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Selective *N*-Acylation and *N*-Alkylation of Biotin

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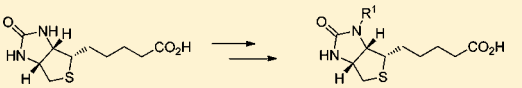
Supporting Information

ABSTRACT: Simple and efficient methodology is presented for the selective acylation and alkylation of biotin at its 3′-nitrogen.



Biotin (**1**, Figure 1) is a water-soluble B vitamin that functions as a prosthetic group for the biotin-dependent

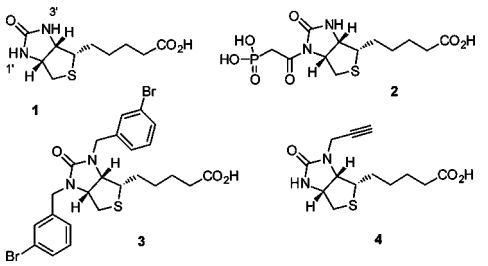


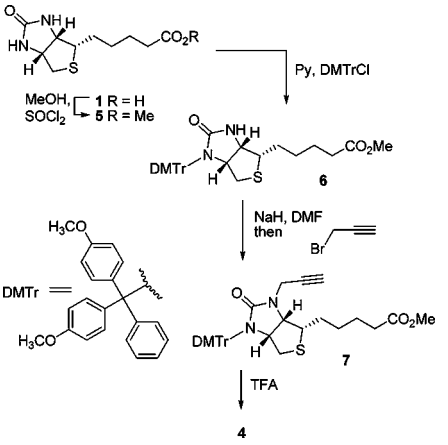
Figure 1. Biotin and its derivatives.

carboxylases, key metabolic enzymes found in all organisms.[1] For this and other reasons, biotin and its derivatives attract considerable synthetic and biochemical interest.[1−4] For example, N1′-substututed derivative **2** is reported to be an inhibitor of biotin CoA carboxylase,[5] **3** is an inhibitor of HIV protease,[6] and N3′-substituted **4** is a substrate for biotin protein ligase.[7] To date, the HIV proteases of type **3** are limited to structures having the same substituents at both N1′ and N3′. In addition, the interaction of biotin with the receptor streptavidin is one of the strongest protein ligand interactions known with a $K_a \approx 10^{15}$ M^{-1}.[8] The key interaction here involves hydrogen bonding between the protein and the ureido nitrogens of biotin. For all these reasons, there is a need for simple and selective methods for functionalizing the two ureido nitrogens of biotin. However, there are relatively few such reports,[6] with a solution being to simply functionalize at both nitrogens and to then separate the resulting isomers by exhaustive HPLC.[7]

What we do know is that the 3′-nitrogen of biotin is significantly more sterically hindered than the 1′-nitrogen due to presence of the 4-carboxybutyl substituent.[9] In addition, the 1′-nitrogen is significantly more nucleophilic than the 3′-nitrogen due to twisted conformation of the bicyclic ring system.[2,10] As such, *N*-acylation or alkylation of biotin, when it occurs, preferentially takes place at the 1′-nitrogen. This has been put to good effect with the preparation of structures such as **2**.[5] However, to our knowledge, there are no specific reports on selective acylation or alkylation at the 3′-nitrogen, despite the need for such methodology to prepare derivatives of type **4**[7] and nonsymmetrical analogues of **3** and other biotin derivatives.

Our approach to this problem is simple but effective. We react biotin with 4,4′-dimethoxytrityl chloride (DMTrCl) to introduce a trityl protecting group at the 1′ nitrogen and then acylate or alkylate at the 3′-nitrogen. Removal of the protecting group gives the desired 3′-substituted product in good yield; see Schemes 1 and Tables 1 and 2 for examples. The methodology

Scheme 1. Selective 3′-Alkylation of Biotin



is simple and generally applicable, while avoiding the current practice[7] of carrying out extensive chromatography to separate isomers.

The sequence is shown in Scheme 1 for the preparation of the known[7] derivative **4**. The methyl ester of biotin (**5**, prepared as shown) was treated with a pyridine solution of DMTrCl containing Et_3N[11] to give **6** in 81% after purification by flash chromatography, without evidence of any N3′-substituted product. Alkylation at N1′ was confirmed by characteristic $^1H-^1H$ ROESY correlations between the NH resonance at 4.67 ppm and 1 proton resonance at 4.35−4.31 (SCHC*H*) and the multiplet at 1.71−1.56 ppm (SCHCH_2). Further characteristic correlations were observed between the

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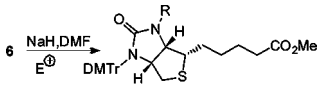
Table 1. 3′-Substitution of Biotin[a]

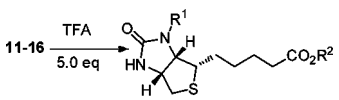


11 - 16

product	electrophile	R	yield[a] (%)
11	$ClCH_2COCl$	$ClCH_2CO$	59[b]
12	$CH_2{=}CH(CH_2)_8COCl$	$CH_2{=}CH(CH_2)_8CO$	60[b]
13	$PhCH_2OCOCl$	$PhCH_2OCO$	80
14	$PO(OEt)_2Cl$	$PO(OEt)_2$	58
15	TsCl	Ts	28[b]
16	$PhCH_2Br$	$PhCH_2$	76

[a]Isolated yield after flash column chromatography on silica gel. [b]Contains 20% DMTrOH by ^{1}H NMR

Table 2. Cleavage of N1′-DMTr



17 - 22

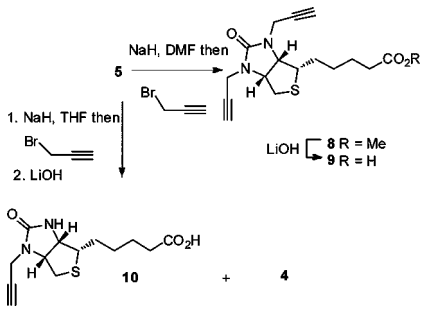
product	R^1	R^2	yield[a] (%)
17	$ClCH_2CO$	Me	84
18	$CH_2{=}CH(CH_2)_8CO$	H	~68[b]
19	$PhCH_2OCO$	Me	90
20	$PO(OEt)_2$	Me	64
21	Ts	H	~58[b]
22	$PhCH_2$	Me	90

[a]Isolated yield after flash column chromatography on silica gel. [b]>90% purity based on ^{1}H NMR

DMTr resonance and the resonances at 4.42−4.37 ppm (SCH_2CH) and 2.47 (SCH_ACH). This material was then treated with sodium hydride in DMF, followed by the addition of propargyl bromide, to give the disubstituted derivative **7** in 85%. Treatment with 5.0 equiv of TFA in dichloromethane then gave the desired derivative **4** in 90% yield.

It is also possible to prepare the 1′-isomer **10** and the doubly alkyated product **9** under the appropriate reaction conditions as shown in Scheme 2. In particular, alkylation of the methyl ester

Scheme 2. Alkylation Alkylation and Dialkylation of Biotin



of biotin (**5**), with propargyl bromide, gave the dialkylated material **9** in 89% yield after hydrolysis of the methyl ester with LiOH. The choice of solvent in the alkylation step is critical, with the use of THF (in place of DMF) under literature conditions,[7] giving a mixture of **4** and its 1′-isomer **10** (2:3, based on ^{1}H NMR) under otherwise identical conditions. These two isomers were separated by reversed-phase HPLC to give **10** and **4** in 30% and 20% yields, respectively.

The conditions shown in Scheme 1 are suitable for reaction of **6** with a variety of electrophiles to give a range of derivatives as shown in Tables 1 and 2. Derivative **13** is particularly significant in that the 1′- and 3′-nitrogens of biotin are differentially protected with trityl and benzyloxycarbonyl (Cbz), respectively. The trityl protecting groups of **11**−**16** were removed on treatment with TFA to give **17**−**22** as summarized in Table 2. Biotin derivative **19**, derived from **13**, is significant in that its 3′-nitrogen is selectively protected with a Cbz group. The protection of biotin at this position is otherwise difficult to achieve. As for the preparation of **4**, the methyl esters were removed under the conditions to give **18** and **21** possibly due to extended time on the rotatory evaporator during workup.

In conclusion, we report a simple and efficient sequence for the selective functionalization of biotin at the 3′-nitrogen. The methodology involves the selective tritylation at the 1′-nitrogen, followed by reaction with the appropriate electrophile and removal of the trityl group. The reported chemistry is significant in that it provides access to the known derivative **4**[7] and also **17**−**22** in good yields. Thus biotin can be selectively protected at the 1′-nitrogen (**6**), 3′-nitrogen (**19**), and also differentially at both sites (**13**). The methodology presented should be amenable to the preparation of a wide range of otherwise inaccessible biotin analogues.

EXPERIMENTAL SECTION

NMR spectra were recorded in the specified solvent at 300 or 600 MHz (^{1}H), 151 MHz (^{13}C), and 121 MHz (^{31}P); chemical shifts are quoted on the δ scale (ppm) with TMS or the solvent signal as the internal standard (^{1}H NMR: $CDCl_3$, TMS = 0.00 ppm and DMSO-d_6, 2.50 ppm; ^{13}C NMR; $CDCl_3$ 77.00 ppm and DMSO-d_6 39.5 ppm. Thin-layer chromatography (TLC) was carried out on commercially available precoated aluminum plates (Merck 60F254) with visualization under 254 nm light or by developing with vanillin or permanganate dip. Flash column chromatography was carried out using Merck Kieselgel 60 (230−400 mesh). Melting points were measured on a microscope hot-stage melting point apparatus and are uncorrected. All yields reported refer to isolated material judged to be homogeneous by TLC and NMR spectroscopy. Oven-dried glassware was used in all reactions carried out under an inert atmosphere (either dry nitrogen or argon). All starting materials and reagents were obtained commercially unless otherwise stated. Removal of solvents "under reduced pressure" refers to the process of bulk solvent removal by rotary evaporation (low vacuum pump) followed by application of high vacuum pump (oil pump) for a minimum of 30 min.

General Procedure A. *Reaction 6 with an Electrophile.* A suspension of sodium hydride (60% dispersion in oil, 5.0 equiv) in dry DMF (4 mL/mmol) was cooled in an ice bath and stirred for 15 min under a nitrogen atmosphere. A solution of **6** (1.0 equiv) in dry DMF (4 mL/mmol) was added dropwise over 30 min, followed by addition of TBAI (0.5 equiv) and dropwise addition of the electrophile (1.2 equiv). The reaction mixture was warmed to rt and stirred for an additional 3 h. The reaction was then quenched by the slow addition of water (20 mL), and the volatiles were removed under reduced pressure. Further water (40 mL) was added, and the aqueous phase was acidified to pH 2 (universal indicator paper) with the addition of aqueous $KHSO_4$. The mixture was extracted with ethyl acetate (3 × 40 mL), and the organic layers were combined, dried over anhydrous Na_2SO_4, filtered, and evaporated under reduced pressure to give a yellow oil that was purified by chromatography on silica gel (1% CH_3OH in CH_2Cl_2). See the individual experiments for details.

General Procedure B. *DMTr Cleavage.* To a solution of disubstituted derivative of biotin (1.0 equiv) in CH_2Cl_2 (20 mL) was added (5.0 equiv) TFA, and the reaction mixture was stirred under argon for 1 h at rt. The solution was concentrated in vacuo, and the

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residue purified by chromatography on silica gel (1% CH_3OH in CH_2Cl_2). See the individual experiments for details.

5-((3a*S*,4*S*,6a*R*)-2-Oxo-3-(prop-2-ynyl)hexahydro-1*H*-thieno-[3,4-*d*]imidazol-4-yl)pentanoic Acid (4).[7] A solution of **7** (0.50 g, 0.83 mmol) in CH_2Cl_2 (20 mL) was treated with TFA according to general method B to give **4** as a white solid (0.21 g, 90%): mp 170−175 °C; ^{1}H NMR (DMSO-d_6, 600 MHz) δ 12.01 (bs, 1H), 6.98 (s, 1H), 4.20−4.34 (m, 3H), 3.60 (dd, J = 17.9, 2.0, 1H), 3.22−3.28 (m, 2H), 2.86 (dd, J = 12.7, 5.3, 1H), 2.60 (d, J = 12.2, 1H), 2.21 (t, J = 7.2, 2H), 1.30−1.81 (m, 6H); ^{13}C NMR (DMSO-d_6, 151 MHz) 174.5, 161.2, 79.4, 75.2, 64.1, 57.3, 55.9, 34.0, 33.5, 28.9, 28.2, 24.4; HRMS (ESI, $[M + Na]^+$) calcd for $C_{13}H_{18}N_2O_3SNa$ 305.0936, found 305.0926.

Methyl 5-((3a*S*,4*S*,6a*R*)-1-(Bis(4-methoxyphenyl)(phenyl)-methyl)-2-oxohexahydro-1*H*-thieno[3,4-*d*]imidazol-4-yl)-pentanoate (6). To a solution of (+)-biotin methyl ester **5** (3.0 g, 11.6 mmol), rendered anhydrous by coevaporation with dry pyridine (three times), in dry pyridine (65 mL) were added 4,4′-dimethoxytrityl chloride (11.8 g, 34.0 mmol), triethylamine (1.95 mL, 13.9 mmol), and DMAP (0.35 g, 2.8 mmol). The mixture was heated to 75 °C, stirred at this temperature for 4 h, and then diluted with $CHCl_3$. The $CHCl_3$ phase was separated and washed three times with 5% aqueous sodium citrate. The organic layer was collected, dried over Na_2SO_4, filtered, and evaporated under reduced pressure. The residue was purified by silica gel chromatography (2% CH_3OH in CH_2Cl_2) to **6** as a yellow foam (5.27 g, 81%): mp 68−71 °C; ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.33−7.23 (m, 5H), 7.21−7.13 (m, 4H), 6.85−6.79 (m, 4H), 4.67 (s, 1H), 4.42−4.37 (m, 1H), 4.35−4.31 (m, 1H), 3.80 (s, 6H), 3.67 (s, 3H), 3.15−3.10 (m, 1H), 2.47 (dd, J = 13.0, 1.8 Hz, 1H), 2.34−2.24 (m, 3H), 1.71−1.56 (m, 4H), 1.50−1.33 (m, 2H); ^{13}C NMR (151 MHz, $CDCl_3$): δ 173.8, 160.9, 158.4, 143.7, 139.5, 135.8, 135.7, 131.3, 129.7, 129.1, 127.6, 127.1, 126.9, 112.8, 72.8, 65.6, 59.7, 55.22, 55.20, 53.9, 51.5, 39.0, 33.6, 28.8, 28.2, 24.6; HRMS (ESI, $[M + Na]^+$) calcd for $C_{32}H_{36}N_2O_5SNa$ 583.2243, found 583.2239.

Methyl 5-((3a*S*,4*S*,6a*R*)-1-(Bis(4-methoxyphenyl)(phenyl)-methyl)-2-oxo-3-(prop-2-ynyl)hexahydro-1*H*-thieno[3,4-*d*]-imidazol-4-yl)pentanoate (7). A suspension of sodium hydride (0.38 g of a 60% dispersion in oil, 16.6 mmol) in dry DMF (15 mL) was cooled in an ice bath and stirred for 15 min under a nitrogen atmosphere. A solution of **6** (2.0 g, 3.5 mmol) in dry DMF (20 mL) was added dropwise over 30 min, followed by addition of TBAI (0.65 g, 1.7 mmol) and dropwise addition propargyl bromide (0.37 mL of 80% solution in toluene, 4.2 mmol). The reaction mixture was warmed to rt and stirred for an additional 3 h. Water (20 mL) was added slowly, and the volatiles were removed under reduced pressure. Further water (40 mL) was added, and the aqueous phase was acidified to pH 2 (universal indicator paper) with the addition of aqueous $KHSO_4$. The mixture was extracted with ethyl acetate (3 × 40 mL), and the organic layers were combined, dried over anhydrous Na_2SO_4, filtered, and evaporated under reduced pressure to give a yellow oil which was purified by chromatography on silica gel (1% CH_3OH in CH_2Cl_2) to give **7** as a low melting white solid (1.81 g, 85%): mp 63−67 °C; ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.34 −7.09 (m, 9H), 6.85−6.79 (m, 4H), 4.61 (dd, J = 17.8, 2.4 Hz, 1H), 4.53−4.45 (m, 1H), 3.80 (s, 6H), 3.67 (s, 3H), 3.55 (dd, J = 17.8, 2.2 Hz, 1H), 3.24−3.19 (m, 1H), 2.37 (t, J = 2.3 Hz, 1H), 2.31−2.34 (m, 2H), 2.26−2.24 (dd, J = 4.2,4.0 Hz, 1H), 1.89 (dd, J = 12.9, 6.0 Hz, 1H), 1.75−1.36 (m, 7H); ^{13}C NMR (151) MHz, $CDCl_3$) δ 173.9, 160.9, 158.5, 143.1, 139.5, 135.2, 131.5, 131.3, 129.8, 129.1, 127.9, 127.7, 127.0, 112.9, 78.5, 73.3, 72.8, 63.1, 62.3, 55.24, 55.22 54.2, 51.6, 37.7, 35.0, 33.9, 28.9, 28.5, 24.7; HRMS (ESI, $[M + Na]^+$) calcd for $C_{35}H_{38}N_2O_5SNa$ 621.2399, found 621.2395.

5-((3a*S*,4*S*,6a*R*)-2-Oxo-1,3-di(prop-2-ynyl)hexahydro-1*H*-thieno[3,4-*d*]imidazol-4-yl)pentanoic Acid (9). A suspension of sodium hydride (0.27 g of a 60% dispersion in oil, 11.6 mmol) in dry DMF (10 mL), under a nitrogen atmosphere, was cooled in an ice bath with stirring. After 15 min, a solution of biotin methyl ester **5** (0.60 g, 2.3 mmol) in DMF (10 mL) was added dropwise over 30 min, followed by TBAI (0.42 g, 1.6 mmol) and propargyl bromide (0.41 mL of 80% solution in toluene, 4.6 mmol). The reaction mixture was warmed to rt and stirred for an additional 3 h. The reaction mixture was quenched by the slow addition of water (10 mL), and the solvents were removed under reduced pressure. Further water (30 mL) was added, and the aqueous phase was acidified to pH 2 (universal indicator paper) with the addition of aqueous $KHSO_4$. The mixture was extracted with ethyl acetate (3 × 30 mL), and the combined organic layers were dried over anhydrous Na_2SO_4, filtered, and evaporated under reduced pressure to give **8** as a yellow oil that was not purified further (0.67 g). This material (0.6 g) was dissolved in THF (1.5 mL) and the solution cooled in an ice bath. Methanol (1.5 mL) followed by a solution of lithium hydroxide (0.06 g 2.66 mmol) in water (4.2 mL) were added. The reaction mixture was stirred for 3 h, allowed to warm to rt, and then concentrated in vacuo. The residue was purified by chromatography on a column of silica gel (1% CH_3OH in CH_2Cl_2) to give **9** as colorless oil (0.50 g, 89%): ^{1}H NMR (600 MHz, DMSO-d_6) δ 12.00 (s, 1H), 4.41 (m, 1H), 4.31−4.20 (m, 2H), 4.13 (dd, J = 17.9, 1.2 Hz, 1H), 3.86 (dd, J = 17.9, 1.0 Hz, 1H), 3.72 (dd, J = 18.0, 0.9 Hz, 1H), 3.31−3.18 (m, 3H), 3.02 (d, J = 12.8 Hz, 1H), 2.85 (dd, J = 12.8, 5.3 Hz, 1H), 2.26−2.18 (m, 2H), 1.81 − 1.72 (m, 1H), 1.60−1.48 (m, 2H), 1.48−1.30 (m, 3H); ^{13}C NMR (151 MHz, DMSO-d_6) δ 174.3, 159.8, 79.1, 79.0, 75.2, 74.6, 62.4, 61.3, 55.1, 34.5, 34.3, 33.4, 31.2, 28.6, 28.0, 24.2; HRMS (ESI, $[M + Na]^+$) calcd for $C_{16}H_{20}N_2O_3SNa$ 343.1092, found 343.1089.

(3a*R*,6*S*,6a*S*)-Benzyl 3-(bis(4-methoxyphenyl)(phenyl)-methyl)-6-(5-methoxy-5-oxopentyl)-2-oxohexahydro-1*H*-thieno[3,4-*d*]imidazole-1-carboxylate (13). Compound **6** (100 mg, 1.0 equiv) and benzyl chloroformate (36 mg, 1.2 equiv) were reacted according to general procedure A to give **13** as a low-melting solid (95 mg, 80%): ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.40−7.09 (m, 14H), 6.81 (dd, J = 9.0, 2.1 Hz, 4H), 5.40 (d, J = 12.3 Hz, 1H), 5.13 (d, J = 12.2 Hz, 1H), 4.75 (m, 2H), 4.38 (m, 1H), 3.81 (s, 6H), 3.74 (s, 3H), 3.44−3.37 (m, 1H), 2.44−2.36 (m, 1H), 2.26 (t, J = 6.7 Hz, 2H), 1.66−1.38 (m, 4H), 1.31−1.13 (m, 2H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 173.9, 158.6, 158.5, 153.0, 152.1, 142.4, 135.7, 134.6, 134.4, 131.5, 131.3, 129.7, 128.6, 128.4, 127.8, 127.6, 127.2, 127.0, 113.1, 73.7, 68.0, 61.4, 61.2, 55.3, 55.2, 51.9, 51.5, 37.0, 33.9, 27.6, 27.4, 24.41; HRMS (ESI, $[M + Na]^+$) calcd for $C_{40}H_{42}N_2O_7SNa$ 717.2610, found 717.2610.

Methyl 5-((3a*S*,4*S*,6a*R*)-1-(Bis(4-methoxyphenyl)(phenyl)-methyl)-3-(diethoxyphosphoryl)-2-oxohexahydro-1*H*-thieno-[3,4-*d*]imidazol-4-yl)pentanoate (14). Compound **6** (120 mg, 0.26 mmol, 1.0 equiv) and diethyl phosphoryl chloride (44 mg, 1.2 equiv) were reacted according to general procedure A to give **14** as a colorless oil (85 mg, 58%): ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.37−7.06 (m, 9H), 6.89−6.75 (m, 4H), 4.78−4.61 (m, 1H), 4.57−4.30 (m, 1H), 4.27−4.07 (m, 4H), 3.79 (s, 6H), 3.67 (s, 3H), 3.41 (m, 1H), 2.56−2.23 (m, 4H), 1.65−1.23 (m, 12H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 174.2, 161.2, 158.7, 158.5, 143.9, 142.8, 135.0, 134.8, 131.7, 131.5, 129.8, 127.7, 127.2, 113.1, 73.5, 65.0 (d, J = 7.0 Hz), 64.1 (d, J = 6.4 Hz), 63.7 (d, J = 7.9 Hz), 63.0 (d, J = 3.0 Hz), 55.4, 52.7, 51.7, 37.0, 34.1, 27.8, 27.5, 24.8, 16.4 (m); ^{31}P NMR (122 MHz, $CDCl_3$) δ −0.93; HRMS (ESI, $[M + Na]^+$) calcd for $C_{36}H_{45}N_2O_8PSNa$ 719.2532, found 719.2533.

Methyl 5-((3a*S*,4*S*,6a*R*)-3-Benzyl-1-(bis(4-methoxyphenyl)-(phenyl)methyl)-2-oxohexahydro-1*H*-thieno[3,4-*d*]imidazol-4-yl)pentanoate (16). Compound **6** (200 mg, 1.0 equiv) and benzyl bromide (61 mg, 1.2 equiv) were reacted according to general procedure A to give **16** as a colorless oil (175 mg, 76%): ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.43−6.82 (m, 14H), 6.82−6.75 (m, 4H), 5.06 (dd, J = 14.6, 5.6 Hz, 1H), 4.31−4.21 (m, 1H), 4.17−4.03 (m, 1H), 3.79 (s, 6H), 3.69 (s, 3H), 3.23−3.13 (m, 1H), 2.44−2.30 (m, 3H), 1.78−1.26 (m, 8H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 173.9, 160.2, 158.4, 143.3, 136.6, 135.5, 135.4, 131.5, 131.3, 129.8, 129.2, 128.7, 128.6, 128.2, 127.9, 127.6, 126.9, 112.8, 72.9, 62.6, 61.8, 55.25, 55.20, 52.4, 51.5, 47.1, 37.4, 33.9, 28.3, 28.0, 24.6; HRMS (ESI, $[M + Na]^+$) calcd for $C_{39}H_{42}N_2O_5SNa$ 673.2712, found 673.2713.

Methyl 5-((3a*S*,4*S*,6a*R*)-3-(2-Chloroacetyl)-2-oxohexahydro-1*H*-thieno[3,4-*d*]imidazol-4-yl)pentanoate (17). Compound **6** (1.0 g, 1.0 equiv) and chloroacetyl chloride (0.16 mL, 1.2 equiv) were reacted according to general procedure A to give **11** as oil (0.71 g, contains 20% DMTrOH, 59%) that was used without further

purification: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.45−7.38 (m, 5H), 7.25 (m, 4H), 6.93 (m, 4H), 4.80 (m, 2H), 4.66−4.32 (m, 2H), 3.79 (s, 6H), 3.66 (s, 3H), 2.55−2.22 (m, 3H), 1.83−1.47 (m, 3H), 1.47−1.07 (m, 3H), 1.01−0.74 (m, 2H); HRMS (ESI, $[M + H]^+$) calcd for $C_{34}H_{38}ClN_2O_6S$ 637.2139, found 637.2133.

Compound **11** (60 mg, 1.0 equiv) and TFA (0.53 mg, 5.0 equiv) were reacted according to general procedure B to give **17** as a colorless oil (0.25 g, 84%): ^{1}H NMR (600 MHz, $CDCl_3$) δ 5.74 (bs, 1H), 5.01−4.91 (m, 1H), 4.80 (d, J = 15.8 Hz, 1H), 4.72 (d, J = 15.8 Hz, 1H), 4.31−4.24 (m, 1H), 3.68 (s, 3H), 3.26−3.19 (m, 1H), 3.14 (d, J = 13.8 Hz, 1H), 3.03 (dd, J = 13.8, 5.4 Hz, 1H), 2.36 (t, J = 7.2 Hz, 2H), 1.80−1.63 (m, 4H), 1.55−1.41 (m, 2H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 173.9, 166.2, 155.9, 62.3, 58.0, 54.9, 51.7, 44.1, 38.0, 33.3, 27.9, 27.8, 24.5; HRMS (ESI, $[M + H]^+$) calcd for $C_{13}H_{19}ClN_2O_4S$ 335.0832, found 335.0822.

Methyl 5-((3a*S*,4*S*,6a*R*)-2-Oxo-3-undec-10-enoylhexahydro-1*H*-thieno[3,4-*d*]imidazol-4-yl)pentanoate (18). Compound **6** (150 mg, 1.0 equiv) and 10-undecenoyl chloride (64 mg, 1.2 equiv) were reacted according to general procedure A to give **12** as a low-melting solid (125 mg, contains 20% DMTrOH, 60%): ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.36−7.06 (m, 9H), 6.86−6.79 (m, 4H), 5.87−5.76 (m, 1H), 5.03−4.90 (m, 2H), 4.43−4.33 (m, 1H), 3.80 (s, 6H), 3.66 (s, 3H), 3.17−3.11 (m, 1H), 2.92−2.76 (m, 1H), 2.44 (t, J = 10.5 Hz, 1H), 2.32 (t, J = 7.5 Hz, 4H), 2.08−1.99 (m, 2H), 1.76−1.53 (m, 6H), 1.42−1.19 (m, 12H); HRMS (ESI, $[M + H]^+$) calcd for $C_{43}H_{54}N_2O_6SNa$ 749.3600, found 749.3607.

Compound **12** (60 mg, 1.0 equiv) and TFA (47 mg, 5.0 equiv) were reacted according to general procedure B to give **18** as a low melting solid (23 mg, >90% pure by ^{1}H NMR, ∼68%): ^{1}H NMR (600 MHz, $CDCl_3$) δ 5.86−5.77 (m, 1H), 5.65 (s, 1H), 4.99 (dd, J = 17.1, 1.7 Hz, 1H), 4.94−4.90 (m, 2H), 4.41−4.33 (m, 1H), 3.57−3.49 (m, 1H), 3.05−2.91 (m, 2H), 2.92−2.74 (m, 2H), 2.34 (t, J = 7.2 Hz, 2H), 2.08−2.00 (m, 2H), 1.73−1.50 (m, 6H), 1.43−1.23 (m, 13H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 177.2, 174.0, 156.5, 139.2, 114.1, 63.7, 55.9, 53.0, 36.8, 35.5, 33.8, 33.6, 29.4, 29.3, 29.1, 28.9, 28.2, 27.8, 24.7, 24.3; HRMS (ESI, $[M + H]^+$) calcd for $C_{21}H_{34}N_2O_4SNa$ 433.2137, found 433.2134.

(3a*R*,6*S*,6a*S*)-Benzyl 6-(5-Methoxy-5-oxopentyl)-2-oxohexahydro-1*H*-thieno[3,4-*d*]imidazole-1-carboxylate (19). Compound **13** (60 mg, 1.0 equiv) and TFA (49 mg, 5.0 equiv) were reacted according to general procedure B to give **19** as white solid (30 mg, 90%): mp 132−135 °C; ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.42 (d, J = 7.2 Hz, 2H), 7.37−7.30 (m, 3H), 6.08 (bs, 1H), 5.35 (d, J = 12.2 Hz, 1H), 5.22 (d, J = 12.2 Hz, 1H), 4.77 (dd, J = 9.2, 6.4 Hz, 1H), 4.39−4.33 (m, 1H), 3.67 (s, 3H), 3.40−3.34 (m, 1H), 3.05−2.98 (m, 1H), 2.78 (dd, J = 12.5, 4.7 Hz, 1H), 2.27−2.19 (m, 2H), 1.60−1.42 (m, 4H), 1.35−1.18 (m, 2H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 173.8, 155.4, 151.7, 135.3, 128.6, 128.5, 128.4, 68.1, 64.4, 55.9, 53.1, 51.5, 36.8, 33.9, 28.2, 27.6, 24.4; HRMS (ESI, $[M + H]^+$) calcd for $C_{19}H_{24}N_2O_5SNa$ 415.1304, found 415.1302.

Methyl 5-((3a*S*,4*S*,6a*R*)-3-(Diethoxyphosphoryl)-2-oxohexahydro-1*H*-thieno[3,4-*d*]imidazol-4-yl)pentanoate (20). Compound **14** (80 mg, 1.0 equiv) and TFA (65 mg, 5.0 equiv) were reacted according to general procedure B to give **20** as a colorless oil (26 mg, 64%): ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.77 (bs, 1H), 4.68−6.60 (m, 1H), 4.50−4.37 (m, 1H), 4.35−4.04 (m, 4H), 3.65 (s, 3H), 3.36−3.25 (m, 1H), 2.95 (dd, J = 12.5, 6.2 Hz, 1H), 2.77 (dd, J = 12.4, 3.8 Hz, 1H), 2.31 (t, J = 7.3 Hz, 2H), 2.10−1.96 (m, 1H), 1.77−1.52 (m, 3H), 1.45−1.28 (m, 8H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 174.2, 159.3, 66.6 (d, J = 3.6 Hz), 64.8 (d, J = 6.2 Hz), 64.1 (d, J = 6.0 Hz), 58.3 (d, J = 7.7 Hz), 54.2, 51.7, 37.0, 34.1, 28.6, 28.0, 24.8, 16.6−16.1 (m).^{31}P NMR (122 MHz, $CDCl_3$) δ −0.30; HRMS (ESI, $[M + H]^+$) calcd for $C_{15}H_{27}N_2O_6PSNa$ 417.1225, found 417.1228.

Methyl 5-((3a*S*,4*S*,6a*R*)-2-Oxo-3-tosylhexahydro-1*H*-thieno-[3,4-*d*]imidazol-4-yl)pentanoate (21). Compound **6** (150 mg, 1.0 equiv) and 4-toluenesulfonyl chloride (60 mg, 1.2 equiv) were reacted according to general procedure A to give **15** as colorless oil (58 mg, contains 20% DMTrOH, 28%): ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.95 (d, J = 8.3 Hz, 2H), 7.38−6.60 (m, 15H), 4.81 (dd, J = 11.1, 6.6 Hz, 1H), 4.36−4.27 (m, 1H), 3.81 (s, 6H), 3.65 (s, 3H), 3.54 (dd, J = 12.0, 6.6 Hz, 1H), 2.55 (s, 3H), 2.49−2.32 (m, 4H), 2.21−1.59 (m, 4H), 1.57−1.30 (m, 2H); HRMS (ESI, $[M + H]^+$) calcd for $C_{39}H_{42}N_2O_7S_2Na$ 737.2331, found 737.2355

Compound **15** (80 mg, 1.0 equiv) and TFA (63 mg, 5.0 equiv) were reacted according to general procedure B to give **21** as a colorless oil (26 mg, >90% pure by ^{1}H NMR, ∼58%): ^{1}H NMR (600 MHz, DMSO-d_6) δ 7.79 (d, J = 8.3 Hz, 2H), 7.74 (s, 1H), 7.39 (d, J = 8.1 Hz, 2H), 4.84 (dd, 1H), 4.34−4.29 (m, 1H), 3.14 (d, J = 5.2 Hz, 1H), 2.85 (dd, J = 12.6, 5.2 Hz, 1H), 2.64 (dd, 1H), 2.37 (s, 3H), 2.20−2.13 (m, 2H), 1.95−0.78 (m, 6H); ^{13}C NMR (151 MHz, DMSO-d_6) δ 174.3, 154.9, 144.4, 135.6, 129.5, 127.7, 65.7, 57.1, 54.4, 36.9, 33.4, 28.5, 28.4, 24.4, 21.0; HRMS (ESI, $[M + H]^+$) calcd for $C_{17}H_{22}N_2O_5S_2Na$ 421.0868, found 421.0866.

Methyl 5-((3a*S*,4*S*,6a*R*)-3-Benzyl-2-oxohexahydro-1*H*-thieno[3,4-*d*]imidazol-4-yl)pentanoate (22). Compound **16** (80 mg, 1.0 equiv) and TFA (70 mg, 5.0 equiv) were reacted according to general procedure B to give **22** as a colorless oil (38 mg, 90%): ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.40−7.24 (m, 5H), 5.55 (bs, 1H), 4.98 (dd, J = 15.2, 9.7 Hz, 1H), 4.39−4.30 (m, 1H), 4.03 (m, 1H), 3.93 (dd, J = 15.2, 6.2 Hz, 1H), 3.68 (s, 3H), 3.16−3.04 (m, 1H), 2.99−2.89 (m, 1H), 2.83−2.75 (m, 1H), 2.40−2.28 (m, 2H), 1.75−1.50 (m, 5H), 1.40−1.30 (m, 1H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 173.9, 162.7, 136.6, 128.8, 128.2, 127.8, 66.2, 65.0, 57.8, 54.4, 51.6, 47.4, 37.9, 33.9, 28.7, 24.6; HRMS (ESI, $[M + H]^+$) calcd for $C_{18}H_{24}N_2O_3SNa$ 371.1405, found 371.1401.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

^{1}H and ^{13}C NMR spectra of all new compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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