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SYNTHESIS OF LSD1 INHIBITOR-PYRROLE-IMIDAZOLE POLYAMIDE CONJUGATES FOR REGION-SPECIFIC ALTERATIONS OF HISTONE MODIFICATION

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Abstract – Synthetic method of LSD1 inhibitor-pyrrole-imidazole polyamide conjugates for region-specific alterations of histone modification is described. A (1*S*,2*R*)-tranylcypromine (PCPA) unit was coupled with an L-lysine part using a nosyl strategy. Conjugation of the inhibitor part with PIP tetramer units was achieved by amide bond formation using PyBOP as a condensation reagent.

INTRODUCTION

Lysine-specific demethylase-1 (LSD1) promotes selective demethylation of mono- and dimethylated lysine 4 residue of histone H3 (H3K4) via a flavin adenine dinucleotide (FAD)-dependent enzymatic oxidation process, which plays an important role in regulating of epigenetic gene expression.[1] LSD1 is upregulated in several human cancer cells.[2] LSD1 inhibitors may, therefore, have high potential as anti-cancer drugs. Suzuki and co-workers recently designed and synthesized a racemic tranylcypromine (PCPA)-L-lysine coupled molecule **1** based on the mechanism of LSD1 inhibition by PCPA (Figure 1). Structure-activity relationship studies resulted in the development of compound **2** (NCD38), which selectively inhibited LSD1 over monoamine oxidases (MAOs).[3] PCPA-lysine coupled molecules cannot inhibit a H3K4 methylation in a genomic region-specific manner, however, due to their non-specific

distribution in cells.

Pyrrole-imidazole polyamides (PIPs) are cell-permeable synthetic molecules that can recognize DNA in a sequence-specific manner by forming antiparallel side-by-side heterodimers in the minor groove of DNA. The specificity is achieved by the rules that an antiparallel Py/Py motif recognizes A·T or T·A base pairs while the Im/Py and Py/Im motifs recognize G·C and C·G base pairs, respectively.[4] A variety of PIP-functional molecule conjugates, such as sequence-specific DNA alkylating agents, have been developed based on this recognition property.[5] We hypothesized that synthesis of PCPA-lysine coupled molecules conjugated with a PIP sequence would allow for genomic region-specific alterations of histone modification. Computational docking studies between **1** and the active site of LSD1 suggested that the phenyl ring of the benzoyl group in **1** is a suitable site for connecting with a PIP sequence through a linker unit. We thus designed and synthesized LSD1 inhibitor-PIP conjugates **3a** and **3b**. Human colon cancer RKO cells were treated with these compounds and ChIP-seq and RNA-seq analyses were performed to investigate the alterations of the epigenomic status. NCD38-activated regions contained several GC-rich DNA sequences and few 5'-$^{A}/_{T}^{A}/_{T}$CG$^{A}/_{T}^{A}/_{T}$-3' sequences. In turn, activated regions induced by **3a** and **3b** significantly included their targeting DNA sequences $^{A}/_{T}^{A}/_{T}^{A}/_{T}^{A}/_{T}^{A}/_{T}^{A}/_{T}$ and $^{A}/_{T}^{A}/_{T}$CG$^{A}/_{T}^{A}/_{T}$, respectively. Thus, activated genomic regions were dramatically switched between NCD38 and LSD1 inhibitor-PIP conjugates.[6] In this report, we describe the details of our synthetic method for LSD1 inhibitor-pyrrole-imidazole polyamide conjugates **3a** and **3b** using a nosyl strategy[7].

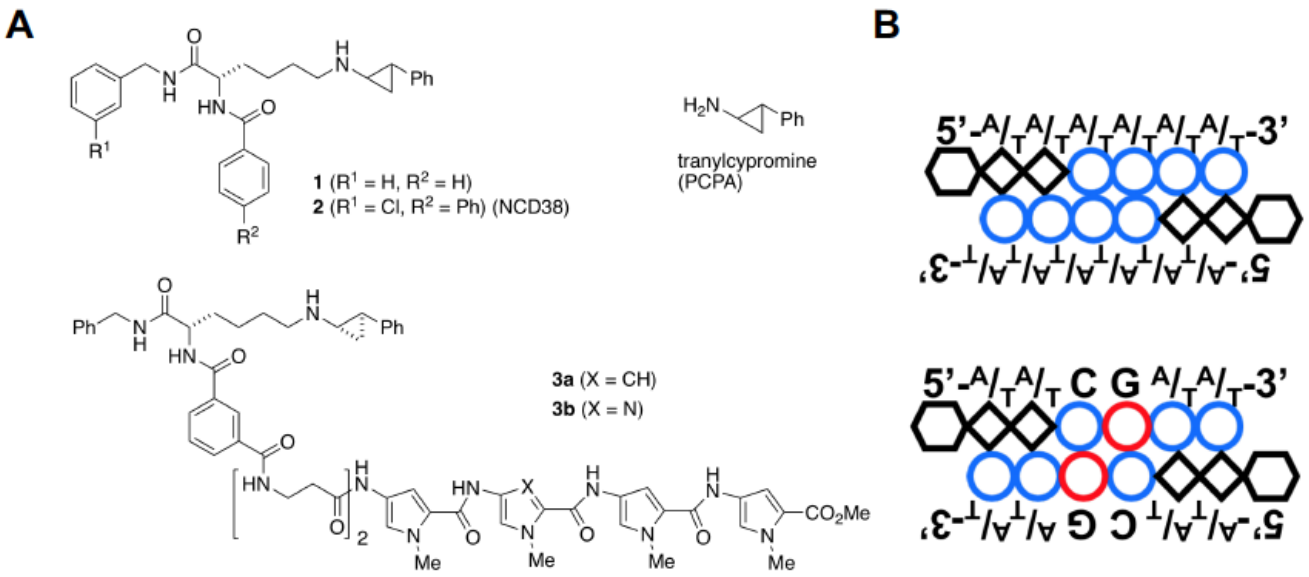
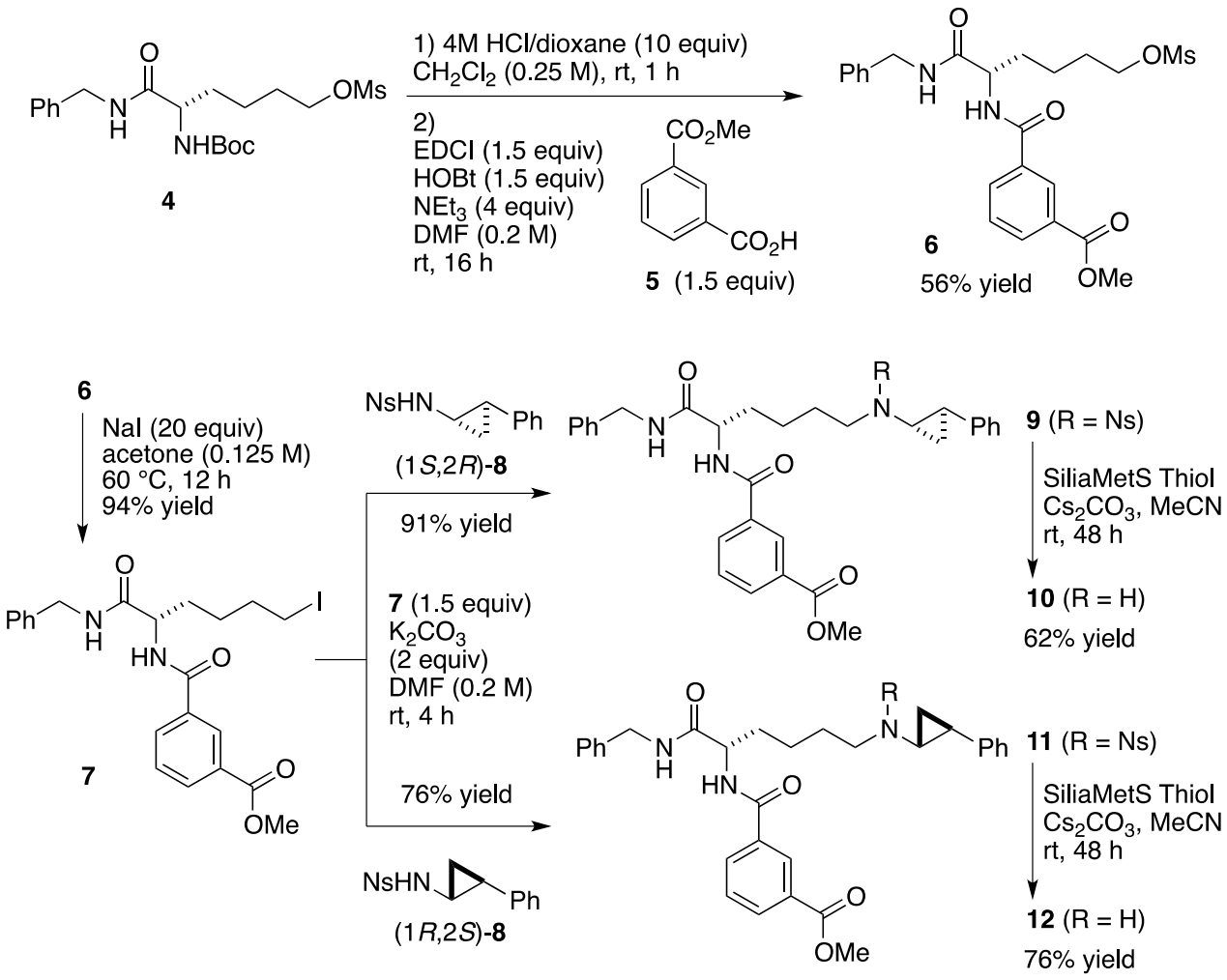


Figure 1. LSD1 Inhibitors and Designed LSD1 Inhibitor-PIP Conjugates. (A) Chemical structure of compounds. (B) Schematic representation for DNA binding of LSD1 Inhibitor-PIP Conjugates. Blue circles, red circles, diamonds and hexagons indicate pyrrole, imidazole, β-alanine and NCD38, respectively.

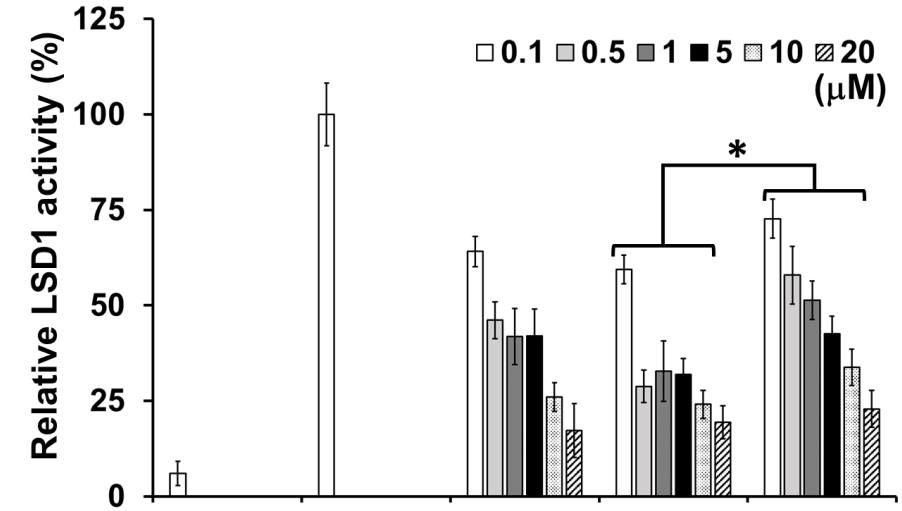
RESULTS AND DISCUSSION

Our synthesis began with known mesylate derivative **4**, which was obtained from L-lysine according to the reported procedure (Scheme 1).[3] After removing the Boc group, the resulting amine was condensed with 3-methoxycarbonylbenzoic acid **5** in the presence of EDCI and HOBt in DMF, producing compound **6** in 56% yield. Mesylate derivative **6** was converted to iodide derivative **7** in 94% yield, which was then reacted with *N*-2-nosyl (Ns) phenylcyclopropylamine **8**. To determine the more potent diastereomer and to facilitate structural analyses of the products using ^{1}H and ^{13}C NMR, both enantiomers (1*S*,2*R*)-**8** and (1*R*,2*S*)-**8** were utilized as nucleophiles.[8] The coupling reaction was performed using 1.5 equiv of **7** and 2 equiv of K_2CO_3 in DMF to give compounds **9** and **11** in 91% yield and 76% yield, respectively.[9] Although compound **9** could also be synthesized from mesylate derivative **6**, the synthetic efficiency was less satisfactory. The Ns group in **9** and **11** could be removed using a silica-supported thiol (SiliaMetS® Thiol) and compounds **10** and **12** were obtained in 62% yield and 76% yield, respectively.



Scheme 1. Synthesis of Inhibitor Parts

The inhibitory activity of LSD1 was evaluated using NCD38 (**2**) and compounds **10** and **12** (Figure 2). LSD1 activities were estimated as 9.6%, 19.4% and 22.9% in the presence of 20 μM of **2**, **10**, and **12**, respectively. At 1 μM of **2**, **10**, and **12**, LSD1 activities were calculated to be 26.2%, 32.7% and 51.4%, respectively. These findings clearly indicated that both **10** and **12** inhibited LSD1 activities as well as the parental **2**. In addition, compound **10** had significantly stronger LSD1 inhibitory activity than compound **12** at 1 μM ($P = 0.029$), 5 μM ($P = 0.036$) and 10 μM ($P = 0.046$), while there was no significant difference at 20 μM ($P = 0.38$). These results indicated that LSD1 inhibitor-PIP conjugates having the same stereochemical relationship as **10** are a more reasonable candidate for this study, and further studies were therefore preformed using compound **9** as a key synthetic intermediate.

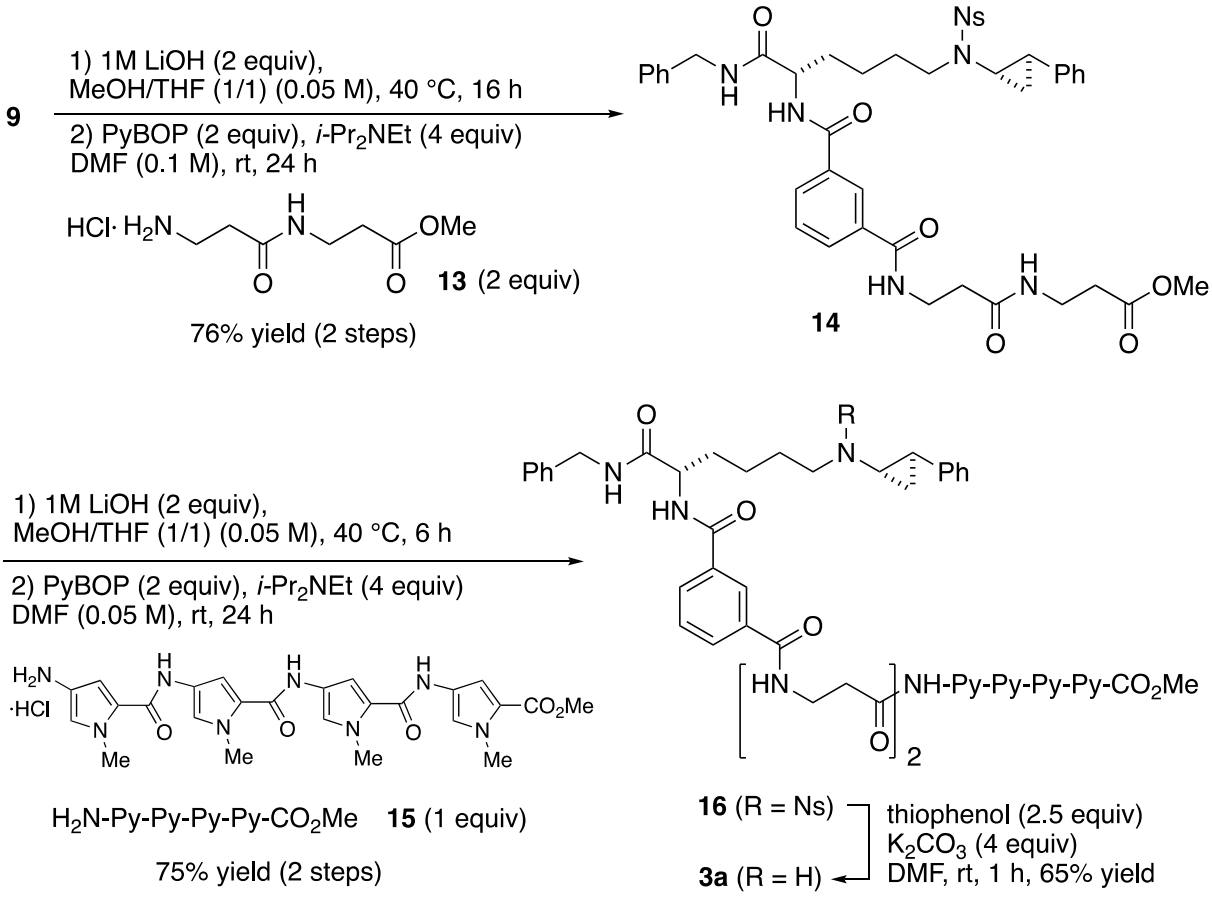


LSD1	–	+	+	+	+
DMSO	1.0%	1.0%	1.0%	1.0%	1.0%
Compound	–	–	**2**	**10**	**12**
Buffer	+	+	+	+	+

Figure 2. Inhibition of LSD1 activity by the compounds *in vitro*. LSD1 activities were assessed using an LSD1 fluorometric drug discovery kit. Relative LSD1 activities are shown as bars filled with white, light grey, grey, black, dots and slashed lines in presence 0.1, 0.5, 1, 5, 10 and 20 μM of the compounds, respectively. LSD1 activities were estimated by comparison with 1% DMSO control as 100%. Data represent the mean of three independent replicates ± SD. * $P < 0.05$.

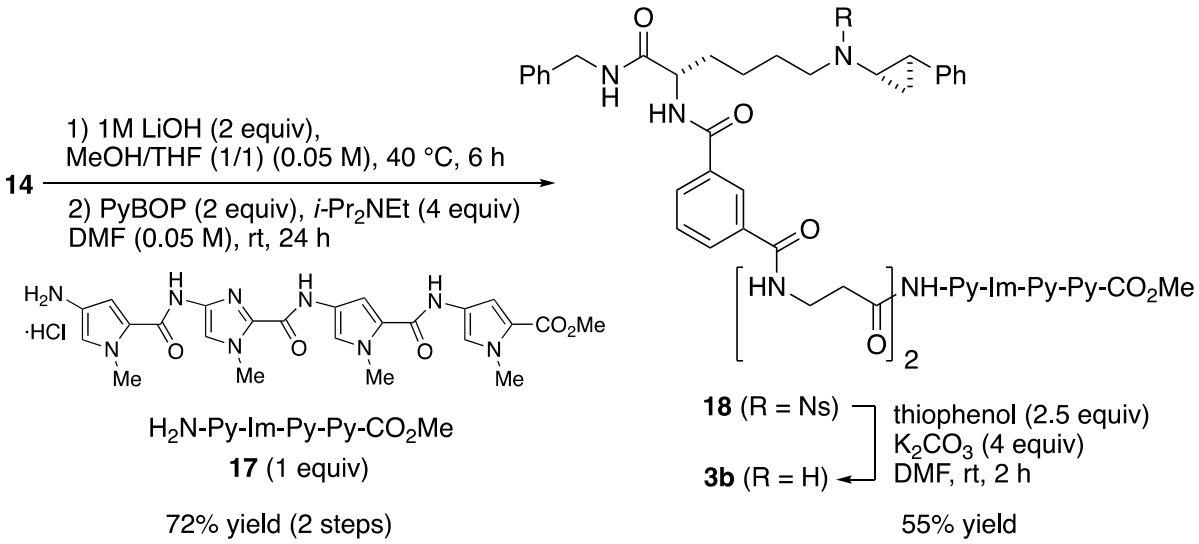
After hydrolysis of the ester part in compound **9** under basic conditions, the obtained carboxylic acid derivative was coupled with a β-alanine dimer **13** to introduce a spacer unit between the LSD1 inhibitor unit and PIP unit. When the reaction was performed using 2 equiv of benzotriazol-1-yl-oxytripyrrolidinophosphonium hexafluorophosphate (PyBOP), 4 equiv of *i*-Pr_2NEt and 2 equiv of **13** in DMF at room temperature, compound **14** was obtained in 76% overall yield from **9**. Hydrolysis of the methyl ester in **14** was performed under basic conditions and then a coupling reaction with pyrrole tetramer (NH_2-Py-Py-Py-Py-CO_2Me·HCl) **15**[10] was examined. The reaction was performed

using 1 equiv of **15** and 2 equiv of PyBOP in DMF at room temperature and compound **16** was produced in 75% yield in 2 steps. Removal of the Ns group using thiophenol and K_2CO_3 in DMF afforded compound **3a** in 65% yield.[11]



Scheme 2. Synthesis of LSD1 Inhibitor-Pyrrole Tetramer Conjugate **3a**

A coupling reaction with an imidazole-containing tetramer, NH_2-Py-Im-Py-Py-CO_2Me·HCl **17**, was also examined under the same reaction conditions as those for the reaction using pyrrole tetramer **15**. After hydrolysis of **14**, the coupling reaction was performed using 2 equiv of PyBOP and **17** (1 equiv) in DMF at room temperature, affording compound **18** in 72% yield in 2 steps from **14**. Removal of the Ns group using thiophenol and K_2CO_3 in DMF afforded compound **3b** in 55% yield. [10]



Scheme 3. Synthesis of LSD1 Inhibitor-Imidazole Containing PIP Tetramer Conjugate **3b**

Finally, the IC_{50} values of compounds were also determined (Table 1). IC_{50} values of LSD1 Inhibitor-PIP conjugates **3a** and **3b** were estimated as 0.808 and 1.02 μM whereas parental inhibitors **2**, **10** and **12** inhibited LSD1 with an IC_{50} value of 0.147, 0.305 and 1.29 μM, respectively. It was implied that LSD1-inhibiting activities of **3a** and **3b** were slightly reduced compared with that of **10**. However, it was not critical reduction because we treated human colon cancer cells at 2 μM of the conjugate compounds, previously.

Table 1. IC_{50} values of compounds against LSD1[1]

Compounds	IC50 (μM)
2	0.147 ± 0.085
10	0.305 ± 0.10
12	1.29 ± 0.15
3a	0.808 ± 0.097
3b	1.02 ± 0.072

[1] Data represent the mean of three independent replicates ± SD.

CONCLUSION

We successfully synthesized LSD1 inhibitor-pyrrole-imidazole polyamide conjugates for region-specific alterations of histone modification. A (1*S*,2*R*)-Tranylcypromine (PCPA) unit was coupled with an L-lysine part using a nosyl strategy. Conjugation of the inhibitor part with PIP tetramer units was achieved by amide bond formation using PyBOP as a condensation reagent.

EXPERIMENTAL

General: Infrared (IR) spectra were recorded on a Fourier transform infrared spectrophotometer, equipped with ATR. NMR spectra were recorded with a 400 MHz spectrometer. Chemical shifts in $CDCl_3$ were reported downfield from TMS (= 0 ppm) for ^1H NMR. For ^{13}C NMR, chemical shifts were reported in the scale relative to the solvent signal [$CHCl_3$ (77.0 ppm)] as an internal reference. Positive-ion mass spectra were recorded by electrospray ionization (ESI-TOF). Column chromatography was performed with silica gel 60N (spherical, neutral, 40–60 μm mesh) (Kanto Chemical) or CHROMATOREX NH-DM1020 (Fuji Silysia Chemical). Reactions were carried out in dry solvent. Other reagents were purified by the usual methods.

Compound 6: To a stirred solution of **4** (809 mg, 1.95 mmol) in CH_2Cl_2 (67.8 mL) at 0 °C was added 4M HCl in dioxane (4.9 mL) and the resulting mixture was stirred at room temperature. After 1 h, organic solvent was removed to give the corresponding amine hydrochloride in a quantitative yield.

To a stirred solution of 3-(methoxycarbonyl)benzoic acid **5** (135.1 mg, 0.75 mmol), 1-hydroxybenzotriazole (HOBt) (101.3 mg, 0.75 mmol), *N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride (EDCI) (143.8 mg, 0.75 mmol), and triethylamine (0.28 mL, 2 mmol) in DMF (2.5 mL) at 0 °C was added the obtained amine hydrochloride (175.4 mg, 0.5 mmol). After 17 h, the reaction was quenched with sat. aq. NH_4Cl solution and diluted with AcOEt. The resulting mixture was washed with 1M aq. HCl solution, sat. aq. $NaHCO_3$ solution, and brine, and then dried over Na_2SO_4. After concentration under reduced pressure, the residue was recrystallized from AcOEt-hexane to give compound **6** as white solid (135 mg, 56% yield). $[\alpha]^{20}_D$ –9.8 (*c* 0.23, $CHCl_3$); Mp 142–143 °C; ^1H NMR (400 MHz, $CDCl_3$) δ 1.47–1.60 (m, 2H), 1.70–2.08 (m, 4H), 2.95 (s, 3H), 3.91 (s, 3H), 4.13–4.25 (m, 2H), 4.36 (dd, *J* = 5.6 Hz, 14.8 Hz, 1H), 4.46 (dd, *J* = 5.6 Hz, 14.8 Hz, 1H), 4.70–4.82 (m, 1H), 7.10–7.37 (m, 7H), 7.43–7.48 (m, 1H), 7.93 (d, *J* = 8.0 Hz, 1H), 8.14 (d, *J* = 8.0 Hz, 1H), 8.38 (d, *J* = 1.2 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 21.5, 28.6, 31.9, 37.3, 43.5, 52.3, 53.4, 69.6, 127.5, 127.6 (2C), 127.9, 128.6 (2C), 128.8, 130.5, 131.7, 132.7, 133.9, 137.8, 166.1, 166.5, 171.3; IR (ATR) ν 1721, 1631, 1532, 1347, 1252, 1163, 956, 821, 694 cm^{-1}; (+)-ESI-HRMS. Calcd for $C_{23}H_{28}N_2NaO_7S^+$ ($M+Na^+$): 499.1509. Found: 499.1529.

Compound 7: A suspension of compound **6** (95.3 mg, 0.2 mmol) and NaI (600 mg, 4.00 mmol) in acetone (4 mL) was refluxed for 12 h. After cooling down to room temperature, the reaction was diluted with $CHCl_3$. The resulting mixture was washed with brine and then dried over Na_2SO_4. After concentration under reduced pressure, the residue was recrystallized from AcOEt-hexane to give compound **7** as white solid (95 mg, 94% yield). $[\alpha]^{20}_D$ –9.5 (*c* 0.14, $CHCl_3$); Mp 153–154 °C; ^1H NMR

(400 MHz, $CDCl_3$) δ 1.49–1.57 (m, 2H), 1.77–1.91 (m, 3H), 1.97–2.06 (m, 1H), 3.17 (t, *J* = 6.8 Hz, 2H), 3.94 (s, 3H), 4.41 (dd, *J* = 5.6 Hz, 14.4 Hz, 1H), 4.51 (dd, *J* = 6.0 Hz, 14.4 Hz, 1H), 4.68–4.74 (m, 1H), 6.71 (dd, *J* = 5.6 Hz, 6.0 Hz, 1H), 7.01 (d, *J* = 8.0 Hz, 1H), 7.20–7.33 (m, 5H), 7.51 (dd, *J* = 8.0 Hz, 8.0 Hz, 1H), 7.97 (dd, *J* = 1.6 Hz, 8.0 Hz, 1H), 8.18 (d, *J* = 8.0 Hz, 1H), 8.40 (d, *J* = 1.6 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 6.4, 26.5, 31.6, 32.9, 43.7, 52.4, 53.5, 127.6, 127.7 (2C), 127.9, 128.7 (2C), 128.9, 130.6, 131.7, 132.8, 134.0, 137.7, 166.2, 166.5, 171.3; IR (ATR) ν 3292, 2927, 1722, 1634, 1538, 1438, 1299, 1260, 729, 697 cm^{-1}; (+)-ESI-HRMS. Calcd for $C_{22}H_{25}IN_2NaO_4^+$ ($M+Na^+$): 531.0751. Found: 531.0756.

Compound (1*S*,2*R*)-8: (1*S*,2*R*)-2-Phenylcyclopropane-1-amine hydrochloride was prepared using the reported procedure. To a stirred solution of (1*S*,2*R*)-2-phenylcyclopropane-1-amine hydrochloride (225 mg, 1.33 mmol) and NEt_3 (0.56 mL, 4.00 mmol) in CH_2Cl_2 (13.3 mL) at room temperature was added *o*-nosyl chloride (354.6 mg, 1.60 mmol). The reaction mixture was kept stirring for 4 h at room temperature. To consume the unreacted 2-nosyl chloride, *N*,*N*-dimethylpropane-1,3-diamine (0.165 mL, 1.33 mmol) was added and the reaction was stirred for 1 h. After dilution with AcOEt, the resulting mixture was washed with 1M aq. HCl, sat. aq. $NaHCO_3$ and brine, and then dried over Na_2SO_4. After concentration under reduced pressure, the residue was purified by silica gel column chromatography (SiO_2, *n*-hexane/AcOEt = 3/1) to give (1*S*,2*R*)-**8** (309 mg, 73% yield). Stereoscopic data was identical to the reported data.

Compound 9: To a stirred suspension of compound (1*S*,2*R*)-**8** (64 mg, 0.2 mmol) and K_2CO_3 (55.3 mg, 0.4 mmol) in DMF (1 mL) at room temperature was added **7** (152.5 mg, 0.3 mmol), and the resulting mixture was stirred for 24 h at the same temperature. The reaction was diluted with AcOEt/*n*-hexane (4/1) and the mixture was washed with water (x2) and brine, and then dried over Na_2SO_4. After concentration in vacuo, the obtained residue was purified by silica gel column chromatography (SiO_2, *n*-hexane/AcOEt = 1/1 to 1/2) to give compound **9** (190.3 mg, 91% yield) as white amorphous.
$[\alpha]^{20}_D$ 13.9 (*c* 2.64, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.10–2.15 (m, 9H), 2.60–2.66 (m, 1H), 3.30–3.40 (m, 1H), 3.43–3.52 (m, 1H), 3.91 (s, 3H), 4.47 (d, *J* = 5.6 Hz, 1H), 4.60–4.68 (m, 1H), 6.56 (broad peak, 1H), 6.92–6.97 (m, 2H), 7.00 (d, *J* = 8.0 Hz, 1H), 7.16–7.33 (m, 8H), 7.50–7.72 (m, 4H), 7.97 (dd, *J* = 1.6 Hz, 8.0 Hz, 1H), 8.04 (d, *J* = 8.0 Hz, 1H), 8.16–8.20 (m, 1H), 8.42–8.45 (m, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 16.0, 22.4, 24.9, 27.8, 31.9, 38.4, 43.5, 49.7, 52.2, 53.6, 124.0, 126.0 (2C), 126.4, 127.4, 127.6 (2C), 128.0, 128.4 (2C), 128.6 (2C), 128.7, 130.5, 131.4, 131.6, 131.7, 132.6, 132.9, 133.7, 134.0, 137.9, 139.2, 147.9, 166.2, 166.5, 171.4; IR (ATR) ν 3285, 1722, 1636, 1542, 1439, 1370, 1259, 1159, 747, 698 cm^{-1}; (+)-ESI-HRMS. Calcd for $C_{37}H_{38}N_4NaO_8S^+$ ($M+Na^+$): 721.2303. Found: 721.2325.

Compound 10: A suspension of compound **9** (33.0 mg, 0.0472 mmol), Cs_2CO_3 (61.6 mg, 0.189 mmol), and SiliaMetS Thiol (1.41 mmol/g) (134 mg, 0.189 mmol) in MeCN (1.2 mL) were stirred for 48 h at room temperature. The yellow suspension was directly purified by silica gel column chromatography (SiO_2, $CHCl_3$/MeOH = 20/1 to 10/1) to give compound **10** (14.9 mg, 62% yield) as colorless oil. $[\alpha]^{20}_D$ 16.2 (*c* 2.4, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.90–1.06 (m, 2H), 1.40–1.60 (m, 4H), 1.80–2.03 (m, 4H), 2.28–2.32 (m, 1H), 2.69–2.76 (m, 2H), 3.94 (s, 3H), 4.41 (dd, *J* = 5.6 Hz, 14.8 Hz, 1H), 4.50 (dd, *J* = 6.0 Hz, 15.2 Hz, 1H), 4.66–4.72 (m, 1H), 6.87 (dd, *J* = 5.6 Hz, 6.0 Hz, 1H), 7.00–7.03 (m, 2H), 7.08 (d, *J* = 7.2 Hz, 1H), 7.11–7.15 (m, 1H), 7.21–7.32 (m, 6H), 7.48–7.54 (m, 2H), 7.98–8.00 (m, 1H), 8.16–8.19 (m, 1H), 8.40–8.42 (m, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 16.8, 23.2, 24.8, 29.4, 32.7, 41.4, 43.6, 49.0, 52.4, 53.6, 125.5, 125.8 (2C), 127.6, 127.7 (2C), 127.9, 128.2 (2C), 128.7 (2C), 128.9, 130.6, 131.7, 132.7, 134.1, 137.8, 142.1, 166.2, 166.3, 171.4; IR (ATR) ν 3282, 2925, 1725, 1635, 1538, 1438, 1258, 729, 696 cm^{-1}; (+)-ESI-HRMS. Calcd for $C_{31}H_{36}N_3O_4^+$ ($M+H^+$): 514.2700. Found: 514.2703.

Compound 11: This compound was prepared using the synthetic procedure for compound **9** (76% yield). White amorphous; $[\alpha]^{20}_D$ –18.6 (*c* 4.04, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.15–2.10 (m, 9H), 2.60–2.65 (m, 1H), 3.30–3.38 (m, 1H), 3.46–3.54 (m, 1H), 3.90 (s, 3H), 4.47 (d, *J* = 6.0 Hz, 1H), 4.60–4.66 (m, 1H), 6.52 (t, *J* = 6.0 Hz, 1H), 6.93 (d, *J* = 7.2 Hz, 2H), 7.00 (d, *J* = 8.0 Hz, 1H), 7.16–7.30 (m, 8H), 7.50–7.72 (m, 4H), 7.97 (d, *J* = 8.4 Hz, 1H), 8.04 (d, *J* = 7.6 Hz, 1H), 8.18 (d, *J* = 7.6 Hz, 1H), 8.45 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 16.0, 22.3, 24.9, 27.7, 31.7, 38.3, 43.6, 49.5, 52.3, 53.6, 124.1, 126.0 (2C), 126.5, 127.4, 127.6 (2C), 128.0, 128.5 (2C), 128.7 (2C), 128.8, 130.5, 131.5, 131.6, 131.7, 132.7, 133.0, 133.7, 134.0, 137.9, 139.2, 147.9, 166.2, 166.5, 171.3; IR (ATR) ν 1724, 1637, 1543, 1261, 1160, 730, 696, 620, 608 cm^{-1}; (+)-ESI-HRMS. Calcd for $C_{37}H_{38}N_4NaO_8S^+$ ($M+Na^+$): 721.2303. Found: 721.2308.

Compound 12: This compound was prepared using the synthetic procedure for compound **10** (76% yield). Colorless oil; $[\alpha]^{20}_D$ –18.9 (*c* 3.6, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.97–1.01 (m, 1H), 1.12–1.17 (m, 1H), 1.40–1.49 (m, 2H), 1.60–1.68 (m, 2H), 1.80–2.03 (m, 3H), 2.30–2.36 (m, 1H), 2.75–2.84 (m, 2H), 3.90 (s, 3H), 4.39 (dd, *J* = 6.8 Hz, 14.8 Hz, 1H), 4.48 (dd, *J* = 6.8 Hz, 14.8 Hz, 1H), 4.70–4.76 (m, 1H), 7.00–7.03 (m, 2H), 7.15 (dd, *J* = 6.8 Hz, 6.8 Hz, 1H), 7.20–7.36 (m, 8H), 7.46–7.50 (m, 2H), 7.96–7.99 (m, 1H), 8.16 (d, *J* = 8.0 Hz, 1H), 8.42 (d, *J* = 2.0 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 16.0, 23.0, 24.1, 28.4, 32.5, 40.8, 43.6, 48.7, 52.4, 53.4, 125.9 (2C), 127.5, 127.7 (2C), 128.0, 128.3 (2C), 128.7 (2C), 128.8, 130.5, 131.7, 132.7, 134.0, 137.8, 141.4, 166.2, 166.5, 171.6; IR (ATR) ν 3297, 2931, 1725, 1637, 1541, 1439, 1264, 732, 697 cm^{-1}; (+)-ESI-HRMS. Calcd for $C_{31}H_{36}N_3O_4^+$ ($M+H^+$): 514.2700. Found: 514.2721.

Compound 14: A solution of **9** (49.0 mg, 0.07 mmol) and 1M LiOH (0.14 mL, 0.14 mmol) in MeOH/THF (1/1) (1.4 mL) was stirred at 40 °C. After 16 h, the resulting mixture was acidified with 1M aq. HCl and the mixture was extracted with $CHCl_3$ and then organic layer was dried over Na_2SO_4. After concentration in vacuo, almost pure carboxylic acid was obtained in quantitative yield. The obtained residue was used for the next reaction without purification.

To a stirred solution of the crude carboxylic acid derivative, benzotriazol-1-yl-oxytripyrrolidinophosphonium hexafluorophosphate (PyBOP) (72.8 mg, 0.14 mmol), and *i*-Pr_2NEt (49 μL mL, 0.28 mmol) in DMF (0.7 mL) at 0 °C was added β-alanine dimer **13** (29.5 mg, 0.14 mmol), and the resulting mixture was stirred for 40 h at room temperature. The reaction was diluted with AcOEt/*n*-hexane (4/1) and the resulting mixture was washed with 1M aq. HCl solution, sat. aq. $NaHCO_3$ solution, water, and brine, and then dried over Na_2SO_4. After concentration under reduced pressure, the obtained residue was purified by silica gel column chromatography (CHROMATOREX NH-DM1020, $CHCl_3$/MeOH = 80/1) to give compound **14** (44.7 mg, 76% yield) as colorless oil. $[\alpha]^{24}_D$ –49.2 (*c* 1.6, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.10–2.11 (m, 9H), 2.43–2.55 (m, 4H), 2.59–2.65 (m, 1H), 3.24–3.38 (m, 1H), 3.40–3.52 (m, 3H), 3.61 (s, 3H), 3.60–3.70 (m, 2H), 4.40 (dd, *J* = 5.6 Hz, 14.8 Hz, 1H), 4.46 (dd, *J* = 6.0 Hz, 14.8 Hz, 1H), 4.63–4.70 (m, 1H), 6.46 (dd, *J* = 5.6 Hz, 6.0 Hz, 1H), 6.88–6.95 (m, 3H), 7.14–7.70 (m, 14H), 7.87–8.00 (m, 3H), 8.22 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 16.0, 22.6, 24.9, 27.9, 31.7, 33.8, 35.0, 35.6, 36.3, 38.3, 43.4, 49.8, 51.8, 53.8, 124.1, 125.4, 126.0 (2C), 126.5, 127.3, 127.5 (2C), 128.4 (2C), 128.6 (2C), 128.8, 130.4, 130.6, 131.5, 131.5, 132.8, 133.7, 133.8, 134.6, 138.0, 139.2, 147.8, 166.9, 166.9, 171.7, 171.9, 172.9; IR (ATR) ν 3316, 1737, 1644, 1542, 1438, 1371, 770, 699, 671, 633 cm^{-1}; (+)-ESI-HRMS. Calcd for $C_{43}H_{48}N_6NaO_{10}S^+$ ($M+Na^+$): 863.3050. Found: 863.3048.

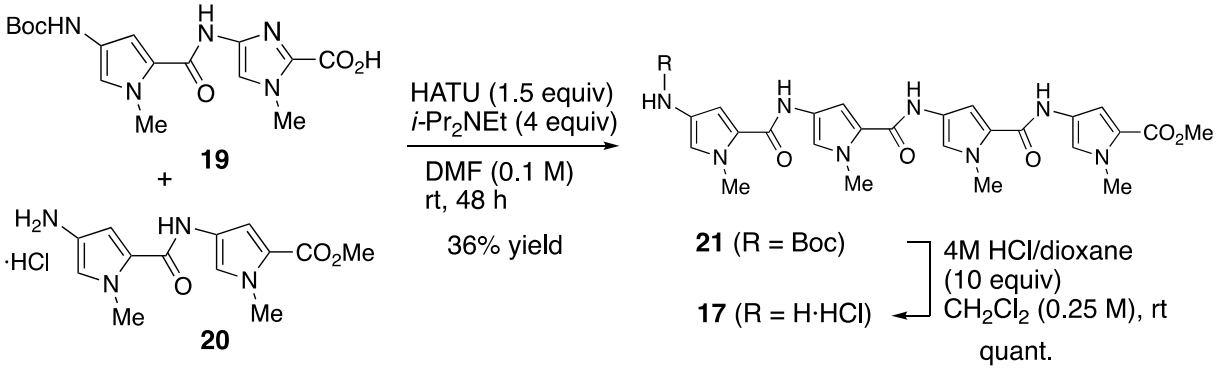
Compound 16: A solution of **14** (44.7 mg, 0.053 mmol) and 1M LiOH (0.106 mL, 0.106 mmol) in MeOH/THF (1/1) (1.06 mL) was stirred at 40 °C. After 16 h, the resulting mixture was acidified with 1M aq. HCl and the mixture was extracted with $CHCl_3$ and then organic layer was dried over Na_2SO_4. After concentration in vacuo, almost pure carboxylic acid was obtained in quantitative yield. The obtained residue was used for the next reaction without purification.

A solution of obtained carboxylic acid derivative (22.1 mg, 0.0267 mmol), **15** (14.9 mg, 0.0267 mmol), benzotriazol-1-yl-oxytripyrrolidinophosphonium hexafluorophosphate (PyBOP) (27.8 mg, 0.0534 mmol), and *i*-Pr_2NEt (0.019 mL, 0.107 mmol) in DMF (0.54 mL) was stirred for 24 h at room temperature. The reaction was diluted with $CHCl_3$/*i*-PrOH (1/1) and the resulting mixture was washed with 1M aq. HCl solution, sat. aq. $NaHCO_3$ solution, and water, and then dried over Na_2SO_4. After concentration under reduced pressure, the obtained residue was purified by silica gel column chromatography (CHROMATOREX NH-DM1020, $CHCl_3$/MeOH = 60/1 to 30/1) to give compound **16** (26.6 mg, 75%

yield) as pale brown solid. Mp 141–142 °C; $[\alpha]^{25}_D$ –29.8 (*c* 0.88, $CHCl_3$); IR (ATR) ν 3292, 2924, 1645, 1543, 1437, 1404, 1252, 1108, 748, 608 cm^{-1}; (+)-ESI-HRMS. Calcd for $C_{67}H_{72}N_{14}NaO_{14}S^+$ ($M+Na^+$): 1351.4971. Found: 1351.4917. Although all signals were detected as broad peaks in the ^1H NMR analysis ($CDCl_3$) probably due to the high molecular weight, an analyzable chart was obtained. See supporting information for the ^1H NMR chart.

Compound 3a: To a stirred solution of **16** (11.0 mg, 0.0083 mmol), K_2CO_3 (4.6 mg, 0.033 μmol) in DMF (0.3 mL) was added thiophenol (2.1 μL, 0.021 mmol) and the resulting solution was stirred for 1 h at room temperature. The yellow solution was directly purified by silica gel column chromatography (SiO_2, $CHCl_3$/MeOH = 20/1 to 10/1) to give compound **3a** (6.5 mg, 65% yield) as colorless solid. $[\alpha]^{20}_D$ –41.4 (*c* 0.48, $CHCl_3$); IR (ATR) ν 3295, 2924, 2853, 1644, 1540, 1436, 1404, 1254, 1205, 1108, 754, 699, 606 cm^{-1}; (+)-ESI-HRMS. Calcd for $C_{61}H_{70}N_{13}O_{10}^+$ ($M+H^+$): 1144.5363. Found: 1144.5350. Although all signals were detected as broad peaks in the ^1H NMR analysis ($CDCl_3$) probably due to the high molecular weight, an analyzable chart was obtained. See supporting information for the ^1H NMR chart.

Synthesis of Compound 17



Compound 21: To a stirred solution of **19**[12] (181.7 mg, 0.5 mmol), **20**[13] (187.7 mg, 0.6 mmol), and HATU (380.2 mg, 1 mmol) in DMF (5 mL) at room temperature was added *i*-Pr_2NEt (0.35 mL, 2 mmol), and the resulting mixture was stirred for 48 h at the same temperature. The reaction was quenched by the addition of 1M aq. $KHSO_4$ solution and the mixture was extracted with CH_2Cl_2. The organic layer was washed with brine and dried over Na_2SO_4. After concentration under reduced pressure, the obtained residue was purified by silica gel column chromatography (SiO_2, *n*-hexane$_3$/AcOEt = 1/3) to give compound **21** (111.9 mg, 36% yield) as yellow amorphous. Mp 155–156 °C; ^1H NMR (400 MHz, $CDCl_3$) δ 1.26 (s, 9H), 3.82 (s, 3H), 3.91 (s, 3H), 3.93 (s, 3H), 3.97 (s, 3H), 4.07 (s, 3H), 6.35 (s, 1H), 6.72 (s, 1H), 6.78 (d, *J* = 2.0 Hz, 1H), 6.84 (s, 1H), 7.00 (s, 1H), 7.43 (d, *J* = 2.0 Hz, 1H), 7.48 (s, 1H), 7.67 (s, 1H),

8.41 (s, 1H), 8.61 (d, *J* = 0.8 Hz, 1H), 8.98 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 28.4 (3C), 29.7, 35.9, 36.7, 36.8, 51.1, 80.3, 103.6, 104.3, 108.4, 114.2, 119.3, 119.5, 119.8, 120.8, 121.0, 121.9, 122.1, 123.4, 133.5, 135.7, 150.1, 153.5, 155.5, 158.6, 158.9, 161.6; IR (ATR) ν 3318, 2926, 1698, 1658, 1587, 1539, 1440, 1404, 1367, 1250, 1205, 1162, 1110, 772, 733 cm^{-1}; (+)-ESI-HRMS. Calcd for $C_{29}H_{35}N_9NaO_7^+$ (M+Na$^+$): 644.2557. Found: 644.2553.

Compound 17: To a stirred solution of **21** (186.5 mg, 0.3 mmol) in CH_2Cl_2 (6.2 mL) at 0 °C was added 4M HCl in dioxane (0.75 mL) and the resulting mixture was stirred at room temperature. After 19 h, organic solvent was removed and the residue was washed with Et_2O to give **17** (168 mg, quant.) as yellow solid, which was used for the next reaction without further purification. Mp 188–189 °C; ^{1}H NMR (400 MHz, CD_3OD) δ 3.79 (s, 3H), 3.89 (s, 3H), 3.92 (s, 3H), 3.98 (s, 3H), 4.09 (s, 3H), 6.93 (d, *J* = 2.4 Hz, 1H), 6.98 (s, 1H), 7.02 (d, *J* = 2.4 Hz, 1H), 7.13 (d, *J* = 2.4 Hz, 1H), 7.36 (d, *J* = 2 Hz, 1H), 7.49–7.53 (m, 2H), 8.43 (dd, *J* = 1.2 Hz, 8.0 Hz, 1H), 8.73 (dd, *J* = 1.2 Hz, 4.0 Hz, 1H). (Protons in NH_2 and one amide NH proton could not be detected in CD_3OD); IR (ATR) ν 3364, 1671, 1633, 1554, 1435, 1404, 1258, 1206, 1110, 769, 638, 611 cm^{-1}; (+)-ESI-HRMS. Calcd for $C_{24}H_{27}N_9O_5^+$ (M+H$^+$): 522.2213. Found: 522.2238.

Compound 18: A solution of **14** (44.7 mg, 0.053 mmol) and 1M LiOH (0.106 mL, 0.106 mmol) in MeOH/THF (1/1) (1.06 mL) was stirred at 40 °C. After 16 h, the resulting mixture was acidified with 1M aq. HCl and the mixture was extracted with $CHCl_3$ and then organic layer was dried over Na_2SO_4. After concentration in vacuo, almost pure carboxylic acid was obtained in quantitative yield. The obtained residue was used for the next reaction without purification.

A solution of obtained carboxylic acid derivative (22.1 mg, 0.0267 mmol), **17** (14.9 mg, 0.0267 mmol), benzotriazol-1-yl-oxytripyrrolidinophosphonium hexafluorophosphate (PyBOP) (27.8 mg, 0.0534 mmol), and *i*-Pr_2NEt (0.019 mL, 0.107 mmol) in DMF (0.54 mL) was stirred for 24 h at room temperature. The reaction was diluted with $CHCl_3$/*i*-PrOH (1/1) and the resulting mixture was washed with 1M aq. HCl solution, sat. aq. $NaHCO_3$ solution, and water, and then dried over Na_2SO_4. After concentration under reduced pressure, the obtained residue was purified by silica gel column chromatography (CHROMATOREX NH-DM1020, $CHCl_3$/MeOH = 60/1 to 30/1) to give compound **18** (25.7 mg, 72% yield) as colorless oil. $[\alpha]^{25}_D$ –35.7 (*c* 0.54, $CHCl_3$); IR (ATR) ν 3296, 2925, 1647, 1542, 1441, 1405, 1255, 1117, 670, 626 cm^{-1}; (+)-ESI-HRMS. Calcd for $C_{67}H_{72}N_{14}NaO_{14}S^+$ (M+Na$^+$): 1352.4923. Found: 1352.4918. Although all signals were detected as broad peaks in the ^{1}H NMR analysis ($CDCl_3$) probably due to the high molecular weight, an analyzable chart was obtained. See supporting information for the ^{1}H NMR chart.

Compound 3b: To a stirred solution of **18** (12.0 mg, 0.00903 mmol), K_2CO_3 (5.0 mg, 0.0361 mmol) in DMF (0.3 mL) was added thiophenol (2.1 μL, 0.0226 mmol) and the resulting solution was stirred for 2 h at room temperature. The yellow solution was directly purified by silica gel column chromatography (SiO_2, $CHCl_3$/MeOH = 20/1 to 10/1) to give compound **3b** (5.7 mg, 55% yield) as colorless solid. $[\alpha]^{20}_D$ 32.1 (*c* 0.57, $CHCl_3$); IR (ATR) ν 3284, 2924, 1643, 1531, 1440, 1404, 1251, 1204, 1107, 750 cm^{-1}; (+)-ESI-HRMS. Calcd for $C_{60}H_{69}N_{14}O_{10}^+$ ($M+H^+$): 1145.5316. Found: 1145.5296. Although all signals were detected as broad peaks in the ^1H NMR analysis ($CDCl_3$) probably due to the high molecular weight, an analyzable chart was obtained. See supporting information for the ^1H NMR chart.

Inhibition of LSD1 activity: LSD1 activities were assessed using an LSD1 fluorometric drug discovery kit (Enzo Life Sciences, New York, NY, USA) according to the manufacturer's instruction. Ten microliters of reaction mixture containing LSD1 (5 ng/mL), H3K4Me2 peptide-fragments (20 μM), CELLestial Red (1×), horseradish peroxidase (1×), and compounds (0.1–20 μM with 1% DMSO) were incubated at room temperature for 30 min. Relative fluorescence units at 590 nm were detected by a NanoDrop 3300 (Thermo Fisher Scientific, Waltham, MA, USA) with a white-light source. The concentration of compounds that resulted in 50% inhibition was determined by plotting log [ln h] against the logit function of the % inhibition. IC_{50} values were determined by regression analysis of the concentration/inhibition data.

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