Convenient Synthesis and Evaluation of Biological Activity of Benzyl (2*S*)-2-[(*R*)-1-hydroxy-2-oxo-(1-phenethyl)prop-3-ylcarbamoyl]-4-oxopiperidine- (or -4-oxopyrrolidine)-1-carboxylate as Novel Histone Deacetylase Inhibitors

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A simple synthesis, involving a key coupling reaction, and the biological activity of the title compounds **16** and **17** are described. The key fragments are the amine·HCl salt **6** and the acids **9** and **13**, which were smoothly coupled by using ethyl(dimethylaminopropyl)carbodiimide (EDCI) and 1-hydroxybenzotriazole (HOBt) in high yield. We have found that the *in vitro* growth inhibitory potency of the new compounds **16** and **17** exhibits good histone deacetylase (HDAC) activity.

Key words: 4-Oxopiperidine-1-carboxylate, 4-Oxopyrrolidine-1-carboxylate, Acetylation, Coupling Synthesis

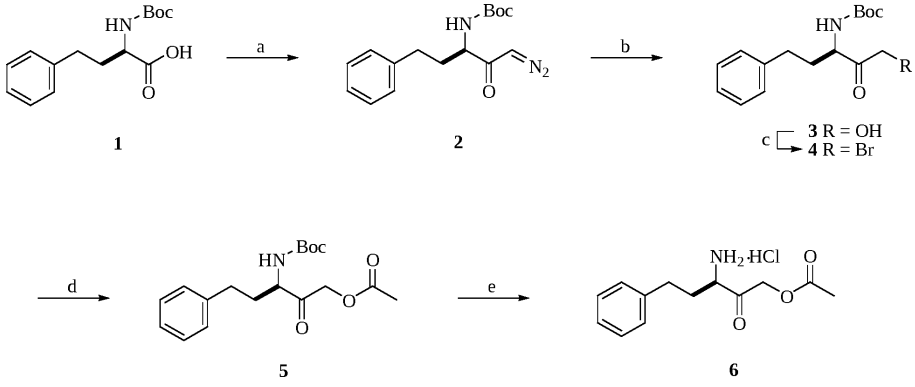
Introduction

The naturally occurring anticancer substances and various synthetic agents for cancer treatment have evoked a great deal of interest in recent years due to their characteristic therapeutic effects [1]. Histone deacetylase (HDAC) inhibitors are a new class of potential therapeutic cancer agents. They are categorized into two classes according to the enzymes responsible for reversible acetylation or deacetylation processes as histone acetyltransferases (HAT) and histone deacetylases (HDAC), respectively [2]. It is well known that the HATs act as transcriptional coactivators, and HDACs are part of transcriptional corepressor complexes [3]. Recently, the Schaefer group [4] reported the synthesis of biarylalanine-containing hydroxamic acids and investigated their action on immunoprecipitated HDAC1 and HDAC6; a subtype selectivity for HDAC6 was confirmed in cells by Western blot. The Angibaud group [5] developed the synthesis of a series of pyrimidyl-5-hydroxamic acids as potent HDAC inhibitors, which can be used as a linker to provide HDAC inhibitors of good enzymatic potency. The Delorme group [6] reported the synthesis and evaluation of sulfonamide hydroxamic acids as new potent HDAC inhibitors with good *in vitro* and *in vivo* activities. Pankiewicz *et al*. [7] described the synthesis of dual inhibitors of inosine monophosphate dehydrogenase and HDAC inhibitors for cancer treatment.

As a part of our ongoing medicinal chemistry program dealing with the development of new HDAC inhibitors, we report herein an efficient synthesis of the title compounds **16** and **17** starting from *N*-Boc-L-homophenylalanine (**1**) and the evaluation of their *in vitro* growth inhibitory potency.

Results and Discussion

To generate the key fragment **6** [8], diazoketone **2** was prepared in quantitative yield from commercially available *N*-Boc-L-homophenylalanine (**1**) by reacting it with freshly prepared diazomethane in the presence of *N*-methylmorpholine and isobutyl chloroformate [9]. Compound **2** was submitted without purification to a treatment with sodium hydroxide in THF/H_2O to yield primary alcohol **3** in 33 % two steps yield. Bromination of alcohol **3** with phosphorus tribromide in diethyl ether gave bromide **4** in 84 % yield. Treatment of **4** with sodium acetate and 18-crown-6 as a



1 **2** **5** **6**

Scheme 1. (a) $ClCO_2i$-Bu, *N*-methylmorpholine, CH_2N_2, 20 °C, 2 h; (b) NaOH, THF/H_2O (8 : 2, v/v), r. t., 24 h (33 %); (c) PBr_3, Et_2O, 0 °C to r. t., 2 h (84 %); (d) NaOAc, 18-crown-6, DMF, r. t., 1 h (82 %); (e) 4 M HCl, 1,4-dioxane, 0 °C, 30 min, r. t., 4 h (85 %).

phase transfer catalyst afforded acetate **5** in 82 % yield [10], which was deprotected with 4 M aqueous HCl solution in 1,4-dioxane to give amine · HCl salt **6** in 85 % yield (Scheme 1).

In order to generate compounds **16** and **17**, *trans*-4-hydroxy-L-proline (**7**) was protected with benzyloxycarbonyl chloride (Cbz-Cl) in the presence of chlorotrimethylsilane (TMS-Cl) to generate the Cbz-protected acid **8** in 95 % two steps yield. Oxidation of **8** was accomplished by using Jones reagent at 0 °C in acetone to afford keto acid **9** in 92 % yield, which was subsequently treated with catalytic amounts of isobutylene and sulfuric acid in dichloromethane to give keto ester **10** [11] in 80 % yield. Ring expansion of keto ester **10** was performed with ethyl diazoacetate (EDA) and boron trifluoride-diethyl etherate ($BF_3 \cdot Et_2O$) in diethyl ether to yield β-keto ester **11** (K : E = 9 : 1) [12], which was subsequently treated with sodium chloride in DMSO to give 4-oxopiperidine **12** and the regioisomeric 5-ketone (isolated ratio = 2 : 3) in 75 % combined yield.

Ketone **12** was hydrolyzed by using trifluoroacetic acid in dichloromethane to give acid **13** in high yield. Acids **9** and **13** were then coupled with amine · HCl salt **6** by using ethyl(dimethylaminopropyl)carbodiimide (EDCI) and 1-hydroxybenzotriazole (HOBt) in the presence of triethylamine to give amides **14** and **15** in 80 and 83 % yield, respectively [13]. To explore the reaction scope and generality, we investigated several coupling agents such as DCC, HOAt, HATU, TATU, and BOP-Cl [14]. Although the latter were more convenient in handling, the HOBt/EDCI method afforded a favorable yield. Deacetylation of compounds **14** and **15** was accomplished with $K_2CO_3 \cdot 1.5\ H_2O$ in $MeOH/CH_2Cl_2/H_2O$ (8 : 1 : 1, v/v) to afford esters **16** and **17** in 70 and 75 % yield, respectively (Scheme 2).

Table 1. HDAC and growth inhibiting potency of novel compounds **16** and **17**.

Compound	IC_{50} cells (μM)[a]
14	ND[b]
15	ND
16	16.3
17	> 100
Sodium butyrate[c]	140

[a] Values are means of three experiments; [b] not detected; [c] substance for comparison.

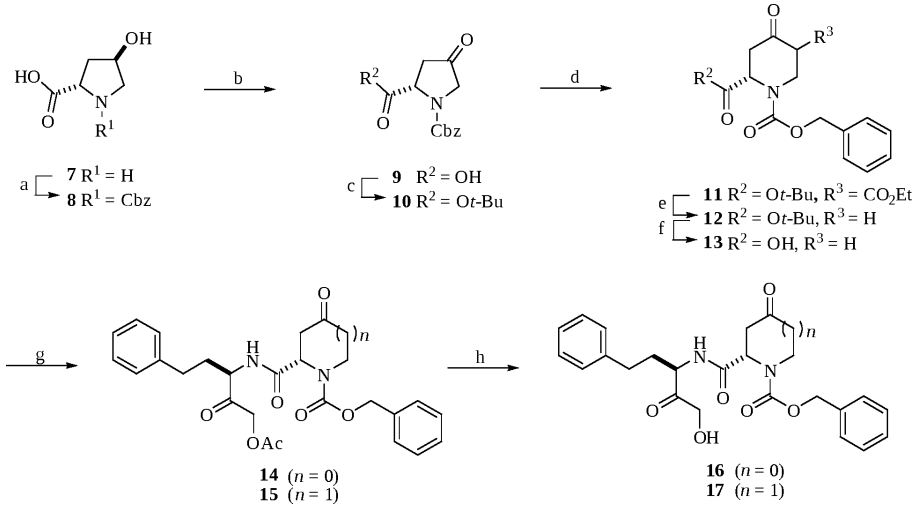
The *in vitro* histone deacetylase (HDAC) activity of **16** and **17** was evaluated in human leukemia K562 cells (2.5×10^8). These compounds exhibited good efficacy (IC_{50}: 16.3 μM for **16**, > 100 μM for **17**) comparable to sodium butyrate *in vitro* histone deacetylase (HDAC) activity, while compounds **14** and **15** were not detected (Table 1).

Conclusion

In conclusion, a simple preparation of the title compounds **16** and **17** as novel histone deacetylase (HDAC) inhibitors has been described. The key fragments were the amine · HCl salt **6**, prepared from *N*-Boc-L-homophenylalanine (**1**), and acids **9** and **13**. We found that compounds **16** and **17** exhibited good efficacy comparable to sodium butyrate as a reference material for *in vitro* HDAC activity. We expect that simple syntheses of new 4-oxopiperidine (or 4-oxopyrrolidine)-1-carboxylates and key fragments will be useful for the modification of histone deacetylase (HDAC) inhibitors.

Experimental Section

Reactions requiring anhydrous conditions were performed with the usual precautions for rigorous exclusion of air and moisture. Tetrahydrofuran was distilled from sodium ben-



Scheme 2. (a) TMS-Cl, DIPEA, CH_2Cl_2, reflux, 2 h; then Cbz-Cl, 0 °C to r. t., 16 h (95 %); (b) CrO_3, H_2SO_4, acetone, 0 °C, 10 min (92 %); (c) isobutylene, H_2SO_4 (cat.), CH_2Cl_2, r. t., 16 h (80 %); (d) EDA, $BF_3 \cdot Et_2O$, Et_2O, r. t., 1 h (90 %); (e) NaCl, DMSO, H_2O (cat.), 140 °C, 4 h (75 %); (f) TFA, CH_2Cl_2, r. t., 2 h (97 %); (g) **6**, HOBt, EDCI, TEA, CH_2Cl_2, r. t., 16 h (80 % for **14**, 83 % for **15**); (h) $K_2CO_3 \cdot 1.5\ H_2O$, MeOH : CH_2Cl_2 : H_2O (8 : 1 : 1, v/v), −10 to 10 °C, 30 min (70 % for **16**, 75 % for **17**).

zophenone ketyl prior to use. Thin layer chromatography (TLC) was performed on precoated silica gel G and GP uniplates from Analtech and visualized with 254 nm UV light. Flash chromatography was carried out on silica gel 60 [Scientific Adsorbents Incorporated (SAI), particle size 32 – 63 μm, pore size 60 Å]. ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker DPX 500 instrument at 500 MHz and 125 MHz, respectively. The chemical shifts are reported in parts per million (ppm) downfield from tetramethylsilane, and *J* values are in Hz. Infrared (IR) spectra were obtained on an ATI Mattson FT/IR spectrometer. Mass spectra were recorded with a Waters Micromass ZQ LC-Mass system, and high-resolution mass spectra (HRMS) were measured with a Bruker BioApex FTMS system by direct injection using an electrospray interface (ESI). When necessary, chemicals were purified according to the reported procedures [15].

Inhibition of histone deacetylase in vitro

Histone deacetylase fraction was prepared as described by theYoshida group [16], human leukemia K562 cells (2.5×10^8) were disrupted in buffer-A [15 mM potassium phosphate buffer (pH 7.5) containing 5 % glycerol and 0.2 mM EDTA] (15 mL). The nuclei were collected by centrifugation (35000 g, 10 min) and resuspended with buffer-A (15 mL) containing 1 M $(NH_4)_2SO_4$. After sonication, the supernatant was collected by centrifugation, and ammonium sulfate was added to make the final concentration 3.5 M. After stirring for 1 h at 0 °C, the precipitate was collected by centrifugation, dissolved with buffer-A (4 mL), and dialyzed against buffer-A (2000 mL). The dialysate was loaded onto a mono Q HR 5/5 column (Pharmacia) equilibrated with buffer-A and eluted with a linear gradient of 0.1 M NaCl in buffer-A (30 mL). A single peak of histone deacetylase activity was eluted with 0.4 M NaCl, and the fraction was stored at −80 °C until use. Inhibition of histone deacetylase was estimated as described by Yoshida *et al*. with slight modifications. [^{3}H$_1$]-labeled histone was prepared by the method of Yoshida *et al*.: 3 K562 cells (108 cells) were incubated in growth medium (25 mL) containing 0.5 mCi mL^{-1} sodium [^{3}H$_1$]acetate (152.8 GBq mmol^{-1}; NEN) and 5 mM sodium butyrate at 37 °C [17]. Histone deacetylase inhibitory activity of a test compound was measured as follows: the mixture (total volume 50 μL) containing the above histone deacetylase fraction (2 μL), [^{3}H$_1$]-labeled histone (100 μg mL^{-1}), and the test compound (5 μL) was incubated for 10 min at 37 °C. [^{3}H$_1$]acetate, which was liberated from [^{3}H$_1$]-labeled histone, was extracted with ethyl acetate, and radioactivity was measured by a liquid scintillation counter.

*N-Benzyloxycarbonyl-4-oxo-(S)-pipecolic acid (**13**)*

To a stirred solution of **12** [18] (0.13 g, 0.38 mmol) in dichloromethane (3 mL) was added trifluoroacetic acid (0.3 mL) at 5 °C, and the mixture was stirred at r. t. for 2 h. The reaction mixture was evaporated *in vacuo*, and the residue was diluted with dichloromethane (20 mL) and washed with 2.5 % aqueous $NaHCO_3$ solution (12 mL) and brine (12 mL). The organic layer was separated, dried over anhydrous $MgSO_4$, filtered and concentrated under reduced pressure. The residue was purified by flash column chromatography (silica gel, 50 % ethyl acetate in *n*-hexanes)

to give **13** (0.085 g, 97 %) as an oil. R_f = 0.2 (ethyl acetate/*n*-hexanes = 1 : 1, v/v). – $[\alpha]_D^{24}$ = −19.6 (*c* = 1.0, $CHCl_3$). – IR (neat, NaCl): *v* = 3433 (OH), 3034, 2961, 1727 (CO), 1704 (COO), 1542, 1423, 1317, 1251, 1186, 1057, 865 cm^{-1}. – ^1H NMR ($CDCl_3$, 500.14 MHz): δ = 9.30 (brs, 1H, OH), 7.42 – 7.24 (m, 5H, Ar-H), 5.23 – 4.98 (m, 3H, CH_2Ph, CH), 4.10 (t, *J* = 6.0 Hz, 1H, CH), 4.68 (t, *J* = 6.0 Hz, 1H, CH), 2.92 – 2.71 (m, 2H, CH_2), 2.55 – 2.43 (m, 2H, CH_2). – ^{13}C NMR ($CDCl_3$, 125.76 MHz): δ = 206.0, 174.1, 156.0, 155.4, 135.8, 128.6, 128.4, 128.0, 68.3, 54.3, 53.5, 40.8, 40.3, 39.4. – HRMS: *m/z* = 278.1032 (calcd. 278.1028 for $C_{14}H_{16}NO_5$, $[M+H]^+$).

*General procedure for the preparation of compounds **14** and **15** via coupling reaction of amine · HCl salt **6** and acids **9** or **13***

To a stirred solution of acids **9** [19] or **13** [20] (0.20 mmol) in dichloromethane (8 mL) was added EDCI (0.24 mmol) and HOBt (0.24 mmol) at 0 °C under argon atmosphere, and then the mixture was stirred at 0 °C for 1.5 h. Triethylamine (0.22 mmol) was added by a syringe to the reaction mixture, followed by addition of amine · HCl salt **6** (0.20 mmol). Then the resulting mixture was stirred at r. t. for 1.5 h, diluted with dichloromethane (10 mL) and washed with 50 % aqueous NH_4Cl solution (10 mL) and brine (10 mL). The aqueous phase was extracted with dichloromethane (6 mL). The combined organic layers were washed with saturated aqueous NH_4Cl solution (15 mL), and the organic phase was separated, dried over anhydrous $MgSO_4$, filtered and concentrated under reduced pressure. The residue was purified by flash column chromatography (silica gel, ethyl acetate/*n*-hexanes/methanol = 22 : 70 : 8, v/v) to afford esters **14** and **15** as viscous oils, respectively.

*Benzyl-(2S)-2-[(R)-1-acetoxy-2-oxo-1-(phenethyl)prop-3-ylcarbamoyl]-4-oxopiperidine-1-carboxylate (**14**)*

R_f = 0.4 (ethyl acetate/*n*-hexanes/methanol = 20 : 75 : 5, v/v). – $[\alpha]_D^{24}$ = −28.4 (*c* = 0.3, $CHCl_3$). – IR (neat, NaCl): *v* = 3024, 2971, 1715 (CO), 1684 (COO), 1520, 1376, 1265, 1112, 728 cm^{-1}. – ^1H NMR ($CDCl_3$, 500.14 MHz): δ = 7.58 – 7.12 (m, 11H, Ar-H, NH), 5.25 – 5.08 (m, 2H, CH_2Ph), 4.71 – 4.45 (m, 3H, CH_2, CH), 4.31 – 3.75 (m, 2H, CH_2), 2.95 – 2.45 (m, 3H, CH_2, CH), 2.38 – 2.21 (m, 2H, CH_2), 2.12 (s, 3H, CH_3), 2.00 – 1.72 (m, 2H, CH_2). – ^{13}C NMR ($CDCl_3$, 125.76 MHz): δ = 206.8, 205.1, 170.7, 170.1, 154.5, 140.1, 136.0, 128.5, 128.4, 128.1, 127.0, 126.1, 67.9, 66.4, 54.9, 54.1, 51.8, 41.2, 40.2, 34.9, 32.1, 31.9, 30.9, 21.2. – HRMS: *m/z* = 481.1975 (calcd. 481.1956 for $C_{26}H_{29}N_2O_7$, $[M+H]^+$).

*Benzyl-(2S)-2-[(R)-1-acetoxy-2-oxo-1-(phenethyl)prop-3-ylcarbamoyl]-4-oxopyrrolidine-1-carboxylate (**15**)*

R_f = 0.4 (ethyl acetate/*n*-hexanes/methanol = 20 : 75 : 5, v/v). – $[\alpha]_D^{24}$ = −21.8 (*c* = 0.3, $CHCl_3$). – IR (neat, NaCl): *v* = 3031, 2976, 1718 (CO), 1696 (COO), 1527, 1458, 1218, 1115, 825 cm^{-1}. – ^1H NMR ($CDCl_3$, 500.14 MHz): δ = 7.51 – 7.03 (m, 11H, Ar-H, NH), 5.21 – 5.01 (m, 2H, CH_2Ph), 4.68 – 4.32 (m, 3H, CH_2, CH), 4.20 – 3.68 (m, 2H, CH_2), 2.98 – 2.42 (m, 5H, CH_2, CH), 2.30 – 2.11 (m, 2H, CH_2), 2.09 (s, 3H, CH_3), 1.98 – 1.61 (m, 2H, CH_2). – ^{13}C NMR ($CDCl_3$, 125.76 MHz): δ = 206.8, 205.1, 170.7, 169.9, 154.5, 140.1, 136.0, 128.5, 128.4, 128.1, 127.0, 126.1, 67.9, 66.4, 54.9, 54.1, 51.8, 41.2, 40.4, 40.2, 34.9, 32.1, 31.9, 30.9, 20.9. – HRMS: *m/z* = 495.2131 (calcd. 495.2145 for $C_{27}H_{31}N_2O_7$, $[M+H]^+$).

*General procedure for the preparation of compounds **16** and **17** via deacetylation of esters **14** and **15***

To a stirred solution of keto esters **14** or **15** (0.13 mmol) in MeOH : CH_2Cl_2 : H_2O (5.4 mL, 8 : 1 : 1, v/v) was added $K_2CO_3 \cdot 1.5\ H_2O$ (0.26 mmol) in H_2O (0.7 mL) at −10 °C, and the mixture was stirred at −10 °C to 0 °C for 30 min. The mixture was evaporated *in vacuo*, and the residue was treated with dichloromethane (15 mL). The mixture was washed with saturated aqueous NH_4Cl solution (6 mL), and the aqueous phase was extracted with dichloromethane (5 mL). The combined organic layers were washed with saturated aqueous NH_4Cl solution (10 mL), and the organic phase was separated, dried over anhydrous $MgSO_4$, filtered and concentrated under reduced pressure. The residue was purified by flash column chromatography (silica gel, ethyl acetate/*n*-hexanes/methanol = 22 : 70 : 8, v/v) to afford primary alcohols **16** and **17** as viscous foams, respectively.

*Benzyl-(2S)-2-[(R)-1-hydroxy-2-oxo-1-(phenethyl)prop-3-ylcarbamoyl]-4-oxopiperidine-1-carboxylate (**16**)*

R_f = 0.3 (ethyl acetate/*n*-hexanes/methanol = 22 : 70 : 8, v/v). – $[\alpha]_D^{24}$ = −16.0 (*c* = 0.5, $CHCl_3$). – IR (neat, NaCl): *v* = 3327 (OH), 3063, 3029, 2926, 1727 (CO), 1695 (COO), 1531, 1454, 1321, 1216, 1061, 752 cm^{-1}. – ^1H NMR ($CDCl_3$, 500.14 MHz): δ = 7.49 – 6.99 (m, 11H, Ar-H, NH), 6.08 (brs, 1H, OH), 5.32 – 4.98 (m, 2H, CH_2Ph), 4.81 – 4.20 (m, 3H, CH_2, CH), 4.12 – 3.51 (m, 2H, CH_2), 2.82 – 2.36 (m, 3H, CH_2, CH), 2.34 – 2.03 (m, 2H, CH_2), 2.00 – 1.65 (m, 2H, CH_2). – ^{13}C NMR ($CDCl_3$, 125.76 MHz): δ = 207.9, 204.9, 170.9, 155.5, 139.9, 135.4, 128.6, 128.4, 128.2, 128.0, 126.4, 68.6, 66.7, 55.2, 54.4, 52.0, 40.6, 40.1, 35.8, 32.9, 31.9, 30.0. – HRMS: *m/z* = 439.4525 (calcd. 439.4810 for $C_{24}H_{27}N_2O_6$, $[M+H]^+$).

*Benzyl-(2S)-2-[(R)-1-hydroxy-2-oxo-1-(phenethyl)prop-3-ylcarbamoyl]-4-oxopyrrolidine-1-carboxylate (**17**)*

R_f = 0.3 (ethyl acetate/*n*-hexanes/methanol = 22 : 70 : 8, v/v). – $[\alpha]_D^{24}$ = −33.8 (*c* = 2.0, $CHCl_3$). – IR (neat, NaCl): *v* = 3331 (OH), 3062, 3029, 2925, 2856, 1725 (CO), 1690 (COO), 1531, 1454, 1357, 1245, 1059, 752 cm^{-1}. – ^1H NMR

($CDCl_3$, 500.14 MHz): δ = 7.48 – 7.00 (m, 11H, Ar-H, NH), 6.11 (brs, 1H, OH), 5.31 – 5.10 (m, 2H, CH_2Ph), 5.50 – 4.89 (m, 1H, CH_2), 4.65 – 4.49 (m, 1H, CH), 4.38 – 4.23 (m, 2H, CH_2), 4.08 – 3.90 (m, 1H, CH), 3.79 – 3.56 (m, 1H, CH_2), 3.34 – 3.03 (m, 1H, CH), 2.82 (d, J = 8.0 Hz, 1H, CH_2), 2.70 – 2.41 (m, 4H, CH_2), 2.20 – 2.01 (m, 1H, CH_2), 1.97 – 1.74 (m, 1H, CH_2). – ^{13}C NMR ($CDCl_3$, 125.76 MHz): δ = 208.2, 205.1, 170.6, 155.6, 140.0, 135.6, 128.7, 128.6, 128.5, 128.3, 126.5, 68.4, 66.6, 55.0, 40.3, 39.9, 38.4, 32.6, 31.5. – HRMS: *m/z* = 453.2005 (calcd. 453.2026 for $C_{25}H_{29}N_2O_6$, $[M+H]^+$).

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