BPTI (58–30).[2] As shown in Scheme I, the prior thiol capture strategy requires that an unsymmetrical disulfide bond be formed between the sulfur of a thiol functionalized rigid dibenzofuran-derived template containing one peptide fragment **1** and the sulfur of a cysteine residue residing at the amino terminus of a second peptide fragment **2**. The resulting aryl cysteinyl disulfide **3** then undergoes intramolecular *O,N*-acyl transfer, forming the new peptide amide **4**. Reaction of **4** with phosphine cleaves the disulfide bond, and protection of the so generated cysteine thiol yields the desired polypeptide **5**, completing one thiol capture cycle. Subsequent activation of **5** to **6** prepares the peptide for another thiol capture cycle. This multistep procedure is designed for the specific purpose of coupling large peptide fragments prepared by the solid-phase method. Synthesis proceeds in a linear fashion beginning at the C-terminus, and fragment couplings are made at each cysteine.

Synthesis of a complex peptide by prior thiol capture thus requires very careful selection of the functions used to protect the cysteine sulfhydryl. Although formation of a disulfide selectively from one sulfur in the presence of others has been accomplished previously in multifunctionalized molecules,[3] there is no widely acceptable method for accomplishing this transformation in either small or large peptides or in proteins.

The linear tactical sequence of ligations abbreviated in Scheme II emphasizes the three types of cysteine S-

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