Synthesis of a *C*-Iminoribofuranoside Analog of the Nicotinamide Phosphoribosyltransferase (NAMPT) Inhibitor FK866

by **Annabelle Gillig**, **Somi Reddy Majjigapu**, **Bernard Sordat**, and **Pierre Vogel***

Laboratory of Glycochemistry and Asymmetric Synthesis (LGSA), Swiss Institute of Technology in Lausanne (EPFL), Batochime, CH-1015 Lausanne
(phone: +41 21 693 93 71; fax: +41 21 693 93 75; e-mail: pierre.vogel@epfl.ch)

FK866 (also named APO866 or WK175) is a potent NAMPT inhibitor being evaluated (Phase II) as a potential anticancer drug. The preparation of the *C*-iminoribofuranoside analog (2*E*)-*N*-[4-(1-benzoylpiperidin-4-yl)butyl]-3-{3-[(2*S*,3*S*,4*R*,5*R*)-3,4-dihydroxy-5-(hydroxymethyl)pyrrolidin-2-yl]phenyl}prop-2-enamide ((−)-**1**) is reported.

Introduction. – Proliferating cells have an increased demand of NAD^+ (nicotinamide adenine dinucleotide), the universal energy- and signal-carrying molecule [1]. Particularly, NAD^+ is involved in the transfer of ADP-ribosyl moiety to proteins, in the calcium-signalling mechanism, in DNA repair, and it acts as a coenzyme of protein deacetylases called sirtuins [2]. NAD^+ participates also in glycolysis and in oxidative phosphorylation [3]. NAD^+ has several precursors [4]. One of them is nicotinamide (NAM) [5] that is glycosylated by α-D-5-phosphoribosyl-1-pyrophosphate (PRPP) and inorganic pyrophosphate (PPi) to NMN (nicotinamide mononucleotide) [6]. This reversible reaction is catalyzed by nicotinamide phosphoribosyltransferase (NAMPT) [7], an enzyme also called visfatin [8], and pre-B cell colony enhancing factor (PBEF), which is a protein with multiple functional properties [9]. NAMPT is overexpressed in cancer cells [10] and in proliferating cells [11]. NAMPT is probably involved in the transformation of dormant form of tumors to malignant ones; it promotes the growth of some types of tumors [9e]. Increased concentration of NAMPT has been reported in colorectal [12], ovarian [13], and prostate [14] cancer. Thus, NAMPT represents an attractive target for cancer chemotherapy. The best explored inhibitor of NAMPT is FK866 (*Fig. 1*; also known as WK175 or APO866) [15]; it has an IC_{50} value of *ca.* 1 nM for cytotoxicity toward several cancer cell lines. It also exhibits promising antitumor activities against both solid tumors and leukemia in preclinical assays [16]. FK866 specifically inhibits human NAD^+ levels in tumors and ultimately induces apoptosis in these cells [17][18]. FK866 enhances the resistivity of mammary carcinoma to radiation [19].

FK866 has a chemosensitivity effect on leukemia cell death induced by antineuroplastin drug such as MNNG (1-methyl-3-nitro-1-nitrosoguanidinium) [20]. Synergistic effects have also been noted for FK866 in combination with TRAIL (tumor necrosis factor-related apoptosis-inducing ligand) [21] or with L-1-methyltryptophan (IDO-specific inhibitor L-1 MT) [22]. FK866 is in Phase-II clinical trials against several forms of human cancers.

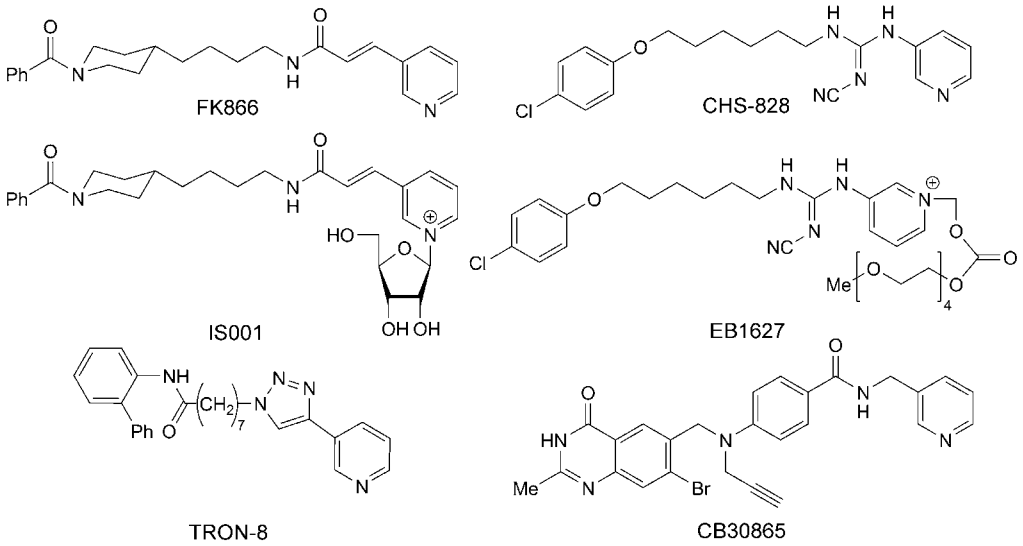


Fig. 1. *Structures of NAMPT inhibitors*

Secondary effects of the drug are lymphopenia, amenia, fatigue, and grade 3 nausea (continuous 96 h infusion, every 28 d). The dose-limiting toxicity of FK866 is thrombocytopenia [19]. This has led to the search for other NAMPT inhibitors such as CHS-828 (*Fig. 1*; also named GMX1778) [23][24], which has been administrated orally in Phase-I clinical trial (solid tumors). Toxicity was dominated by gastro-intestinal symptoms and thrombocytopenia [25]. To improve pharmacokinetics and water solubility, derivative EP1627 (also named GMX1777) was proposed as an alternative to CHS-828 [26]. Similarly, the D-ribofuranosyl derivative of FK866, called IS001, was prepared to improve poor water solubility of FK866. Unfortunately, IS001 has a weaker affinity to NAMPT than FK866. Crystal structures of IS001 complexed with NAMPT showed that the aglycon part adopts the same position as FK866 alone in the active site of dimeric NAMPT [27] (*Fig. 2*) [28][29]. In the latter complex, the site reserved for the ribofuranosyl cation is free.

A novel NAMPT inhibitor, TRON-8, resulting from 'click chemistry' has been shown to have an IC_{50} value of *ca.* 3 nM for NAD^+ depletion but is less cytotoxic than FK866 [30]. Recently, CB30865 (also named MPI-0479626), a subnanomolar and relative cytotoxic agent, was determined to inhibit human NAMPT with an IC_{50} value of *ca.* 0.2 nM [31].

As mentioned above, although FK866 is a potent inhibitor of NAMPT (IC_{50} *ca.* 1 nM) and shows interesting key antitumor activities, its low bioavailability (96 h continuous intravenous infusion) due to the metabolic instability of the pyridine moiety [32] and toxicity toward lymphocytes [33] necessitates the search for analogs with a better therapeutic index. In this report, we present our efforts toward this objective. Inspired by the X-ray crystal structures reported for complexes of FK866 [28][29] and IS001 [27] with NAMPT, we envisioned that the 1,4-dideoxy-1,4-imino-β-D-ribofur-

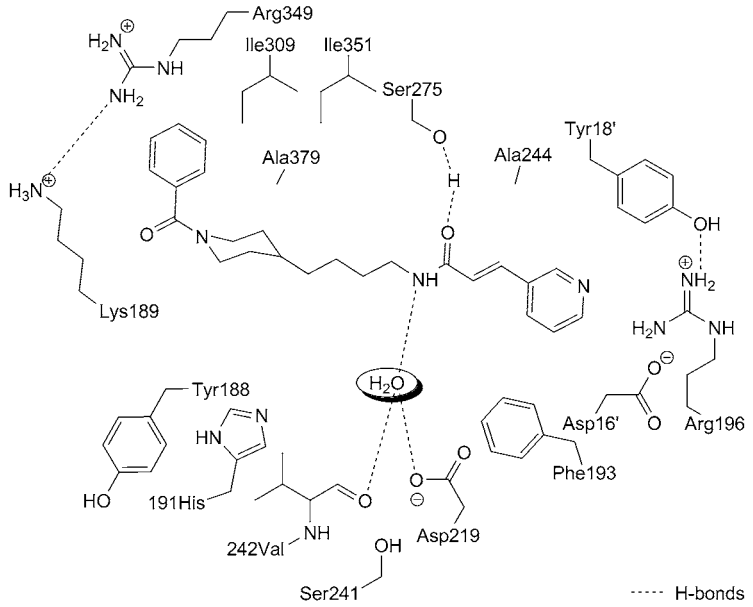


Fig. 2. *Structure of FK866 and schematic representation of its interaction with NAMPT* [28] [29]

anosyl-*C*-aryl derivatives of IS001, compound (−)-**1** (*Fig. 3*), should mimick the transition state or a key intermediate formed in the rate-determining step of the glycosidation process catalyzed by NAMPT, and thus be a good and selective inhibitor of this enzyme. The X-ray structure of the NAMPT–IS001 complex suggested that the iminoribitol moiety of (−)-**1** should occupy the place reserved to the ribofuranosyl cation on its way to be attached to the pyridine moiety of nicotinamide. The conjugate acid (ammonium ion) of the iminoribitol moiety is expected to interact with the active site of NAMPT, as does the D-ribofuranosyl cation intermediate. The observation that IS001 was a weaker ligand than FK866 for NAMPT [27] is not a surprise to us. IS001 is a product-like ligand, and, thus, NAMPT has been tuned up by Nature to remove it from its active site. On the contrary, mimetic of IS001, (−)-**1**, is an intermediate or transition state-like ligand, and thus should interact strongly with the enzyme.

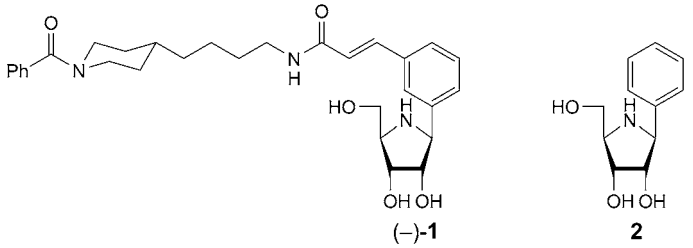
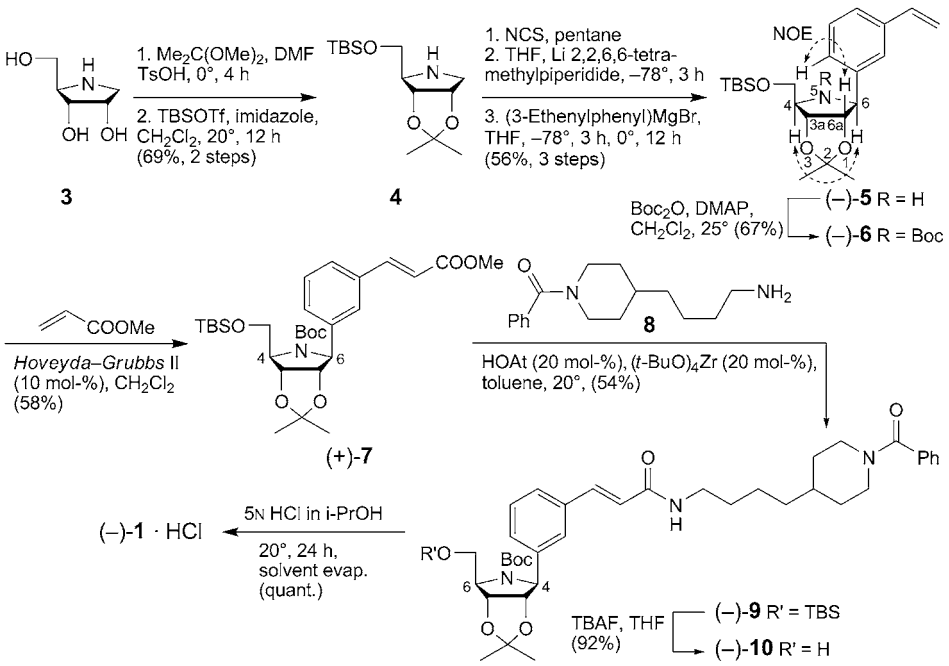


Fig. 3. *Structures of compounds* (−)-**1** *and* **2**

Results and Discussion. – Our synthesis of compound (–)-**1** was inspired by the synthesis of **2**, developed by *Schramm* and co-workers in 1993 [34], and started from 1,4-dideoxy-1,4-imino-D-ribitol (**3**), prepared according to the procedure of *Witte* and *McClard* [35]. The 2,3-*cis*-diol moiety of **3** was protected as an acetonide and the 5-OH group as a silyl ether applying standard conditions (*Scheme*) to give **4** [36]. *N*-Chlorination of **4** with *N*-chlorosuccinimide (NCS) and basic treatment afforded an imine that reacted with (3-ethenylphenyl)magnesium bromide to afford the styrene derivative (–)-**5** (56%). The (1*S*)-configuration of (–)-**5** was established by NOEs in its 2D-^{1}H-NMR spectrum that showed correlation peaks H–C(4)/H–C(6) of the pyrrolidine moiety and for the aromatic C–H and H–C(6a) pair. After protection of amine (–)-**5** as a *N*-Boc derivative (–)-**6** (67%), metathesis with methyl acrylate catalyzed by *Grubbs*-II catalyst provided (+)-**7** in 58% yield. Catalytic ((*t*-BuO)$_4$Zr) ester/amide exchange [37] with primary amino derivative **8** furnished acrylamide (–)-**9**. Desilylation of (–)-**9** with Bu_4NF, followed by acidic hydrolysis of the carbamate and acetonide moieties (i-PrOH/5N HCl), provided (–)-**1** (quant.; *Scheme*). Compound **8** was prepared from commercially available 4-(piperidin-4-yl)butanoic acid, reduction of which with $LiAlH_4$ [38] gave the corresponding aminoalcohol, which was amidified with PhCOCl [39]. *Mitsunobu* substitution with phthalimide [40] and subsequent aminolysis of the phthalimide moiety with $NH_2NH_2 \cdot H_2O$ [41] provided **8**.

Scheme. *Synthesis of* C-*Iminoribofuranoside Analog* (–)-**1** · HCl. For abbreviations, *cf.* the *Exper. Part.*



Conclusion. – An efficient synthesis of a *C*-iminoribofuranoside analog of the NAMPT inhibitor FK866 has been developed. The method should allow generation of

a library of further analogs (*e.g.*, acrylamides from (+)-**7**). Their syntheses will be reported together with their biological activities in a forthcoming article.

Experimental Part

General. All commercially available reagents and solvents (*Fluka*, *Aldrich*, *Acros*) were used without further purification. For reactions requiring anh. conditions, dry solvents were bought (*Fluka*, *Aldrich*). Reactions were monitored by TLC (*Merck*; silica gel *60* F_{254} plates); detection with UV light, $KMnO_4$, or *Pancaldi* reagent ($(NH_4)_6MoO_4$, $Ce(SO_4)_2$, H_2SO_4, H_2O). Purifications used flash chromatography (FC) on silica gel (SiO_2; *Merck* No. *9385* silica gel *60*, 240–400 mesh). Optical rotations: at 25° with a *Jasco P-1020* polarimeter; $[\alpha]$ values in 10^{-1} deg cm^2 g^{-1}. UV Spectra: *Perkin-Elmer Lambda 10* UV/VIS spectrophotometer. IR Spectra: *Perkin-Elmer Paragon 1000* FT-IR spectrometer. ^{1}H-NMR Spectra: *Bruker ARX-400* and a *DPY-400* spectrometer at 400 MHz; chemical shifts in ppm rel. to residual ^{1}H-solvent signal (CHD_2OD: δ(H) 3.34 ppm; $CHCl_3$: δ(H) 7.27 ppm; C_6HD_5: δ(H) 7.30 ppm) as the internal reference; assignments confirmed by 2D-COSY spectra; the multiplicities reflect apparent signal patterns; ^{13}C-NMR spectra: with the same instrument as above at 100.6 MHz; chemical shifts in ppm rel. to residual ^{13}C-solvent signal (CD_3OD: δ(C) 49 ppm; $CDCl_3$: δ(C) 77 ppm; C_6D_6: δ(C) 128.5 ppm); assignments confirmed by 2D-HSQC spectra; coupling constants J(H,H), J(C,H) in Hz. MALDI-TOF-MS: *Axima-CFR*$^+$ spectrometer, *Kratos*. ESI-Q-MS: *Finnigan SSQ 710C* spectrometer, *Thermoquest*. ESI-HR-MS: *Q-TOF Ultima* spectrometer, *Microman*. Elemental analyses: performed by Mr. *Euro Solari*, EPFL. *Abbreviations*. Boc, (*tert*-Butoxy)carbonyl; DMAP, 4-(dimethylamino)pyridine; HOAt, 1-hydroxy-7-azabenzotriazole; NCS, *N*-chlorosuccinimide; TBAF, Bu_4NF; TBSOTf, (*tert*-butyl)dimethylsilyl triflate (=trifluoromethanesulfonate); TsOH, *p*-toluenesulfonic acid.

5-O-*[(*tert-*Butyl)dimethylsilyl]-1,4-dideoxy-1,4-imino-2,3*-O-*isopropylidene*-D-*ribitol* (=*(3a*R*, 4*R*,6a*S*)-4-({[(*tert-*Butyl)(dimethyl)silyl]oxy}methyl)tetrahydro-2,2-dimethyl-3a*H*-[1,3]dioxolo[4,5-*c*]-pyrrole*; **4**). To a stirred suspension of *1,4-dideoxy-1,4-imino*-D-*ribitol* (**3**; 5 g, 37.5 mmol) in 70 ml of DMF was added 30 ml of 2,2-dimethoxypropane and 2 g of TsOH as granula. After 3 h at 20°, TsOH granula was filtered, the mixture was concentrated (50° water bath), and the crude product was dissolved in dry CH_2Cl_2 (150 ml). TBSOTf (25 ml, 100 mmol) and 1*H*-imidazole (7 g, 100 mmol) were added. The mixture was stirred 12 h and washed with a sat. aq. soln. of $NaHCO_3$ (250 ml) and brine (250 ml), dried ($MgSO_4$), and concentrated. Purification by FC (SiO_2; 10 to 100% AcOEt in petroleum ether (PE) containing 0.03% of Et_3N) provided **4** (7.9 g, 69%). Yellow oil. Data were in accordance with those published in [36].

*(3a*R*,4*R*,6*S*,6a*S*)-4-({[(*tert-*Butyl)(dimethyl)silyl]oxy}methyl)-6-(3-ethenylphenyl)tetrahydro-2,2-dimethyl-3a*H*-[1,3]dioxolo[4,5-c]pyrrole* ((−)-**5**). Compound **4** (2.3 g, 7.8 mmol) was dissolved in pentane (112 ml), and NCS (4.2 g, 31.3 mmol) was added at 20°. The mixture was stirred for 30 min, filtered, and solvents were evaporated. The crude product was dissolved in dry THF (50 ml), and a soln. of lithium 2,2,6,6-tetramethylpiperidide (1.2 equiv., prepared previously with 2,2,6,6-tetramethylpiperidine (1.7 ml, 9.8 mmol) and BuLi (1.6M in hexane, 5.9 ml, 9.4 mmol) in dry THF (62.5 ml)) was added dropwise over 3 h at −78°. The mixture was stirred for 2 h at −78° and concentrated. The crude product was dissolved in dry THF (112.5 ml), and (3-ethenylphenyl)magnesium bromide (0.28M, 116.6 ml, 62.6 mmol) was added dropwise during 1 h at −78°. The mixture was stirred for 3 h at −78° and 12 h at 0°, then, 500 ml of Et_2O were added, followed by 200 ml of a 5% aq. soln. of NH_4Cl. The org. phase was sequentially washed with H_2O (500 ml) and brine (500 ml), dried ($MgSO_4$), and solvents were evaporated. Purification by FC (SiO_2; 5% AcOEt in PE) provided (−)-**5** (1.7 g, 56%). Yellow oil. $[\alpha]_D^{25} = -13$, $[\alpha]_{577}^{25} = -13$, $[\alpha]_{435}^{25} = -25$, $[\alpha]_{405}^{25} = -39$ ($c = 0.10$ g/100 ml, MeCN). UV (MeCN): 246 (8820). IR (neat): 2955, 2925, 2855, 1380, 1370, 1255, 1070, 835, 630. ^{1}H-NMR (400 MHz, $CDCl_3$): 7.46, 7.27 (2*m*, 4 arom. H); 6.72 (*dd*, $^3J(1'',2_a'') = 10.8$, $^3J(1'',2_b'') = 17.6$, H–C(1'')); 5.76 (*dd*, $^3J(2_b'',1'') = 17.6$, $^2J(2_b'',2_a'') = 0.9$, H_b–C(2'')); 5.25 (*dd*, $^3J(2_a'',1'') = 10.8$, $^2J(2_a'',2_b'') = 0.9$, H_a–C(2'')); 4.51 (*dd*, $^3J(3a,6a) = 7.1$, $^3J(3a,4) = 4.7$, H–C(3a)); 4.45 (*dd*, $^3J(6a,3a) = 7.1$, $^3J(6a,6) = 5.3$, H–C(6a)); 4.19 (*d*, $^3J(6,6a) = 5.3$, H–C(6)); 3.88 (*dd*, $^2J(1_a',1_b') = 10.2$, $^3J(1_a',4) = 3.7$, H_a–C(1')); 3.78 (*dd*, $^2J(1_b',1_a') = 10.2$, $^3J(1_b',4) = 5.3$, H_b–C(1')); 3.35 (*ddd*,

$^3J(4,1'_b) = 5.3$, $^3J(4,3a) = 4.7$, $^3J(4,1'_a) = 3.7$, H–C(4)); 1.59, 1.34 (2*s*, Me_2C); 0.91 (*s*, *t*-Bu); 0.10, 0.09 (2*s*, Me_2Si). ^{13}C-NMR (100.6 MHz, $CDCl_3$): 141.6; 137.8; 136.7; 128.7; 126.1; 125.2; 124.4; 114.1; 114.0; 87.6; 81.2; 67.9; 65.6; 63.7; 29.7; 27.5; 25.4; 25.9; −5.5. HR-ESI-TOF-MS: 390.2451 ($[M+H]^+$, $C_{22}H_{36}NO_3Si^+$; calc. 390.2464).

tert-*Butyl (3a*R,*4*R,*6*S,*6a*S*)-4-({[(*tert-*Butyl)(dimethyl)silyl]oxy}methyl)-6-(3-ethenylphenyl)tetrahydro-2,2-dimethyl-5*H*-[1,3]dioxolo[4,5-*c*]pyrrole-5-carboxylate* ((−)-**6**). Di(*tert*-butyl) dicarbonate (2 g, 8.46 mmol) and DMAP (1.7 g, 460.6 mmol) were dissolved in dry CH_2Cl_2 (10 ml) at r.t. for 5 min. Then, (−)-**5** (1.7 g, 4.4 mmol) was added, followed by pyridine (3 ml) in dry CH_2Cl_2 (20 ml). The mixture was stirred for 12 h. Et_2O (20 ml) was then added, and the org. layer was washed with a 5% aq. soln. of NH_4Cl (20 ml), dried ($MgSO_4$), filtered, and concentrated *in vacuo*. Purification by FC (SiO_2; 2% AcOEt in PE) provided (−)-**6** (1.3 g, 60%). Yellow oil. $[\alpha]_D^{25} = -30$, $[\alpha]_{577}^{25} = -37$, $[\alpha]_{435}^{25} = -57$, $[\alpha]_{405}^{25} = -60$ ($c = 0.03$ g/100 ml, MeCN). UV (MeCN): 205 (600), 285 (890), 250 (14′000). IR (neat): 2955, 2930, 1695, 1365, 1250, 1065, 835, 775. ^{1}H-NMR (400 MHz, $CDCl_3$): 7.28 (*m*, 4 arom. H); 6.71 (*dd*, $^3J(1'',2''_a) = 17.6$, $^3J(1'',2''_b) = 10.9$, H–C(1″)); 5.72 (*dd*, $^3J(2''_a,1'') = 17.6$, $^3J(2''_a,2''_b) = 0.8$, H_a–C(2″)); 5.23 (*dd*, $^3J(2''_b,1'') = 10.9$, $^3J(2''_b,2''_a) = 0.8$, H_b–C(2″)); 5.00 (*m*, H–C(6)); 4.70 (*d*, $^3J(6a,6) = 5.6$, H–C(6a)); 4.64 (*dd*, $^3J(3a,6a) = 5.6$, H–C(3a)); 4.26 (*m*, H–C(4)); 3.88 (*m*, $CH_2(1')$); 1.58, 1.37 (2*s*, Me_2C); 1.37 (br. *s*, CO_2CMe_3); 0.86 (*s*, *t*-Bu); 0.07, 0.04 (2*s*, Me_2Si). ^{13}C-NMR (100.6 MHz, $CDCl_3$): 154.7; 136.9; 136.6; 128.4; 125.7; 125.3; 124.6; 124.0; 113.7; 87.2; 81.4; 80.0; 69.3; 65.3; 63.1; 28.1; 27.8; 18.3; 25.8; 25.6; −5.5. HR-ESI-TOF-MS: 490.3007 ($[M+H]^+$, $C_{27}H_{44}NO_5Si^+$; calc. 490.4877). Anal. calc. for $C_{27}H_{43}NO_5Si$ (489.72): C 66.22, H 8.85, N 2.86; found: C 66.46, H 8.95, N 2.73.

tert-*Butyl (3a*R,*4*R,*6*S,*6a*S*)-4-({[(*tert-*Butyl)(dimethyl)silyl]oxy}methyl)tetrahydro-6-{3-[(1*E*)-3-methoxy-3-oxoprop-1-en-1-yl]phenyl}-2,2-dimethyl-5*H*-[1,3]dioxolo[4,5-*c*]pyrrole-5-carboxylate* ((+)-**7**). A soln. of (−)-**6** (887 mg, 1.8 mmol) in dry CH_2Cl_2 (1.8 ml) and methyl acrylate (312 mg, 3.6 mmol) were added to a soln. of *Hoveyda–Grubbs* second-generation catalyst (113 mg, 0.2 mmol) in dry CH_2Cl_2 (7.2 ml) under Ar. The mixture was stirred for 20 h at r.t., and solvents were evaporated. Purification by FC (SiO_2; 4 to 6% AcOEt in PE) provided the starting material (−)-**6** (127.2 mg, 14%) and the desired cross product (+)-**7** (561.3 mg, 58%). Yellow oil. $[\alpha]_D^{25} = +33$, $[\alpha]_{577}^{25} = -30$, $[\alpha]_{435}^{25} = -75$, $[\alpha]_{405}^{25} = -91$ ($c = 0.10$ g/100 ml, MeCN). UV (MeCN): 278 (18′800). IR (neat): 2930, 1720, 1695, 1375, 1170, 1065, 835, 775. ^{1}H-NMR (400 MHz, $CDCl_3$): 7.67 (*d*, , $^3J(1'',2'') = 16.0$, H–C(1″)); 7.55 – 7.27 (*m*, 4 arom. H); 6.41 (*d*, $^3J(2'',1'') = 16.0$, H–C(2″)); 5.14 – 4.69 (*m*, H–C(6)); 4.65 (*d*, $^3J = 5.5$, H–C(6a)); 4.57 (*m*, H–C(3a)); 4.21 (br. *s*, H–C(4)); 4.06 – 3.61 (*m*, $CH_2(1')$); 3.79 (*s*, MeO); 1.58, 1.34 (2*s*, Me_2C); 1.28 – 1.12 (*m*, CO_2CMe_3); 0.8 (*s*, *t*-Bu); 0.02, 0.01 (2*s*, Me_2Si). ^{13}C-NMR (100.6 MHz, $CDCl_3$): 167.4; 154.6; 144.9; 143.1; 134.3; 128.8; 127.8; 126.5; 125.7; 117.7; 112.0; 87.0; 81.4; 80.0; 69.3; 65.2; 63.3; 51.6; 29.7; 28.1; 25.8; 25.6; −5.5. HR-ESI-TOF-MS: 548.3028 ($[M+H]^+$, $C_{29}H_{46}NO_7Si^+$; calc. 548.3044). Anal. calc. for $C_{29}H_{45}NO_7Si \cdot 0.33$ hexane: C 64.59, H 8.68, N 2.43; found: C 64.45, H 8.95, N 2.17.

[4-(4-Aminobutyl)piperidin-1-yl](phenyl)methanone (**8**). A suspension of 4-(piperidin-4-yl)butanoic acid hydrochloride (9 g; 43 mmol) in dry THF (250 ml) was cooled in an ice bath and stirred during the dropwise addition of $LiAlH_4$ (6.6 g; 173 mmol). Stirring was continued at 20° for 10 min, and the mixture was heated under reflux for 6 h. The mixture was cooled to 0°, and 40% aq. KOH was added slowly under vigorous stirring. After stirring at 20° for 1 h, the suspension was filtered on a *Celite* pad that was washed with THF (150 ml) and MeOH (150 ml). After evaporation of the solvent, the crude was purified by FC (SiO_2; AcOEt/Et_3N/MeOH 18 : 2 : 80) to give 77% of the reduced product as a yellow oil. Under Ar, Et_3N (1.79 ml; 12.7 mmol) and PhCOCl (0.74 ml; 6.36 mmol) were subsequently added to a cooled (0°) soln. of the previously reduced compound (1 g, 6.36 mmol) in dry CH_2Cl_2 (20 ml), and the mixture was stirred for 10 min. After stirring at 20° for 2 h, the mixture was diluted with a sat. aq. soln. of NH_4Cl until a clear soln. was obtained. It was then extracted with CH_2Cl_2 (3 × 50 ml). The combined org. extracts were washed with brine (100 ml), dried ($MgSO_4$), and the solvents were evaporated. Purification by FC (SiO_2; AcOEt/PE 7 : 3) gave 1.32 g of the protected product (80%) as a light yellow oil. Under Ar, diethyl azodicarboxylate (243 ml; 1.3 mmol) was added dropwise to a soln. of the previous protected compound (1.34 g; 5 mmol), phthalimide (840 mg; 5 mmol), and Ph_3P (1.4 g; 5 mmol) in THF (15 ml) at 20°. After 1 h, the mixture was concentrated. Purification by FC (85% Et_2O in PE) afforded *2-{4-[1-(phenylcarbonyl)piperidin-4-yl]butyl}-1*H*-isoindole-1,3(2*H*)dione* (1.87 g; 91%). White solid. M.p. 96 – 100°. IR (neat): 2930, 1705, 1625, 1394, 708. ^{1}H-NMR (400 MHz, 323 K, $CDCl_3$): 7.96, 7.75 (2*m*, 4 H,

phthalimido); 7.33 (*m*, 5 arom. H); 4.68, 3.70 (2 br. *m*, H_a–C(2″,6″)); 3.70 (*m*, H_b–C(2″,6″), CH_2(1′)); 2.95, 2.73 (2 br. *t*, CH_2(3″,5″)); 1.86 – 1.00 (*m*, CH_2(2′), CH_2(3′), CH_2(4′), H–C(4″)). ^{13}C-NMR (100.6 MHz, $CDCl_3$): 170.2; 168.4; 136.4; 133.9; 132.0; 129.3; 128.3; 126.8; 123.1; 62.1; 48.0; 37.8; 36.0; 35.8; 28.7; 23.8. HR-ESI-TOF-MS: 391.2004 ($[M + H]^+$, $C_{24}H_{27}N_2O_3^+$; calc. 391.2022).

Under Ar, $NH_2NH_2 \cdot H_2O$ (0.51 ml; 10.38 mmol) was added to a soln. of the above product (1.6 g; 4.15 mmol) in dry EtOH (50 ml), and the mixture was stirred at 20° for 10 min and then heated under reflux for 2 h. The mixture was filtered over *Celite*, and the solvents were evaporated. The crude product was dissolved in CH_2Cl_2 (100 ml), and the soln. was washed with an aq. soln. of K_2CO_3 (100 ml). The aq. layer was extracted with CH_2Cl_2 (3 × 50 ml), and the combined org. extracts were washed with brine, dried ($MgSO_4$), filtered, and solvents were evaporated. Purification by FC (SiO_2; MeOH/AcOEt/Et_3N 80 : 18 : 2) afforded 760 mg (70%) of **8**. Yellow oil. IR (neat): 2919, 1623, 1430, 1276, 707. ^{1}H-NMR (400 MHz, 323K, $CDCl_3$): 7.45 – 7.33 (*m*, 5 arom. H); 4.68, 3.72 (2*m*, 2 H_a–C(2)); 2.96 (*m*, H_b–C(2)); 2.71 (*m*, H_b–C(2), CH_2(4′)); 1.90 (br. *s*, NH_2); 1.81, 1.63 (2*m*, 2 H_a–C(3)); 1.57 – 1.42 (*m*, H–C(4), CH_2(3′)); 1.40 – 1.24 (*m*, CH_2(1′), CH_2(2′)); 1.20, 1.11 (2*m*, 2 H_b–C(3)). ^{13}C-NMR (100.6 MHz, $CDCl_3$): 170.2; 136.3; 129.4; 128.4; 126.7; 48.0; 41.2; 36.0; 35.9; 32.8; 31.7; 23.7. HR-ESI-TOF-MS: 261.1975 ($[M+H]^+$, $C_{16}H_{25}N_2O^+$; calc. 261.1967. Anal. calc. for $C_{16}H_{24}N_2O \cdot 0.5\ H_2O$: C 71.34, H 9.35, N 10.40; found: C 70.96, H 9.22, N 10.61.

tert-*Butyl (3a*S,*4*S,*6*R,*6a*R*)-4-{3-[(1*E*)-3-{[4-(1-Benzoylpiperidin-4-yl)butyl]amino}-3-oxoprop-1-en-1-yl]phenyl}-6-({[(*tert*-butyl)(dimethyl)silyl]oxy}methyl)tetrahydro-2,2-dimethyl-5*H*-[1,3]dioxolo[4,5-*c*]pyrrole-5-carboxylate* ((−)-**9**). To a soln of **8** (92.2 mg, 354 μmol) in dry toluene (550 μl), (+)-**7** (135 mg, 246.5 μmol) and HOAt (7 mg, 55 μmol) were added, followed by the addition of $(t\text{-BuO})_4Zr$ (21 μl, 55 μmol) under Ar. The mixture was stirred at 100° for 12 h and cooled down to r.t. MeOH (1 ml) and CH_2Cl_2 (1 ml) were added, and the soln. was filtered through SiO_2. Solvents were evaporated, and the residue was purified by FC (SiO_2; 50% AcOEt in PE) to afford (−)-**9** (116 mg, 60%). White foam. $[\alpha]_D^{25} = -9.5$, $[\alpha]_{577}^{25} = -21$, $[\alpha]_{435}^{25} = -34$, $[\alpha]_{405}^{25} = -53$ ($c = 0.052$ g/100 ml, MeCN). UV (MeCN): 271 (26′800). IR (neat): 3300, 2925, 1695, 1610, 1365, 1260, 1065, 800. ^{1}H-NMR (400 MHz, 323 K, $CDCl_3$): 7.58 (*d*, $^3J(1'',2'') = 15.6$, H–C(1″)); 7.41 – 7.27 (*m*, 9 arom. H); 6.34 (*d*, $^3J(2'',1'') = 15.6$, H–C(2″)); 5.63 (*m*, NH); 4.90 (*m*, H–C(4)); 4.67 (*d*, $^3J = 5.5$, H–C(3a)); 4.60 (*dd*, $^3J = 5.5$, 3.2, H–C(6a)); 4.25 (*m*, H–C(6)); 3.85 (*m*, CH_2(1′)); 3.37 (*dd*, $^3J = 6.9$, 13.1, $NHCH_2$); 2.85, 1.72 (2*m*, $(CH_2)_2NCO$); 1.58, 1.35 (2*s*, Me_2C); 1.62 – 1.06 (*m*, CO_2CMe_3, $NHCH_2CH_2CH_2CH(CH_2)_2$); 0.84 (*s*, *t*-Bu); 0.04, 0.02 (2*s*, Me_2Si). ^{13}C-NMR (100.6 MHz, $CDCl_3$): 170.1; 165.8; 154.5; 140.4; 136.2; 129.3; 128.3; 126.6; 126.0; 125.4; 120.8; 111.8; 87.0; 81.2; 79.9; 69.2; 65.0; 63.0; 47.9; 39.4; 35.9; 31.8; 29.7; 23.8; 32.7; 27.9; 25.7; 25.5; 18.1; −5.6. HR-ESI-TOF-MS: 776.4665 ($[M+H]^+$, $C_{44}H_{66}N_3O_7Si^+$; calc. 776.4670). Anal. calc. for $C_{44}H_{65}N_3O_7Si$: C 68.09, H 8.44, N 5.41, found: C 67.97, H 8.34, N 5.37.

tert-*Butyl (3a*S,*4*S,*6*R,*6a*R*)-4-{3-[(1*E*)-3-{[4-(1-Benzoylpiperidin-4-yl)butyl]amino}-3-oxoprop-1-en-1-yl]phenyl}tetrahydro-6-(hydroxymethyl)-2,2-dimethyl-5*H*-[1,3]dioxolo[4,5-*c*]pyrrole-5-carboxylate* ((−)-**10**). TBAF (1M in THF, 193 μl, 193 μmol) was added dropwise to a soln. of (−)-**9** (83.3 mg, 107.3 μmol) in THF (550 μl). The mixture was stirred for 1 h at r.t., and MeOH (550 μl) was added. Solvents were evaporated, and the residue was purified by FC (SiO_2; 60 to 100% AcOEt in PE) to afford (−)-**10** (65.3 mg, 92%). Light pink solid. M.p. 98 – 104°. $[\alpha]_D^{25} = -8$, $[\alpha]_{577}^{25} = 0$, $[\alpha]_{435}^{25} = -10$, $[\alpha]_{405}^{25} = -17$ ($c = 0.052$ g/100 ml, MeCN). UV (MeCN): 271 (14′900). IR (neat): 2925, 1610, 1260, 1015, 790. ^{1}H-NMR (400 MHz, 323 K, $CDCl_3$): 7.56 (*d*, $^3J(1',2') = 15.6$, H–C(1′)); 7.50 – 7.23 (2*m*, 9 arom. H); 6.40 (*d*, $^3J(2',1') = 15.6$, H–C(2′)); 6.01 (br. *s*, NH); 4.98 (*m*, H–C(4)); 4.62 – 4.51 (2*m*, H–C(3a), H–C(6a)); 4.29 (*m*, H–C(6)); 3.87, 3.74 (2*dd*, CH_2OH); 3.34 (*dd*, $^3J = 6.7$, 12.5, $NHCH_2$); 2.86, 1.65 (2*m*, $(CH_2)_2NCO$); 1.57, 1.32 (2*s*, Me_2C); 1.56 – 1.06 (*m*, CO_2CMe_3, $NHCH_2CH_2CH_2CH_2CH(CH_2)_2$). ^{13}C-NMR (100.6 MHz, $CDCl_3$): 170.3; 165.9; 155.8; 142.0; 140.4; 136.2; 135.1; 129.4; 128.9; 128.3; 127.0; 126.7; 124.4; 121.1; 112.2; 86.6; 81.2; 80.8; 68.7; 65.9; 64.2; 42.5; 39.5; 35.9; 32.8; 31.9; 29.8; 29.6; 23.9; 28.0; 27.5; 25.4. HR-ESI-TOF-MS: 662.3802 ($[M+H]^+$, $C_{38}H_{52}N_3O_7^+$; calc. 662.3805). Anal. calc. for $C_{38}H_{51}N_3O_7 \cdot H_2O$: C 67.13, H 7.86, N 6.18; found: C 67.42, H 7.92, N 5.94.

*(2*E*)-*N*-[4-(1-Benzoylpiperidin-4-yl)butyl]-3-{3-[(2*S,*3*S,*4*R,*5*R*)-3,4-dihydroxy-5-(hydroxymethyl)-pyrrolidin-2-yl]phenyl}prop-2-enamide Hydrochloride* ((−)-**1** · HCl). To a soln. of (−)-**10** (53 mg, 80 μmol) in i-PrOH (1 ml) was added a 5N soln. of HCl in i-PrOH (800 μl) dropwise. The mixture was stirred at 20° for 24 h, and the solvents were evaporated to afford (−)-**1** · HCl (42 mg, 94%). White solid.

M.p. 104–107°. ^{1}H-NMR (400 MHz, CD_3OD): 7.83–7.32 (*m*, 10 arom. H, CONH), 7.55 (*d*, $^3J(1',2') = 15.8$, H–C(1′)); 6.69 (*d*, $^3J(2',1') = 15.8$, H–C(2′)); 4.70–4.48 (*m*, H–C(2), H–C(3)); 4.59, 2.83 (2*m*, $NHCH_2$); 4.27 (*m*, H–C(4)); 3.92 (*m*, CH_2OH), 3.70 (*m*, H–C(5)); 3.70, 3.11, 1.86, 1.69 (4*m*, $(CH_2)_2NCO$); 1.65–1.06 (*m*, $NHCH_2CH_2CH_2CH_2CH(CH_2)_2$). ^{13}C-NMR (100.6 MHz, CD_3OD): 172.3; 168.2; 140.5; 137.3; 137.1; 135.2; 131.0; 130.9; 130.8; 129.7; 129.6; 129.2; 127.8; 123.3; 112.2; 76.1; 73.1; 67.8; 65.5; 60.2; 49.0; 43.8; 40.5; 37.1; 33.9; 30.1; 25.2; 25.0. HR-ESI-TOF-MS: 522.2960 ($[M+H]^+$, $C_{30}H_{40}N_3O_5^+$; calc. 522.2968). Anal. calc. for $C_{30}H_{39}N_3O_5 \cdot HCl \cdot 1.5\ H_2O$: C 61.58, H 7.41, N 7.18; found: C 61.84, H 7.27, N 6.88.

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