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Microwave-Assisted Formation of Peptide–Vitamin Conjugates

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Amino acid and peptide conjugates with vitamin B_3, H, E and D_3 were synthesized by using microwave irradiation in good to satisfactory yields. In addition, a novel benzotriazole-activated biotin intermediate has been introduced, which could serve as an alternative biotinylation agent.

Introduction

Peptides are of considerable interest as therapeutic agents, but many suffer from low oral availability, because their physicochemical characteristics do not allow diffusion through the lipid bilayer cell wall,[1,2] as well as rapid biliary clearance. In the development of peptide-based drugs, common approaches to circumvent these major shortcomings have involved absorption enhancers,[3] enzyme inhibitors[4] and complex emulsion systems.[5]

More recently, new approaches to enhance uptake have involved vitamins covalently bound to peptides. Water-soluble vitamins are usually transported into cells by potocytosis.[6] Zhang and McCormick have proposed the use of vitamin B_6 in a co-delivery strategy: receptor-mediated transport in eukaryotic cells, with acceptance of the amine of a peptide–vitamin conjugate, facilitates the cell uptake of peptide and transport into the cytosol.[7,8] Orally administered peptide–cobalamin (vitamin B_{12}) conjugates generated high levels of peptide absorption.[9,10] A similar strategy for "vitamin-cloaked" biotin (vitamin H) (**1b**) covalently bound to peptidic HIV-1 protease inhibitors increased the serum concentration, suggesting an improved resistance to clearance.[11]

In the present study, amino acids and peptides are coupled through amide or ester linkages to vitamins B_3 (**1a**), H (**1b**), E (**7**) and D_3 (**10**), which cover a range of different hydro/lipophilicities. For the hydrophilic vitamins B_3 (**1a**) and H (**1b**) such conjugation increases the solubility of the bioconjugate in a physiological medium. Whereas for the lipophilic vitamins E (**7**) and D_3 (**10**) absorption through membranes is eased and transport into cells is facilitated, thus leading to an enhancement in anti-cancer activity.[12,13]

Vitamin H undergoes strong noncovalent interaction with (strept)avidin,[14] as shown by enzymatic reactions, radiolabeling techniques, and fluorescence assays.[15] Recently, novel conjugates of biotin with amino acids showed remarkable gelation properties in aqueous media with a biotinyl hydrogel potential to act as efficient drug carriers.[16]

α-Tocopherol (vitamin E) (**7**) is an active lipophilic antioxidant in biological membranes, which protects skin against oxidative damage, and by coupling with amino acids to form pro-vitamins provides both anti-oxidant and moisturizing effects on skin.[17,18] Vitamin E attached to a taxol-based drug exhibits a therapeutic efficiency higher than taxol and camptothecin due to its better solubility in fats and better retention in membranes.[19] Furthermore, Wang et al. observed the suppression of breast cancer cells by peptide conjugates of vitamin E.[20]

We previously prepared diverse peptide conjugates using benzotriazole methodology,[21] this approach is now expanded by coupling amino acids and peptides with vitamins, utilizing microwave irradiation to shorten reaction times[22] and minimize epimerization. The present work represents the first microwave-assisted formation of peptide–vitamin bioconjugates.

Results and Discussion

Preparation of Benzotriazole Derivatives 3a,b of Niacin and Biotin

Niacin (vitamin B_3) (**1a**) and biotin (vitamin H) (**1b**) were activated by treatment with 1-(methylsulfonyl)-1*H*-benzotriazole under microwave irradiation at 50 W irradiation power and 70 °C in the presence of triethylamine to form the benzotriazolide of niacin **3a** and of biotin **3b**, respectively, in yields of 75–78% (Scheme 1, Table 1).

Compound **3a** was earlier prepared under reflux conditions for 12 h,[23] and we now found microwave irradiation shortened the reaction time to 1 h. Compounds **3a**,**b** were used as active intermediates for further coupling reactions.

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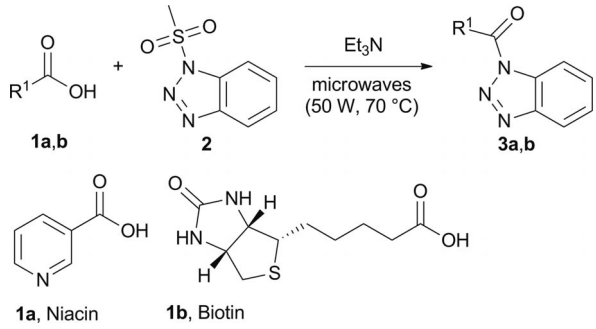


Scheme 1.

Table 1. Preparation of benzotriazole derivatives of niacin and biotin.

Entry	Reactant	Product	Conditions	Yield	M.p. [°C]
1	**1a**	**3a**	THF, 1 h	78%	101–102[a]
2	**1b**	**3b**	DMF, 15 min	75%	216–218

[a] Ref.[23] 87–89 °C.

Preparation of Peptide–Niacin Bioconjugates 5a–e

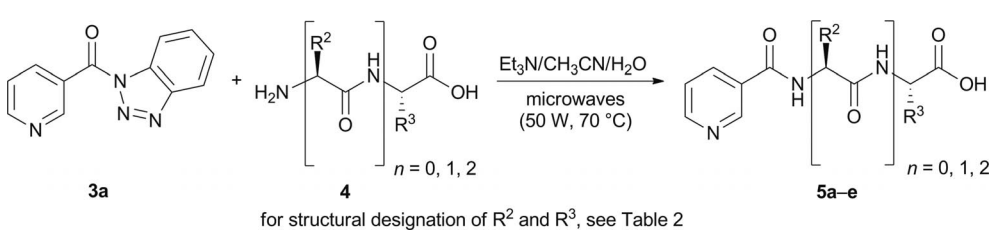
Benzotriazole-activated niacin **3a** coupled with free amino acids, dipeptides and tripeptides (**4**, n = 0, 1 and 2) under microwave irradiation at 70 °C in the presence of 0.5 equiv. of triethylamine to give **5a**–**e** (43–81%).

The reactions of free phenylalanine and lysine with **3a** take place in 5 min, whereas those with the other substrates occur in 20 min. All the amino acid–niacin conjugates were isolated by adjusting the reaction mixture pH to 4–5 by using 4 N HCl (Scheme 2, Table 2) The purity of the compounds was verified by ^{1}H and ^{13}C NMR spectroscopy and elemental analysis.

Table 2. Preparation of compounds **5a**–**e**.

Product	n	R^2	R^3	Yield	M.p. [°C] (ref. m.p.)
5a	0	–	benzyl	66%	183–184 (177–178)[24]
5b	0	–	$(CH_2)_4NH(Z)$	81%	215–217 (175–176)[25]
5c	0	–	$(CH_2)_2SCH_3$	51%	198–200
5d	1	isobutyl	isobutyl	45%	159–160
5e	2	H	*sec*-butyl	43%	147–149

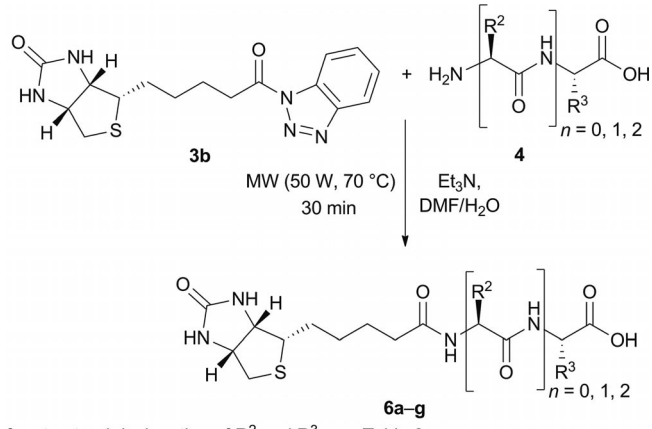
Amino acid–niacin bioconjugates were previously prepared either (i) by reaction of nicotinyl chloride with esters of amino acids followed by hydrolysis and purification by column chromatography (overall yields 33–85%),[26,27] or (ii) by reaction of *N*-hydroxysuccinimide (NHS) ester of nicotinic acid followed by coupling with an amino acid at room temperature (yields 61–76%).[28,29] Our preparative methodology provides procedures, which are more simple, reduces reaction time, and allows purification by crystallization thus avoiding column chromatography.



Scheme 2.

Preparation of Peptide–Biotin Bioconjugates 6a–g

Biotin activated with a benzotriazole moiety **3b** coupled with free amino acids in DMF under microwave irradiation at 70 °C for 30 min in the presence of 2 equiv. of triethylamine to give compounds **6a**–**d** (35–82%). The products were isolated by diluting the DMF solution with water and acidifying the reaction mixture to pH = 2–3 with 4 N HCl (Scheme 3, Table 3). Under the same reaction conditions, free dipeptides Gly-Val-OH and Leu-Leu-OH as well as



Scheme 3.

Table 3. Preparation of compounds **6a**–**g**.

Products	n	R^2	R^3	Yield	M.p. [°C] (ref. m.p.)
6a	0	–	*sec*-butyl	82%	224–226 (230–233)[16]
6b	0	–	benzyl	71%	204–206 (205)[16]
6c	0	–	$(CH_2)_4NH(Z)$	78%	148–150
6d	0	–	$(CH_2)_2SCH_3$	70%	205–208 (207)[16]
6e	1	H	isopropyl	35%	141–144
6f	1	isobutyl	isobutyl	75%	240–241
6g	2	H	*sec*-butyl	48%	156–159

free tripeptide Gly-Gly-Ile-OH coupled with **3b** to give biotin-Gly-Val-OH **6e**, biotin-Leu-Leu-OH **6f** and biotin-Gly-Gly-Ile-OH bioconjugates **6g** in 35, 75 and 48% yield, respectively. The low isolated yield of **6e** and **6g** is ascribed to their hydrophilic nature. The purity of compounds **6a**–**g** was checked by ^{1}H and ^{13}C NMR spectroscopy and elemental analysis.

The importance of biotinylation led earlier workers to devise many procedures for coupling biotin to diverse scaffolds through amide linkages. The most common involve: (i) The formation of *N*-hydroxysuccinimide ester of biotin by reaction with either *N*,*N*′-disuccinimidyl carbonate (DSC)[30] or *N*-hydroxysuccinimide (NHS);[31,32] both require the use of coupling reagents such as EDC or DCC and reaction times of up to 24 h. (ii) The preparation of moisture- and temperature-sensitive intermediate biotinyl chloride by the reaction of biotin with oxalyl chloride, which requires careful regulation of the temperature at 0 °C to avoid complex by-products.[33] (iii) The in situ use of coupling reagents without isolation of the active intermediate (DCC Steglich conditions),[34] EDC/HCl,[35] TBTU or PyBOP or DICPDI generally used for the solid-phase synthesis of biotin–peptide conjugates and always needing additives such as HOBt and HOAt, strong bases as NMM, DIEA and DMAP and excesses of up to 5 equiv. of biotin and coupling reagents.[36–38] (iv) The formation of the pentafluorophenyl ester of biotin through initial coupling with DCC for 6 h followed by the reaction with an azide under hydrogenation conditions to form the coupled amide in 56% overall yields[39a] or with an amino acid derivative in the presence of DIEA for 48 h in 34–58% overall yields.[39b] (v) The formation in solution phase of amino acid–biotin conjugates from amino acid esters by coupling with moisture-sensitive EDC for 4 h, which requires column chromatography for purification and a further hydrolysis step to generate the product as acid.[16] As all the previous methods suffer from long reaction times and/or moderate yields, the present procedures allow shorter reaction times and purification by simple crystallization.

Preparation of α-Tocopherol–Peptide Conjugates 9a–e

Amino acid and peptide ester conjugates **9a**–**e** linked to α-tocopherol by an ester, were prepared (44–68%) as microcrystals or waxes by *O*-acylation of α-tocopherol (**7**) with Cbz-protected acylbenzotriazoles **8** under microwave irradiation (20 W, 50 °C) for 20 min in anhydrous DMF in the presence of 2 equiv. of potassium carbonate (Scheme 4, Table 4). Compounds **9a**–**e** were characterized by ^{1}H and ^{13}C NMR spectroscopy and elemental analysis. Lack of duplication of the ^{13}C NMR peaks of **9a**–**e** supports their chiral purity.

Table 4. Preparation of compounds **9a**–**e**.

Products	R^4	R^5	Yield	M.p. [°C]
9a	$(CH_3)_2CH$	Z	68%	63–64
9b	benzyl	Z	60%	wax
9c	benzyl	Z-L-Val	44%	116–118
9d	H	Z-L-Val-L-Phe	55%	86–89
9e	H	Z-L-Ala-L-Phe	51%	wax

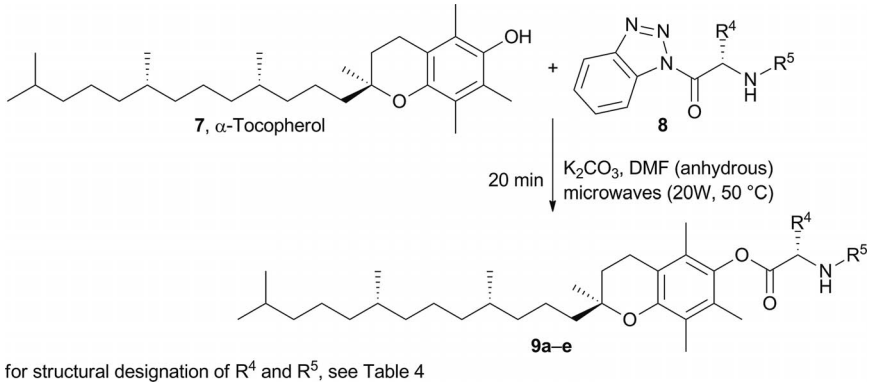
The most common previous method for coupling protected amino acids with α-tocopherol used DCC with either DMAP[39a,40] or anhydrous pyridine as base[18] at 20 °C. Our alternative method for α-tocopherol–peptide conjugates applies microwave irradiation.

Preparation of Cholecalciferol–Peptide Conjugates 11a–d

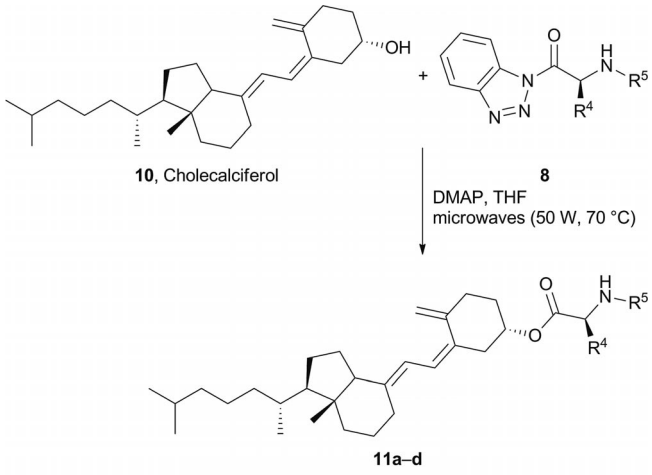
We prepared amino acid and peptide conjugates of vitamin D_3 **11a**–**d** by *O*-acylation of cholecalciferol (**10**) with Cbz-protected acylbenzotriazoles **8** in the presence of DMAP in freshly distilled THF and under microwave irradiation (50 W, 70 °C) for 1–2 h (Scheme 5, Table 5). Compounds **11a**–**d** were purified by column chromatography and characterized by ^{1}H and ^{13}C NMR spectroscopy and HRMS analysis.

Based on ^{1}H and ^{13}C NMR spectroscopy, it appeared that no epimerization occurred during the preparation of compounds **11a**,**b**. However, in the case of **11c**,**d** duplication of peaks was observed. We therefore carried out 2D NMR experiments (COSY, HSQC and HMBC) for compound **11d** to be able to assign all peaks. This resulted in the confirmation of the presence of a mixture of diastereomers in a 1:3 ratio and thus epimerization during *O*-acylation under these reaction conditions.

We have located[39a] only two previous couplings of amino acid units with cholecalciferol: (i) with Boc-protected



Scheme 4.



Scheme 5.

Table 5. Preparation of compounds **11a**–**d**.

Products	R^4	R^5	Yield	M.p. [°C]
11a	isopropyl	Z	56%	oil
11b	CH_2-indolyl	Z	64%	oil
11c	benzyl	Z-L-Ala	52%	oil
11d	benzyl	Z-L-Val	58%	166–169

ALA (aminolevulinic acid) and (ii) with azido-protected ALA by using the EDC coupling reagent at room temperature (yields of 29 and 30%, respectively). Our benzotriazole-mediated *O*-acylation under microwave irradiation gives cholecalciferol–peptide conjugates **11a**–**d** in yields of 56–64% after purification by column chromatography.

Conclusions

We described here the microwave-assisted synthesis of new peptide–vitamin conjugates. Vitamins B_3, H, E and D_3 were used in this work for their diverse applicability and activity and the ease of linking them to peptides through amide or ester bonds. This simple method with relatively short reaction times has proved to be an alternative and sometimes better way to form these building blocks in comparison to literature reports. Moreover, we have prepared a new, activated and fairly stable form of biotin **3b** that could be used in the various biotinylation studies. The retention of chirality in the peptide conjugates of vitamins B_3, H and E is demonstrated by NMR spectroscopy.

Experimental Section

General: Melting points were determined with a hot-stage apparatus and are uncorrected. ^{1}H NMR (300 MHz) spectra were recorded in $CDCl_3$, $(CD_3)_2CO$ or [D_6]DMSO with TMS as the internal standard and $CDCl_3$, $(CD_3)_2CO$ or [D_6]DMSO as the internal standard for ^{13}C NMR (75 MHz). Column chromatography was conducted on flash silica gel (200–425 mesh). Visualization of TLC plates was done by UV light and phosphomolybdic acid staining. Anhydrous THF was obtained by distillation immediately prior to use from sodium/benzophenone ketyl. A single-mode cavity microwave synthesizer with a continuous irradiation at 2450 MHz and an infrared temperature control system was used.

General Procedure for the Synthesis of Benzotriazole-Derivatized Niacin 3a: A modified procedure to the established one[23] was employed where microwave irradiation replaced the conventional heating to shorten the reaction time. Nicotinic acid (2.00 g, 16.3 mmol) and 1-(methylsulfonyl)-1*H*-benzo[*d*][1,2,3]triazole ($BtSO_2Me$, 3.20 g, 16.3 mmol) were dissolved in anhydrous THF (4 mL). Et_3N (1.64 g, 2.28 mL, 16.3 mmol) was added to the reaction mixture, and then the reaction mixture was exposed to microwave irradiation (60 W, 70 °C) for 1 h until completion of the reaction (monitored by TLC). THF was then evaporated, and the residue was dissolved in ethyl acetate and washed with 5% sodium carbonate, then water, and dried with anhydrous magnesium sulfate. The solvent was evaporated under reduced pressure, and the compound was recrystallized from a mixture of dichloromethane/hexanes to yield the product (2.84 g, 12.7 mmol).

(1*H*-Benzo[*d*][1,2,3]triazol-1-yl)(pyridin-3-yl)methanone (Nia-Bt) (3a): Beige microcrystals (78%); m.p. 101–102 °C. ^{1}H NMR ($CDCl_3$): δ = 9.34 (d, J = 2.1 Hz, 1 H), 8.82 (dd, J = 4.8, 1.5 Hz, 1 H), 8.48 (dt, J = 8.1, 1.9 Hz, 1 H), 8.32 (d, J = 8.4 Hz, 1 H), 8.10 (d, J = 8.1 Hz, 1 H), 7.66 (t, J = 7.5 Hz, 1 H), 7.54–7.43 (m, 2 H) ppm. ^{13}C NMR ($CDCl_3$): δ = 165.3, 153.9, 152.4, 146.0, 139.2, 132.1, 131.0, 127.9, 126.9, 123.3, 120.5, 114.9 ppm. $C_{12}H_8N_4O$ (224.22): calcd. C 64.28, H 3.60, N 24.99; found C 63.95, H 3.45, N 24.65.

General Procedure for the Synthesis of Benzotriazole-Derivatized Biotin 3b: In a dried heavy-walled Pyrex tube containing a small stir bar, D-biotin (**1b**) (1.00 g, 4.1 mmol) and $BtSO_2Me$ (0.81 g, 4.1 mmol) were dissolved in anhydrous DMF (4 mL). Triethylamine (0.41 g, 4.1 mmol, 0.57 mL) was added to the reaction mixture, which was then exposed to microwave irradiation (50 W, 70 °C) for 15 min with simultaneous cooling until completion of the reaction. Upon cooling to 5 °C, a white precipitate started to form. The solid was filtered and washed with chloroform. The solid was further washed with 5% sodium carbonate, then water, and dried to yield the product (1.01 g, 2.9 mmol).

(3a*S*,4*S*,6a*R*)-4-[5-(1*H*-Benzo[*d*][1,2,3]triazol-1-yl)-5-oxopentyl]-tetrahydro-1*H*-thieno[3,4-*d*]imidazol-2(3*H*)-one (Biotin-Bt) (3b): White microcrystals (75%); m.p. 216–218 °C. ^{1}H NMR ([D_6]-DMSO): δ = 8.22 (d, J = 8.7 Hz, 2 H), 7.76 (t, J = 7.7 Hz, 1 H), 7.59 (t, J = 7.5 Hz, 1 H), 6.51 (s, 1 H), 6.39 (s, 1 H), 4.35–4.28 (m, 1 H), 4.20–4.12 (m, 1 H), 3.45–3.40 (m, 2 H), 3.18–3.10 (m, 1 H), 2.89–2.80 (m, 1 H), 2.59 (d, J = 12.6 Hz, 1 H), 1.80–1.40 (m, 6 H) ppm. ^{13}C NMR ([D_6]DMSO): δ = 172.3, 162.8, 145.4, 130.7, 130.6, 126.3, 120.0, 114.0, 61.0, 59.2, 55.4, 39.9, 34.7, 28.1, 27.9, 23.6 ppm. $C_{16}H_{19}N_5O_2S$ (345.42): calcd. C 55.63, H 5.54, N 20.27; found C 55.39, H 5.55, N 20.05.

General Procedure for the Preparation of Peptide–Niacin Bioconjugates 5a–e: A dried heavy-walled Pyrex tube containing a small stir bar was charged with nicotinylbenzotriazole (1 equiv.) dissolved in acetonitrile (5.0 mL), and a solution of free amino acid or dipeptide (1 equiv.) and base Et_3N (0.5 equiv.) in water (1 mL) was added. The reaction mixture was exposed to microwave irradiation (50 W, 70 °C) for specified times. The reaction mixture was allowed to cool until the temperature fell below 30 °C. The acetonitrile was evaporated, and the pH of the aqueous layer was adjusted at 4 by adding 4 N HCl drop by drop. The mixture was extracted with ethyl acetate and dried with anhydrous magnesium sulfate. The solvent was removed under reduced pressure, and the residue was washed with

diethyl ether; the solid thus obtained was recrystallized from ethyl acetate to give the product.

(*S*)-2-(Nicotinamido)-3-phenylpropanoic Acid (Nia-Phe-OH) (5a): White shiny crystals (66%); m.p. 183–184 °C. ^{1}H NMR ([D$_6$]-DMSO): δ = 9.00–8.92 (m, 2 H), 8.70 (s, 1 H), 8.12 (d, J = 7.5 Hz, 1 H), 7.52–7.46 (m, 1 H), 7.30–7.16 (m, 5 H), 4.66–4.63 (m, 1 H), 3.26–3.18 (m, 1 H), 3.10–3.02 (m, 1 H) ppm. ^{13}C NMR ([D$_6$]-DMSO): δ = 173.0, 165.0, 152.1, 148.5, 138.0, 135.1, 129.4, 129.1, 128.3, 126.5, 123.5, 54.3, 36.3 ppm. $C_{15}H_{14}N_2O_3$ (270.29): calcd. C 66.66, H 5.22, N 10.36; found C 66.64, H 5.22, N 10.35.

(*S*)-6-{[(Benzyloxy)carbonyl]amino}-2-(nicotinamido)hexanoic Acid [Nia-Lys(*Z*)-OH] (5b): White microcrystals (81%); m.p. 215–218 °C. ^{1}H NMR ([D$_6$]DMSO): δ = 9.03 (d, J = 1.5 Hz, 1 H), 8.80 (d, J = 7.5 Hz, 1 H), 8.72 (dd, J = 4.8, 1.5 Hz, 1 H), 8.24–8.20 (m, 1 H), 8.20–7.48 (m, 1 H), 7.36–7.22 (m, 6 H), 4.98 (s, 2 H), 4.36–4.30 (m, 1 H), 3.02–2.90 (m, 2 H), 1.84–1.74 (m, 2 H), 1.50–1.28 (m, 4 H) ppm. ^{13}C NMR ([D$_6$]DMSO): δ = 173.6, 165.2, 156.1, 152.0, 148.6, 137.3, 135.2, 129.5, 128.4, 127.7, 123.5, 65.1, 52.7, 30.2, 29.0, 23.2 ppm.

(*S*)-4-(Methylthio)-2-(nicotinamido)butanoic Acid (Nia-Met-OH) (5c): White microcrystals (51%); m.p. 198–200 °C. ^{1}H NMR ([D$_6$]-DMSO): δ = 9.03 (d, J = 1.5 Hz, 1 H), 8.87 (d, J = 7.8 Hz, 1 H), 8.72 (dd, J = 4.8, 1.5 Hz, 1 H), 8.24–8.19 (m, 1 H), 7.55–7.50 (m, 1 H), 4.56–4.48 (m, 1 H), 2.62–2.48 (m, 2 H), 2.10–2.03 (m, 5 H) ppm. ^{13}C NMR ([D$_6$]DMSO): δ = 173.2, 165.2, 152.0, 148.5, 135.2, 129.4, 123.4, 51.6, 30.2, 30.0, 14.6 ppm. $C_{11}H_{14}N_2O_3S$ (254.30): calcd. C 51.95, H 5.55, N 11.02; found C 52.25, H 5.29, N 11.17.

(*S*)-4-Methyl-2-[(*S*)-4-methyl-2-(nicotinamido)pentanamido]-pentanoic Acid (Nia-Leu-Leu-OH) (5d): White microcrystals (45%); m.p. 159–160 °C ^{1}H NMR ([D$_6$]DMSO): δ = 9.03 (s, 1 H), 8.72–8.62 (m, 2 H), 8.25–8.16 (m, 2 H), 7.50 (dd, J = 9.0, 4.5 Hz, 1 H), 4.60–4.50 (m, 1 H), 4.254.16 (m, 1 H), 1.76–1.40 (m, 6 H), 0.95–0.87 (m, 9 H), 0.83 (d, J = 6.6 Hz, 3 H) ppm. ^{13}C NMR ([D$_6$]-DMSO): δ = 174.0, 172.0, 164.8, 151.9, 148.6, 135.2, 129.7, 123.4, 51.5, 50.2, 24.4, 24.3, 23.2, 22.9, 21.4, 21.4 ppm. HRMS: calcd. for $C_{18}H_{28}N_3O_4$ [M + H]$^+$ 350.2074; found 350.2069.

(2*S*,3*S*)-3-Methyl-2-{2-[2-(nicotinamido)acetamido]acetamido}-pentanoic Acid (Nia-Gly-Gly-Ile-OH) (5e): White microcrystals (43%); m.p. 147–149 °C. ^{1}H NMR ([D$_6$]DMSO): δ = 12.62 (br. s, 1 H), 9.12–9.03 (m, 2 H), 8.73 (dd, J = 4.8, 1.8 Hz, 1 H), 8.28–8.20 (m, 2 H), 7.91 (d, J = 8.4 Hz, 1 H), 7.54 (ddd, J = 8.2, 5.0, 0.8 Hz, 1 H), 4.19 (dd, J = 8.4, 6.0 Hz, 1 H), 3.91 (d, J = 5.7 Hz, 2 H), 3.79 (d, J = 5.7 Hz, 2 H), 1.84–1.72 (m, 1 H), 1.47–1.32 (m, 1 H), 1.24–1.10 (m, 1 H), 0.92–0.75 (m, 6 H) ppm. ^{13}C NMR ([D$_6$]-DMSO): δ = 172.8, 168.9, 168.7, 165.1, 151.7, 148.4, 135.3, 129.5, 123.5, 56.2, 42.7, 41.7, 36.4, 24.6, 15.5, 11.3 ppm. HRMS: calcd. for $C_{16}H_{22}N_4O_5Na$ [M + Na]$^+$ 373.1482; found 373.1493.

General Procedure for the Preparation of Peptide–Biotin Conjugates 6a–g: A dried heavy-walled Pyrex tube containing a small stir bar was charged with a mixture of free amino acid **4** (1 equiv.) in DMF (3 mL). Et_3N (2 equiv.) was added and 2 drops of water to dissolve the suspension. D-Biotin-Bt (**3b**) (1 equiv.) was added, and the reaction mixture was exposed to microwave irradiation (50 W, 70 °C) for 30 min. The reaction mixture was allowed to cool to 40 °C. Once the reaction was complete, the mixture was quenched with ice, then acidified to pH = 2–3 with 4 N solution of HCl, and a precipitate was formed. This solid was then filtered and washed with water, then dried under reduced pressure to give the desired product.

(2*S*)-3-Methyl-2-{5-[(3a*S*,4*S*,6a*R*)-2-oxohexahydro-1*H*-thieno[3,4-*d*]-imidazol-4-yl]pentanamido}pentanoic Acid (Biotin-Ile-OH) (6a): White microcrystals (82%); m.p. 224–226 °C. ^{1}H NMR ([D$_6$]-DMSO): δ = 12.50 (br. s, 1 H), 7.95 (d, J = 8.4 Hz, 1 H), 6.43 (s, 1 H), 6.38 (s, 1 H), 4.34–4.28 (m, 1 H), 4.21–4.10 (m, 2 H), 3.13–3.05 (m, 1 H), 2.82 (dd, J = 12.5, 5.0 Hz, 1 H), 2.58 (d, J = 12.6 Hz, 1 H), 2.21–2.08 (m, 2 H), 1.81–1.70 (m, 1 H), 1.65–1.10 (m, 8 H), 0.87–0.80 (m, 6 H) ppm. ^{13}C NMR ([D$_6$]DMSO): δ = 173.2, 172.3, 162.7, 61.1, 59.2, 56.1, 55.5, 36.2, 34.7, 28.1, 28.0, 25.4, 24.7, 15.6, 11.3 ppm. $C_{16}H_{27}N_3O_4S$ (357.47): calcd. C 53.76, H 7.61, N 11.75; found C 53.46, H 7.79, N 11.69.

(*S*)-2-{5-[(3a*S*,4*S*,6a*R*)-2-Oxohexahydro-1*H*-thieno[3,4-]imidazol-4-yl]pentanamido}-3-phenylpropanoic Acid (Biotin-Phe-OH) (6b): White microcrystals (71%); m.p. 204–206 °C. ^{1}H NMR ([D$_6$]-DMSO): δ = 12.62 (s, 1 H), 8.12 (d, J = 8.7 Hz, 1 H), 7.30–7.18 (m, 5 H), 6.40 (s, 1 H), 6.37 (s, 1 H), 4.47–4.37 (m, 1 H), 4.33–4.27 (m, 1 H), 4.12–4.07 (m, 1 H), 3.09–2.99 (m, 2 H), 2.89–2.77 (m, 2 H), 2.57 (d, J = 12.3 Hz, 1 H), 2.04 (t, J = 7.2 Hz, 2 H), 1.63–1.35 (m, 4 H), 1.24–1.13 (m, 2 H) ppm. ^{13}C NMR ([D$_6$]DMSO): δ = 173.2, 172.1, 162.7, 137.7, 129.0, 128.1, 126.3, 61.0, 59.2, 55.4, 53.3, 36.8, 34.8, 28.0, 25.2 ppm. $C_{19}H_{25}N_3O_4S$ (391.48): calcd. C 58.29, H 6.44, N 10.73; found C 58.20, H 6.42, N 10.68.

(*S*)-6-{[(Benzyloxy)carbonyl]amino}-2-{5-[(3a*S*,4*S*,6a*R*)-2-oxo-hexahydro-1*H*-thieno[3,4-*d*]imidazol-4-yl]pentanamido}hexanoic Acid [Biotin-Lys(Z)-OH] (6c): Off-white microcrystals (78%); m.p. 148–151 °C. ^{1}H NMR ([D$_6$]DMSO): δ = 12.34 (br. s, 1 H), 8.02 (d, J = 7.5 Hz, 1 H), 7.40–7.27 (m, 5 H), 7.24 (t, J = 5.4 Hz, 1 H), 6.45–6.28 (m, 2 H), 5.00 (s, 2 H), 4.32–4.26 (m, 1 H), 4.17–4.08 (m, 2 H), 3.12–3.03 (m, 1 H), 3.01–2.91 (m, 2 H), 2.81 (dd, J = 12.2, 5.0 Hz, 1 H), 2.57 (d, J = 12.3 Hz, 1 H), 2.12 (t, J = 7.4 Hz, 2 H), 1.75–1.23 (m, 12 H) ppm. ^{13}C NMR ([D$_6$]DMSO): δ = 173.9, 172.3, 162.7, 156.2, 156.1, 137.3, 128.4, 127.7, 65.1, 61.0, 59.2, 55.5, 51.7, 34.8, 30.7, 29.0, 28.1, 28.0, 25.2, 22.8 ppm. HRMS: calcd. for $C_{24}H_{34}N_4O_7SNa$ [M + Na]$^+$ 529.2091; found 529.2091.

(*S*)-4-(Methylthio)-2-{5-[(3a*S*,4*S*,6a*R*)-2-oxohexahydro-1*H*-thieno-[3,4-*d*]imidazol-4-yl]pentanamido}butanoic Acid (Biotin-Met-OH) (6d): In this case, after acidification with 4 N HCl, barely some solids started to precipitate. For that reason, NaCl powder was added to saturate the aqueous solution and force the product to precipitate as white microcrystals (70%); m.p. 205–208 °C. ^{1}H NMR ([D$_6$]DMSO): δ = 12.6 (s, 1 H), 8.09 (d, J = 7.8 Hz, 1 H), 6.42 (s, 1 H), 6.37 (s, 1 H), 4.35–4.25 (m, 2 H), 4.16–4.09 (m, 1 H), 3.09–3.05 (m, 1 H), 2.82 (dd, J = 12.6, 4.8 Hz, 1 H), 2.57 (d, J = 12.3 Hz, 1 H), 2.48–2.38 (m, 2 H), 2.12 (t, J = 7.2 Hz, 2 H), 2.03 (s, 3 H), 1.97–1.79 (m, 2 H), 1.63–1.42 (m, 4 H), 1.36–1.24 (m, 2 H) ppm. ^{13}C NMR ([D$_6$]DMSO): δ = 173.5, 172.3, 162.7, 61.0, 59.2, 55.5, 50.8, 34.8, 30.7, 29.8, 28.1, 25.2, 14.6 ppm. $C_{15}H_{25}N_3O_4S_2$ (375.50): calcd. C 47.98, H 6.71, N 11.19; found C 48.37, H 6.96, N 11.10.

(*S*)-3-Methyl-2-(2-{5-[(3a*S*,4*S*,6a*R*)-2-oxohexahydro-1*H*-thieno-[3,4-*d*]imidazol-4-yl]pentanamido}acetamido)butanoic Acid (Biotin-Gly-Val-OH) (6e): White microcrystals (35%); m.p. 141–144 °C. ^{1}H NMR ([D$_6$]DMSO): δ = 12.7 (s, 1 H), 8.03 (t, J = 5.7 Hz, 1 H), 7.92 (d, J = 8.4 Hz, 1 H), 6.43 (s, 1 H), 6.37 (s, 1 H), 4.30 (dd, J = 7.8, 4.8 Hz, 1 H), 4.18–4.10 (m, 2 H), 3.75 (d, J = 5.7 Hz, 2 H), 3.13–3.05 (m, 1 H), 2.82 (dd, J = 12.5, 5.0 Hz, 1 H), 2.57 (d, J = 12.6 Hz, 1 H), 2.12 (t, J = 7.4 Hz, 2 H), 2.08–1.99 (m, 1 H), 1.66–1.40 (m, 4 H), 1.37–1.25 (m, 2 H), 0.86 (d, J = 6.9 Hz, 6 H) ppm. ^{13}C NMR ([D$_6$]DMSO): δ = 172.9, 172.4, 169.2, 162.7, 61.0, 59.2, 57.0, 55.4, 41.7, 35.0, 30.0, 28.2, 28.0, 25.2, 19.1, 17.9 ppm. HRMS: calcd. for $C_{17}H_{28}N_4O_5S_2Na$ [M + Na]$^+$ 423.1667; found 423.1673.

(*S*)-4-Methyl-2-[(*S*)-4-methyl-2-{5-[(3a*S*,4*S*,6a*R*)-2-oxohexahydro-1*H*-thieno[3,4-*d*]imidazol-4-yl]pentanamido}pentanamido]pentanoic Acid (Biotin-Leu-Leu-OH) (6f): White microcrystals (75%); m.p.

240–243 °C. ^{1}H NMR ([D$_6$]DMSO): δ = 8.03 (d, J = 8.1 Hz, 1 H), 7.90 (d, J = 7.8 Hz, 1 H), 6.45 (s, 1 H), 6.38 (s, 1 H), 4.40–4.27 (m, 2 H), 4.23–4.08 (m, 2 H), 3.12–3.04 (m, 1 H), 2.81 (dd, J = 12.6, 5.1 Hz, 1 H), 2.57 (d, J = 12.3 Hz, 1 H), 2.10 (t, J = 6.9 Hz, 2 H), 1.68–1.38 (m, 11 H), 1.35–1.24 (m, 2 H), 0.88 (d, J = 6.6 Hz, 3 H), 0.87 (d, J = 6.3 Hz, 3 H), 0.83 (d, J = 6.6 Hz, 3 H), 0.82 (d, J = 6.6 Hz, 3 H) ppm. ^{13}C NMR ([D$_6$]DMSO): δ = 174.0, 172.2, 171.9, 162.7, 61.1, 59.2, 55.5, 50.6, 50.2, 40.9, 35.0, 28.1, 25.4, 24.3, 24.2, 23.2, 22.9, 21.6, 21.4 ppm. $C_{22}H_{38}N_4O_5S$ (470.63): calcd. C 56.15, H 8.14, N 11.90; found C 56.16, H 8.44, N 11.96.

(2*S*)-3-Methyl-2-[2-(2-{5-[(3a*S*,4*S*,6a*R*)-2-oxohexahydro-1*H*-thieno-[3,4-*d*]imidazol-4-yl]pentanamido}acetamido)acetamido]pentanoic Acid (Biotin-Gly-Gly-Ile-OH) (6g): This compound is water soluble. For that reason, the mixture was not quenched with ice, but instead 4 N HCl (0.5 mL) was added to neutralize the reaction mixture, then diethyl ether. The aqueous phase was collected, then concentrated under reduced pressure. The resulting residue was washed with ethanol, then acetone to yield the product as white microcrystals (48%); m.p. 156–159 °C. ^{1}H NMR ([D$_6$]DMSO): δ = 12.60 (br. s, 1 H), 8.10 (t, J = 5.9 Hz, 1 H), 8.04 (t, J = 6.3 Hz, 1 H), 7.96 (d, J = 7.8 Hz, 1 H), 6.44 (s, 1 H), 6.37 (s, 1 H), 4.34–4.26 (m, 1 H), 4.19–4.10 (m, 2 H), 3.76 (d, J = 6.0 Hz, 2 H), 3.68 (d, J = 5.7 Hz, 2 H), 3.14–3.05 (m, 1 H), 2.82 (dd, J = 12.5, 5.3 Hz, 1 H), 2.57 (d, J = 12.3 Hz, 1 H), 2.13 (t, J = 7.1 Hz, 2 H), 1.85–1.72 (m, 1 H), 1.66–1.10 (m, 8 H), 0.90–0.80 (m, 6 H) ppm. ^{13}C NMR ([D$_6$]-DMSO): δ = 172.8, 172.6, 169.3, 168.8, 162.7, 61.0, 59.2, 56.2, 55.4, 42.1, 41.7, 36.4, 35.0, 28.2, 28.1, 25.1, 24.6, 15.6, 11.3 ppm. $C_{20}H_{33}N_5O_6S$ (471.57): calcd. C 50.94, H 7.05, N 14.85; found C 50.90, H 7.58, N 14.80.

General Procedure for the Preparation of α-Tocopherol–Peptide Conjugates 9a–e: A dried heavy-walled Pyrex tube containing a small stir bar was charged with a mixture of Z-protected acylbenzotriazole **8** (1 equiv.) and α-tocopherol (**7**) (1.1 equiv.) dissolved in anhydrous DMF (2 mL). K_2CO_3 (2 equiv.) was added, and the mixture was exposed to microwave irradiation (20 W, 50 °C) for 20 min until completion of the reaction (monitored by TLC). The mixture was quenched with ice, then it was extracted with ethyl acetate (2 × 15 mL). The combined organic layers were concentrated under reduced pressure, then purified by column chromatography to yield the corresponding product.

(2*R*)-2,5,7,8-Tetramethyl-2-[(4*R*,8*R*)-4,8,12-trimethyltridecyl]-chroman-6-yl (*S*)-2-{[(Benzyloxy)carbonyl]amino}-3-methylbutanoate (Z-Val-*O*-Tocopherol) (9a): Purified by column chromatography using hexanes/ethyl acetate in a 9.5:0.5 ratio to yield the product as off-white microcrystals (68%); m.p. 63–64 °C. ^{1}H NMR (CDCl$_3$): δ = 7.40–7.31 (m, 5 H), 5.32 (d, J = 9.3 Hz, 1 H), 5.17–5.10 (m, 2 H), 4.64 (dd, J = 9.3, 3.9 Hz, 1 H), 2.57 (t, J = 6.6 Hz, 2 H), 2.46–2.42 (m, 1 H), 2.08 (s, 3 H), 2.00 (s, 3 H), 1.95 (s, 3 H), 1.84–1.70 (m, 2 H),1.56–1.00 (m, 30 H), 0.87–0.83 (m, 12 H) ppm. ^{13}C NMR (CDCl$_3$): δ = 171.1, 156.6, 149.8, 140.5, 136.4, 128.7, 128.4, 128.3, 126.8, 125.0, 123.4, 117.7, 75.3, 67.3, 59.3, 39.6, 37.6, 37.5, 33.0, 32.9, 31.1, 28.2, 25.0, 24.7, 23.0, 22.9, 21.2, 20.8, 20.0, 19.9, 17.3, 13.3, 12.5, 12.1 ppm. HRMS: calcd. for $C_{42}H_{65}NO_5Na$ [M + Na]$^+$ 686.4755; found 686.4786.

(*R*)-2,5,7,8-Tetramethyl-2-[(4*R*,8*R*)-4,8,12-trimethyltridecyl]-chroman-6-yl (*S*)-2-[(Benzyloxy)carbonyl]amino}-3-phenylpropanoate (Z-Phe-*O*-Tocopherol) (9b): Purified by column chromatography using hexanes/ethyl acetate in a 9.5:0.5 ratio to yield the product as wax (60%).^{1}H NMR (CDCl$_3$): δ = 7.40–7.27 (m, 10 H), 5.33 (d, J = 8.4 Hz, 1 H), 5.11 (s, 1 H), 5.03–4.70 (m, 1 H), 3.50–3.38 (m, 1 H), 2.60 (t, J = 6.5 Hz, 3 H), 2.11 (s, 3 H), 1.96 (s, 3 H), 1.93 (s, 3 H), 1.90–1.70 (m, 3 H), 1.62–1.52 (m, 2 H), 1.48–1.32 (m, 14 H), 1.25–1.05 (m, 8 H), 1.04–0.86 (m, 12 H) ppm. ^{13}C NMR (CDCl$_3$): δ = 170.81, 156.0, 149.8, 140.4, 136.3, 136.0, 129.5, 128.9, 128.7, 128.3, 128.2, 127.4, 126.7, 125.0, 123.3, 117.6, 75.3, 67.2, 55.1, 39.6, 38.3, 37.6, 37.5, 33.0, 32.9, 31.2, 28.2, 25.0, 24.6, 22.9, 22.9, 21.2, 20.8, 20.0, 19.9, 13.2, 12.3, 12.0 ppm. HRMS: calcd. for $C_{46}H_{65}NO_5Na$ [M + Na]$^+$ 734.4755; found 734.4775.

(2*R*)-2,5,7,8-Tetramethyl-2-[(4*R*,8*R*)-4,8,12-trimethyltridecyl]-chroman-6-yl (*S*)-2-[(*S*)-2-{[(benzyloxy)carbonyl]amino}-3-methylbutanamido]-3-phenylpropanoate (Z-Val-Phe-*O*-Tocopherol) (9c): Purified by column chromatography using hexanes/ethyl acetate in a 8:2 ratio to yield the product as off-white microcrystals (44%); m.p. 116–118 °C. ^{1}H NMR (CDCl$_3$): δ = 7.35–7.25 (m, 10 H), 6.38 (d, J = 7.8 Hz, 1 H), 6.27 (d, J = 7.8 Hz, 1 H), 5.28–5.12 (m, 2 H), 5.09–5.04 (m, 2 H), 4.05–3.93 (m, 1 H), 3.50–3.37 (m, 1 H), 3.20–3.06 (m, 1 H), 2.56 (t, J = 6.5 Hz, 2 H), 2.07 (s, 3 H), 1.96–1.85 (m, 6 H), 1.82–1.70 (m, 2 H), 1.57–1.47 (m, 2 H), 1.45–1.04 (m, 22 H), 0.95–0.78 (m, 18 H) ppm. ^{13}C NMR (CDCl$_3$): δ = 171.2, 170.5, 156.4, 149.8, 140.4, 135.7, 129.5, 129.0, 128.7, 128.4, 128.3, 127.5, 125.0, 123.4, 117.7, 77.7, 77.2, 76.8, 75.3, 67.3, 60.5, 53.3, 39.6, 38.1, 37.7, 37.5, 33.0, 31.3, 28.2, 25.0, 24.7, 24.2, 23.0, 22.9, 21.3, 20.8, 20.0, 19.9, 19.3, 19.1, 13.2, 12.4, 12.1 ppm. HRMS: calcd. for $C_{51}H_{74}N_2O_6Na$ [M + Na]$^+$ 833.5439; found 833.5479.

(*R*)-2,5,7,8-Tetramethyl-2-[(4*R*,8*R*)-4,8,12-trimethyltridecyl]-chroman-6-yl (5*S*,8*S*)-8-Benzyl-5-isopropyl-3,6,9-trioxo-1-phenyl-2-oxa-4,7,10-triazadodecan-12-oate (Z-Val-Phe-Gly-*O*-Tocopherol) (9d): Recrystallized from diethyl ether to yield the product as off-white microcrystals (55%); m.p. 86–89 °C. ^{1}H NMR (CDCl$_3$): δ = 7.44–7.28 (m, 5 H), 7.24–7.12 (m, 5 H), 6.85–6.75 (br. s, 1 H), 6.67 (d, J = 8.4 Hz, 1 H), 5.24 (d, J = 6.9 Hz, 1 H), 5.11–4.98 (m, 3 H), 4.85–4.74 (m, 1 H), 4.40–4.26 (m, 1 H), 4.23–4.10 (m, 1 H), 3.97 (t, J = .5 Hz, 1 H), 3.23–3.12 (m, 1 H), 3.10–3.00 (m, 1 H), 2.62–2.50 (m, 2 H), 2.07 (s, 3 H), 1.98 (s, 3 H), 1.94 (s, 3 H), 1.83–1.70 (m, 3 H), 1.51–1.48 (m, 2 H), 1.45–1.02 (m, 21 H), 0.88–0.73 (m, 18 H) ppm. ^{13}C NMR (CDCl$_3$): δ = 171.4, 171.2, 168.6, 156.9, 149.8, 140.3, 136.6, 136.1, 129.4, 128.9, 128.8, 128.5, 128.4, 127.2, 126.7, 125.0, 123.4, 117.7, 75.3, 67.5, 61.1, 54.3, 41.3, 39.6, 38.1, 37.7, 37.5, 33.0, 31.2, 30.7, 28.2, 25.0, 24.7, 23.0, 22.9, 21.2, 20.8, 20.0, 19.9, 19.4, 17.6, 13.2, 12.4, 12.0 ppm. HRMS: calcd. for $C_{53}H_{77}N_3O_7Na$ [M + Na]$^+$ 890.5654; found 890.5667.

(*R*)-2,5,7,8-Tetramethyl-2-[(4*R*,8*R*)-4,8,12-trimethyltridecyl]-chroman-6-yl (5*S*,8*S*)-8-Benzyl-5-methyl-3,6,9-trioxo-1-phenyl-2-oxa-4,7,10-triazadodecan-12-oate (Z-Ala-Phe-Gly-*O*-Tocopherol) (9e): Purified by column chromatography using hexanes/ethyl acetate in a 8:2 ratio to yield the product as wax (51%). ^{1}H NMR (CDCl$_3$): δ = 7.35–7.28 (m, 5 H), 7.20–7.16 (m, 5 H), 6.82–6.58 (m, 2 H), 5.20 (d, J = 6.3 Hz, 1 H), 5.11–4.96 (m, 2 H), 4.81–4.72 (m, 1 H), 4.40–4.26 (m, 1 H), 4.20–4.10 (m, 2 H), 3.18 (dd, J = 13.5, 6.0 Hz, 1 H), 3.06 (dd, J = 13.8, 7.0 Hz, 1 H), 2.56 (t, J = 6.2 Hz, 2 H), 2.07 (s, 3 H), 1.99 (s, 3 H), 1.95 (s, 3 H), 1.81–1.70 (m, 3 H), 1.59–1.48 (m, 3 H), 1.43–1.02 (m, 23 H), 0.92–0.80 (m, 12 H) ppm. ^{13}C NMR (CDCl$_3$): δ = 172.4, 171.2, 168.6, 156.4, 149.8, 140.3, 136.6, 136.1, 129.4, 128.8, 128.5, 128.3, 127.2, 126.7, 125.0, 123.4, 117.7, 75.3, 67.5, 54.2, 51.2, 41.3, 39.6, 38.0, 37.7, 37.5, 33.0, 32.9, 31.2, 29.9, 28.2, 25.0, 24.7, 23.0, 22.9, 21.2, 20.8, 20.0, 19.9, 18.3, 13.2, 12.4, 12.0 ppm. HRMS: calcd. for $C_{51}H_{73}N_3O_7Na$ [M + Na]$^+$ 862.5357; found 862.5341.

General Procedure for the Preparation of Cholecalciferol–Peptide Conjugates 11a–d: A dried heavy-walled Pyrex tube containing a small stir bar was charged with a mixture of Z-protected acylbenzotriazole **8** (1 equiv.), cholecalciferol (**10**) (1.2 equiv.) and DMAP (0.2 equiv.) dissolved in freshly distilled THF (5 mL). The reaction mixture was exposed to microwave irradiation (50 W, 70 °C) for

2.5 h until complete. The reaction mixture was allowed to cool until the temperature fell below 30 °C when the mixture was quenched with water and extracted with ethyl acetate. The combined organic layers were washed with Na_2CO_3 (10%), water and dried with anhydrous magnesium sulfate. The solvent was removed under reduced pressure, and the residue was chromatographed on a silica gel column to give the product **11**.

(*Z*)-3-[(*E*)-2-{(1*R*,3a*S*,7a*R*)-7a-Methyl-1-[(*R*)-6-methylhept-2-yl]hexahydro-1*H*-inden-4(2*H*)-ylidene}ethylidene]-4-methylenecyclohexyl (2*S*)-2-{[(Benzyloxy)carbonyl]amino}-3-methylbutanoate (Z-Val-*O*-Cholecalciferol) (11a): Purified by column chromatography using hexanes/ethyl acetate in a 9.5:0.5 ratio to yield the product as oil (56%). ^{1}H NMR ($CDCl_3$): δ = 7.36–7.31 (m, 5 H), 6.19 (d, *J* = 11.4 Hz, 1 H), 6.02 (d, *J* = 11.4 Hz, 1 H), 5.30 (d, *J* = 8.7 Hz, 1 H), 5.11 (s, 2 H), 5.07 (s, 1 H), 5.05–5.02 (m, 1 H), 4.86–4.84 (m, 1 H), 4.32–4.24 (m, 1 H), 2.84–2.75 (m, 1 H), 2.62–2.51 (m, 1 H), 2.44–2.34 (m, 2 H), 2.26–2.12 (m, 2 H), 2.06–1.92 (m, 3 H), 1.86–1.75 (m, 2 H), 1.68–1.60 (m, 3 H), 1.58–1.42 (m, 4 H), 1.38–1.20 (m, 6 H), 1.18–1.06 (m, 3 H), 0.96 (d, *J* = 6.6 Hz, 3 H), 0.92 (d, *J* = 6.3 Hz, 3 H), 0.89–0.85 (m, 9 H), 0.54 (s, 3 H) ppm. ^{13}C NMR ($CDCl_3$): δ = 171.6, 156.4, 144.6, 142.8, 136.5, 133.9, 128.7, 128.3, 123.0, 117.5, 113.0, 73.0, 67.2, 59.2, 56.8, 56.5, 46.1, 42.2, 40.7, 39.7, 36.3, 32.2, 32.0, 31.6, 29.2, 28.2, 27.9, 24.1, 23.8, 23.0, 22.8, 22.4, 19.2, 19.1, 17.6, 12.2 ppm. HRMS: calcd. for $C_{40}H_{59}NO_4Na$ [M + Na]$^+$ 640.4336; found 640.4331.

(1*S*,*Z*)-3-[(*E*)-2-{(1*R*,7a*R*)-7a-Methyl-1-[(*R*)-6-methylhept-2-yl]hexahydro-1*H*-inden-4(2*H*)-ylidene}ethylidene]-4-methylenecyclohexyl (2*S*)-2-{[(Benzyloxy)carbonyl]amino}-3-(1*H*-indol-2-yl)propanoate (Z-Trp-*O*-Cholecalciferol) (11b): Purified by column chromatography using hexanes/ethyl acetate in a 8:2 ratio to yield the product as oil (64%). ^{1}H NMR ($CDCl_3$): δ = 8.12 (br. s, 1 H), 7.53 (d, *J* = 7.5 Hz, 1 H), 7.40–7.27 (m, 5 H), 7.18 (dt, *J* = 7.5, 1.1 Hz, 1 H), 7.07 (dt, *J* = 7.6, 1.1 Hz, 1 H), 6.98–6.96 (m, 1 H), 6.14 (d, *J* = 11.1 Hz, 1 H), 6.01 (d, *J* = 11.4 Hz, 1 H), 5.33 (d, *J* = 8.4 Hz, 1 H), 5.15–5.01 (m, 3 H), 4.96–4.85 (m, 1 H), 4.82 (d, *J* = 2.4 Hz, 1 H), 4.72–4.62 (m, 1 H), 3.34–3.17 (m, 2 H), 2.82–2.74 (m, 1 H), 2.45–1.80 (m, 9 H), 1.70–1.43 (m, 6 H), 1.40–1.08 (m, 11 H), 0.92 (d, *J* = 6.3 Hz, 3 H), 0.88 (s, 3 H), 0.86 (d, *J* = 1.5 Hz, 3 H), 0.53 (s, 3 H) ppm. ^{13}C NMR ($CDCl_3$): δ = 171.8, 156.0, 144.6, 142.8, 136.5, 136.3, 134.1, 128.7, 128.3, 127.8, 123.0, 122.9, 122.4, 119.8, 118.9, 117.6, 113.0, 111.3, 110.3, 73.3, 67.1, 56.8, 56.5, 54.9, 46.1, 42.0, 40.7, 39.7, 36.3, 34.8, 32.2, 31.9, 29.2, 28.2, 27.8, 24.1, 23.8, 23.0, 22.8, 22.4, 19.0, 12.2 ppm. HRMS: calcd. for $C_{46}H_{60}N_2O_4Na$ [M + Na]$^+$ 727.4445; found 727.4479.

(*S*,*Z*)-3-[(E)-2-{(1*R*,3a*S*,7a*R*)-7a-Methyl-1-[(*R*)-6-methylhept-2-yl]hexahydro-1*H*-inden-4(2*H*)-ylidene}ethylidene]-4-methylenecyclohexyl (*S*)-2-[(*S*)-2-{[(Benzyloxy)carbonyl]amino}propanamido]-3-phenylpropanoate (Z-Ala-Phe-*O*-Cholecalciferol) (11c): Purified by column chromatography using hexanes/ethyl acetate in a8:2 ratio to yield the product as oil (52%). ^{1}H NMR ($CDCl_3$): δ = 7.28–7.23 (m, 5 H), 7.22–7.15 (m, 3 H), 7.10–6.98 (m, 2 H), 6.41–6.30 (m, 1 H), 6.15–5.40 (m, 2 H), 5.26–5.10 (m, 1 H), 5.06–4.94 (m, 3 H), 4.94–4.88 (m, 1 H), 4.78–4.70 (m, 2 H), 4.20–4.08 (m, 1 H), 3.06–2.94 (m, 2 H), 2.78–2.60 (m, 1 H), 2.49–2.36 (m, 1 H), 2.30–2.06 (m, 2 H), 1.98–1.72 (m, 3 H), 1.68–1.52 (m, 2 H), 1.50–1.34 (m, 3 H), 1.30–1.10 (m, 9 H), 1.10–1.00 (m, 3 H), 0.98–0.88 (m, 2 H), 0.84 (d, *J* = 6.1 Hz, 3 H), 0.80 (d, *J* = 6.6 Hz, 9 H), 0.46 (d, *J* = 7.8 Hz, 3 H) ppm. ^{13}C NMR ($CDCl_3$): δ = 171.9, 170.8, 156.0, 144.4, 143.2, 142.9, 136.3, 135.9, 134.2, 133.9, 129.6, 128.7, 128.4, 128.3, 127.3, 123.0, 122.7, 117.6, 113.2, 73.5, 73.2, 67.3, 56.8, 56.5, 53.3, 53.2, 50.6, 46.1, 42.1, 40.7, 39.7, 38.0, 36.3, 32.0, 31.7, 29.3, 28.2, 27.9, 24.1, 23.8, 23.0, 22.8, 22.4, 19.1, 18.7, 12.2 ppm. HRMS: calcd. for $C_{47}H_{64}N_2O_5Na$ [M + Na]$^+$ 759.4707; found 759.4738.

(*S*,*Z*)-3-[(*E*)-2-{(1*R*,3a*S*,7a*R*)-7a-Methyl-1-[(*R*)-6-methylhept-2-yl]-hexahydro-1*H*-inden-4(2*H*)-ylidene}ethylidene]-4-methylenecyclohexyl (*S*)-2-[(*S*)-2-{[(Benzyloxy)carbonyl]amino}-3-methylbutanamido]-3-phenylpropanoate (Z-Ala-Phe-*O*-Cholecalciferol) (11d): Purified by column chromatography using hexanes/ethyl acetate in a 8:2 ratio to yield the product as white microcrystals (58%); m.p. 166–169 °C. ^{1}H NMR ($CDCl_3$): δ = 7.40–7.24 (m, 5 H), 7.17–6.85 (m, 5 H), 6.29 (d, *J* = 7.8 Hz, 1 H), 6.19 (d, *J* = 8.4 Hz, 1 H), 6.15–6.08 (m, 1 H), 6.00–5.90 (m, 1 H), 5.22 (dd, *J* = 20.7, 8.1 Hz, 1 H), 4.99–4.86 (m, 3 H), 4.85–4.83 (m, 1 H), 4.83–4.73 (m, 2 H), 4.00–3.50 (m, 1 H), 3.01 (d, *J* = 6.0 Hz, 2 H), 2.77–2.67 (m, 1 H), 2.51–2.40 (m, 1 H), 2.30–2.17 (m, 2 H), 2.16–1.75 (m, 6 H), 1.69–1.54 (m,4 H), 1.50–1.35 (m, 4 H), 1.34–1.15 (m, 6 H), 1.14–1.00 (m, 4 H), 0.95–0.70 (m, 16 H) ppm. ^{13}C NMR ($CDCl_3$): δ = 170.9, 156.5, 144.4, 143.0, 135.9, 133.9, 129.5, 128.7, 128.4, 128.3, 127.3, 122.9, 117.5, 113.2, 73.5, 67.3, 60.4, 56.8, 56.6, 53.3, 46.1, 42.2, 40.7, 39.7, 38.4, 36.4, 32.2, 32.0, 31.0, 29.3, 28.2, 27.9, 24.1, 23.8, 23.1, 22.8, 22.4, 19.5, 19.1, 18.0, 17.5, 12.2 ppm. HRMS: calcd. for $C_{49}H_{68}N_2O_5Na$ [M + Na]$^+$ 787.5020; found 787.5033.

Supporting Information (see footnote on the first page of this article): Copies of ^{1}H and ^{13}C NMR spectra of compounds **3a**,**b**, **5a**–**e**, **6a**–**g**, **9a**–**e** and **11a**–**d**.

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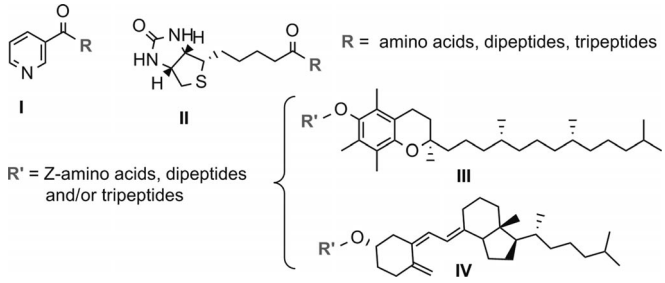

Amino acid and peptide bioconjugates of niacin **I**, biotin **II**, α-tocopherol **III** and cholecalciferol **IV** are prepared in good to satisfactory yields by using microwave assistance. In addition, a new benzotriazole-activated biotin intermediate is synthesized, which may serve as an advantageous alternative biotinylation reagent.

Keywords: Microwave chemistry / Peptides / Amino acids / Vitamins

