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Synthesis of MEN11420, a glycosylated bicyclic peptide, by intramolecular double cyclization using a chloroimidazolinium coupling reagent

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Abstract—The synthesis of MEN11420, a potent tachykinin receptor antagonist, has been achieved. The bicyclic glycosylated structure of MEN11420 was constructed via intramolecular double cyclization using CIP-mediated activation. The head to tail cyclization of the linear precursor, which contained an α-amino acid at its C-terminus, proceeded so rapidly that no serious racemization was apparent at the activated carboxyl function. The desired product was obtained without the need for purification of the intermediates throughout the synthesis.

1. Introduction

Tachykinins (TKs) comprise a family of peptides that are widely distributed in the central and peripheral nervous system and play a key role in neuronal stimulation.[1] Of the three mammalian's TKs (Substance P, Neurokinin A, and Neurokinin B), Neurokinin A (NKA) is known to play a role in bronchoconstriction, smooth muscle contraction and inflammation. Thus, antagonists of the Tachykinin NK_2 receptor which recognize Neurokinin A have a wide range of potential therapeutic applications.[2] MEN11420, which is derived from the bicyclic Tachykinin analogue MEN10627, is a potent and selective tachykinin NK_2 receptor antagonist as has been reported by Renzetti et al.[3] in 1998. Structurally, MEN11420, cyclo{[Asn(β-D-GlcNAc)-Asp-Trp-Phe-Dap-Leu]cyclo(2β–5β)} (Fig. 1), is a bicyclic peptide with a (2-acetylamino-2-desoxy-β-D-glucopyranosyl)-L-asparaginyl residue in the place of the Met residue, which is normally present in MEN10627. In spite of its unique structure and specific activity for the NK_2 receptor, no detailed procedure for the synthesis of MEN11420 has, to date, been reported, although, a Boc-based classical synthesis of bicyclic MEN10627 was reported by Pavone et al.[4] in 1995.

Recently, we have developed a new coupling reagent, 2-chloro-1,3-dimethyl-2-imidazolinium hexafluorophosphate (CIP),[5] which in the presence of an additive (HOAt[6] or DMAP[7]) permits the effective coupling of sterically hindered α-dialkylamino acids or *N*-methylamino acids (Fig. 2). Using this coupling reagent, we were able to

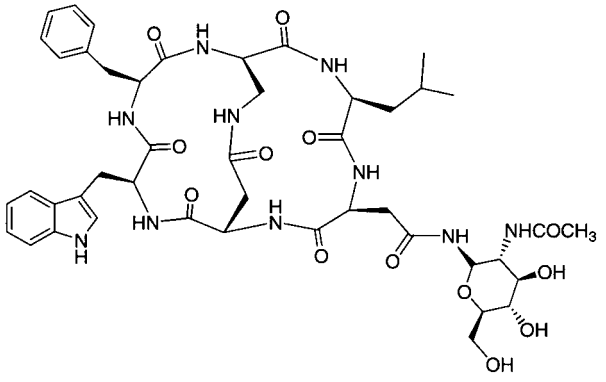

Figure 1. Structure of MEN11420.

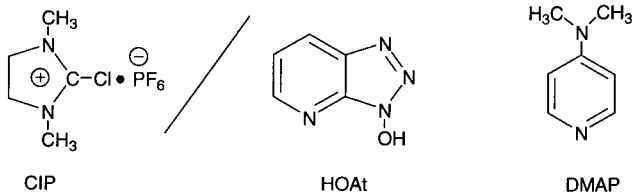

Figure 2. Structures of CIP, HOAt and DMAP.

Keywords: CIP; coupling reagent; intramolecular cyclization; MEN11420; tachykinin antagonist.

Abbreviations: Boc=*tert*-butoxycarbonyl; Bu^t=*tert*-butyl; CIP=2-chloro-1,3-dimethyl-2-imidazolium hexafluorophosphate; Dap=2,3-diaminopropionic acid; DIEA=*N*,*N*-diisopropylethylamine; DIPCDI=*N*,*N'*-diisopropylcarbodiimide; DMAP=4-dimethylaminopyridine; Fmoc=fluoren-9-ylmethyloxycarbonyl; HOAt=1-hydroxy-7-azabenzotriazole; HOBt=*N*-hydroxybenzotriazole; MALDI-TOF=matrix-assisted laser desorption ionization time-of-flight; SPPS=solid phase peptide synthesis; TFA=trifluoroacetic acid; TFE=2,2,2-trifluoroethanol.

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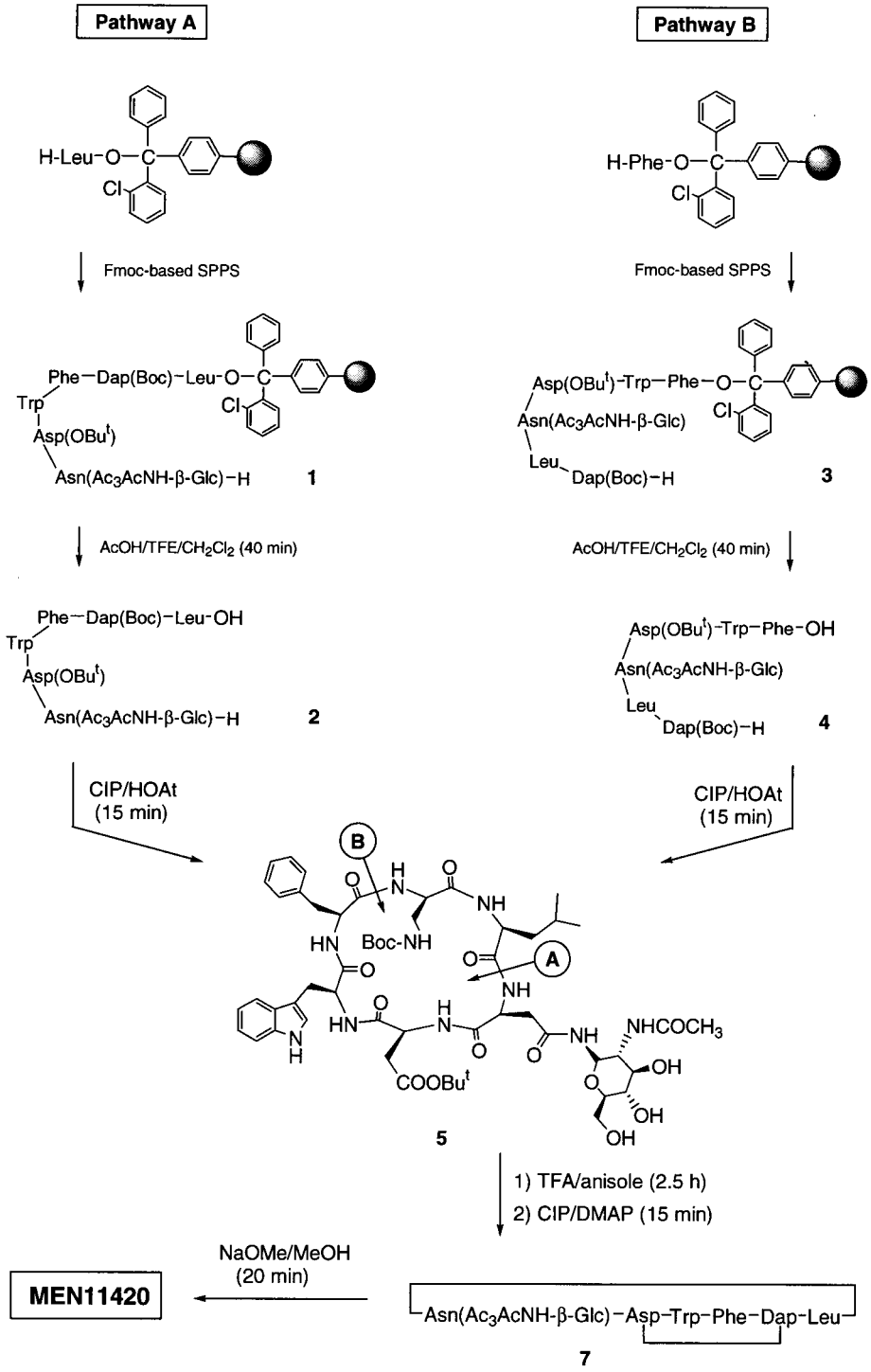
achieve the convergent syntheses of polythiazoline alkaloids,[8] peptaibols[9] and cytostatic depsipeptide.[10] These synthetic studies suggest that the intramolecular coupling of compounds, including sterically hindered glycosylated amino acids, might be feasible using the CIP-additive as a coupling reagent. Here, we describe the synthesis of MEN11420 based on a general synthetic scheme via the CIP-mediated intramolecular double cyclization of a glycosylated linear precursor.

2. Results and discussion

The overall strategy for the synthesis is shown in Scheme 1. A double intramolecular cyclization using the CIP reagent is achieved by combining a head to tail cyclization and a subsequent cyclization between the side chain functional groups. In the first cyclization of the precursor, which contains an optically active α-amino acid at its C-terminus, racemization at the activated carboxyl function presents a



Scheme 1.

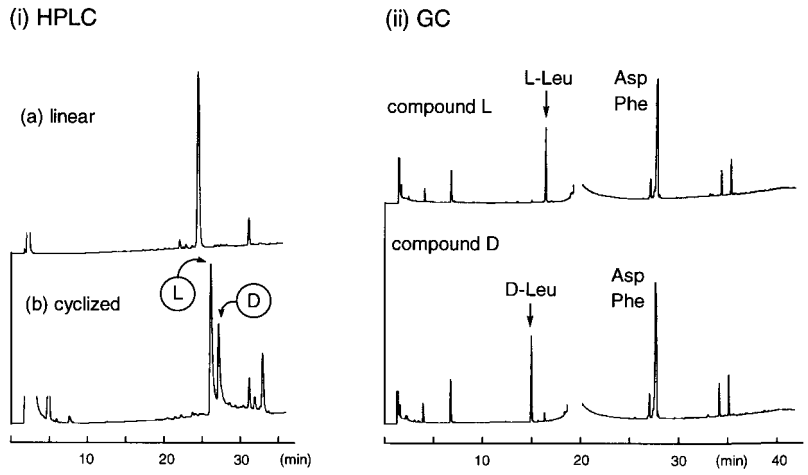


Figure 3. HPLC and GC profiles of the products obtained by pathway A. (i) HPLC profiles of the crude glycosylated precursor **2** (a) and the monocylized crude intermediate **5** (b). (ii) GC spectra of the monocyclized compound L and compound D.

serious problem. A low reaction rate for the condensation would be expected to be one of the major reasons for racemization.[11] Thus, two different schemes for the first cyclization step were selected; one, which involved cyclization at the most hindered site between the glycosylated Asn and Leu (Scheme 1, pathway A) and the other, which involved the less hindered site between Dap(Boc) and Phe (Scheme 1, pathway B). Each required linear precursor for the above synthetic scheme was prepared by Fmoc-based SPPS, since sugar residues, when covalently linked to the peptide are not stable in anhydrous HF or the other strong acids used for cleavage from the resin under classical Boc-based procedures.[12] As a solid support, an acid labile 2-chlorotritylchloride (Clt) resin[13] was selected instead of the conventional benzylalcohol-based Wang resin.

Based on the above scheme, the protected linear precursor resin **1**, which was used in pathway A, was first constructed starting from the Fmoc-Leu-Clt resin prepared by published procedures.[13] Successive incorporation using a 2.5 equiv. excess of Fmoc-Dap(Boc)-OH, Fmoc-Phe-OH, Fmoc-Trp-OH and Fmoc-Asp(OBu^t)-OH were conducted by a combination of piperidine-mediated deprotection and DIPCDI-mediated coupling. The N-terminal glycosylated residue, Fmoc-Asn(Ac_3AcNH-β-Glc)-OH,[14] was introduced by the same procedure, except that a 1.3 equiv. excess of the glycosylated amino acid and an extended coupling reaction (6 h) were employed. At each condensation step, the Kaiser ninhydrin test was negative. After the construction of the peptide chain, the N-terminal Fmoc group was removed by treatment with piperidine to yield the side chain protected precursor resin **1**.

Another precursor resin **3**, used in the pathway B, was then constructed starting from the Fmoc-Phe-Clt resin. The successive incorporation of Fmoc-amino acids, except for Fmoc-Leu-OH, was conducted similarly as above. The condensation of Fmoc-Leu-OH to the glycosylated Asn was achieved by the CIP/HOAt method instead of the conventional DIPCDI method because of the provable low reactivity of sterically hindered glycosylated amino acid at the N-terminus. At each condensation step, the Kaiser ninhydrin test was negative and the desired precursor resin **3** was obtained without difficulty.

Each protected precursor peptide was then cleaved from the corresponding resin by treatment with AcOH/TFE/CH_2Cl_2 (1:1:8) to yield the linear precursors **2** or **4** as powders. Each

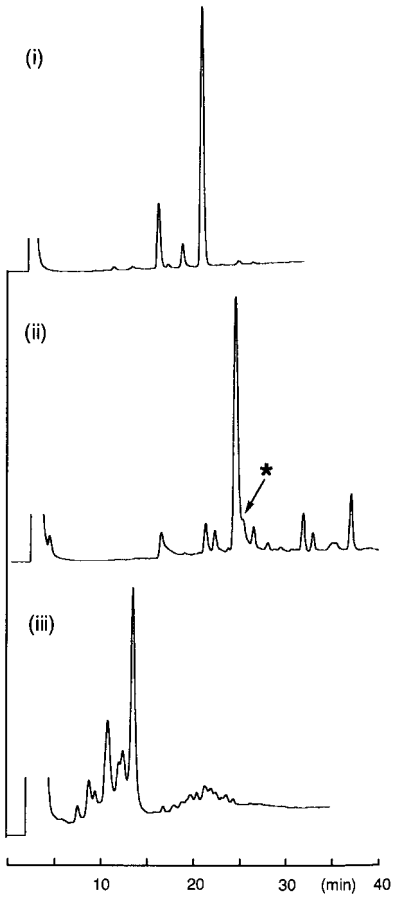


Figure 4. HPLC profiles of the products obtained by pathway B. (i) Crude glycosylated precursor **4**. (ii) Mono cyclized crude intermediate **5**. * denotes the corresponding D-isomer. (iii) Double cyclized crude intermediate **7**.

crude product showed a single main peak on analytical HPLC and was used for the next double cyclization without further purification (Figs. 3(i, part a) and 4(i)).

For the initial cyclization step, based on pathway A, the head to tail cyclization of precursor **2** was conducted by CIP-mediated activation in DMF in the presence of HOAt. When the reaction was monitored by HPLC in the presence of an excess amount of CIP (9 equiv.), the starting material had disappeared within 15 min. Incomplete conversion to the cyclized product was observed when a slight excess of CIP was used, even when the reaction time was extended. The use of HOBt as an additive also gave poor results and gave a complex mixture.

The cyclized product, however, was found to be a mixture of two compounds, as determined by HPLC analysis (Fig. 3(i, part b)). Each compound had an amino acid composition and mass value which was consistent with the theoretical values, and there was no significant difference between the values of the two compounds. These results strongly suggest that any difference in the two products would be reflected in their optical purity. Thus, the optical purity of each compound was examined by GC analysis using a chiral column. The (pentafluoropropionyl) amino acid *n*-butyl esters, derived from a total hydrolysate of the each compound, were analyzed in a Chirasil Val-L capillary column. The analysis indicated that the compound L contained all the expected L-amino acids, while the compound D contained D-Leu instead of a L-Leu residue (Fig. 3(ii)); this clearly shows that the racemization occurred during the initial cyclization between the Leu and glycosylated Asn residue. The relative content of the racemized product to the desired product was estimated to be 33% by HPLC. The content of the racemized product decreased to 30% when the cyclization was conducted with an additional additive, $CuCl_2$,[15] but no remarkable suppression of racemization was observed.

Initial cyclization between the less hindered Phe and Dap(Boc) residues was then examined (Scheme 1, pathway B). The coupling reaction was conducted with CIP/HOAt as described for pathway A and the progress of the reaction was monitored on HPLC. The linear precursor disappeared within 15 min using a smaller amount of CIP (3 equiv.) compared with those used in pathway A. The product showed a single main peak, which contained a small shoulder peak derived from the corresponding D-Phe isomer (Fig. 4(ii)). The relative content of the racemized product was estimated to be less than 6% by HPLC- and GC-analysis. Thus, the CIP-mediated head to tail cyclization between the general α-amino acid proceeded so rapidly that no serious racemization had occurred, whereas the cyclization of the precursor having a glycosylated amino acid at its N-terminus proceeded more slowly, resulting in a racemized product in a moderate yield. The crude cyclized product was then treated with TFA/anisole to remove the side chain protecting groups to yield monocyclized hexapeptide derivative **6**.

The second intramolecular cyclization was conducted using the crude deprotected product without further purification: the monocyclized product **6** was treated with CIP in the presence of DMAP under the same conditions as described for the first cyclization step. The reaction, which was monitored by HPLC, also proceeded within 15 min in the presence of an excess amount of CIP (Fig. 4(iii)). The desired product was obtained as a major product on HPLC, although several side reaction products were observed in this second cyclization step. These side products probably arose from the formation of oligomers resulting from the intermolecular coupling reaction. The *O*-acetyl groups of the crude double cyclized product were finally removed by treatment with sodium methoxide in methanol. The crude product, which was isolated by adsorption to Diaion HP20, was purified by preparative reverse phase HPLC to yield the desired product in 4% overall yield (calculated from the starting Clt resin, total 11 steps). The integrity of the purified product was determined by analytical HPLC, amino acid analysis after acid hydrolysis, MALDI-TOF MS and by ^{1}H and ^{13}C NMR. The optical purity of the constituent amino acids in the synthetic MEN11420 was confirmed by gas chromatography using a chiral column. The content of each D-amino acid was less than 0.5%, a result of the hydrolysis conditions.

3. Conclusion

We report herein the synthesis of MEN11420, a potent tachykinin NK_2 receptor antagonist, based on a general scheme for the synthesis of bicyclic glycopeptides. The CIP-mediated intramolecular double cyclizations proceeded within 15 min in the presence of HOAt or DMAP. The CIP-mediated intramolecular cyclization between general α-amino acids proceeded so rapidly that no serious racemization occurred at the activated carboxyl function. Thus, the entire procedure, which includes the synthesis of a glycosylated peptide on a solid support and the double cyclization–deprotection steps in solution, could be conducted in a reasonably short period of time (several days) with no need for purifying the intermediates. A single step HPLC purification at the final step of the synthesis was sufficient to obtain the optically pure MEN11420.

4. Experimental

4.1. General

Solvents were reagent grade and were dried prior to use. Fmoc amino acid derivatives, except for the diaminopropionic acid (Dap) derivative, and the 2-chlorotrityl chloride resin were obtained from Calbiochem Novabiochem and were used without further purification. Fmoc-Dap(Boc)-OH was prepared according to the procedure for Z-Dap(Boc)-OH[16] with several modifications. For the quantification of Fmoc amino acids on the resin, the absorbance at 301 nm after cleavage of the Fmoc group with piperidine was measured according to the procedure described by Meienhofer et al.[17]

Melting points are uncorrected. The ^{1}H and ^{13}C NMR spectra were recorded on a 270 MHz (JEOL) or 400 MHz (Bruker) spectrometer with TMS as an internal standard. FAB-MS was obtained on a JEOL JMS-SX102A

spectrometer equipped with a JMA-DA7000 data system. MALDI-TOF MS was obtained on a Voyager-DE Biospectrometry Workstation of PerSeptive Biosystems. Gas chromatography (GC) using Chirasil Val-L capillary column was conducted according to published procedures.[8,18] HPLC was carried out on a reversed phase column which was eluted with CH_3CN in 0.1% aq. TFA and products were detected at OD 220 nm: Rt_1, YMC AM302 (4.6×150 mm), flow rate 0.9 ml/min; Rt_2, YMC-Pack ProC18 AS 323 (10×250 mm), flow rate 2.5 ml/min.

4.1.1. Fmoc-Dap(Boc)-OH. To a stirred solution of bis[trifluoroacetoxy]-phenyliodine (6.5 g, 15 mmol) in DMF/H_2O (2:1, 60 ml), Fmoc-Asn-OH (3.37 g, 10 mmol) was added. After 15 min, pyridine (1.6 ml, 20 mmol) was added and the mixture was stirred for an additional 10 h. Solvent of the mixture was removed by evaporation and the residue was dissolved in H_2O (100 ml). The solution was acidified with 6N HCl and then washed with Et_2O. The pH of the aqueous phase was adjusted to 6 with 4N NaOH and the solution was allowed to stand at 4°C. The resulting precipitate was filtered, washed with Et_2O and dried to yield 2.5 g (81%) of Fmoc-Dap-OH as a solid.

To a solution of Fmoc-Dap-OH (2.5 g, 8 mmol) in DMF (10 ml), DIEA (2.8 ml, 16 mmol) and $(Boc)_2O$ (3.5 g, 16 mmol) were added and the mixture was stirred for 4 h at 25°C. The solvent was removed by evaporation and the residue was extracted with AcOEt (50 ml). The organic phase was washed with 5% aq. citric acid and then H_2O, dried over $MgSO_4$, and evaporated. The residue was triturated with hexane to yield Fmoc-Dap(Boc)-OH as an amorphous powder. For storage, the product was converted to its DCHA salt to yield 3.5 g (71%) of Fmoc-Dap(Boc)-OH DCHA as a solid. For characterization, a part of the product was treated with excess amount of $TMSCHN_2$ in hexane to yield Fmoc-Dap(Boc)-OMe as a powder: mp 150–152°C, $[\alpha]_D^{23}=+16.8$ (c=0.5, $CHCl_3$), ^{1}H NMR (270 MHz, $CDCl_3$) δ 7.76 (d, J=7.3 Hz, 2H), 7.61 (d, J=6.9 Hz, 2H), 7.40 (t, J=6.9 Hz, 2H), 7.31 (t, J=7.3 Hz, 2H), 5.93 (brd, J=6.4 Hz, 1H), 4.89 (brt, J=5.6 Hz, 1H), 4.50 (brs, 1H), 4.40 (d, J=6.0 Hz, 2H), 4.22 (t, J=6.6 Hz, 1H), 3.76 (s, 3H), 3.55 (brs, 2H), 1.44 (s, 9H). ^{13}C NMR (67.8 Hz, $CDCl_3$) δ 170.91, 156.40, 155.94, 143.63, 141.24, 127.66, 127.01, 125.07, 119.91, 80.00, 67.03, 54.90, 52.71, 47.06, 42.09, 28.20. FAB-MS, m/z 441.2031 for $[M+H]^+$ (Calcd 441.2026 for $C_{24}H_{29}N_2O_6$).

4.1.2. H-Asn(Ac_3AcNH-β-Glc)-Asp(OBu^t)-Trp-Phe-Dap-(Boc)-Leu-OH, 2. *(a) Anchoring to Clt resin*. To 2.0 g (2.1 mmol) of 2-chlorotrityl chloride (Clt) resin, Fmoc-Leu-OH (0.19 g, 0.54 mmol) in CH_2Cl_2/DMF (2:1, 10 ml) and DIEA (0.8 ml, 4.6 mmol) were added. The mixture was agitated for 2 h at 25°C and the solvent was removed by filtration. MeOH/DIEA (9:1, 8 ml) was added to the resin and the mixture was agitated for a further 30 min at 25°C. The resin was filtered, washed with MeOH and then dried in vacuo to yield 2.1 g of Fmoc-Leu-Clt resin, substituted at a level of 0.271 mmol/g.

(b) Condensation on Clt resin. Piperidine (20%) in DMF was added to the above resin and the mixture was agitated for 20 min at 25°C. The resin was filtered and washed with DMF. To this resin, Fmoc-Dap(Boc)-OH (2.5 equiv.) in DMF, HOBt (2.5 equiv.), DIEA (2.5 equiv.) and DIPCDI (2.5 equiv.) were added, and the mixture was agitated for 2 h at 25°C. After washing the resin with DMF, the same deprotection and condensation procedure was repeated for the incorporation of Fmoc-Phe-OH, Fmoc-Trp-OH and Fmoc-Asp(OBu^t)-OH. For the incorporation of Fmoc-Asn(Ac_3AcNH-β-Glc)-OH, a 1.3 equiv. excess of the amino acid and other reagents were used. At each condensation step, the results of a Kaiser ninhydrin test were negative after a single coupling reaction. The N-terminal Fmoc group was removed by treatment with 20% piperidine/DMF. The obtained resin was filtered, washed with DMF and MeOH, and dried to yield 2.4 g of the desired hexapeptide resin **1**.

(c) Cleavage. To 0.5 g (0.1 mmol) of the above peptide resin **1** (0.207 mmol/g), AcOH/TFE/CH_2Cl_2 (1:1:8, 20 ml) was added and the mixture was agitated for 40 min at 25°C. The resin was filtered and washed with AcOH/TFE/CH_2Cl_2 (1:1:8, 5 ml). The filtrate was evaporated to dryness and the residue was dissolved in 2N AcOH. The solution was lyophilized to yield 110 mg (84%) of **2** as a white powder: Rt_1 25.47 min [CH_3CN (30–60%/30 min)], MALDI-TOF MS, m/z 1266.47 for $[M+H]^+$ (Calcd 1266.36 for $C_{60}H_{85}N_{10}O_{20}$). Amino acid analysis after 6N HCl hydrolysis; Asp 1.45, Leu 1.00, Phe 1.06.

4.1.3. H-Dap(Boc)-Leu-Asn(Ac_3AcNH-β-Glc)-Asp(OBu^t)-Trp-Phe-OH, 4. *(a) Anchoring to Clt resin*. To 2.0 g (2.4 mmol) of 2-chlorotrityl chloride (Clt) resin, Fmoc-Phe-OH (0.46 g, 1.2 mmol) in CH_2Cl_2/DMF (2:1, 10 ml) and DIEA (1 ml, 5.7 mmol) were added. The mixture was agitated for 1.5 h at 25°C and the solvent was removed by filtration. MeOH/DIEA (9:1, 15 ml) was added to the resin and the mixture was agitated for a further 30 min at 25°C. The resin was filtered, washed with MeOH and then dried to yield 2.4 g of Fmoc-Phe-Clt resin, substitution at a level of 0.37 mmol/g.

(b) Condensation on Clt resin. Starting from the above Fmoc-Phe-Clt resin, Fmoc-Trp-OH and Fmoc-Asp(OBu^t)-OH were incorporated using DIPCDI by the same procedure described for precursor **1**. 1.51 g of the resulting Fmoc-Asp(OBu^t)-Trp-Phe-Clt resin (3.03 g) was used for the further chain elongation. For the incorporation of Fmoc-Asn(Ac_3AcNH-β-Glc)-OH, a 1.7 equiv. excess of the amino acid and DIPCDI were used. For the coupling of Fmoc-Leu-OH (2.5 equiv.) to the N-terminal glycosylated amino acid, CIP/HOAt (2.5–2.0 equiv.) were employed. Finally, Fmoc-Dap(Boc)-OH (2.5 equiv.) was incorporated using DIPCDI (2.5 equiv.) and the N-terminal Fmoc group was removed by treatment with 20% piperidine/DMF. At each condensation step, the results of a Kaiser ninhydrin test were negative after single coupling reaction. The obtained resin was filtered, washed with DMF and MeOH, and dried to yield 1.4 g of the desired hexapeptide resin **3**.

(c) Cleavage. To 0.32 g (0.083 mmol) of the above peptide resin **3** (0.26 mmol/g), AcOH/TFE/CH_2Cl_2 (1:1:8, 13 ml) was added and the mixture was agitated for 40 min at 25°C. The resin was filtered and washed with AcOH/TFE/CH_2Cl_2 (1:1:8, 5 ml). The filtrate was evaporated to dryness

and the residue was treated with H_2O to afford 95 mg (90%) of **4** as a powder: Rt_1 21.19 min [CH_3CN(30–60%/30 min)], MALDI-TOF MS, *m/z* 1266.08 for $[M+H]^+$ (Calcd 1266.36 for $C_{60}H_{85}O_{20}N_{10}$). Amino acid analysis after 6N HCl hydrolysis; Asp 1.44, Leu 0.98, Phe 1.00.

4.1.4. Monocyclized intermediate, 5, by pathway A. To the solution of linear precursor **2** (5 mg, 4 μmmol) in DMF (1 ml), HOAt (5 mg, 40 μmol), CIP (13 mg, 47 μmol) and DIEA (14 μl, 80 μmol) were added, and the mixture was stirred for 15 min at 25°C. HPLC analysis of the mixture showed a major product (Compound L, Rt_1 26.70 min) accompanied with a side product (Compound D, Rt_1 27.79 min) (Fig. 3(i)). Relative content of the compound D was 33% on the HPLC. Both products were isolated on a semipreparative column YMC-Pac ProC18AS323 (10×250 mm), which was eluted with CH_3CN/0.1% TFA [CH_3CN 30–60% (60 min)]. Each product was then hydrolyzed for amino acid analysis and GC analysis. Amino acid analysis after 6N HCl hydroloysis: Compound L; Asp 1.47, Leu 1.03, Phe 1.00; Compound D; Asp 1.49, Leu 1.05, Phe 1.00. MALDI-TOF MS: Compound L; *m/z* 1362.68 for $[M+H]^+$ (Calcd 1362.34 for $C_{60}H_{38}N_{10}O_{19}\cdot CF_3COOH$); Compound D; *m/z* 1362.45 for $[M+H]^+$ (Calcd 1362.34 for $C_{60}H_{38}N_{10}O_{19}\cdot CF_3COOH$). GC-spectra of the acid hydrolysate derived from Compound L and D [80°C for 3 min, then 80 to 190°C (3°C/min)] are shown in Fig. 3(ii).

4.1.5. Double cyclization by pathway B. *(a) Monocyclized intermediate,* ***5***. To the solution of linear precursor **4** (70 mg, 55 μmol) in DMF (9 ml), HOAt (19 mg, 0.14 mmol) and CIP (46 mg, 0.17 mmol) were added. DIEA (43 μl, 0.25 mmol) was then added in three portions to the mixture and the resulting solution was stirred for 15 min at 25°C. HPLC analysis of the mixture showed a single major peak with a small shoulder (relative content, 6%) (Fig. 4(ii)). The content of D-Phe isomer in the major product was confirmed by GC analysis using the same procedure as described in pathway A synthesis. AcOH (0.12 ml) was added to the reaction mixture. The solvent of the reaction mixture was then removed by evaporation and the resulting residue was dissolved in AcOEt (20 ml). The organic phase was washed with H_2O, dried over $MgSO_4$ and evaporated to dryness. The crude product was used for the next deprotection reaction without any further purification: Rt_1 24.30 min [CH_3CN (30–60%/30 min)], MALDI-TOF MS, *m/z* 1362.79 for $[M+H]^+$ (Calcd 1362.34 for $C_{60}H_{83}N_{10}O_{19}\cdot CF_3COOH$). Amino acid analysis after 6N HCl hydrolysis; Asp 1.54, Leu 1.00, Phe 1.00.

(b) Double cyclized intermediate, ***7***. TFA-anisole (7.0–0.24 ml) was added to the above mono cyclized product **5** and the mixture was stirred for 2.5 h at 25°C. The TFA was evaporated in vacuo and the residue was extracted with 2N AcOH. The aqueous phase was washed with Et_2O and lyophilized to yield the side chain deprotected product **6** as a hygroscopic powder: MALDI-TOF MS, *m/z* 1205.94 for $[M+H]^+$ (Calcd 1206.118 for $C_{51}H_{67}N_{10}O_{17}\cdot CF_3COOH$). Amino acid analysis after 6N HCl hydrolysis; Asp 1.71, Leu 1.03, Phe 1.00.

To the solution of the deprotected product **6** (55 μmol) in DMF (9 ml), DIEA (48 μl, 0.27 mmol), DMAP (0.13 g, 1.1 mmol) and CIP (0.38 g, 1.3 mmol) were added, and the mixture was stirred for 15 min at 25°C. AcOH (0.16 ml) was added to the reaction mixture. The solvent was removed by evaporation and the residue was dissolved in AcOEt (20 ml). The organic phase was washed with H_2O, dried over $MgSO_4$, and then evaporated to dryness. The crude product was used for the next deacetylation reaction without any further purification: Rt_1 13.58 min [CH_3CN (30–60%/30 min)], MALDI-TOF MS, *m/z* 1188.83 for $[M+H]^+$ (Calcd 1188.10 for $C_{51}H_{65}N_{10}O_{16}\cdot CF_3COOH$). Amino acid analysis after 6N HCl hydrolysis; Asp 1.71, Leu 0.82, Phe 1.00.

4.1.6. MEN11420, 1. To a solution of the double cyclized crude product **7** (55 μmol) in MeOH (5.0 ml), 28% NaOMe/MeOH (0.32 ml, 0.17 mmol) was added and the mixture was stirred for 20 min at 25°C. The pH of the mixture was adjusted to ca. 6 with AcOH and the solvent of the mixture was evaporated in vacuo. The residue was dissolved in 0.1% aq. TFA (10 ml). Diaion HP20 (ca. 5 g) was added to the solution and the mixture was gently stirred for 30 min at 25°C. The resin was filtered and washed with H_2O. The adsorbed product was then eluted with 80% CH_3CN in 0.1% aq. TFA and the filtrate was lyophilized. The crude product was purified by preparative HPLC [YMC-Pack ProC18AS323 (10×250 mm), CH_3CN (25–32%/65 min)] to yield 2.6 mg (overall 4.4%) of **1** as a white powder: $[\alpha]_D^{25}=-41.2°$ (c=0.1, H_2O). Rt_1 17.10 min [CH_3CN (25–35%/30 min)], ^1H NMR (400 MHz, D_2O at 45°C) δ 7.68 (d, J=7.5 Hz, 1H), 7.65 (d, J=7.5 Hz, 1H), 7.57–7.48 (m, 3H), 7.43–7.39 (m, 3H), 7.34 (t, J=7.5 Hz, 1H), 7.00 (s, 1H), 5.02 (d, J=9.8 Hz, 1H), 4.89 (m, 1H), 4.84 (m, 1H), 4.60 (m, 1H), 4.50 (m, 1H), 4.11 (dd, J=14.3, 3.4 Hz, 1H), 3.97–3.95 (m, 1H), 3.89–3.86 (m, 2H), 3.84–3,68 (m, 1H), 3.59–3.47 (m, 2H), 3.53 (dd, J=14.3, 3.4 Hz, 1H), 3.41 (dd, J=14.2, 5.4 Hz, 1H), 3.21 (dd, J=16.0, 3.9 Hz, 1H), 2.77 (dd, J=16.0, 3.9 Hz, 1H), 2.11 (s, 3H), 1.80 (m, 2H), 1.07 (d, J=5.6 Hz, 3H), 1.04 (d, J=5.6 Hz, 3H). ^{13}C NMR (100 MHz, D_2O at 25°C) δ 21.01, 22.43, 22.61, 24.78, 26.67, 34.19, 36.33, 36.61, 38.82, 39.72, 40.39, 49.22, 51.34, 54.37, 54.54, 55.17, 56.64, 60.72, 60.84, 69.62, 71.99, 74.29, 77.91, 78.89, 109.23, 112.26, 118.60, 119.93, 122.58, 123.82, 127.07, 127.55, 129.18, 129.65, 136.47, 137.40, 159.67, 163.56, 171.25, 171.60, 172.23, 173.17, 173.20, 174.58, 174.95, 175.04, 177.09. MALDI-TOF MS, *m/z* 1062.32 for $[M+H]^+$ (Calcd 1061.99 for $C_{45}H_{59}N_{10}O_{13}\cdot CF_3COOH$). Amino acid analysis after 6N HCl hydrolysis; Asp 1.95, Leu 1.07, Phe 1.00, Trp 0.56. The GC chromatogram obtained after acid hydrolysis showed that the content of each D-amino acid was less than 0.5%.

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