

A Novel Protecting Group for Constructing Combinatorial Peptide Libraries

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(Received September 13, 2000)

3,4,5-Tris(octadecyloxy)benzyl alcohol, HO–Bzl(OC_{18})$_3$, was prepared from gallic acid and stearyl bromide. Using conventional step-wise elongation, *N*,*C*-protected peptides, Fmoc–AA_n–...–AA_1–OBzl(OC_{18})$_3$, were synthesized. The substituted benzyl esters were selectively cleaved by a treatment with 4 M hydrogen chloride in ethyl acetate to give Fmoc–AA_n–...–AA_1–OH and HO–Bzl(OC_{18})$_3$. Thus, the substituted benzyl group is effective for the protection of *C*-terminal carboxyl groups in liquid-phase peptide synthesis. Because the substituted benzyl group has a moderately high molecular weight, Fmoc–AA_n–...–AA_1–OBzl(OC_{18})$_3$ can be easily purified by size-exclusion chromatography; all protected peptides are eluted in the void fraction of a Sephadex LH-20 gel-filtration column. The combination of the carboxyl-protecting group Bzl(OC_{18})$_3$ with simple purification by the gel-filtration gives a novel route for constructing combinatorial peptide libraries in the solution phase.

Combinatorial chemistry has attracted much attention in various fields from lead compound searches in drug discovery[1] to host–guest chemistry as a means of chemical evolution.[2] Peptide libraries are the most classic example of combinatorial libraries, and synthetic peptide libraries are usually prepared in the solid phase.[3] Peptide synthesis on a polymer support is well established and the procedures are simple, including easy separation of the desired peptides from the reaction mixture by washing with a solvent. However, solid-phase synthesis has unavoidable problems: the difficulties in reacting the soluble reagents with all the reactive sites on the solid support and in monitoring the heterogeneous reaction. To overcome these problems, soluble supports have been proposed, such as a polyethylene glycol[4] and a dendrimer.[5] As polyethylene glycol is a mixture of homologs with a range of molecular weights, this heterogeneity makes monitoring reactions difficult: typically, chromatographic separation as well as mass spectral analysis is complicated. A dendrimer has a uniform molecular structure, but has multiple reactive sites in the molecule, which make completing as well as monitoring reactions somewhat difficult.

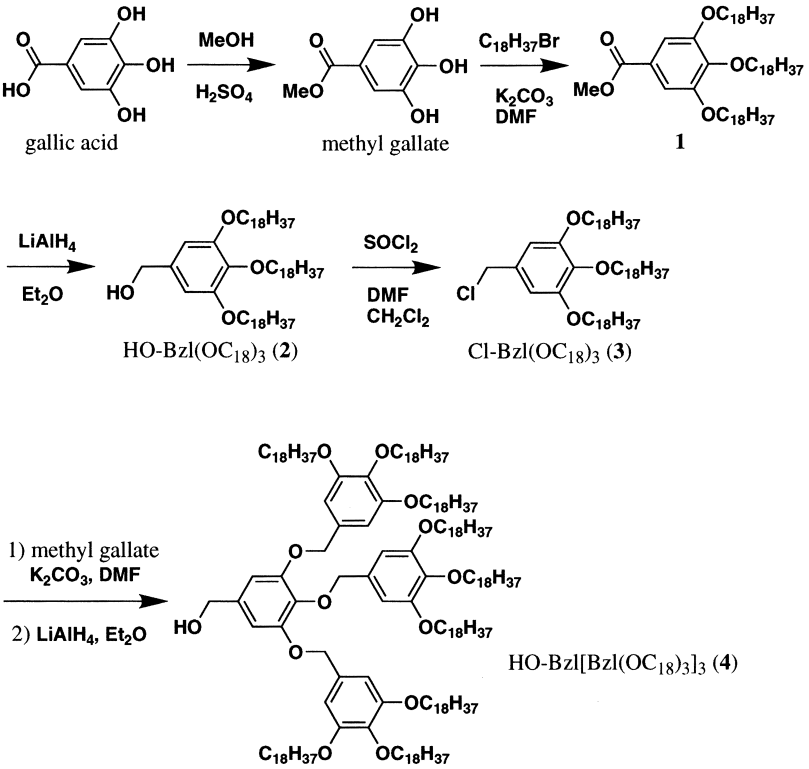
Here, we report on the preparation of a novel protecting group, 3,4,5-tris(octadecyloxy)benzyl alcohol **2**, for peptide synthesis in the solution phase. The substituted benzyl alcohol **2** is effective for protecting the carboxyl group in a conventional peptide synthesis using the Fmoc strategy. The *N,C*-protected peptides have well-defined molecular structures and the reaction is easily monitored by standard techniques used in organic synthesis, including chromatography, mass and NMR spectroscopies. Moreover, the protected peptides are fairly large molecules (MW > 1000) and are able to be easily purified by gel filtration. The desired peptides are eluted in the void fraction using size-exclusion chromatography. The combination of **2** as a carboxyl protecting group with simple purification by gel filtration gives a novel route for constructing synthetic peptide libraries.[6]

Results and Discussion

Several protecting groups for the carboxyl group in liquid-phase peptide synthesis are available:[7] the acid-labile *t*-butyl group and the benzyl group cleavable by hydrogenation are generally used. Substituted benzyl esters have also been investigated as protecting groups. The stability is dependent upon the substituents:[7] electron-withdrawing groups on the benzene ring stabilize the esters to acidic conditions and electron-donating groups make the esters more acid-labile. To prepare an acid-labile carboxyl protecting group with a fairly high molecular weight, a 3,4,5-trialkoxybenzyl alcohol was investigated. The starting material, gallic acid, is inexpensive and has many reactive sites on the benzene ring. Saturated straight alkyl chains, –$(CH_2)_{n-1}CH_3$, are chemically stable and the long alkyl chains increase the molecular weight. Octadecyl groups (n = 18) were used in the present study. Inexpensive and commercially available stearyl bromide is effective as an octadecyl-introducing reagent to all three phenolic hydroxy groups of gallic acid.

3,4,5-Tris(octadecyloxy)benzyl alcohol (HO–Bzl(OC_{18})$_3$, **2**) was prepared from gallic acid and stearyl bromide as shown in Scheme 1. Percec and his colleagues have already reported the preparation of 3,4,5-tris(dodecyloxy)benzyl alcohol,[8] and we slightly modified their procedures (see Experimental section). The synthetic procedures are so simple that the large-scale preparation of **2** is possible without any difficulty.

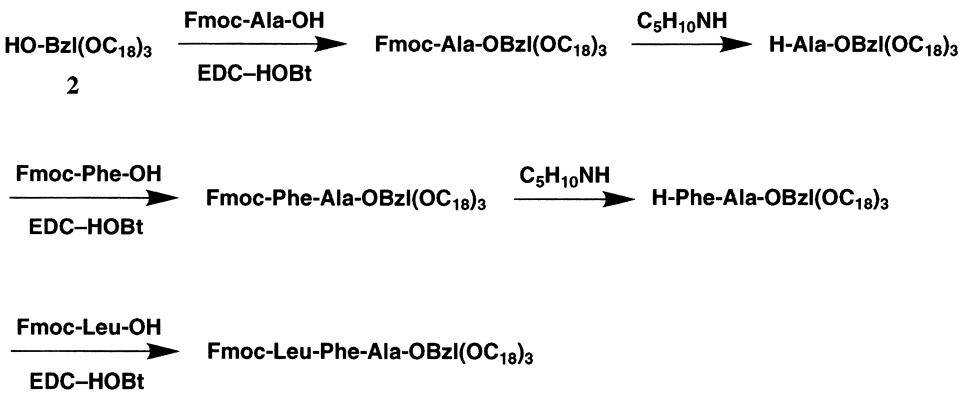
Because 3,4,5-tris(octadecyloxy)benzyl esters have elec-



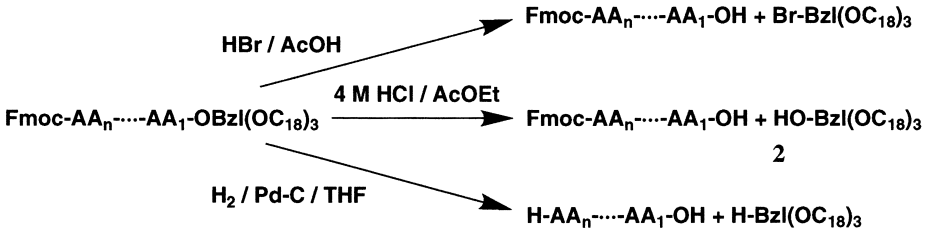
Scheme 1. Synthesis of carboxyl-protecting substituted benzyl alcohols.

tron-donating groups, they are expected to be hydrolyzed by a treatment with acid, as described above. Therefore, the base-labile and acid-resistant 9-fluorenylmethyloxycarbonyl (Fmoc) group was used for protection of the amino groups in the peptide syntheses. Fmoc-Ala-OH reacted with HO–Bzl(OC_{18})$_3$ (**2**) in dichloromethane with 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide (EDC) as the coupling reagent in the presence of 1-hydroxybenzotriazole (HOBt) to give Fmoc–Ala–O–Bzl(OC_{18})$_3$ in 63% isolated yield (Scheme 2). The Fmoc protecting group was selectively cleaved by the action of piperidine in dichloromethane at 0 °C to give H–Ala–OBzl(OC_{18})$_3$ in 92% isolated yield. The reaction of Fmoc–Phe–OH with H–Ala–OBzl(OC_{18})$_3$ by EDC–HOBt afforded Fmoc–Phe–Ala–OBzl(OC_{18})$_3$ in 67% isolated yield. Fmoc-deprotection of the dipeptide and successive peptide formation with Fmoc–Leu–OH similarly yielded the protected tripeptide Fmoc–Leu–Phe–Ala–OBzl(OC_{18})$_3$. All of the synthetic compounds were purified by silica-gel chromatography and/or recrystallization and characterized by their ^{1}H NMR, infra-red and/or mass spectra. All of the compounds were pure enantiomers or diastereomers, indicating that no racemization occurred. It is to be noted that all of the reactions were clean, and the yields estimated from TLC and ^{1}H NMR analyses were higher (> 90%) than the isolated yields described above.

Cleavage of the ester bond of the 3,4,5-tris(octadecyloxy)benzyl esters was examined under several conditions (see Scheme 3). The treatment of Fmoc–Ala–OBzl(OC_{18})$_3$ by hydrogen bromide in acetic acid induced a selective cleavage of the ester to give Fmoc–Ala–OH without any loss of the Fmoc group. Rather than hydrolyzed HO–Bzl(OC_{18})$_3$, the corresponding bromide Br–Bzl(OC_{18})$_3$ was isolated. When Fmoc–Ala–OBzl(OC_{18})$_3$ was subjected to less acidic conditions, such



Scheme 2. Synthesis of peptides Fmoc–AA_n–...–AA_1–OBzl(OC_{18})$_3$.



Scheme 3. Cleavage of protecting groups in Fmoc–AA_n–...–AA_1–OBzl(OC_{18})$_3$.

as 4 M hydrogen chloride in ethyl acetate (1 M = 1 mol dm^{-3}),[9] the quantitative hydrolysis afforded a 1:1 mixture of Fmoc–Ala–OH and HO–Bzl(OC_{18})$_3$. After evaporation, the resulting residue was treated with hexane and insoluble Fmoc–Ala–OH was isolated as a pure white solid by filtration. The filtrate was evaporated to give pure HO–Bzl(OC_{18})$_3$, which could be re-used for protection as it stood. The acidic hydrolysis procedure is very simple and this acidic cleavage was applied to other peptides. Fmoc–Phe–Ala–OBzl(OC_{18})$_3$ and Fmoc–Leu–Phe–Ala–OBzl(OC_{18})$_3$ were hydrolyzed by stirring in 4 M HCl–AcOEt overnight and successively evaporated in vacuo to give Fmoc–Phe–Ala–OH and Fmoc–Leu–Phe–Ala–OH, respectively, plus HO–Bzl(OC_{18})$_3$. Hydrogenation of Fmoc–Ala–OBzl(OC_{18})$_3$ on palladium–charcoal in tetrahydrofuran induced deprotection of both the Fmoc and Bzl(OC_{18})$_3$ groups to give unprotected alanine in one step. After hydrogenation, 3,4,5-tris(octadecyloxy)toluene, H–Bzl(OC_{18})$_3$, was isolated, and thus could not be re-used as a protecting reagent. The Fmoc–Ala–OH and H–Ala–OH formed were both in their L-forms and no racemization was observed during the above-mentioned deprotections. Thus, the 3,4,5-tris(octadecyloxy)benzyl group has been proven to be an effective carboxyl protecting group in peptide synthesis.

In the peptide synthesis described above, the resulting *N*,*C*-protected peptides (or amino acid) Fmoc–AA_n–…–AA_1–O–Bzl(OC_{18})$_3$ were purified by silica-gel chromatography. These peptides have fairly large molecular weights because of the *C*-protected Bzl(OC_{18})$_3$ group. We therefore examined the idea of purifying the peptides by size-exclusion chromatography with Sephadex LH-20 from Amersham Pharmacia Biotech, a hydroxypropylated cross-linked dextran gel in organic solvents. After complete consumption of an amino component (checked by TLC), the dichloromethane solution containing the reaction mixture was loaded onto a LH-20 gel column. The column was eluted with dichloromethane and the first void fraction was collected and evaporated to give a white solid. The ^{1}H NMR and mass spectra showed that the solid was solely the desired peptides, indicating that such a gel filtration was an extremely simple purification for all the Fmoc–AA_n–…–AA_1–OBzl(OC_{18})$_3$ peptides prepared.

We examined the preparation of a potential combinatorial peptide library by using Bzl(OC_{18})$_3$ group and gel filtration. A mixture of Fmoc–Ala–OH (0.5 mol amt.) and Fmoc–Phe–OH (0.5 mol amt.) was coupled with H–Ala–OBzl(OC_{18})$_3$ (1 mol amt.) by EDC–HOBt in dichloromethane. The reaction mixture was directly purified by LH-20 gel-filtration and the void fraction was a 1:1 mixture of Fmoc–Ala–Ala–OBzl(OC_{18})$_3$ and Fmoc–Phe–Ala–OBzl(OC_{18})$_3$. The purification procedure is so simple that the methodology should provide a new strategy for constructing combinatorial peptide libraries.

At 914, the molecular weight of HO–Bzl(OC_{18})$_3$ (**2**) is not very high. A larger benzyl alcohol, 3,4,5-tris[3,4,5-tris(octadecyloxy)benzyloxy]benzyl alcohol (HO–Bzl[OBzl(OC_{18})$_3$]$_3$, **4**, MW 2871), was prepared from the coupling of methyl gallate and Cl–Bzl(OC_{18})$_3$ (**3**) with successive $LiAlH_4$-reduction in 90% isolated yield for the two steps by a slight modification of the reported procedures.[8] Fmoc–Ala–OH reacted with HO–Bzl[OBzl(OC_{18})$_3$]$_3$ (**4**) by EDC–HOBt to give Fmoc–Ala–OBzl[OBzl(OC_{18})$_3$]$_3$ in 30% isolated yield. The low yield is ascribed to the low reactivity of the sterically hindered alcohol. The reactivity should increase by using spacers, as in commercially available polymer supports.[10]

Because the benzyl group is effective for the protection of hydroxy and mercapto groups,[7] the Bzl(OC_{18})$_3$ group should also be useful for the protection of OH and SH as well as COOH. Indeed, Cl–Bzl(OC_{18})$_3$ reacted smoothly with methyl gallate, as described above. Moreover, 3,4,5-tris(octadecyloxy)benzoic acid, prepared by the hydrolysis of methyl 3,4,5-tris(octadecyloxy)benzoate (**1**) with KOH (89%), should be useful for protection of hydroxy groups through the formation of an ester bond and 3,4,5-tris(octadecyloxy)benzaldehyde, prepared by the oxidation of HO–Bzl(OC_{18})$_3$ (**2**) with pyridinium chlorochromate (87%), should be useful for the protection of amino groups through the formation of an imino bond. All of the protected compounds proposed above would be purified by a simple gel filtration as well. Although we have demonstrated only peptide syntheses, the Bzl(OC_{18})$_3$ group together with these derivatives described above should be applicable to the construction of many small molecule libraries.

Experimental

General. All melting points were measured with a Yanagimoto micro melting apparatus and are uncorrected. Infrared absorption spectra were measured with a Shimadzu FTIR-8600 spectrophotometer. ^{1}H NMR spectra were measured with a Bruker AC-300 spectrometer; δs are expressed in parts per million relative to $CHCl_3$ (7.26 ppm) as an internal reference. MALDI-TOF MS were recorded on a Thermoquest Finnigan Lasermat spectrometer; samples were dissolved in $CHCl_3$ and 2,5-dihydroxybenzoic acid or α-cyano-4-hydroxycinnamic acid was used as the matrix. The elementary analyses were performed at the Microanalysis Center of Kyoto University.

General Synthetic Procedures. Coupling: Fmoc–AA_n–OH (3.0 mmol), H–AA_{n-1}–…–AA_1–OBzl(OC_{18})$_3$ (1.5 mmol), and HOBt (3.0 mmol) were dissolved in 60 mL of dry CH_2Cl_2 under N_2. EDC•HCl (3.3 mmol) and Et_3N (0.3 mL) were added to an ice-chilled solution and the mixture was stirred at room temperature. After the disappearance of H–AA_{n-1}–…–AA_1–OBzl(OC_{18})$_3$

was checked by TLC (about 1 day), the mixture was washed with brine six times and dried over Na_2SO_4. The solvent was evaporated and a(n) (oily) residue was solidified from methanol to give crude white Fmoc–AA_n–…–AA_1–OBzl(OC_{18})$_3$. In the case of $n = 1$, HO–Bzl(OC_{18})$_3$ was not completely consumed, even after 3-days reaction, and was removed by washing the crude product with hexane. Further purification by flash column chromatography (FCC) with silica gel (Merck, Kieselgel 60, 9385) and recrystallization afforded the pure desired coupled compound.

Fmoc-Deprotection: To an ice-chilled dry CH_2Cl_2 solution (5 mL) of Fmoc–AA_n–…–AA_1–OBzl(OC_{18})$_3$ (0.1 mmol) was added dropwise an ice-chilled dry CH_2Cl_2 solution (5 mL) of piperidine (3 mL) under N_2. The solution was stirred at room temperature for 40 min and ice-chilled methanol (40 mL) was added dropwise with cooling in an ice–water bath. The resulting white precipitate was filtered and washed with methanol to give pure H–AA_n–…–AA_1–OBzl(OC_{18})$_3$.

Ester-Cleavage: Fmoc–AA_n–…–AA_1–OBzl(OC_{18})$_3$ (15 μmol) was dissolved in 4 M HCl–AcOEt (10 mL) and the solution was stirred at room temperature overnight. After evaporation in vacuo, the residue was a 1:1 mixture of Fmoc–AA_n–…–AA_1–OH and HO–Bzl(OC_{18})$_3$. The white solid was treated with a small amount of hexane and the insoluble acid was separated from the hexane-soluble alcohol by filtration. The separated solid was a pure *N*-protected peptide. The filtrate was evaporated to give pure HO–Bzl(OC_{18})$_3$. In the case of $n = 1$ and AA_1 = Ala, the chirality of the resulting Fmoc–Ala–OH was checked: Fmoc–Ala–OH was first transformed to H–Ala–OH by treatment with piperidine in dichloromethane as described above and then the unprotected alanine was analyzed by the chiral HPLC as described below.

Fmoc/Ester-Cleavage: Fmoc–AA_n–…–AA_1-OBzl(OC_{18})$_3$ (15 μmol) was dissolved in THF (10 mL) and 5% palladium–charcoal (10 mg) was added to the solution. The suspension was stirred at room temperature overnight under a hydrogen atmosphere. After filtration and evaporation in vacuo, the residue was a 1:1 mixture of H–AA_n–…–AA_1–OH and H–Bzl(OC_{18})$_3$. In the case of $n = 1$ and AA_1 = Ala, the chirality of the resulting H–Ala–OH was checked as follows. After treatment of the residue with water, the aqueous solution was analyzed by a chiral HPLC: SUMICHIRAL OA-6100, 4.6ϕ × 250 mm, Sumika Chemical Analysis Service, aqueous 1 mM $CuSO_4$, 1.0 mL/min, at room temperature. The peaks of H–D–Ala–OH and H–L–Ala–OH were completely resolved and their retention times were 7.7 (D) and 14.7 min (L).

Gel-Filtration: Peptide syntheses were performed at a 60-times smaller scale (1 mL CH_2Cl_2 solution) than the coupling procedures described above. After the reaction, the resulting solution was loaded onto a 9.1 ϕ × 193 mm column of hydroxypropylated cross-linked dextran gel (Amersham Pharmacia Biotech, Sephadex LH-20) and CH_2Cl_2 was used as the solvent, eluting at 0.40 mL / min. The desired peptides were eluted at 4.2–5.2 mL of eluent volume as the void fraction.

Methyl 3,4,5-Tris(octadecyloxy)benzoate (1). A freshly distilled DMF suspension (400 mL) of K_2CO_3 (39 g, 290 mmol) was fully purged with N_2, to which was added stearyl bromide (30.9 g, 93 mmol). The mixture was heated to 70 °C under N_2 and then methyl gallate (5.4 g, 30 mmol; commercially available and also prepared from gallic acid and methanol in the presence of sulfuric acid, 81%) was added with stirring. After 5-h heating, the cooled mixture was poured into ice water (1.5 L) and the resulting precipitate was filtered and washed with methanol. The pale-brown solid was dissolved in CH_2Cl_2 and filtered to remove any undissolved brown products. The filtrate was concentrated in vacuo and the residue was recrystallized from methanol to give crude **1** as a pale-yellow powder (24.3 g). Further purification by FCC with CH_2Cl_2 afforded pure **1**; white powder; mp 63–64 °C; IR (KBr) 1717 (C=O), 1587 (C=C), 860 cm^{-1} ($C^{2,6}$–H); ^{1}H NMR ($CDCl_3$) δ 0.87 (9H, t, J = 7 Hz, 3,4,5-O–C_{17}–CH_3), 1.25 (84H, m, 3,4,5-O–C_3–$(CH_2)_{14}$), 1.47 (6H, m, 3,4,5-O–C_2–CH_2), 1.81 (6H, m, 3,4,5-O–C–CH_2), 3.88 (3H, s, 1-$COOCH_3$), 4.00 (6H, t, J = 6 Hz, 3,4,5-OCH_2), 7.23 (2H, s, 2,6-H). TOFMS found: *m/z* 943. Calcd for $C_{62}H_{117}O_5$: MH^+, 942. Found: C; 78.81, H; 12.69%. Calcd for $C_{62}H_{116}O_5$: C; 79.09, H; 12.42%.

3,4,5-Tris(octadecyloxy)benzyl Alcohol (HO–Bzl(OC_{18})$_3$, 2). To an ice-chilled dry Et_2O solution (120 mL) of the above crude **1** (3.9 g, < 4.1 mmol) was added $LiAlH_4$ (0.19 g, 5.0 mmol) under N_2 and the mixture was then refluxed for 2 h. After the mixture was cooled, water (0.2 mL) and aq 15% NaOH (0.2 mL) were added dropwise to give precipitates. The filtered solid was treated with THF and the undissolved products were removed. The filtrate was concentrated in vacuo and the residue was recrystallized from 1-propanol to give pure **2** (3.21 g, 76% for the above two steps); white powder; mp 67–68 °C; IR (KBr) 3540, 3480 (O–H), 1595 (C=C), 814 cm^{-1} ($C^{2,6}$–H); ^{1}H NMR ($CDCl_3$) δ 0.88 (9H, t, J = 7 Hz, 3,4,5-O–C_{17}–CH_3), 1.25 (84H, m, 3,4,5-O–C_3–$(CH_2)_{14}$), 1.46 (6H, m, 3,4,5-O–C_2–CH_2), 1.79 (6H, m, 3,4,5-O–C–CH_2), 3.93 (2H, t, J = 6.5 Hz, 4-OCH_2), 3.97 (4H, t, J = 6.5 Hz, 3,5-OCH_2), 4.59 (2H, s, 1-CH_2), 6.56 (2H, s, 2,6-H). TOFMS found: *m/z* 913. Calcd for $C_{61}H_{116}O_4$: M^+, 913. Found: C; 79.93, H; 13.07%. Calcd for $C_{61}H_{116}O_4$: C; 80.20, H; 12.80%.

***N*-(9-Fluorenylmethyloxycarbonyl)alanine 3,4,5-Tris(octadecyloxy)benzyl Ester (Fmoc–Ala–OBzl(OC_{18})$_3$).** Coupling of Fmoc–Ala–OH with **2** by EDC–HOBt gave crude Fmoc–Ala–OBzl(OC_{18})$_3$. Further purification by FCC with CH_2Cl_2 and recrystallization from methanol afforded the pure protected alanine (63% as isolated yield and 80% based on consumed **2**); white powder; mp 97–99 °C; IR (KBr) 3310 (N–H), 1734 (ester C=O), 1690 (amide C=O), 1591 (C=C), 822 cm^{-1} ($C^{2,6}$–H); ^{1}H NMR ($CDCl_3$) δ 0.88 (9H, t, J = 6 Hz, 3,4,5-O–C_{17}–CH_3), 1.25 (87H, m, 3,4,5-O–C_3–$(CH_2)_{14}$, Ala–α-CH_3), 1.46 (6H, m, 3,4,5-O–C_2–CH_2), 1.79 (6H, m, 3,4,5-O–C–CH_2), 3.92 (2H, t, J = 6 Hz, 4-OCH_2), 3.94 (4H, t, J = 6 Hz, 3,5-OCH_2), 4.23 (1H, t, J = 7 Hz, Fmoc–9-H), 4.38 (2H, d, J = 6 Hz, Fmoc–9-CH_2), 4.46 (1H, m, Ala–α-H), 5.08 (2H, s, 1-CH_2), 5.38 (1H, d, J = 8 Hz, Ala–NH), 6.52 (2H, s, 2,6-H), 7.31, 7.40 (each 2H, t, J = 7 Hz, Fmoc–2,3,6,7-H), 7.59, 7.76 (each 2H, d, J = 7 Hz, Fmoc–1,4,5,8-H). TOFMS found: *m/z* 1207. Calcd for $C_{79}H_{132}NO_7$: MH^+, 1207.

Alanine 3,4,5-Tris(octadecyloxy)benzyl Ester (H–Ala–O–Bzl(OC_{18})$_3$). Deprotection of the Fmoc group in Fmoc–Ala–OBzl(OC_{18})$_3$ by piperidine gave H–Ala–OBzl(OC_{18})$_3$ (92%); white powder; mp 62–65 °C; IR (KBr) 1734 (ester C=O), 1589 (C=C), 818 cm^{-1} ($C^{2,6}$–H); ^{1}H NMR ($CDCl_3$) δ 0.88 (9H, t, J = 6 Hz, 3,4,5-O–C_{17}–CH_3), 1.25 (87H, m, 3,4,5-O–C_3–$(CH_2)_{14}$, Ala–α-CH_3), 1.46 (6H, m, 3,4,5-O–C_2–CH_2), 1.79 (6H, m, 3,4,5-O–C–CH_2), 3.59 (1H, q, J = 7 Hz, Ala–α-H), 3.93 (2H, t, J = 6 Hz, 4-OCH_2), 3.95 (4H, t, J = 6 Hz, 3,5-OCH_2), 5.04 (2H, s, 1-CH_2), 6.52 (2H, s, 2,6-H).

***N*-(9-Fluorenylmethyloxycarbonyl)phenylalanylalanine 3,4,5-Tris(octadecyloxy)benzyl Ester (Fmoc–Phe–Ala–OBzl–(OC_{18})$_3$).** Coupling of Fmoc–Phe–OH with H–Ala–OBzl–(OC_{18})$_3$ by EDC–HOBt gave crude Fmoc–Phe–Ala–OBzl(OC_{18})$_3$. Further purification by FCC with 5% Et_2O–CH_2Cl_2 and recrystallization from methanol afforded the pure protected dipeptide (67%); white powder; mp 102–104 °C; IR (KBr) 3294 (N–H),

1736 (ester C=O), 1692 (urethane amide C=O), 1653 (amide C=O), 1591 (C=C), 820 cm^{-1} ($C^{2,6}$–H); ^1H NMR ($CDCl_3$) δ 0.88 (9H, t, J = 6 Hz, 3,4,5-O–C_{17}–CH_3), 1.25 (87H, m, 3,4,5-O–C_3–$(CH_2)_{14}$, Ala–α-CH_3), 1.46 (6H, m, 3,4,5-O–C_2-CH_2), 1.79 (6H, m, 3,4,5-O–C–CH_2), 3.07 (2H, m, Phe–α-CH_2), 3.93 (2H, t, J = 6 Hz, 4-OCH_2), 3.95 (4H, t, J = 6 Hz, 3,5-OCH_2), 4.19 (1H, t, J = 7 Hz, Fmoc–9-H), 4.33 (1H, m, Ala–α-H), 4.43 (2H, m, Fmoc–9-CH_2), 4.54 (1H, m, Phe–α-H), 5.04 (2H, s, 1-CH_2), 5.30 (1H, br-d, Phe–NH), 6.30 (1H, br-d, Ala–NH), 6.50 (2H, s, 2,6-H), 7.18–7.25 (5H, m, Phe–β-C_6H_5), 7.30, 7.40 (each 2H, t, J = 7 Hz, Fmoc–2,3,6,7-H), 7.54, 7.76 (each 2H, d, J = 7 Hz, Fmoc–1,4,5,8-H). TOFMS found: m/z 1377. Calcd for $C_{88}H_{140}N_2NaO_8$: MNa^+, 1377.

***N*-(9-Fluorenylmethyloxycarbonyl)alanylalanine 3,4,5-Tris(octadecyloxy)benzyl Ester (Fmoc–Ala^1–Ala^2–OBzl(OC_{18})$_3$).** Coupling of Fmoc–Ala–OH with H–Ala–OBzl(OC_{18})$_3$ by EDC–HOBt gave crude Fmoc–Ala–Ala–OBzl(OC_{18})$_3$. Further purification by FCC with 5% Et_2O–CH_2Cl_2 and recrystallization from methanol afforded the pure protected dipeptide (70%); white powder; mp 88–92 °C; IR (KBr) 3294 (N–H), 1732 (ester C=O), 1693 (urethane amide C=O), 1651 (amide C=O), 1589 (C=C), 820 cm^{-1} ($C^{2,6}$–H); ^1H NMR ($CDCl_3$) δ 0.87 (9H, t, J = 6 Hz, 3,4,5-O–C_{17}-CH_3), 1.25 (90H, m, 3,4,5-O–C_3–$(CH_2)_{14}$, Ala^{1,2}–α-CH_3), 1.45 (6H, m, 3,4,5-O–C_2-CH_2), 1.77 (6H, m, 3,4,5-O–C–CH_2), 3.93 (2H, t, J = 6 Hz, 4-OCH_2), 3.95 (4H, t, J = 6 Hz, 3,5-OCH_2), 4.22 (1H, t, J = 7 Hz, Fmoc–9-H), 4.24 (1H, m, Ala^2–α-H), 4.40 (2H, d, J = 7 Hz, Fmoc–9-CH_2), 4.60 (1H, m, Ala^{1}α-H), 5.06 (2H, s, 1-CH_2), 5.37 (1H, br, Ala^1–NH), 6.47 (1H, br, Ala^2–NH), 6.51 (2H, s, 2,6-H), 7.31, 7.40 (each 2H, t, J = 7 Hz, Fmoc–2,3,6,7-H), 7.58, 7.76 (each 2H, d, J = 7 Hz, Fmoc–1,4,5,8-H).

Phenylalanylalanine 3,4,5-Tris(octadecyloxy)benzyl Ester (H–Phe–Ala–OBzl(OC_{18})$_3$). Deprotection of Fmoc group in Fmoc–Phe–Ala–OBzl(OC_{18})$_3$ by piperidine gave H–Phe–Ala–OBzl(OC_{18})$_3$ (92%); white powder; ^1H NMR ($CDCl_3$) δ 0.88 (9H, t, J = 6 Hz, 3,4,5-O–C_{17}–CH_3), 1.25 (87H, m, 3,4,5-O–C_3–$(CH_2)_{14}$, Ala–α-CH_3), 1.46 (6H, m, 3,4,5-O–C_2–CH_2), 1.78 (6H, m, 3,4,5-O–C–CH_2), 2.71 (1H, dd, J = 9, 14 Hz, Phe–α-CH), 3.25 (1H, dd, J = 4, 14 Hz, Phe–α-CH), 3.63 (1H, dd, J = 4, 9 Hz, Phe–α-H), 3.93 (2H, t, J = 6 Hz, 4-OCH_2), 3.96 (4H, t, J = 6 Hz, 3,5-OCH_2), 4.62 (1H, quintet, J = 7.5 Hz, Ala–α-H), 5.06 (2H, s, 1-CH_2), 6.52 (2H, s, 2,6-H), 7.20–7.34 (5H, m, Phe–β-C_6H_5), 7.79 (1H, d, J = 7.5 Hz, Ala–NH).

N-(9-Fluorenylmethyloxycarbonyl)leucylphenylalanylalanine 3,4,5-Tris(octadecyloxy)benzyl Ester (Fmoc–Leu–Phe–Ala–OBzl(OC_{18})$_3$). Coupling of Fmoc–Leu–OH with H–Phe–Ala–OBzl(OC_{18})$_3$ by EDC–HOBt gave crude Fmoc–Leu–Phe–Ala–OBzl(OC_{18})$_3$. Further purification by FCC with 15% Et_2O–CH_2Cl_2 and recrystallization from methanol afforded the pure protected tripeptide (52%); white powder; mp 147–149 °C; IR (KBr) 3279 (N–H), 1740 (ester C=O), 1693 (urethane amide C=O), 1647 (amide C=O), 1591 (C=C), 818 cm^{-1} ($C^{2,6}$–H); ^1H NMR ($CDCl_3$) δ 0.88 (15H, t, J = 6 Hz, 3,4,5-O–C_{17}–CH_3, Leu–γ-CH_3), 1.25 (87H, m, 3,4,5-O–C_3–$(CH_2)_{14}$, Ala–α-CH_3), 1.46 (6H, m, 3,4,5-O–C_2–CH_2), 1.57 (3H, m, Leu–α-CH_2, γ-H), 1.79 (6H, m, 3,4,5-O–C–CH_2), 3.07 (2H, d, J = 6 Hz, Phe–α-CH_2), 3.93 (2H, t, J = 6 Hz, 4-OCH_2), 3.95 (4H, t, J = 6 Hz, 3,5-OCH_2), 4.12 (1H, m, Leu–α-H), 4.19 (1H, t, J = 7 Hz, Fmoc–9-H), 4.34 (1H, m, Ala–α-H), 4.42–4.53 (2H, m, Fmoc–9-CH_2), 4.64 (1H, q, J = 7 Hz, Phe–α-H), 5.03 (2H, s, 1-CH_2), 5.05 (1H, br, Leu–NH), 6.46, 6.55 (each 1H, d, J = 6 Hz, Ala, Phe–NH), 6.51 (2H, s, 2,6-H), 7.16–7.23 (5H, m, Phe–β-C_6H_5), 7.31, 7.41 (each 2H, t, J = 7 Hz, Fmoc–2,3,6,7-H), 7.57, 7.77 (each 2H, d, J = 7 Hz, Fmoc–1,4,5,8-H). TOFMS found: m/z 1490. Calcd for $C_{94}H_{151}N_3NaO_9$: MNa^+, 1490.

3,4,5-Tris(octadecyloxy)benzyl Chloride (Cl–Bzl(OC_{18})$_3$, 3). Following the reported procedure,[8] reaction of **2** with $SOCl_2$ in the presence of a catalytic amount of DMF in dry CH_2Cl_2 and recrystallization from methanol afforded pure **3** (92%); white powder; mp 65–66 °C; IR (KBr) 1593 (C=C), 833 cm^{-1} ($C^{2,6}$–H); ^1H NMR ($CDCl_3$) δ 0.88 (9H, t, J = 7 Hz, 3,4,5-O–C_{17}–CH_3), 1.25 (84H, m, 3,4,5-O–C_3–$(CH_2)_{14}$), 1.46 (6H, m, 3,4,5-O–C_2–CH_2), 1.79 (6H, m, 3,4,5-O–C–CH_2), 3.93 (2H, t, J = 6.5 Hz, 4-OCH_2), 3.96 (4H, t, J = 6.5 Hz, 3,5-OCH_2), 4.51 (2H, s, 1-CH_2), 6.56 (2H, s, 2,6-H). TOFMS found: m/z 932. Calcd for $C_{61}H_{116}{}^{35}ClO_3$: MH^+, 932. Found: C; 78.69, H; 12.51%. Calcd for $C_{61}H_{115}ClO_3$: C; 78.61, H; 12.44%.

3,4,5-Tris[3′,4′,5′-tris(octadecyloxy)benzyloxy]benzyl Alcohol (HO–Bzl[OBzl(OC_{18})$_3$]$_3$, 4). Similarly to the synthesis of **1**, a reaction of methyl gallate and **3** at 74 °C for 4 h afforded crude methyl 3,4,5-tris[3′,4′,5′-tris(octadecyloxy)benzyloxy]benzoate. Further purification by FCC with 30% hexane–CH_2Cl_2 gave the pure ester (95%); white powder; ^1H NMR ($CDCl_3$) δ 0.88 (27H, t, J = 6 Hz, 3′,4′,5′-O–C_{17}–CH_3), 1.25 (252H, m, 3′,4′,5′-O–C_3–$(CH_2)_{14}$), 1.44 (18H, m, 3′,4′,5′-O–C_2–CH_2), 1.72 (18H, m, 3′,4′,5′-O–C–CH_2), 3.75 (4H, t, J = 6.5 Hz, 3′,5′-OCH_2 on the 4-position), 3.87 (8H, t, J = 5 Hz, 3′,5′-OCH_2 on the 3,5-positions), 3.87 (3H, s, 1-$COOCH_3$), 3.97 (6H, m, 4′-OCH_2), 5.02 (6H, s, 3,4,5-OCH_2), 6.59 (2H, s, 2′,6′-H on the 4-position), 6.62 (4H, s, 2′,6′-H on the 3,5-position), 7.37 (2H, s, 2,6-H).

Similarly to the synthesis of **2**, the reduction of the above crude ester with $LiAlH_4$ afforded **4** (90% for the two steps); white powder; mp 75–77 °C; IR (KBr) 1589 (C=C), 814 cm^{-1} ($C^{2,6,2',6'}$-H); ^1H NMR ($CDCl_3$) δ 0.88 (27H, t, J = 6 Hz, 3′,4′,5′-O–C_{17}–CH_3), 1.25 (252H, m, 3′,4′,5′-O–C_3–$(CH_2)_{14}$), 1.44 (18H, m, 3′,4′,5′-O–C_2–CH_2), 1.72 (18H, m, 3′,4′,5′-O–C–CH_2), 3.75 (4H, t, J = 6.5 Hz, 3′,5′-OCH_2 on the 4-position), 3.87 (8H, t, J = 5 Hz, 3′,5′-OCH_2 on the 3,5-positions), 3.97 (6H, m, 4′-OCH_2), 4.58 (2H, s, 1-CH_2), 5.02 (6H, s, 3,4,5-OCH_2), 6.56 (2H, s, 2,6-H), 6.59 (2H, s, 2′,6′-H on the 4-position), 6.62 (4H, s, 2′,6′-H on the 3,5-position). Found: C; 79.43, H; 12.77%. Calcd for $C_{190}H_{350}O_{13}$•H_2O: C; 79.77, H; 12.40%.

***N*-(9-Fluorenylmethyloxycarbonyl)alanine 3,4,5-Tris[3′,4′,5′-tris(octadecyloxy)benzyloxy]benzyl Ester (Fmoc–Ala–O–Bzl[OBzl(OC_{18})$_3$]$_3$).** Coupling of Fmoc–Ala–OH with **4** by EDC–HOBt gave crude Fmoc–Ala–OBzl[OBzl(OC_{18})$_3$]$_3$. Further purification by FCC with CH_2Cl_2 and recrystallization from methanol afforded the pure protected alanine (30%); white powder; ^1H NMR ($CDCl_3$) δ 0.88 (27H, t, J = 6 Hz, 3′,4′,5′-O–C_{17}–CH_3), 1.25 (255H, m, 3′,4′,5′-O–C_3–$(CH_2)_{14}$, Ala–α-CH_3), 1.44 (18H, m, 3′,4′,5′-O–C_2–CH_2), 1.72 (18H, m, 3′,4′,5′-O–C–CH_2), 3.75 (4H, t, J = 6.5 Hz, 3′,5′-OCH_2 on the 4-position), 3.87 (8H, t, J = 5 Hz, 3′,5′-OCH_2 on the 3,5-positions), 3.97 (6H, m, 4′-OCH_2), 4.23 (1H, t, J = 7 Hz, Fmoc–9-H), 4.38 (2H, d, J = 6 Hz, Fmoc–9-CH_2), 4.46 (1H, m, Ala–α-H), 5.02 (6H, s, 3,4,5-OCH_2), 5.08 (2H, s, 1-CH_2), 5.38 (1H, d, J = 8 Hz, Ala–NH), 6.52 (2H, s, 2,6-H), 6.59 (2H, s, 2′,6′-H on the 4-position), 6.62 (4H, s, 2′,6′-H on the 3,5-position), 7.31, 7.40 (each 2H, t, J = 7 Hz, Fmoc–2,3,6,7-H), 7.59, 7.76 (each 2H, d, J = 7 Hz, Fmoc–1,4,5,8-H).

3,4,5-Tris(octadecyloxy)benzoic Acid. To an H_2O–EtOH (1:3) solution (133 mL) of methyl ester **1** (12.52 g) was added KOH (7.53 g). The mixture was refluxed for 4 h, and then left standing at room temperature overnight. The precipitated solid was separated by decantation, and was stirred vigorously for several days in 0.6 M HCl (250 mL) and CH_2Cl_2 (150 mL). After

evaporation of CH_2Cl_2, the residue was filtered, washed with water and methanol, and dried to give the crude acid. Recrystallization from 1-propanol (400 mL), washing with methanol and drying over P_2O_5 gave the pure carboxylic acid (11.01 g, 89%); white powder; mp 86–87 °C; IR (KBr) 1682 (C=O), 1587 (C=C), 864 cm^{-1} ($C^{2,6}$–H); ^1H NMR ($CDCl_3$) δ 0.88 (9H, t, J = 6 Hz, 3,4,5-O–C_{17}–CH_3), 1.25 (84H, m, 3,4,5-O–C_3–$(CH_2)_{14}$), 1.47 (6H, m, 3,4,5-O–C_2–CH_2), 1.79 (6H, m, 3,4,5-O–C–CH_2), 4.01 (4H, t, J = 6 Hz, 3,5-OCH_2), 4.03 (2H, t, J = 6 Hz, 4-OCH_2), 7.29 (2H, s, 2,6-H). Found: C; 78.70, H; 12.54%. Calcd for $C_{61}H_{114}O_5$: C; 78.99, H; 12.39%.

3,4,5-Tris(octadecyloxy)benzaldehyde. To a dry CH_2Cl_2 solution (80 mL) of substituted benzyl alcohol **2** (2.0 g, 2.2 mmol) was added pyridinium chlorochromate (0.71 g, 3.3 mmol) and the reaction mixture was stirred at room temperature for 3 h. After evaporation, the residue was treated with Et_2O and the undissolved materials were removed. The filtrate was concentrated in vacuo and the residue was purified by FCC with CH_2Cl_2 to give the pure aldehyde (1.78 g, 87%); white powder; mp 75–76 °C; IR (KBr) 2731 (OC–H), 1693 (C=O), 1585 (C=C), 824 cm^{-1} ($C^{2,6}$–H); ^1H NMR ($CDCl_3$) δ 0.88 (9H, t, J = 7 Hz, 3,4,5-O–C_{17}–CH_3), 1.25 (84H, m, 3,4,5-O–C_3–$(CH_2)_{14}$), 1.46 (6H, m, 3,4,5-O–C_2–CH_2), 1.79 (6H, m, 3,4,5-O–C–CH_2), 4.03 (4H, t, J = 6 Hz, 3,5-OCH_2), 4.05 (2H, t, J = 6 Hz, 4-OCH_2), 7.08 (2H, s, 2,6-H), 9.82 (1H, s, CHO). TOFMS found: m/z 912. Calcd for $C_{61}H_{115}O_4$: MH^+, 912. Found: C; 79.97, H; 12.79%. Calcd for $C_{61}H_{114}O_4$: C; 80.37, H; 12.61%.

We thank Mr. Atsushi Tanaka of Asahi Chemical Industry, Co., Ltd. for his measurements of MALDI-TOF MS and Mr. Koji Kumon of Ritsumeikan University for his assistance with chiral HPLC analyses.

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10 For example, see "The Combinatorial Chemistry Catalog," Novabiochem (http://www.nova.ch).