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The Synthesis of a GH-Releasing Compound NNC 26-1089

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Abstract:

The synthesis of trimethylhydrazine hydrochloride provides a stable and safe-to-handle reactant for multigram-scale synthesis making the preparation of the tripeptidelike Novo Nordisk growth hormone secretagogue NNC 26-1089 in multigram scale feasible. NNC 26-1089 was synthesised over seven steps in an overall yield of 33%, with a stereoisomeric purity of more than 99% measured by chiral capillary electrophoresis (CE).

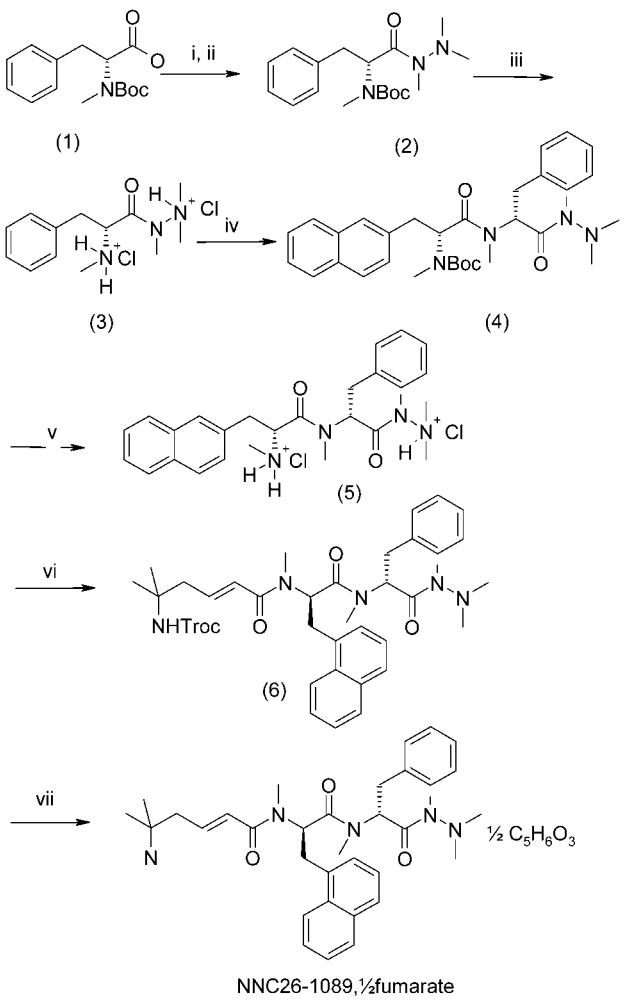
Introduction

The growth hormone secretagogue NNC 26-1089 posseses two chiral centers. It is formed using trimethylhydrazine and three unnatural amino acid building blocks, (2*R*)-2-(*tert*-butoxycarbonylmethylamino)-3-phenyl propionic acid, 2(*R*)-(*N*-*tert*-butoxycarbonyl-*N*-methylamino)-3-(naphth-2-yl)propionic acid, and (5*E*)-amino-5-methylhex-2-enoic acid. Recently, Ankersen et al. have published the synthesis of NNC 26-1089,[1] but the synthesis suffers from a very low overall yield and tedious workup procedures often involving purification by column chromatography. The stereoisomeric purity was not determined.

Now, we present a novel method of producing NNC 26-1089, which can be used to synthesize multigram quantities of material in high sterioisomeric purity. The final compound NNC 26-1089 is isolated as its fumarate salt as shown below in Scheme 1. We report here a new method of the NNC 26-1089 synthesis and the determination of the stereoisomeric purity.

One of the difficulties with the formation of NNC 26-1089 was the synthesis of trimethylhydrazine. Several procedures for the synthesis of trimethylhydrazine are described in the literature,[2−5] but in the last step, they all suffer from low yield, low product purity, and tedious handling procedures both from a health, safety, and technical perspective. One has to consider the carcinogenic nature,[6,7] the low boiling point,[8] and limited stability of trimethylhydrazine. We found a novel technique of isolating trimethylhydrazine hydrochloride.[9]

Scheme 1. **Synthesis of NNC 26-1089, ½fumarate**[a]



[a] i) Isobutylchloroformate, NMM, EtOAc, ii) trimethylhydrazine-HCl, DMF (80%), iii) EtOAc, HCl (80%), iv) Boc-*N*-Me-2-D-Nal, EDC·HCl, HOOBt, NMM, DMA (91%), v) HCl, EtOAc (86%), vi) (**10**), NMM, EtOAc, water (100%), vii) Zn, AcOH, EtOAc (100%), viii) fumaric acid, EtOAc (82%).

Trimethylhydrazine was synthesized according to a modified version of Bisselle and Beltrami.[4] Bisselle and Beltrami described the formation of trimethylhydrazine by the addition of $LiAlH_4$ to 1,1-dimethyl-2-formylhydrazine in ether. After reduction of 1,1-dimethyl-2-formylhydrazine this method involved a quench of the reaction mixture with 6 M HCl, forming trimethylhydrazine hydrochoride in water. The organic phase was then either removed by distillation or by

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phase separation. Next, the acidic solution containing trimethylhydrazine was concentrated and added to a hot solution of 6 M NaOH, which liberated the trimethylhydrazine salt. The formed trimethylhydrazine was then distilled two to three times over solid NaOH or CaH_2 to remove water and to purify the product. The isolated, volatile product quickly turned yellow and had to be kept under nitrogen. In our hands, the use of trimethylhydrazine stored as the free base afforded poorer acylation results over time. The formation of dimethylhydrazine and tetramethylhydrazine is suspected.

To avoid unnecessary handling and decomposition of trimethylhydrazine, the completed $LiAlH_4$/1,1-dimethyl-2-formylhydrazine reaction was instead quenched directly with MeOH, and the reaction mixture was placed into a Vigreux condenser. The receiving flask was charged with HCl in MeOH and precooled to −70 °C. Then the reaction mixture was distilled, and the collected distillate was concentrated in vacuo, affording trimethylhydrazine as its monohydrochloride salt in 90% yield and in high purity. Trimethylhydrazine hydrochloride appears to be a very hygroscopic, semicrystalline oil. When protected from moisture, trimethylhydrazine hydrochloride has been stored in the refrigerator more than 12 months without loss of acylating potential.[10] Compared to known carboxylic acid salts, as stated in ref 9, the hydrochloride salt of trimethyhydrazine will not have the possibility to interfere with its counterion (the carboxylic acid) during amide bond-forming reactions.

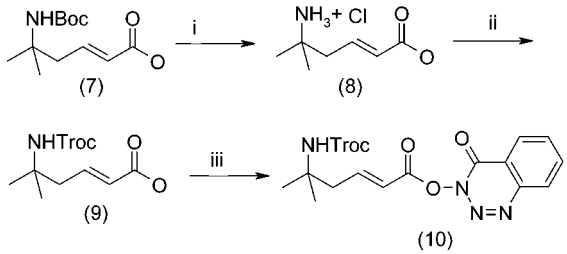
The addition of trimethylhydrazine to *N*-Boc-*N*-Me-D-Phe (**2**) was performed with isobutyl chloroformate in the usual manner instead of utilizing the far more expensive *N*-ethyl-*N*′-(3-dimethylaminopropyl)carbodiimide·HCl(EDAC)/1-hydroxy-7-azabenzotriazol(HOAt) peptide-coupling method. Trimethylhydrazine was used, as its hydrochloride salt was liberated in situ with *N*-methylmorpholine (NMM).

Conveniently, the Boc-group of **2** was removed with HCl in EtOAc, and **3** could easily be isolated as a white, crystalline precipitate in high purity. Inspired by Nozaki,[11] we discovered that the usage of 3,4-dihydro-3-hydroxy-4-oxo-1,2,3-benzotriazine (HOOBt) as coupling agent was as effective as HOAt in the formation of **4**. Here, 1 mol equiv of HOOBt was used. When compared to HOOBt, HOAt is very expensive. It is also worth noting that Applied Biosystems recently has suspended general shipping of HOAt products, pending completion of testing to determine whether HOAt should be reclassified as an explosive category substance pursuant to U.S. Department of Transportation (DOT) regulations and United Nations guidelines. HOAt is currently classified as a highly flammable solid.

The Boc-group of **4** was removed with HCl in EtOAc as used before, furnishing white crystals of **5** in high purity.

We decided to isolate the active ester of the N-terminal amino acid **10** before final coupling as given in Scheme 2. Thereby, we obtained a very crystalline and easy to purify intermediate and a purer end product. The N-terminal amino

Scheme 2. **Synthesis of the N-terminal amino acid 10**[a]



[a] i) HCl, EtOAc (94%), ii) TrocOSu, $NaHCO_3$−NaOH buffer pH 9.5, 2-PrOH (100%), iii) DCC, HOOBt, EtOAc, then 2-PrOH (70%).

acid **10** was synthesised from **7**[12,13] as shown below in overall 66% yield. It can be recrystallised in MeOH, EtOH, or 2-PrOH without reacting with the solvent.

One of the other reasons for using **10** instead of **9** for the next condensation reaction was the nature of **9**. The N-protected amino acid **9** possessed one major technical drawback for larger-scale synthesis: It was difficult to crystallise/recrystallise. Either it appeared to be too soluble in most solvents, or it gave an oily precipitate upon cooling (which then crystallised slowly).

The carboxylic acid **9** was also prepared by addition of TrocCl instead of succinimidyl 2,2,2-trichloroethyl carbonate (TrocOSu), but 2 equiv was necessary to complete the reaction. We observed a tendency towards oligomerisation of **9**, when applying TrocCl. When synthesising **10** from **9**, we found it to be crucial not to use DCC in excess, since this led to impurities, which were difficult to remove. We observed the dimeric product of HOOBt as described by König and Geiger.[14] Furthermore, we discovered it to be important not to warm the reaction mixture during EtOAc removal in the work up. Otherwise, we noticed the buildup of acetic acid HOOBt ester. As an alternative to EtOAc, DCM may be used.

The condensation of **5** and **10** was performed simply by adding the two components into a 1:1 mixture of water and EtOAc containing NMM. We observed no hydrolysis of **10**, even though the reaction was performed at 50−60 °C. The Troc-protected compound **6** appeared to be a very sticky oil, which made the handling and further operations difficult. However, the addition of **6** in EtOAc to Zn/AcOH had the advantage that **6** did not need to be isolated. Zn in AcOH/EtOAc removed the Troc-group of **6** as well as the utilization of pure AcOH. The rate of reaction in pure AcOH was slightly faster. To increase the rate of reaction, EtOAc can be distilled off after addition of **6** is completed. Finally, NNC 26-1089 was isolated as its fumarate salt in good yield. We tested the deprotection of **6** containing a Boc group (instead of a Troc group) as described by Ankersen et al. An extensive degradation of the wanted product under usual Boc deprotection conditions (TFA/CH_2Cl_2 or EtOAc/HCl) was observed. On a larger scale the yield was usually poor. Previously, peptides containing tertiary amide bonds have

(10) ^{1}H NMR in DMSO after 1 year was identical with the original spectra.
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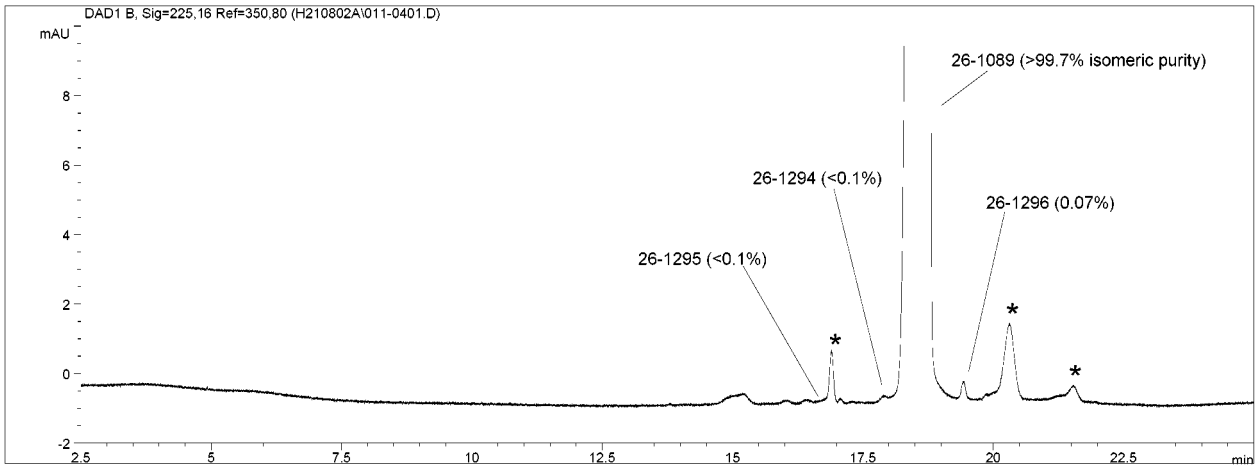

Figure 1. **Electropherogram of NNC 26-1089 and isomers. The expansion of an electropherogram shows the chiral capillary electrophoresis analysis of NNC 26-1089. Electrolyte and conditions are given in the Experimental Section. To identify the position (migration time) of the opposite enantiomer (26-1295) and the two isomers (26-1294 and 26-1296) NNC 26-1089 was spiked with appropriate amounts of each. The stereoisomeric purity of NNC 26-1089, as indicated in the figure, was calculated as area % with the assumption of equal UV molar absorption of all four isomers. The peaks marked with * are unidentified impurities.**

been shown to undergo rapid degradation during removal of a Boc group.[15] This was also the case here.

Finally, the chiral purity of **6** was determined using capillary electrophoresis (CE) as separation technique. CE has over the past decade proven to be an analytical technique that can achieve rapid and high-resolution separations of a variety of compounds in small sample volumes.[16] CE can be particularly useful in chiral analysis, and the inherent simplicity of the CE approach offers great potential for chiral resolutions.[17]An electropherogram of NNC 26-1089 and its position compared to those of the isomers is given in Figure 1. The result was a stereoisomeric purity of 99.7%.

Experimental Section

General Methods. All chemicals were purchased from Sigma Aldrich, Fluka, and Synthetech. 1,1-Dimethyl-2-formylhydrazine was prepared according to the procedure by Bisselle and Beltrami. Melting points were detected on a Buchi melting point B-545 and were uncorrected. ^{1}H and ^{13}C NMR were recorded on a Bruker 400 MHz, and chemical shifts (δ) are in ppm relative to TMS. Elemental analyses were obtained from Novo Nordisk Microlab. The LC−MS analyses were performed on a PE Sciex API 100 LC/MS System using a YMC 3 mm × 50 mm YMC-pack ODS-A column, which was eluted at 1 mL/min and positive ionspray with a flow rate of 20 μL/min. The column was equilibrated with 5% (ACN + 0.1% TFA) in an aqueous solution of TFA in water (0.1%). After injection, the sample was eluted by a gradient from 5 to 90% ACN in 0.1% aqueous TFA during 10 min.

HPLC (Method A1). The RP analysis was performed using a Waters 2690 systems fitted with a Waters 996 diode array detector. UV detections were collected at 214, 254, 276, and 301 nm on a 218TP54 4.6 mm × 250 mm 5μ C-18 silica column (The Separations Group, Hesperia), which was eluted at 1 mL/min at 42 °C. The column was equilibrated with 5% acetonitrile in a buffer consisting of 0.1 M ammonium sulfate, which was adjusted to pH 2.5 with 4 M sulfuric acid. After injection, the sample was eluted by a gradient of 5 to 60% acetonitrile in the same aqueous buffer during 50 min.

HPLC (Method B1). The RP analysis was performed using a Waters 2690 systems fitted with a Waters 996 diode array detector. UV detections were collected at 214, 254, 276, and 301 nm on a 218TP54 4.6 mm × 250 mm 5 μ C-18 silica column (The Separations Group, Hesperia), which was eluted at 1 mL/min at 42 °C. The column was equilibrated with 5% acetonitrile (+ 0.1% TFA) in an aqueous solution of TFA in water (0.1%). After injection, the sample was eluted by a gradient of 5 to 60% acetonitrile (+ 0.1% TFA) in an aqueous solution of TFA in water (0.1%) during 50 min.

HPLC (Method h8). The RP analysis was performed using a Waters 600S system fitted with a Waters 996 diode array detector. UV detections were collected using a Waters 3 mm × 150 mm 3.5 μ C-18 Symmetry column. The column was heated to 42 °C and eluted with a linear gradient of 5−90% acetonitrile, 85−0% water, and 10% trifluoroacetic acid (0.5%) in water over 15 min at a flow rate of 1 mL/min.

HPLC (Method A: pH3). The RP analysis was performed using a Merck Hitachi system using a RP-18 4 mm × 250 mm column, packed in-house with YMC 120 Å, 5 μm. The column was heated to 35 °C and eluted at a flow rate of 0.6 mL/min or 0.9 mL/min (individually described for each chromatogram) with eluent A: water (175 mL) and triethylamine (1.1 mL), mixed while stirring. The pH was adjusted to 3 with 10% phosphoric acid. Water was added to a total of 200 mL, followed by methanol (1800 mL) while stirring.

HPLC (Method B: pH3). The RP analysis was performed using a Merck Hitachi system using a RP-18 4 mm × 250 mm column, packed in-house with YMC 120 Å, 5 μm. The column was heated to 35 °C and eluted at a flow rate of 0.6 or 0.9 mL/min (individually described for each chromato-

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gram) with eluent B: water (600 mL) and triethylamine (5.5 mL), mixed while stirring. The pH was adjusted to 3 with 10% phosphoric acid. Water was added to a total of 700 mL followed by ACN (1300 mL) while stirring.

The sterioisomeric purity of NNC 26-1089 was determined by a chiral capillary electrophoresis (CE) analytical system developed for the purpose.

Capillary Electrophoresis. The CE analyses were performed on a $HP^{3D}CE$ capillary electrophoresis instrument (Agilent, Waldborn, Germany) equipped with an auto sampler, a capillary cartridge, a high-voltage power supply, a diode array detector, electrodes, and a hydrostatic injection system. The electrophoretic data system was the HP Chemstation software, and the data were collected with a frequency of 10 Hz. The CE separations were carried out with untreated fused-silica capillaries from Agilent with the following dimensions: 80.5 cm total length with 72.5 cm effective length, 50 μm inner diameter, and extended light path with an inner diameter of 150 μm at the detector window. The electrolyte was prepared by dissolving 2.0% (w/v) sulfobuthyl ether-β-cyclodextrin (Advasep 4, Cydex, Inc., Overland Park, KS) in phosphate buffer pH 2.5 (Agilent) followed by filtering through a 0.45 μm polypropylene filter. To this solution 10% (v/v) acetonitrile was added to give the final electrolyte. Since the electrophoretic mobility of the complex between the analyzed compounds and the negatively charged cyclodextrin was stronger than the analyzed compounds alone the polarity had to be reversed in order to make them pass the detector.

The electrophoretic conditions were the following: voltage −30 kV, current −47 μA, capillary temperature controlled at 30 °C, injection 50 mbar for 8.0 s, detection UV at 225 nm with reference of 350 nm. The sample concentration was 2.0 mg/mL in 50% acetonitrile/5 mM phosphate buffer pH 2.5. The capillary was conditioned with 0.1 N NaOH for 20 min daily and flushed with electrolyte for 2.5 min between each run.

For analysis purposes, the following isomers of NNC 26-1089 were synthesized according to the procedures described below.

26-1294: (2*E*)-5-amino-5-methylhex-2-enoic acid *N*-methyl-*N*-((1*S*)-1-{*N*-methyl-*N*-[(1*R*)-2-phenyl-1-(*N*,*N*′,*N*′-trimethylhydrazinocarbonyl)ethyl]carbamoyl}-2-(2-naphthyl)ethyl)-amide, 26-1295: (2*E*)-5-Amino-5-methylhex-2-enoic acid *N*-methyl-*N*-((1*S*)-1-{*N*-methyl-*N*-[(1*S*)-2-phenyl-1-(*N*,*N*′,*N*′-trimethylhydrazinocarbonyl)ethyl]carbamoyl}-2-(2-naphthyl)ethyl)amide, and 26-1296: (2*E*)-5-Amino-5-methylhex-2-enoic acid *N*-methyl-*N*-((1*R*)-1-{*N*-methyl-*N*-[(1*S*)-2-phenyl-1-(*N*,*N*′,*N*′-trimethylhydrazinocarbonyl)ethyl]carbamoyl}-2-(2-naphthyl)ethyl)amide. All isomers were purified by reverse phase HPLC. (2*R*)-2-(*tert*-Butoxycarbonylmethylamino)-3-phenyl propionic acid and 2(*R*)-(*N*-*tert*-butoxycarbonyl-*N*-methylamino)-3-(naphth-2-yl)propionic acid were provided by Synthetech.

***N*,*N*,*N*′-Trimethylhydrazine Hydrochloride (1).** A reactor was charged with $LiAlH_4$ (19.0 g, 499 mmol) and dry THF (250 mL) To the vigorously stirred suspension, a solution of 1,1-dimethyl-2-formylhydrazine (40.0 g, 454 mmol) in dry THF (250 mL) was added over 1 h. The reaction mixture was stirred overnight at room temperature. The reactor was equipped with a Vigreux condenser precharged with 4.8 M HCl in MeOH (350 mL) in the receiving flask. The flask was cooled to −70 °C. Quenching with MeOH (200 mL) in THF (200 mL) and subsequent distillation of the mixture at atmospheric pressure at 60−65 °C afforded a distillate, which was concentrated in vacuo and dried under vacuum overnight to afford 45.2 g (409 mmol, 90%) of **1** as semicrystalline colourless oil. The very hygroscopic salt is stable for minimum 1 year if kept cool and dry in a closed container. Anal. Calcd for $C_3H_{11}ClN_2$: C, 32.58; H, 10.03; Cl, 32.06; N, 25.33. Found: C, 29.68; H, 9.91; Cl, 32.39; N, 23.10. ^{1}H NMR (DMSO): $\delta = 2.61$ (s, 3H, CH_3), 2.74 (s, 6H, 2 × CH_3), 7.50−12.0 (b, 1.8H). ^{13}C NMR (DMSO): $\delta = 30.57$, 42.77 (2C).

***N*-Methyl-*N*-[(1*R*)-2-phenyl-1-(*N*,*N*′,*N*′-trimethylhydrazinocarbonyl)ethyl]carbamic Acid *tert*-Butyl Ester (2).** A reactor was charged with (67.0 g, 239.9 mmol) of (2*R*)-2-(*tert*-butoxycarbonylmethylamino)-3-phenylpropionic acid dissolved in EtOAc (330 mL). The solution was cooled to −15 °C, and NMM (26.4 mL, 239.9 mmol) was added. After 10 min, −15 °C cold isobutylchloroformate (31.4 mL, 239.9 mmol) was added dropwise, maintaining a temperature between −15 and −13 °C. The mixture was stirred for 10 min at −15 °C. Then NMM (39.6 mL, 359.8 mmol) was added immediately, followed by a solution of **1** (39.8 g, 359.8 mmol) in dry DMF (330 mL), which was added over a few minutes. The temperature was kept below −10 °C during addition. The reaction mixture was stirred for 3 h at −15 °C, then overnight at room temperature. The next day the reaction mixture was cooled to −10 °C, and precipitation was removed by filtration. The filtrate was diluted with EtOAc (1300 mL) and washed with 10% aqueous $NaHSO_4$ (700 mL). The aqueous layer was extracted with EtOAc (1300 mL). The combined organic phases were washed with water (700 mL), saturated $NaHCO_3$ (700 mL), water (700 mL), and saturated NaCl (700 mL). The organic phase was dried over $MgSO_4$, filtered, and then concentrated in vacuo to afford yellow oil. Addition of heptane (700 mL) to the oil and cooling for 1 h in the freezer furnished white crystals. The crystals were removed by filtration, washed with cold heptane, and dried under vacuum at 40 °C to give 64.4 g (192.3 mmol, 80%; mp 79−81 °C) of **2**. Anal. Calcd for $C_{18}H_{29}N_3O_3$: C, 64.45; H, 8.71; N, 12.53. Found: C, 64.75; H, 8.93; N, 12.55. ^{1}H NMR (DMSO) major rotamer: $\delta =$ 1.16 (s, 9H, *tert*-butyl), 2.37 (s, 3H, CH_3), 2.45 (s, 3H, CH_3), 2.68 (s, 3H, CH_3), 2.79 (s, 3H, CH_3), 2.84 (m, 2H, CH_2), 5.38 (t, 1H, $J = 7.0$, 7.5, CH), 7.13−7.31 (m, 5H). ^{13}C NMR ($CDCl_3$), major rotamer: $\delta = 22.01$, 27.63 (3C), 29.55, 34.69, 42.61 (2C), 56.58, 77.99, 125.95, 128.01 (2C), 129.08 (2C), 138.70, 154.21, 171.48. ESI-MS: $m/z = 358.2$ $[M + Na]^+$, 336.0 $[M + H]^+$. LC−MS: $R_t = 6.24$ min. $[M + H]^+ = 336$. HPLC (h8): $R_t = 13.10$ min.

(2*R*)-2-Methylamino-3-phenylpropionic Acid Trimethylhydrazide, Dihydrochloride (3). A reactor was charged

with **2** (63.3 g, 189 mmol) in EtOAc (800 mL). HCl gas was added for 3 min via the gas inlet. The addition of HCl gas was repeated after 20 and 40 min. Stirring was continued for 4 h. White crystals of product were formed. The reactor was then flushed with nitrogen, and the reaction mixture was concentrated in vacuo to remove excess of HCl. The product was removed by filtration, washed with EtOAc, and dried under vacuum at 40 °C to give 46.8 g (80%; mp 216−220 °C dec) of **3** as white crystals. Anal. Calcd for $C_{13}H_{23}Cl_2N_3O$: C, 50.65; H, 7.52; Cl, 23.00; N, 13.63. Found: C, 50.56; H, 7.75; Cl, 22.58; N, 13.53. ^{1}H NMR (DMSO) major rotamer: δ = 1.74 (s, 3H, CH_3), 2.44−2.47 (m, 6H, 2 × CH_3), 2.75 (s, 3H, CH_3), 3.02 (dd, 1H, J = 9.0, 13.1 Hz), 3.28 (dd, 1H, J = 4.5 Hz, 13.1), 4.80 (m, 1H, J = 4.5, 8.5 Hz, CH), 6.75−7.15 (b, 3H), 7.19−7.35 (m, 5H). ^{13}C NMR (DMSO) major rotamer: δ = 22.57, 31.27, 35.59, 42.01, 43.33, 57.71, 127.28, 128.73 (2C), 129.62 (2C), 135.18, 168.49. ESI-MS: m/z = 236.1 $[M + H]^+$. LC−MS: R_t = 3.70 min, $[M + H]^+$ = 236. HPLC (h8): R_t = 6.80 min.

***N*-Methyl-*N*-((1*R*)-1-{*N*-methyl-*N*-[(1*R*)-2-phenyl-1-(*N*,*N*′,*N*′-trimethylhydrazinocarbonyl)ethyl]carbamoyl}-2-(2-naphthyl)ethyl)carbamic Acid *tert*-Butyl Ester (4).** In a reactor, 2-(*R*)-(*N*-*tert*-butoxycarbonyl-*N*-methylamino)-3-(naphth-2-yl)-propionic (44.9 g, 136 mmol) acid was dissolved in DMA (250 mL). Then HOOBt (22.2 g, 136 mmol) and NMM (15.0 mL, 136 mmol) was added to the solution at room temperature followed immediately by EDAC (26.1 g, 136 mmol), NMM (31.5 mL, 286 mmol), and **3** (42.0 g, 136 mmol). The reaction mixture was stirred overnight at room temperature. The next day, EtOAc (750 mL) was added and washed with 10% $NaHSO_4$ (300 mL). The aqueous layer was extracted with EtOAc (500 mL). The combined organic extracts were washed with water (100 mL), saturated $NaHCO_3$ (300 mL), water (100 mL), and saturated NaCl. (300 mL). The resulting organic phase was dried over $MgSO_4$, filtered, and concentrated in vacuo to afford 67.9 g (91%) of crude **4** as yellow oil. The material was used in the next step without further purification. Anal. Calcd for $C_{32}H_{42}N_4O_4$: C, 70.30; H, 7.74; N, 10.25. Found: C, 70.19; H, 7.90; N, 10.12. LC−MS: R_t = 8.21 min, $[M + H]^+$ = 547. HPLC (h8): R_t = 16.08 min. ^{1}H NMR (DMSO) major rotamer: δ = 0.85 (s, 9H, *tert*-butyl), 2.10 (s, 3H, CH_3), 2.43 (s, 3H, CH_3), 2.46 (s, 3H, CH_3), 2.77 (s, 3H, CH_3), 2.91 (m, 2H, CH_2), 2.95 (s, 3H, CH_3), 3.05 (m, 2H, CH_2), 5.00 (dd, 1H, CH, J = 4.6, 10.6 Hz), 6.19 (dd, 1H, J = 5.1, 10.7 Hz), 7.18−7.84 (m, 12H). ^{13}C NMR (DMSO), selected peaks: δ = 21.88, 27.16 (3C), 27.64, 30.65, 34.27, 34.72, 42.61, 42.71, 53.89, 57.63, 78.51, 125.24, 125.78, 126.34, 127.31, 127.66, 128.12, 128.18, 128.30 (2C), 128.58 (2C), 128.70, 131.78, 132.90, 135.90, 138.04, 153.27, 169.80, 172.10.

(2*R*)-*N*-Methyl-2-(methylamino)-3-(2-naphthyl)-*N*-[(1*R*)-2-phenyl-1-(*N*,*N*′,*N*′-Trimethylhydrazinocarbonyl)ethyl]-propionamide, Dihydrochloride (5). A reactor was charged with **4** (67.9 g, 124 mmol) in EtOAc (700 mL). HCl gas was added for 10 min. Addition of HCl gas was repeated again after 30 min. Stirring was continued for further 30 min, and a white precipitate formed. After completion of the reaction, the mixture was flushed with nitrogen and concentrated in vacuo to remove excess of HCl gas. The product was removed by filtration, washed with EtOAc, and dried under vacuum at 40 °C to afford 55.5 g (86%, mp 221−223 °C dec) of **5**. Anal. Calcd for $C_{27}H_{36}Cl_2N_4O_2$: C, 62.46; H, 6.98; Cl, 13.65; N, 10.78. Found: C, 62.16; H, 7.10; Cl, 13.32; N, 10.68. LC−MS: R_t = 5.00 min, $[M + H]^+$ = 447. HPLC (h8): R_t = 9.76 min, purity 98.2% (214 nm). ^{1}H NMR (DMSO) selected peaks: δ = 2.02 (t, 3H, CH_3, J = 3.9 Hz), 2.34 (s, 3H, CH_3), 2.40 (s, 3H, CH_3), 2.75 (s, 3H, CH_3), 2.93 (s, 3H, CH_3), 2.97 (m, 2H, CH_2), 3.28 (m, 2H, CH_2), 4.60 (m, 1H, CH), 6.34 (dd, 1H, J = 5.9, 9.8 Hz), 7.18−7.89 (m, 12H), 8.63 (b, 1H, NH), 9.78 (b, 1H, NH). ^{13}C NMR (DMSO) selected peaks: δ = 22.78, 31.90, 31.92, 34.90, 36.17, 43.56, 43.75, 53.49, 59.47, 126.76, 126.97, 127.32, 128.37, 128.40, 128.57, 128.73, 129.20 (2C), 129.34, 129.56 (2C), 132.38, 133.07, 133.72, 138.40, 168.31, 172.07.

{(3*E*)-1,1-Dimethyl-4-[*N*-methyl-*N*-((1*R*)-1-{*N*-methyl-*N*-[(1*R*)-2-phenyl-1-(*N*,*N*′,*N*′-trimethylhydrazinocarbonyl)ethyl]carbamoyl}-2-(2-naphthyl)ethyl)carbamoyl]but-3-enyl}carbamic Acid 2,2,2-Trichloroethyl Ester (6). A reactor was charged with a suspension of **5** (55.6 g, 107 mmol) in water (350 mL) and EtOAc (350 mL). Then NMM (24.8 mL, 225 mmol) was added, and after 5 min **10** (49.7 g, 107 mmol) was added. The reaction mixture was stirred for 10 min, and additional NMM (11.8 mL, 107 mmol) was added. The next day, the reaction mixture was diluted with EtOAc (200 mL). The phases were separated, and the aqueous layer was extracted with EtOAc (350 mL). The combined organic extracts were washed with 10% $NaHSO_4$ (300 mL), water (150 mL), saturated $NaHCO_3$ (300 mL), and saturated NaCl (150 mL). The washed organic phase was dried over $MgSO_4$, filtered, and concentrated in vacuo to afford crude **6** as yellow oil. The material was taken to the next step without further purification. A yield of 100% was anticipated. LC−MS: R_t = 8.07 min, $[M + H]^+$ = 748. HPLC (h8): R_t = 15.54 min, purity 90.8% (214 nm).^{1}H NMR (DMSO) selected peaks: δ = 1.17 (s, 6H, 2 × CH_3), 2.18 (s, 3H, CH_3), 2.34 (s, 3H, CH_3), 2.38 (s, 3H, CH_3), 2.49 (m, 2H, CH_2), 2.69 (s, 3H, CH_3), 2.82 (m, 1H, CH), 2.83 (s, 3H, CH_3), 3.00 (m, 2H, CH_2, J = 5.4, 14.2 Hz), 3.20 (dd, 1H, CH, J = 6.9, 13.7 Hz), 5.62 (t, 1H, J = 7.3 Hz), 4.75 (s, 2H, CH_2), 6.04 (dd, 1H, CH, J = 5.4, 10.3 Hz), 6.12 (d, 1H, J = 15.2 Hz), 6.45 (dt, 1H, J = 7.6, 15.2 Hz), 7.16−7.89 (m, 12H). ^{13}C NMR (DMSO) selected peaks: δ = 22.82, 27.48, 27.55, 30.46, 31.77, 35.20, 35.71, 42.54, 43.15, 43.51, 43.63, 53.02, 55.02, 55.23, 73.67, 97.35, 123.83, 126.18, 126.70, 127.15, 128.17, 128.25, 128.40, 128.84, 129.06 (2C), 129.69 (2C), 132.56, 133.78, 136.50, 139.03, 142.54, 153.52, 165.60, 170.05, 172.42.

(2*E*)-5-Amino-5-methylhex-2-enoic Acid *N*-Methyl-*N*-((1*R*)-1-{*N*-methyl-*N*-[(1*R*)-2-phenyl-1-(*N*,*N*′,*N*′-trimethylhydrazinocarbonyl)ethyl]carbamoyl}-2-(2-naphthyl)-ethyl)amide·$^1/_2$Fumarate (NNC 26-1089·Fumarate). A reactor was charged with a suspension of activated Zn-dust

(21.0 g, 321 mmol) in AcOH (125 mL). The flask was immersed in an ambient temperature water bath, and a solution of crude **6** (as given above) in EtOAc (125 mL) was added dropwise over 30 min while stirring rigorously. Stirring was continued overnight. The next day, the reaction mixture was diluted with EtOAc (200 mL) and water (500 mL). A weak hydrogen evolution was seen. Within 1 h, the mixture was filtered to remove Zn-dust, and the filtrate was extracted with 1 M aqueous HCl (6 × 100 mL). The combined aqueous extracts were washed with EtOAc (50 mL). Then EtOAc (500 mL) was added to the washed aqueous phase and the pH adjusted to 9.5 with 25% aqueous NH_3 while stirring. The layers were allowed to separate, and the aqueous layer was extracted with EtOAc (500 mL). The combined organic extracts were washed with of 1% aqueous NH_3 (200 mL) and saturated NaCl (200 mL). The organic phase was dried over $MgSO_4$, filtered, and concentrated in vacuo to afford pale yellow oil. The oil was dissolved in EtOAc (1000 mL) and the solution heated to 50 °C. A suspension of fumaric acid (12.4 g, 110 mmol) in EtOAc (200 mL) was added while stirring. The formed clear solution was allowed to cool to room temperature, which resulted in the formation of a white precipitate. The next day, the product was removed by filtration and washed with EtOAc. Drying under vacuum at 40 °C to constant weight afforded 60.0 g (82% for three steps, mp 184−86 °C) of NNC 26-1089·$^1/_2$fumarate as white crystals. LC−MS: R_t = 5.09 min, $[M + H]^+$ = 572. Anal. Calcd for $C_{34}H_{45}N_5O_3C\cdot(C_4H_4O_4)$: C, 68.66; H, 7.52; N, 11.12. Found: C, 68.57; H, 7.70; N, 10.97. HPLC (h8): R_t = 10.37 min, purity 98.3% (214 nm). HPLC (A1): R_t = 34.44 min, purity 100.0% (214 nm). HPLC (B1): R_t = 36.30 min, purity 100.0% (214 nm). Stereoisomeric purity (CE): 99.7% (see Figure 1). ^{1}H NMR (DMSO) selected peaks: δ = 1.05 (s, 6H, 2 × CH_3), 2.21 (s, 3H, CH_3), 2.36 (s, 3H, CH_3), 2.29 (m, 2H, CH_2), 2.39 (s, 3H, CH_3), 2.70 (s, 3H, CH_3), 2.81 (dd, 1H, CH, J = 7, 14 Hz), 2.85 (dd, 1H, CH, J = 10.3, 13 Hz), 2.85 (s, 3H, CH_3), 3.01 (dd, 1H, CH, J = 5.3, 14 Hz), 3.20 (dd, 1H, CH, J = 7, 14 Hz), 5.65 (t, 1H, J = 7 Hz), 6.07 (dd, 1H, CH, J = 5.3, 10.3 Hz), 6.13 (d, 1H, J = 15 Hz), 6.38 (s, 1H, fumaric acid), 6.45 (dt, 1H, J = 7, 15 Hz), 7.14−7.86 (m, 12H). ^{13}C NMR (DMSO) selected peaks: δ = 22.81, 27.92, 28.07, 30.49, 31.79, 35.18, 35.71, 43.19, 43.53, 45.10, 52.06, 55.02, 55.29, 124.67, 136.51 (fumaric acid), 141.43, 165.72, 169.36 (fumaric acid), 170.12, 172.59.

5-Methyl-5-amino-hex-(2*E*)-enoic Acid, Hydrochloride (8). A reactor was charged with **7** (125 g, 510 mmol) in EtOAc (975 mL). The mixture was cooled to 0−5 °C, and addition of HCl gas started while stirring rigously. The temperature was kept below 25 °C, while adding HCl gas (160 g) over a period of 40 min. A white precipitate formed. The ice bath was removed, and the mixture was allowed to equilibrate to room temperature. The next day, the reaction mixture was evaporated to half volume to remove excess HCl and filtered. The filter cake was washed with EtOAc (2 × 100 mL) and dried to constant weight in vacuo at 30 °C. Fine, white crystals of **8** were isolated: 86 g (94%, mp 156−159 °C). Anal. Calcd for $C_7H_{14}ClNO_2$: C, 46.80; H, 7.86; Cl, 19.73; N, 7.80. Found: C, 46.83; H, 7.88; Cl, 19.65; N, 7.78. IR (KBr) selected peaks: 985 and 1661 cm^{-1} (trans double bond), 2979 cm^{-1} (C−H stretch), 1699 cm^{-1} (C═O stretch). HPLC (BpH3): R_t = 1.9 min (**7**, 5.4 min). ^{1}H NMR ($CDCl_3$): δ = 1.3 (s, 6H, 2× CH_3), 2.5 (d, 2H, J = 9 Hz), 5.9 (d, 1H, J = 13 Hz), 6.8 (dt, 1H, J = 9, 13 Hz), 8.3 (broad s, 3H), 12 (broad s, 1H). ^{13}C NMR ($CDCl_3$): δ = 171.6, 154.6, 147.7, 53.3, 42.6, 28.8, 28.0.

5-Methyl-5-(*N*-2,2,2-trichloroethyloxycarbonyl)amino-hex-(2*E*)-enoic Acid (9). A reactor was charged with **8** (18 g, 100 mmol) of dissolved in saturated $NaHCO_3$ (180 mL) solution (pH adjusted to 11 with 2 M NaOH) and 2-propanol (180 mL). The mixture was cooled to 0−5 °C, and TrocOSu (30 g, 100 mmol) was added in three portions over 2 h while stirring rigorously. The pH was kept between 9.5 and 9.75 by occasional addition of 2 M NaOH. The cooling facility was removed, and stirring was continued overnight at room temperature. The next day, the reaction mixture was filtered and evaporated to one-third volume. The pH of the residue was adjusted to 9.5 with 2 M NaOH and washed with MTBE (100 mL). The MTBE phase was extracted with 2 M NaOH (100 mL). The combined aqueous phase was acidified with 4 M HCl to pH 2 and extracted with MTBE (2 × 150 mL). The combined organic phase was dried over $MgSO_4$, filtered, and concentrated in vacuo to afford 32 g (100%) of **9** as colourless oil, which crystallised upon standing. The crude product was used without further purification. However, **9** can be recrystallised in a 50/50 mixture of isopropyl acetate and petrol ether. Then **9** (1.00 g) of was dissolved in isopropyl acetate (2 mL) upon heating, and petrol ether (2 mL) was added. The mixture was allowed to equilibrate to room temperature, and **9** crystallised upon standing. The mixture was filtered, and the crystals were washed with petrol ether and dried in vacuo to constant weight to afford 0.33 g (33%, mp 90−91 °C) of **9**. Anal. Calcd for $C_{10}H_{14}Cl_3NO_4$: C, 37.70; H, 4.43; Cl, 33.38; N, 4.40. Found: C, 38.12; H, 4.69; Cl, 33.37; N, 4.35. IR (KBr) selcted peaks: 3284 cm^{-1} (carbamate), 996, 1648 cm^{-1} (trans-double bond), 2984 cm^{-1} (C−H stretch), 1694 cm^{-1} (C═O stretch). The ^{13}C and ^{1}H NMR was in accordance with ref 14. HPLC (BpH3): R_t = 7.6 min, (HOSu −2.1 min).

5-Methyl-5-(*N*-1,1,1-trichloroethyloxycarbonyl)amino-hex-(2*E*)-enoic Acid-4-oxo-4*H*-benzo[*d*][1,2,3]-triazin-3-yl Ester (10). A reactor was charged with **9** (10 g, 31.4 mmol) and HOOBt (5.1 g, 31.4 mmol) dissolved in EtOAc (100 mL). The mixture was cooled to −5 °C. Then DCC (6.5 g, 29.9 mmol) was dissolved in EtOAc (25 mL) and added over 20 min. Precipitation of DCU occurred, and heat was developed. Then DCC (0.3 g, 1.5 mmol) dissolved in EtOAc (2 mL) was added, if the reaction mixture contained more than 2% of **9** after stirring for 6 h at −5 to 0 °C, and stirring at −5 to 0 °C was continued for additional 1 h. Otherwise, the cooling facility was removed, and the reaction mixture was allowed to equilibrate to room temperature. The next day, the reaction mixture was filtered and the filtrate concentrated on a rotavapor (without applying heat!!), stripped with 2-propanol (2 × 50 mL) to afford 16 g of yellow crystals. The crude product was recrystallised in

2-PrOH (130 mL), and 10.2 g (70%, mp 158−159 °C) of pure **10** was isolated as white crystals. Anal. Calcd for $C_{17}H_{17}Cl_3N_4O_5$: C, 44.03; H, 3.70; Cl, 22.94; N, 12.08. Found: C, 44,06; H, 3,76; Cl, 23,01; N, 12,05. IR (KBr) selected peaks: 3353 cm^{-1} (carbamate), 968, 1648 cm^{-1} (trans double bond), 2980 cm^{-1} (C−H stretch), 1516, 1602 cm^{-1} (aromatic). HPLC (BpH3): R_t = 37.5min. ^{1}H NMR ($CDCl_3$): δ = 8.4 (d, 1H), 8.25 (d, 1H, J = 7 Hz), 8.05 (t, 1H, J = 7 Hz), 7.85 (t, 1H, J = 7 Hz), 6.75−6.9 (d triplet, 1H, J = 7 Hz, J = 14 Hz), 6.25 (d, 1H, J = 14 Hz), 5.0 (s, 1H), 4.7 (s, 1H), 2.85 (d, 2H, J = 7 Hz), 1.4(s, 6H). ^{13}C NMR ($CDCl_3$) δ = 161.8, 152.6, 151.1, 150.3, 144.3, 135.4, 132.7, 129.0, 125.8, 122.2, 118.9, 95.6, 74.0, 53.1, 42.4, 27.5.

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