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Novel Tetrahydropyran-Based Peptidomimetics from a Bioisosteric Transformation of a Tripeptide. Evidence of Their Activity at Melanocortin Receptors

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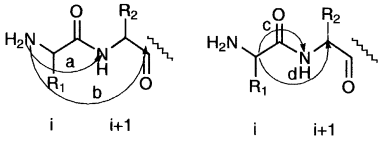
Abstract—We have prepared novel peptidomimetics based on a 2,4,6-trisubstituted tetrahydropyran. This scaffold was constructed in an isosteric transformation using conceptual constraints imposed on a tripeptide moiety involving O_i'–C^{γ}_{i+1} and O_i'–N_{i+2} formal cyclization modes. A series of regioselective transformations commencing with a substituted dihydropyran-4-one readily provided the required analogues. Specific tetrahydropyrane analogues modeled on PheArgTrp as a truncated version of the melanocortin receptor message sequence, showed activity at the melanocortin receptors MC4R and MC1R. Thus, the 2,4,6-trisubstituted tetrahydropyran scaffold has provided a potentially useful peptidomimetic lead, and conceptual cyclization of peptide moieties can offer a valuable design strategy in peptidomimetic research.

Introduction

While nitrogen-containing saturated ring systems constitute fundamental peptidomimetic scaffolds, the corresponding oxacycles are less frequently used for this purpose. Hirschmann[1] reported the first glucose-based peptidomimetic somatostatin agonist and several efforts have subsequently been reported for utilization of the pyranose structure to mimick peptide motifs.[2–10] However, these approaches involved mainly transformations of carbohydrate structures, in particular including capping the hydroxyl groups with alkyl or acyl groups. This methodology leaves excess oxygen atoms in the molecule because their removal would usually involve more elaborate transformations. Consequently, the pyranose structure merely serves as an attachment center for spatial display of a few or several substituents, but the structures do not have a clear topological relationship to their peptide counterpart. On the other hand, more direct translations have been accomplished with many nitrogen-containing heterocycles designed by conceptual bridging of the peptide backbone as illustrated in Scheme 1.



Scheme 1. Examples of frequently used modes of conceptual bridging atoms in dipeptide moieties leading to cyclic peptidomimetics. Modes of bridging: a. N_i–N_{i+1}; b. N_i–$C_{i+1}'N_i$–C^{α}_i; c. C^{α}_i–N_{i+1}; d. C^{α}_i–C^{α}_{i+1}.

In this report, we want to present two aspects of our petidomimetic research. One, concerns the development of oxacyclic peptidomimetics. These structures differ from the earlier pyranose-based systems by having closer topological relationships to the constrained peptide structure and by the fact that they are derived from amino acids not carbohydrates. The other aspect of our report is the demonstration of conceptual bridging of peptide structures as a low-cost technique in the arsenal of drug discovery tools. This methodology can rapidly produce early peptidomimetic leads from the initial peptide structure, without prior knowledge of the active conformation.

The bridging operations, presented in Scheme 1 have generated many heterocyclic structures including

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oxopiperazines and diazepines (mode a, b),[11] Freidinger lactams[12,13] (mode c), piperidines (mode c, d), and so on. Toniolo[14] reviewed early work regarding the design of peptidomimetics using 18 modes of constraints within the tripeptide moiety. In most cases, these various modes of cyclization have been used to design peptidomimetics addressing specific secondary structures of the parent peptides, mostly the β- and γ-turn motifs.[15] Nonetheless, a large number of possible cyclization modes, as well as a multitude and diversity of associated chemical structures, brought us to the use of this method, as a conceptual and mnemonic device in the discovery of peptidomimetics, without a prior detailed elucidation of the exact secondary structures of the corresponding peptides.

Results

Design of oxacylic peptidomimetics

Herein, we present a design and an initial evaluation of 2,4,6-trisubstituted tetrahydropyrans based on the conceptual bridging of the carbonyl group C_i' in a tripeptide. Our design strategy is shown in Charts 1 and 2. It involves topological transformation of the tripeptide moiety into the tetrahydropyran system that is isosteric to the parent peptide. In addition, the designed tetrahydropyran analogues can also be viewed as isosteres of a constrained amide bond.

In the first case (Chart 1), the tripeptide **(A)** was conceptually bridged by connecting the backbone $C_i'{=}O_i'$ group to C^{γ}_{i+1} of the side chain. The tetrahydropyran structures **(C)** and **(D)** are obtained from **(A)** by replacing N_{i+1} with carbon and oxygen, respectively. The bridging function becomes either oxygen in **(C)** or a methylene group in **(D)**.

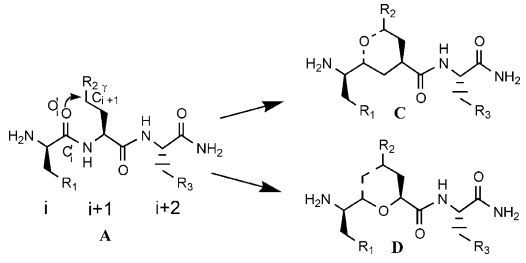


Chart 1. Design of tetrahydropyran analogues using $C_i'{=}O_i'\cdots C^{\gamma}_{i+1}$ mode of peptide bridging.

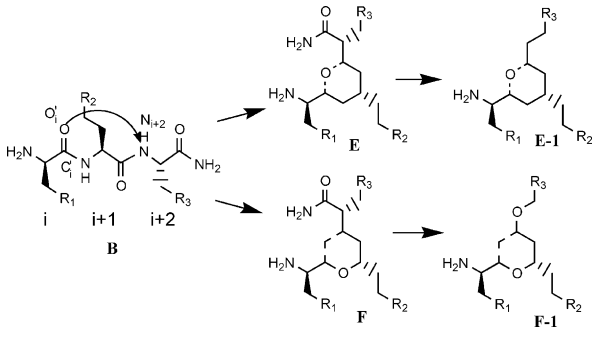


Chart 2. Design of tetrahydropyran analogues using $C_i'{=}O_i'\cdots N_{i+2}$ mode of peptide bridging.

In the second case (Chart 2), the bridging in **(B)** connects the backbone $C_i'{=}O_i'$ and N_{i+1}. The centers O_i' and N_{i+1} become either O in **(E)** or CH_2 in **(F)** while the amide bond C'_{i+1}–N_{i+2} is replaced by CH_2–CH in both constructs. Structures **(E-1)** and **(F-1)** represent further divergence from peptide moieties as well as a structural simplification to facilitate synthesis.

In order to determine if the described transformations of the tripeptide can actually produce bioisosteric structures, we have applied this concept to finding nonpeptide ligands for the melanocortin receptors (MCR), as a part of our ongoing efforts in this area.[16–18] Among five subtypes of these receptors, MC1R has been linked to skin pigmentation[19] while MC4R has been associated with feeding behavior[20,21] and modifying sexual activity in humans.[22]

As a model tripeptide for **(A)** and **(B)**, we have selected AcDPhe-Arg-TrpNH$_2$ **(1)**, a truncated version of the message sequence His-Phe-Arg-Trp of peptide agonists at the melanocortin receptors.[23] The peptide **(1)** is known to demonstrate weak but significant activity at MC4R. We also chose 2-naphthyl moiety to be an isosteric replacement for indole in Trp as a synthetically more accessible starting benchmark for peptidomimetic synthesis.[24]

The specific compounds that were made and tested in our in vitro assays are listed in Chart 3. Analogues **10** and **13** correspond to the construct **D** from Chart 1. Structurally, these two peptidomimetics differ from each other by a number of CH_2 groups in the arginine-related side chain. The three remaining structures **18**, **23**, and **24**

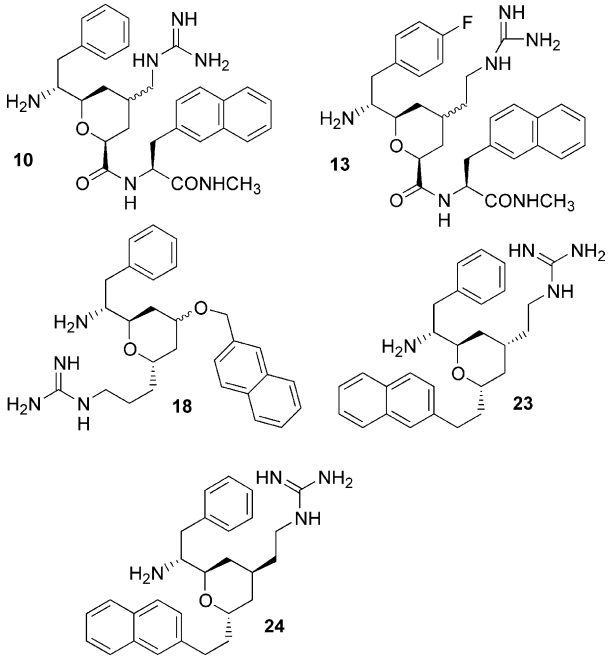


Chart 3. Compounds tested at the melanocortin receptors MC4R and MC1R.

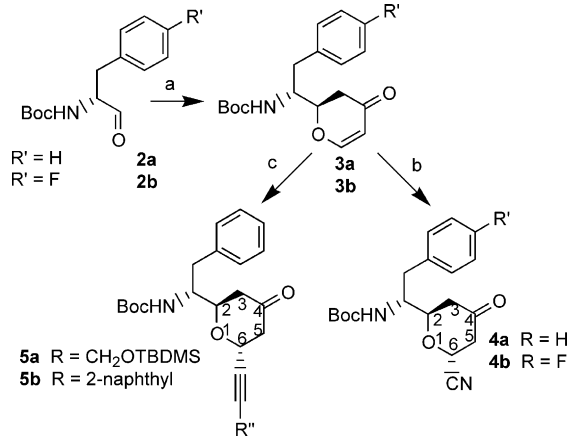
originated from the design illustrated in Chart 2. The structure **18** corresponds to **F-1** while **23** and **24** have been derived from **E-1**. As compared to the original constructs, **F** and **E**, all three synthesized compounds are lacking the carboxamide terminus and **18** is a mixture of diatereoisomers at C-4. Also, analogues **23** and **24** contain the chain corresponding to the arginine side chain in **B** shortened by one CH_2. They also represent separate isomers that would relate respectively to L- and D-isomers of arginine in **B**.

Synthesis

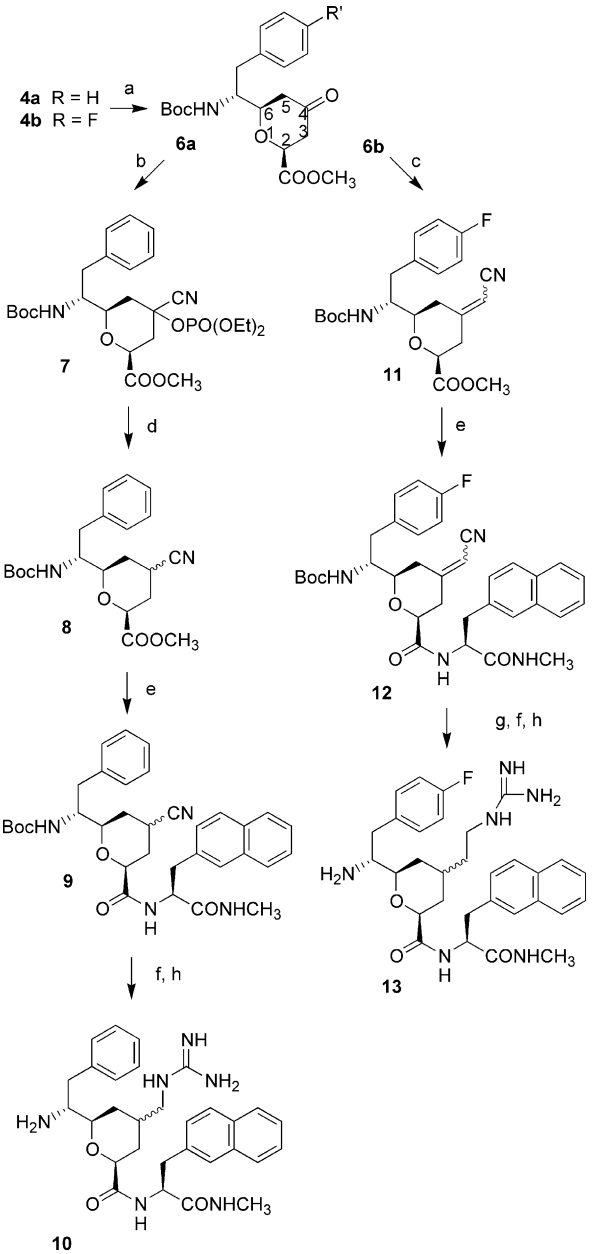
The key intermediates for the preparation of all described 2,4,6-trisubstituted tetrahydropyrane analogues have been 3,4-dihydro-2H-pyran-4-ones **3a** and **3b**. These are derived from a convenient hetero Diels–Alder cyclization approach.[25] Amino acid aldehydes used for this reaction ascertained the first substitution in the pyran ring. Two remaining substituents were introduced sequentially utilizing properties of the conjugated ketone system in **3a** and **3b**. The reaction sequence started with 1,4-additions of the nucleophiles such as the cyano group[26] (step b) and alkynes[27] (step c) resulting in analogues **4a**, **4b** and **5a**, **5b.** All these reactions took place with high diastereoselectivity.

While **5a** and **5b** contained the required stereochemistry at C-6, the configuration of this center in **4a** and **4b** had to be reversed. We discovered that basic hydrolysis of the cyano group (Scheme 3) occurs with epimerization thus furnishing the correct diastereoisomers of **6a** and **6b**. The configurations of the center at C-6 for **4b** and **6b** were determined by difference Nuclear Overhauser Effect (NOE) NMR spectroscopy at 500 MHz. In the case of **4b**, irradiation of the H-2 resonance showed a significant NOE to a proton at the carbon adjacent to the NH Boc group. Additionally, irradiation of H-6 showed NOEs to both protons at position 5, but no significant NOE was detected to H-2. For **6b**, irradiation of H-2 showed a strong NOE to H-6 indicating that these two protons are in close proximity on the same side of the ring. These data confirmed the proposed structures. Prior to the hydrolysis of CN group, the 4-keto group in **4a** and **4b** had to be protected in the form of a dimethyl acetal (step a, i) to prevent decomposition of the material. The acetal was subsequently hydrolyzed with acetic acid (step a, iii) and the free carboxyl group was esterified with TMS diazomethane (step a, iv).

The next stage in the conversions of **6a** and **6b** involved installation of the cyano and cyanomethylene groups at C-4 as precursors of the guanidine function relating to the tripetide arginine site.



Scheme 2. Reagents and conditions: (a) CH_2=C(OSi-C(OSiMe$_3$)CH=CHOMe, $LiClO_4$, Et_2O, rt; (b) TMSCN, BF_3Et_2O, DCM, 0 °C; (c) R-C≡CH, BuLi, TMSI, CuI*DMS, THF.



Scheme 3. Reagents and conditions: (a) (i) $HC(OMe)_3$, TsOH, MeOH; (ii) KOH, EtOH, H_2O; (iii) AcOH, THF; (iv) $TMSCH_2N_2$, MeOH; (b) $(EtO)_2OPCN$, NaCN, THF, rt; (c) $(EtO)_2OPCH_2CN$, NaH, THF, −40 °C; (d) SmI_2, *t*-BuOH, THF, rt; (e) (i) LiOH, H_2O, THF, rt; (ii) 2-Nal-CONHMe, HOBt, NMM, EDCI, DMF, rt; (f) H_2, Ni/Ra, MeOH/NH_4OH (1:4); (g) H_2, $NaBH_4$, $PdCl_2$, MeOH; (h) (i) BocNHC(=NBoc)SMe, $HgCl_2$, Et_3N, DMF, 0 °C; (ii) TFA, DCM.

Thus, the 4-keto group in **6a** was converted to the cyano intermediate **8,** constituting a mixture of isomers, by means of cyanohydrin phosphate **7** (Scheme 3, step b) followed by reduction of the latter with samarium iodide.[28] The ester function in **8** was replaced with L-naphthylalanine methylcarboxamide to produce **9** using standard methodologies of ester hydrolysis and amide bond formation. The cyano group in **9** was reduced to the corresponding amine with Raney nickel and subsequently converted to **10** by a typical guanidinylation technique[29] followed by TFA cleavage of all Boc protections.

The cyanomethylene group was introduced to **6b** using a Horner–Emmons reaction[30] (Scheme 3, step c) resulting in **11**. A transformation of **11** into **13** essentially followed the same methodology as the one applied for making **10** from **8** with one additional step (Scheme 3, step g) involving reduction of the conjugated double bond with $NaBH_4/PdCl_2$.[31] This hydrogenation method was used for a chemoselective reduction of carbon–carbon double bond in α,β-unsaturated carbonyl compounds.[32]

The reactions listed in Scheme 4 represent the conversion of **5a** to **18**. En route, reduction of ketone **14** produced a mixture of diasteroisomeric alcohols in the ratio of about 2:1. This mixture was carried to the final product **18**.

Analogues relating to the construct **E-1** in Chart 2, were derived from ketone **5b** (Scheme 5). A Horner–Emmons reaction of **5b** followed by hydrogenation of the double bond in **18** produced two diastereoisomers **19a** and **19b** in a 2:1 ratio. Using NOESY and NOE techniques, we assigned configuration **19b** to the major and **19a** to the minor isomer. We carried out the subsequent steps c and d with diastereoisomeric mixtures of starting materials and separated the products at each step to confirm the structure of each isomer. In the final purification, we obtained a single compound **24** but **23** was not completely isolated from the other isomer and contained about 20% of **24.**

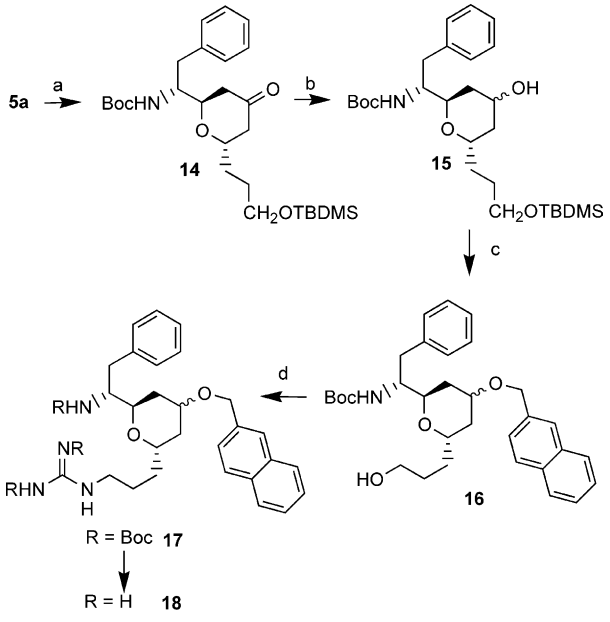
Biological testing

Table 1 shows the biological activity of 2,4,6-trisubstituted tetrahydropyran peptidomimetics. The compounds were evaluated in the binding and functional cell-based assays. Analogues **10** and **13**, corresponding to the construct **D**, have binding affinity and agonist potency at MC4R similar to those of **1**. On the other hand, E_{max} values of **10** and **13** indicate that they may be only partial agonists at this receptor. One particular difference between these two peptidomimetics is the emergence of MC1R binding in **13**. Over all, the peptidomimetics **10** and **13** appear to be bioisosteric with respect to the model tripeptide **1**.

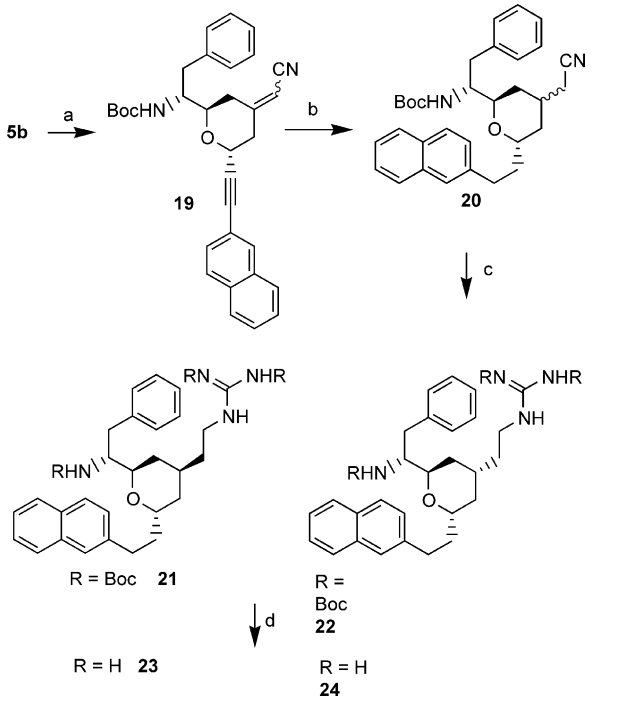
The analogues **17**, related to **F**, as well as **23**, and **24**, related to the construct **E** do not show the agonist

Table 1. Affinity and functional activity of investigated analogues at the melanocortin receptors MC4R and MC1R

	MC4R		MC1R	
	K_i (nM)	EC_{50} (nM) (E_{max}%)	K_i (nM)	EC_{50} (nM) (E_{max}%)
1	1188	2803 (49)	> 5000	
10	3395	2463 (45)	> 5000	
13	1163	4269 (52)	1445	> 25 000
18	797	> 100000	2858	1636 (69)
23	1701	> 100000	3310	1498 (64)
24	5472	> 100000	> 5000	



Scheme 4. Reagents and conditions: (a) H_2, Pd/C 10%, AcOEt; (b) L-Selectride, THF, −78 °C; (c) (i) 2-(bromomethyl)naphthalene, NaH, NaI, THF; (ii) TBAF, THF (d) BocHNC(=NH)NHBoc, DEAD, PPh_3, THF, 0 °C; (e) TFA, DCM.



Scheme 5. Reagents and conditions: (a) $(EtO)_2OPCH_2CN$, NaH, THF, −40 °C; (b) H_2, 40PSI, Wilkinson cat., toluene; (c) (i) H_2, Ni/Ra, MeOH/NH_4OH (1:4); (ii) BocNHC(=NBoc)SMe, $HgCl_2$, Et_3N, DMF, 0 °C; (d) TFA, DCM.

activity at MC4R. Compounds **18** and **23** have similar binding affinity at MC4R to that of **1** with **18** appearing somewhat more potent. The key difference in the measured biological activity of **18** and **23** versus **10** and **13** is an agonist effect at MC1R of the two former compounds.

Discussion and Conclusions

Even though compared to other recently published results[33] we have obtained only low activity at the melanocortin receptors, this work does illustrate that 2,4,6-trisubstituted tetrahydropyrans designed by conceptual bridging the tripeptide moiety between O_i' and C^{γ}_{i+1} or N_{i+2} are ligands of the melanocortin receptors MC1R and MC4R. Therefore, we can consider this transformation as being bioisosteric with respect to the model peptide. It should be noted that our design hasn't been based on attempts to mimic a particular secondary structure of the peptide ligand, for example, β-turn but only on a topological relationship between the peptide and 2,4,6-trisubstituted tetrahydropyrans. Whether the reported structures correspond to any potential secondary peptide structures can possibly be determined by conformational analysis of **10**, **13**, **18, 23** and **24**. This analysis could also aid determination of conformational characteristics of ligands required for the activity at the melanocortin receptors.

While it will remain important to analyze peptide secondary structures and continue designing non-peptide mimetics using conformational information from peptide leads, a systematic review of possible peptide constraint modes and structures associated with them may also provide a fertile ground for the design of diverse non-peptide analogues. As a result of this formal analysis, one can envision initiation of a peptidomimetic strategy through the systematic design of a library of surrogates for constraint modes of peptide ligands. When screened against biological targets, these libraries may provide access to initial hits. Thus, such an approach can be particularly effective in early discovery of non-peptide ligands from peptide leads. It can supplement other methods such as computer modeling and high throughput screening.

In summary, a conceptual bridging of the tripeptide moiety led to the design of novel 2,4,6-tetrahydropyran compounds. A sound synthetic methodology was subsequently developed to make these analogues. Testing on the melanocortin receptors confirmed the hypothesis that this bridging approach could produce bioisosteric peptidomimetics based on the tetrahydropyran moiety.

Experimental

Biology

In vitro assays. In vitro binding assay for the MCRs. The binding activity of MCR ligands was evaluated at three hMCRs using a cell-based assay that is specific for each subtype of MCRs (MC1R, MC3R, MC4R). Each subtype was stably transfected into HEK293 cells seeded in 96 well poly-l-lysine coated plates (VWR) with DMEM culture media containing 10% FBS, 1% Minimum Essential Amino Acids, 0.1% Penn/Strep and 0.1% L-glutamine (Gibco). NDP-MSH-Eu was dissolved in MEM (Gibco) + 10% Seablock (Pierce) + 1% DMSO (Sigma) mixture to attain a final concentration of 25 nM. This is to be added fresh each assay.

Binding activity (calculated as IC_{50} and K_i values) was determined in these cell lines by measuring the displacement of a constant concentration of europium labeled NDP-α-MSH with competing unlabeled ligands. Europium activity was detected using time resolve fluorometry on a Wallac plate reader under the Bioworks Europium program.

In vitro functional assay for the MCRs. The agonist activity of MCR ligands was evaluated at three hMCRs using the above MCR expressing HEK293 cells which were stably transfected with a reporter system consisting of a c-AMP responsive element (CRE) coupled to a luciferase reporter gene. Agonist activity was determined by assaying cells for luciferase activity in Packard 96-well view plate using luciferase assay buffer containing tricine (20 mM), $MgCO_3Mg(OH)_2 5H_2O$ (1.07 mM), $MgSO_4 7H_2O$ (2.67 mM), EDTA (0.1 mM) and luciferase substrate containing: DTT (5 mg/mL), ATP (0.292 mg/mL), co-enzyme A (0.208 mg/mL), D-luciferin (0.142 mg/mL). Responses were compared to the effect of NDP-MSH (MT-I) and expressed as a % of maximum activity of MT-I (E_{max}) which is considered to be a full agonist at each of the three MCRs.

Chemistry

General information

^1H and ^{13}C NMR spectra were recorded with Varian Inova 300 and 500 spectrometers; the data are reported as follows: chemical shift in ppm from Me_4Si line as external standard, multiplicity (b = broad, s = singlet, d = doubled, t = triplet, q = quartet, m = multiplet) and coupling constants. Normal resolution mass spectra and LC–MS were performed on a Micromass ZMD-400 spectrometer. High resolution mass spectra were obtained with AutoSpec Spectrometer at the Nebraska Center for Mass Spectrometry, University of Nebraska. Flash chromatography was carried out on a Biotage system using Flash 40 silica cartridges. Analytical HPLC was performed using a ThermoQuest system equipped with MetaChem, Polaris, C18 3μ 4.6×250 mm column, using linear gradient elution starting from CH_3CN/0.1% phosphoric acid in water (20:80) to 100% CH_3CN over 20 min (1 mL/min). Preparative HPLC was done on Rainin Dynamax and Varian ProStar systems equipped with MetaChem, Polaris, C18 10μ column 50×250 mm using gradient elution starting from CH_3CN/0.1% trifluoroacetic acid in water to 100% CH_3CN over 50 min. All reactions were carried out

under argon atmosphere. Anhydrous solvents and reagents were purchased from commercial sources. Reactions were monitored by analytical HPLC and thin-layer chromatography using Baker-flex silica plates 1B2-F. In addition to characterization data provided below, we obtained for each compound normal resolution MS and LC–MS that were in agreement with the proposed structures.

A general method for the cyano group addition to 3a and 3b (Scheme 2b). To a stirred solution of TMSCN (3.6 mL, 26.6 mmol) and ketone (8.7 mmol) in DCM (125 mL) was added BF_3Et_2O (0.12 mL, 0.87 mmol) at 0 °C. The reaction mixture was stirred for 2 h at 0 °C, quenched with NH_4Cl and extracted with DCM. Combined organic extracts were washed with $NaHCO_3$ dried over $MgSO_4$ and concentrated under reduced pressure. The residue containing TMS enol ether was dissolved in THF and treated with 1 M HCl (1:1 mixture) then extracted and dried over $MgSO_4$. Purification by flash chromatography (hexanes/EtOAc, 6:4) afforded the product.

[1-(6*R*-Cyano-4-oxo-tetrahydro-pyran-2*R*-yl)-2*R*-phenyl-ethyl]-carbamic acid *tert*-butyl ester 4a. (2.4 g, 79%). ^{1}H NMR (300 MHz; $CDCl_3$) δ 7.38–7.22 (m, H), 5.29 (d, J = 6.9 Hz, 1H), 4.85 (d, 10.2 Hz, 1H), 4.20–4.10 (m, 1H), 3.97–3.86 (m, 1H), 3.00–2.81 (m, 3H), 2.70–2.58 (m, 2H), 2.42–2.35 (m, 1H), 1.4 (s, 9H); ^{13}C NMR (300 MHz, $CDCl_3$) δ 201.4, 155.8, 137.1, 129.6, 129.0, 127.1, 116.2, 80.2, 74.4, 64.5, 54.9, 44.5, 43.5, 38.8, 28.5.

[1*R*-(6*R*-Cyano-4-oxo-tetrahydro-pyran-2*R*-yl)-2-(4-fluoro-phenyl)-ethyl]-carbamic acid *tert*-butyl ester 4b. ^{1}H NMR (300 MHz; $CDCl_3$) δ 7.28–7.10 (m, 2H), 7.05–6.90 (m, 2H), 5.27 (d, J = 7.5 Hz), 4.90 (d, J = 10.2 Hz, 1H), 4.18–4.04 (m, 1H), 3.92–3.78 (m, 1H), 2.96–2.78 (m, 3H), 2.72–2.50 (m, 2H), 2.42–2.28 (m, 1H), 1.4 (s, 9H); ^{13}C NMR (300 MHz, $CDCl_3$) δ 201.4, 163.7, 155.9, 132.9, 131.2, 116.3, 115.9, 115.6, 80.3, 74.5, 64.6, 54.9, 44.5, 43.5, 38.0, 28.5; HRFAB (positive) *m*/*e* 363.1720 calculated for $C_{19}H_{24}N_2O_4F$ $(M+H)^+$, found 363.1718.

A general method for addition of acetylenes to enone 3a (Scheme 2c.). Preparation of a copper iodide-dimethylsulfide complex. Copper(I) iodide was dissolved in Et_2O/DMS mixture and filtered. The filtrate was diluted with hexanes to precipitate CuI*1.5DMS complex as white crystals. This complex was dried at room temp. under vacuum for 1–2 h to give CuI*0.75DMS as white powder.

Typical procedure for conjugate addition of alkynylcopper reagents. *n*-Butyllithium (1.5 mL, 2.4 mmol, 1.6 M) was added at −10C to a stirred solution of an acetylene derivative (2.4 mmol) in THF (5 mL). After 20 min, CuI*0.75DMS complex (0.63 g, 2.7 mmol) was added in one batch. The stirring was continued for 45 min at −10 °C, the temperature was lowered to −78 °C and TMSI (0.335 mL, 2.4 mmol) was added. After 5 min, a solution of enone **3a** (1.6 mmol) in THF (5 mL) was added and the reaction mixture was stirred at −30 °C for 1 h. The reaction was quenched by addition of saturated NH_4Cl at −30 °C and the mixture was stirred for 30 min at room temperature. The layers were separated and the aqueous phase was extracted with Et_2O. The combined ether layer was washed with 1M $Na_2S_2O_3$, brine and dried over $MgSO_4$. Evaporation of the solvents gave product that was purified on a silica column (hexane:EtOAc, 8:2). This procedure gave product with the yields 60–80%.

(1*R*-{6*R*-[3-(*tert*-Butyl-dimethyl-silanyloxy)-prop-1-ynyl]-4-oxo-tetrahydro-pyran-2R-yl}-2-phenyl-ethyl)-carbamic acid *tert*-butyl ester 5a. HPLC Rt. 22.22, ^{1}H NMR (300 MHz; $CDCl_3$) δ 7.36–7.18 (m, 5H), 5.19 (d, 1H, J = 6.6 Hz), 4.97 (d, 1H, J = 10.2 Hz), 4.23 (s, 2H), 4.23–4.04 (m, 1H), 3.88–3.72 (m, 1H), 3.02–2.82 (m, 2H), 2.80–2.70 (m, 1H), 2.61–2.38 (m, 2H), 2.24–2.12 (m, 1H), 1.4 (s, 9H), 0.92 (s, 9H), 0.11 (s, 6H); ^{13}C NMR (300 MHz, $CDCl_3$) δ 204.9, 156.0, 137.8, 130.0, 128.6, 126.7, 88.3, 80.7, 80.0, 71.0, 65.6, 55.2, 51.7, 46.7, 45.0, 39.0, 28.6, 26.0, 18.3, −5.0; HRFAB (positive) *m*/*e* 488.2832 calculated for $C_{27}H_{41}NO_5Si$ $(M+H)^+$, found 488.2829.

[1-(6*R*-Naphthalen-2-ylethynyl-4-oxo-tetrahydro-pyran-2*R*-yl)-2-phenyl-ethyl]-carbamic acid *tert*-butyl ester 5b. HPLC Rt. 21.32 ^{1}H NMR (300 MHz; $CDCl_3$) δ 7.96–7.78(m, 3H), 7.61–7.51 (m, 2H), 7.45–7.39 (m, 1H), 7.38–7.17 (m, 3H), 7.17–7.04 (m, 3H), 5.45 (d, 1H, J = 6.6 Hz), 5.09 (d, 1H, J = 10.2 Hz), 4.45–4.35 (m, 1H), 3.96–3.98 (m, 1H), 3.12–2.96 (m, 2H), 2.95–2.82 (m, 1H0, 2.81–2.46 (m, 2H), 2.38–2.18 (m, 1H), 1.5 (s, 9H); ^{13}C NMR (300 MHz, $CDCl_3$) δ 205.0, 156.0, 137.9, 133.3, 133.0, 12.5, 129.9, 128.8, 128.6, 128.2, 128.1, 127.3, 127.0, 126.7, 119.1, 89.8, 85.3, 80.0, 71.0, 66.3, 55.5, 47.1, 45.2, 39.0, 28.7; HRFAB (positive) *m*/*e* 470.2331 calculated for $C_{30}H_{32}NO_4$ $(M+H)^+$, found 470.2329.

A general procedure for a transformation of the cyano group in 6a and 6b to the methyl ester. Formation of dimethylacetal and hydrolysis of cyano group (Scheme 3a, i and ii). A solution of ketone (6.7 mmol) in MeOH (70 mL), trimethylortoformate (20 mL) and *p*-toluenesulfonic acid (0.2 g) was heated for 12 h at 80 °C. After cooling to room temperature, the reaction mixture was neutralized with 1 M KOH and evaporated under reduced pressure. The residue was refluxed for 1 h in a mixture of EtOH (30 mL) and 1 M KOH (30 mL, 30 mmol), the reaction mixture was diluted with water and extracted with ether. Then water phase, containing the product, was acidified with 10% HCl and extracted with EtOAc. The organic phase was dried over $MgSO_4$ and concentrated under reduced pressure. The crude product was dissolved (Scheme 3a, ii) in a solution of THF (50 mL) and 2.5 M acetic acid (50 mL). The reaction mixture was heated at 60 °C for 12 h, extracted with ether to remove impurities, and the aqueous phase was acidified to pH 2–3. The product was extracted with EtOAc, the organic phase was dried over $MgSO_4$ and concentrated under reduced pressure A solution of this crude product in MeOH (30 mL) (Scheme 3a, iv) was exposed to five cycles of esterifications. Each cycle con-

sisted of cooling the reaction mixture to 0 °C, dropwise addition of $TMSCH_2N_2$ (1.8 mL) and stirring at room temperature for 1 h. After the last cycle, the excess of $TMSCH_2N_2$ was decomposed by careful treatment with acetic acid and solvents were removed under reduced pressure. The residue was dissolved in ether, washed with saturated aqueous $NaHCO_3$ and dried over $MgSO_4$. Purification on a silica column (hexanes/EtOAc 7:3) afforded products with the overall yields of 45–55%.

6*R*-(1*R*-*tert*-Butoxycarbonylamino-2-phenyl-ethyl)-4-oxo-tetrahydro-pyran-2*S*-carboxylic acid methyl ester 6a. ^{1}H NMR (300 MHz; $CDCl_3$) δ 7.33–7.17 (m, 5H), 5.15 (d, $J = 10.2$ Hz, 1H), 4.14 (dd, $J_1 = 4.5$ Hz, $J_2 = 10.8$, 1H), 3.99–43.90 (m, 1H), 3.85 (s, 3H), 3.63–3.54 (m, 1H), 3.04–2.88 (m, 2H), 2.70–2.52 (m, 2H), 2.28–2.00 (m, 1H), 2.00–1.80 (m, 1H), 1.42 (s, 9H); ^{13}C NMR (300 MHz, $CDCl_3$) δ 204.8, 170.0, 156.0, 137.9, 129.6, 129.5, 128.9, 126.9, 79.9, 75.8, 75.0, 54.8, 52.9, 44.8, 43.8, 38.8, 28.6; HRFAB (positive) *m*/*e* 400.1736 calculated for $C_{20}H_{27}NO_6Na$ $(M + Na)^+$, found 400.1729.

6*R*-[1*R*-*tert*-Butoxycarbonylamino-2-(4-fluoro-phenyl)-ethyl]-4-oxo-tetrahydro-pyran-2*S*-carboxylic acid methyl ester 6b. ^{1}H NMR (300 MHz; $CDCl_3$) δ 7.24–7.15 (m, 2H), 7.06–6.95 (m, 2H), 5.04 (d, $J = 9.9$ Hz, 1H), 4.16 (dd, $J_1 = 4.5$ Hz, $J_2 = 10.8$ Hz, 1H), 3.87 (s, 3H), 3.86–3.74 (m, 1H), 3.68–3.52 (m, 1H), 3.04–2.88 (m, 2H), 2.70–2.50 (m, 3H), 2.34–2.20 (m, 1H), 1.4 (s, 9H); ^{13}C NMR (300 MHz, $CDCl_3$) δ 204.6, 170.8, 169.9, 163.5, 160.3, 156.0, 133.7, 131.2, 130.9, 115.7, 115.4, 79.9, 77.4, 76.0, 75.0, 54.7, 52.8, 44.2, 43.7, 38.0, 28.5; HRFAB (positive) *m*/*e* 396.1822 calculated for $C_{20}H_{27}NO_6F$ $(M + H)^+$, found 396.1827.

6*R*-(1*R*-*tert*-Butoxycarbonylamino-2-phenyl-ethyl)-4-cyano-4-(diethoxy-phosphoryloxy)-tetrahydro-pyran-2S-carboxylic acid methyl ester 7 (Scheme 3b). To a solution of ketone **6a** (0.66 g 1.8 mM) and diethylcyanophosphate (0.88 g 5.4 mM) in dry THF (30 mL) was added 0.5 M solution of NaCN in DMF (10.8 mL) and the mixture was stirred at room temperature for 2 h. The reaction mixture was quenched with water and extracted with a mixture of hexane and ethyl acetate (1:1). The organic layer was washed with brine several times, dried over $MgSO_4$ and concentrated under reduced pressure affording crude product that was subjected to flash chromatography on a silica column (hexane/EtOAc, 2:1). Yield 0.45. (90%) of **6*R*-(1*R*-*tert*-butoxycarbonylamino-2-phenyl-ethyl)-4-cyano-4-(diethoxy-phosphoryloxy)-tetrahydro-pyran-2S-carboxylic acid methyl ester 7.** ^{1}H NMR (300 MHz; $CDCl_3$) δ 7.40–7.10 (m, 5H), 4.95 (d, $J = 9.9$ Hz, 1H), 4.17(q, $J = 7.2$ Hz, 4H), 4.14–1.05 (m, 1H), 3.98–3.86 (m, 1H), 3.83 (s, 3H), 3.68–3.54 (m, 1H), 2.98.286 (m, 2H), 2.84–2.74 (m, 1H), 2.34–2.24 (m, 1H), 2.12–1.90 (m, 2H), 1.4 (s, 9H), 1.36 (s, 6H); ^{13}C NMR (300 MHz, $CDCl_3$) δ 169.3, 155.8, 137.6, 129.7, 128.8, 126.9, 117.4, 79.9, 74.2, 73.2, 65.0, 54.6, 52.9, 39.0, 28.5, 16.3; HRFAB (positive) *m*/*e* 563.2134 calculated for $C_{25}H_{37}N_2O_9PNa$ $(M + Na)^+$, found 563.2159.

6*R*-(1*R*-*tert*-Butoxycarbonylamino-2-phenyl - ethyl) - 4 - cyano - tetrahydro-pyran-2*S*-carboxylic acid methyl ester 8 (Scheme 3d). To a solution of crude **7** (0.5 g, 0.93 mmol) in THF (7 mL) and *t*-BuOH (0.09 mL, 0.93 mmol) was degassed with argon and solution of SmI_2 (28 mL, 2.8 mmol, 0.1 M in THF) was added at room temperature. Progress of the reaction was followed by HPLC and SmI_2 was added. After 4 h, reaction mixture was quenched with 10% HCl and extracted with ethyl ether. Extracts were washed with 5% $Na_2S_2O_3$, H_2O and brine and dried over $MgSO_4$. After removal of solvent under reduced pressure, the crude product of was purified by flash chromatography (hexane/EtOAc, 2:1) to give 0.25 g, (69%) of **6*R*-(1*R*-*tert*-butoxycarbonylamino-2-phenyl-ethyl)-4-cyano-tetrahydro-pyran-2*S*-carboxylic acid methyl ester 8**. ^{1}H NMR (300 MHz; $CDCl_3$) δ 7.41–7.07 (m, 5H), 5.05–4.80 (m, 1H), 4.4–4.20 (m, 1H), 4.2–4.05 (m, 1H), 4.98–4.80 (m, 1H), 3.81 (s, 3H), 3.68–3.60 (m, 1H), 3.30–3.3.08 (m, 1H), 3.00–2.87 (m, 2H), 2.40–2.08 (m, 1H), 1.9–1.60 (m, 2H), 1.4 (s, 9H); ^{13}C NMR (300 MHz, $CDCl_3$) δ 170.5, 156.0, 138.2, 129.7, 128.8, 126.6, 120.6, 79.9, 75.0, 73.0, 55.1, 52.7, 38.8, 31.1, 30.7, 28.6.

{1R-[4-Cyano-6S-(1R-methylcarbamoyl-2-naphthalen-2-yl - ethylcarbamoyl) - tetrahydro-pyran-2R-yl]-2-phenyl-ethyl}-carbamic acid *tert*-butyl ester 9 (Scheme 3e, i). Methyl ester **8** (0.1 g, 0.26 mmol) was dissolved in THF/H_2O (2 mL/0.6 mL) and water solution of LiOH (0.014 g, 0.6 mmol) was added. The reaction was stirred for 2 h at room temperature, concentrated under reduced pressure to about 1/3 volume and treated with 1 N HCl to pH 2–3. The product (acid) was extracted with ethyl acetate. The organic phase was washed with water until pH 4 and concentrated (Scheme 3e, ii). To a solution of crude acid in DMF (1 mL) were added HOBt (0.07 g, 0.52 mmol), NMM (0.23 mL, 2.1 mmol) and L-Nal-NH_2 (0.093 g, 0.28 mmol, TFA salt) following by addition of EDCI (0.061 g, 0.32 mmol). The reaction mixture was stirred overnight at room temperature, diluted with water, extracted with ethyl acetate. Purification on a preparative HPLC afforded 0.14 g (92%) of **{1*R*-[4-Cyano-6*S*-(1*R*-methylcarbamoyl-2-naphthalen-2-yl-ethylcarbamoyl)-tetrahydro-pyran-2r-yl]-2-phenyl-ethyl} - carbamic acid *tert*-butyl ester 9** as a mixture of two diastereoisomers in a ratio 1:1 with HPLC retention times 16.73 and 16.94 min, respectively. ^{1}H NMR (300 MHz; $CDCl_3$) δ 7.85–7.80 (m, 2H), 7.73 (s, 1H), 7.53–7.35 (m, 3H), 7.34–7.20 (m, 3H), 7.17–7.12 (m, 2H), 5.01 (d, $J = 9.9$ Hz, 1H), 4.80–4.4.68 (m, 2H), 4.16–4.06 (m, 1H), 4.00–3.89 (m, 1H), 3.82–3.66 (m, 1H), 3.34–3.26 (m, 2H), 3.00 (s, 1H), 2.84–2.74 (m, 3H), 2.19–2.08 (m, 1H), 1.80–1.58 (m, 2H), 1.4 (s, 9H), 1.20–1.00 (m, 1H); ^{13}C NMR (300 MHz, $CDCl_3$) δ 171.8, 170.6, 156.1, 137.5, 134.3, 133.7, 132.7, 129.3, 128.8, 128.7, 128.5, 128.0, 127.8, 127.5, 127.0, 126.7, 126.2, 120.4, 80.0, 74.8, 73.7, 54.7, 54.5, 38.6, 38.5, 30.2, 29.8, 28.7, 26.7, 24.8.

6*R*-(1*R*-Amino-2-phenyl-ethyl)-4-guanidinomethyl-tetrahydro-pyran-2*S*-carboxylic acid (1*R*-methylcarbamoyl-2-naphthalen-2-yl-ethyl)-amide 10 (Scheme 3f). A solution of **9** (0.034 g, 0.06 mmol) in MeOH/NH_4OH (4:1) (10

mL) was hydrogenated overnight in the presence of Raney nickel at 40 psi, room temperature. The catalyst was removed by filtration; the filtrate was concentrated to an oily residue (Scheme 3h). A solution of crude amine, 1,3-bis(benzyloxycarbonyl)-2-methyl-2-thiopseudourea (CAS# 25508-20-7) (0.24 g, 0.08 mmol) and triethylamine (0.015 mL) in DMF (1 mL) was treated with $HgCl_2$ (0.021 g, 0.08 mmol) at 0 °C and stirred at this temperature for 2 h. The reaction mixture was diluted with ethyl acetate (15 mL), the solids were removed by filtration, the filtrate evaporated and the residue was purified on a silica column using hexanes/ethyl acetate 1/1 affording 0.03 g of the product which was subsequently treated with a solution of dichloromethane, trifluoroacetic acid and water in 2:1:0.1 ratio (1 mL) for 3 h at room temperature. The reaction mixture was diluted with 1,2-dichloroethane and repeatedly evaporated with fresh portions of this solvent to remove residual trifluoroacetic acid. The final residue was purified by a preparative HPLC affording 0.022 g (70%) of **6*R*-(1*R*-Amino-2-phenyl-ethyl)-4-guanidinomethyl-tetrahydro-pyran-2*S*-carboxylic acid (1*R*-methylcarbamoyl-2-naphthalen-2-yl-ethyl)-amide 10**. ^{1}H NMR (300 MHz; CD_3OD) δ 7.88–7.70 (m, 3H), 7.50–7.26 (m, 8H), 7.14–7.08 (m, 1H), 4.08–3.98 (m, 1H), 3.74–3.44 (m, 2H), 3.42–3.38 (m, 1H), 3.33–3.20 (m, 2H), 3.29–2.86 (m, 3H), 2.75 (s, 3H), 2.30–2.06 (m, 1H), 1.90–1.50 (m, 4H), 1.38–1.22 (m, 1H); ^{13}C NMR (300 MHz, $CDCl_3$) δ 174.4, 173.4, 159.2, 136.7, 136.0, 134.4, 130.9, 130.8, 130.7, 130.5, 129.7, 129.4, 129.3129.1128.9, 128.8, 128.7, 127.7, 127.2, 74.5, 71.8, 57.4, 43.0, 39.9, 37.3, 36.9, 31.9, 31.0, 30.2, 29.9, 26.8; HRFAB (positive) *m*/*e* 531.3084 calculated for $C_{30}H_{39}N_6O_3$ $(M+H)^+$, found 531.3078.

6*R*-[1*R*-*tert*-Butoxycarbonylamino-2-(4-fluoro-phenyl)-ethyl]-4-cyanomethylene-tetrahydro-pyran-2*S*-carboxylic acid methyl ester 11 (Scheme 3c). Diethylcyanomethylphosphate (0.1 mL, 0.61 mmol) was added dropwise into a mixture of NaH (0.03 g, 0.61 mmol, 60% in mineral oil) in THF (5 mL) (mineral oil was removed with hexane prior to addition of THF) at 0 °C and the reaction mixture was stirred for 1 h. The mixture was cooled to −40 °C, and ketone **6b** (0.2 g, 0.51 mmol) in THF (0.5 mL) was added. After stirring for 3 h, the reaction mixture was quenched with saturated NH_4Cl. The product was extracted with Et_2O and purified on a silica column (hexanes/EtOAc, 2:1) affording (0.2 g, 94%), **6*R*-[1*R*-*tert*-butoxycarbonylamino-2-(4-fluoro-phenyl)-ethyl]-4-cyanomethylene-tetrahydro-pyran-2*S*-carboxylic acid methyl ester 11**. HPLC retention time. 16.27 min. ^{1}H NMR (300 MHz; $CDCl_3$) δ 7.25–7.10 (m, 2H), 7.06–6.94 (m, 2H), 5.32–5.18 (m, 1H), 5.10–4.94 (m, 1H), 3.87–3.81 (m, 5H), 3.4–3.22 (m, 1H), 3.20–2.82 (m, 2H), 2.78–2.56 (m, 1H), 2.54–2.28 (m, 2H), 2.26–2.10 (m, 1H), 1.4 (s, 9H); ^{13}C NMR (300 MHz, $CDCl_3$) δ 169.9, 163.6, 160.2, 156.0, 155.8, 133.7, 131.0, 130.9, 116.0, 115.8, 115.5, 96.1, 80.1, 76.9, 76.8, 76.7, 76.0, 75.8, 54.8, 52.8, 38.2, 38.0, 37.5, 37.3, 35.2, 35.1, 28.6; HRFAB (positive) *m*/*e* 441.1802 calculated for $C_{22}H_{27}N_2O_5FNa$ $(M+Na)^+$, found 441.1787.

[1*R*-[4-Cyanomethylene-6*S*-(1*R*-methylcarbamoyl-2-naphthalen-2-yl-ethylcarbamoyl)-tetrahydro-pyran-2*R*-yl]-2-(4-fluoro-phenyl)-ethyl]-carbamic acid *tert*-butyl ester 12. This was obtained from **11** (0.15 g) analogously to **9** from **8**. ^{1}H NMR (300 MHz; $CDCl_3$) δ 7.90–7.64 (m, 3H), 7.62–7.34 (m, 4H), 7.12–7.88 (m, 4H), 5.32 (s, 1H), 5.15 (d, $J=9.0$ Hz, 1H), 4.84–4.64 (m, 1H), 4.02–3.83 (m, 1H), 3.82–3.64 (m, 1H), 3.48–3.10 (m, 3H), 2.96–2.54 (m, 7H), 2.43–2.02 (m, 2H), 1.91–1.70 (m, 1H), 1.4 (s, 9H); ^{13}C NMR (300 MHz, $CDCl_3$) δ 171.9, 170.0, 163.6, 159.8, 156.1, 134.2, 133.7, 133.3, 132.7, 130.8, 128.7, 128.4, 128.0, 127.7, 127.5, 126.7, 126.3, 115.8, 115.6, 96.2, 80.24, 77.5, 76.9, 76.6, 54.9, 54.5, 53.7, 38.6, 37.9, 37.6, 37.2, 35.2, 28.7, 26.7; HRFAB (positive) *m*/*e* 639.2959 calculated for $C_{35}H_{41}N_4O_5F$ Na $(M+Na)^+$, found 639.2986.

6*R*-[1*R*-Amino-2-(4-fluoro-phenyl)-ethyl]-4-(2-guanidino-ethyl)-tetrahydro-pyran-2*S*-carboxylic acid (1*R*-methylcarbamoyl-2-naphthalen-2-yl-ethyl)-amide 13 (Scheme 3g). Sodium borohydride (0.02 g, 0.52 mmol) was added to a mixture of $PdCl_2$ (0.04 g, 0.22 mmol) in methanol (1 mL). Subsequently, **12** (0.5 g, 0.8 mmol) was added and the mixture was stirred under normal hydrogen pressure for 3 h. After filtration of solids, the filtrate was concentrated under reduced pressure and the residue was converted to **13** with procedures (Scheme 3f, h) analogues to those used for making **10** from **9**, including final purification on a preparative HPLC affording 0.27 g (60%) of **6*R*-[1*R*-amino-2-(4-fluoro-phenyl)-ethyl]-4-(2-guanidino-ethyl)-tetrahydro-pyran-2*S*-carboxylic acid (1*R*-methylcarbamoyl-2-naphthalen-2-yl-ethyl)-amide 13**. HPLC retention time. 7.71 min; ^{1}H NMR (300 MHz; $CDCl_3$) δ 7.90–7.76 (m, 3H), 7.72 (s, 1H), 7.54–7.38 (m, 3H), 7.34–7.25 (m, 2H), 7.16–7.07 (m, 2H), 5.00–4.84 (m, 1H), 3.84–3.76 (m, 1H), 3.58–3.34 (m, 3H), 3.22–2.98 (m, 3H), 2.96–2.85 (m, 2H), 2.82–2.70 (m, 3H), 1.83–1.50 (m, 4H), 1.50–1.20 (m, 2H), 1.03–0.90 (m, 1H), 1.57–1.28 (m, 1H); HRFAB (positive) *m*/*e* 563.3136 calculated for $C_{31}H_{39}FN_6O_3Na$ $(M+H)^+$, found 563.3146.

(1*R*-{6*S*-[3-(*tert*-Butyl-dimethyl-silanyloxy)-propyl]-4-oxo-tetrahydro-pyran-2*R*-yl}-2-phenyl-ethyl)-carbamic acid tert-butyl ester 14 (Scheme 4a). Raney nickiel was added to a solution of **5a** (0.15 g, 0.31 mmol) in EtOAc and the mixture was stirred at 50 °C for 0.5 h to deactivate possible traces of sulfur contamination left from the Michael addition. The solids were removed by filtration and the filtrate was hydrogenated using Pd/C 10% at normal conditions for 1 h. After filtration, the solvent was evaporated under reduced pressure and the residue was chromatographed (hexane/EtOAc, 7:3) to give 0.14 g (92%) **(1*R*-{6*S*-[3-(*tert*-Butyl-dimethyl-silanyloxy)-propyl]-4-oxo-tetrahydro-pyran-2*R*-yl}-2-phenyl-ethyl)-carbamic acid *tert*-butyl ester 14**. ^{1}H NMR (300 MHz; $CDCl_3$) δ 7.40–7.15 (M, 5H), 4.97 (d, 1H, $J=9.6$ Hz), 4.44–4.26 (m, 1H), 3.99–3.90 (m, 1H), 3.89–3.80 (m, 1H), 3.74–3.56 (m, 2H), 3.03–2.82 (m, 2H), 2.79–2.49 (m, 2H), 2.36–2.16 (m, 2H), 1.72–1.55 (m, 4H), 1.4 (s, 9H), 0.92 (s, 9H), 0.85 (m, 6H); ^{13}C NMR (300 MHz, $CDCl_3$) δ 207.6, 156.0, 138.1, 129.5, 128.7, 126.7, 79.8, 74.1, 70.0, 62.7, 55.1, 46.0, 44.8, 39.1, 28.6, 26.2, 18.6,-5.1; HRFAB (positive) *m*/*e* 492.3145 calculated for $C_{27}H_{46}NO_5Si$ $(M+H)^+$, found 492.3137.

(1*R*-{6*S*-[3-(*tert*-Butyl-dimethyl-silanyloxy) - propyl] - 4 - hydroxy - tetrahydro - pyran - 2*R* - yl} - 2 - phenyl-ethyl)-carbamic acid *tert*-butyl ester 15 (Scheme 4b). A solution of ketone **14** (0.2 g, 0.41 mmol) in THF at −78 °C was treated dropwise with L-Selectride stirred 0.5 h. The reaction was quenched with 10% aq NH_4Cl, the product extracted with ether, dried with $MgSO_4$ and the extract was concentrated under reduced pressure. The residue was purified by silica gel chromatography (hexane/EtOAc 8:2) giving 0.16 g (yield 80%) of **15**, two diastereoisomers. HPLC retention times 21.92 and 22.58 min with the ratio 2.2:1. ^{1}H NMR (300 MHz; $CDCl_3$) δ 7.35–7.17 (m, 5H), 5.01 (d, 0.31H, J = 9.6 Hz), 4.81 (d, 0.69H, J = 8.4 Hz), 4.28–4.02 (m, 1H), 4.01–3.79 (m, 2H), 3.78–3.48 (m, 3H), 3.04–2.72 (m, 2H), 2.04–1.42 (m, 8H), 1.4 (s, 9H), 0.93 (s, 9H), 0.08 (s, 6H); ^{13}C NMR (300 MHz, $CDCl_3$) δ 156.0, 138.8, 138.2, 129.8, 129.6, 128.6, 126.5, 79.4, 73.5, 71.3, 68.6, 67.8, 64.7, 64.4, 63.7, 63.0, 55.6, 53.2, 38.7, 38.1, 35.5, 31.4, 29.9, 28.6, 26.2, 18.6, −5.0; HRFAB (positive) m/e 494.3302 calculated for $C_{27}H_{48}NO_5Si$ $(M+H)^+$, found 494.3298.

{1R-[6S-(3-Hydroxy-propyl)-4-(naphthalen-2-ylmethoxy) -tetrahydro-pyran-2*R*-yl]-2-phenyl-ethyl}-carbamic acid *tert*-butyl ester 16 (Scheme 4c, i). Sodium hydride (0.032 g, 0.82 mmol) was placed in the dried flask under argon and washed with hexane to remove mineral oil, the THF (1.0 mL) was added followed by dry sodium iodide and the solution was cooled to 0 °C. To this mixture, alcohol **15** (0.12 g, 0.41 mmol) dissolved in THF (0.5 mL) was added and the mixture was stirred at 0 °C for 0.5 h. Then bromide (0.09 g, 0.43 mmol) dissolved in DMF (0.2 mL) was added dropwise and the reaction mixture was stirred at room temperature for 12 h. The work-up involved addition of 10% aq NH_4Cl, extracted of the product with ether. Purification on a silica column (hexane/EtOAc: 95:5) furnished (0.08 g, 52%) of **{1*R*-[6*S*-[3-(*tert*-Butyl-dimethyl-silanyloxy)-propyl]-4-(naphthalen-2-ylmethoxy)-tetrahydro-pyran-2*R*-yl]-2-phenyl-ethyl}-carbamic acid *tert*-butyl ester** that was dissolved in a mixture of acetic acid (Scheme 4c, ii), water and THF in ratio 3:1:1. After overnight stirring the reaction mixture wa evaporated and chromatographed on a silica column (hexane/EtOAc, 8:2) producing 0.04 g, (32%) in two steps of **{1*R*-[6*S*-(3-Hydroxy-propyl)-4-(naphthalen-2-ylmethoxy)-tetrahydro-pyran-2*R*-yl]-2-phenyl-ethyl}-carbamic acid *tert*-butyl ester 16.** HPLC retention time. 18.85 min; ^{1}H NMR (300 MHz; $CDCl_3$) δ 7.91–7.81 (m, 3H), 7.78 (s, 0.57H), 7.75 (s, 0.43H), 7.56–7.38 (m, 3H), 7.34–7.12 (m, 5H), 4.98 (d, 1H, J = 9 Hz), 4.80–4.58 (m, 2H), 4.22–3.72 (m, 3H), 3.71–3.62 (m, 2H), 3.62–3.50 (m, 1H), 2.96–2.72 (m, 2H), 2.08–1.84 (m, 2H), 1.83–1.50 (m, 6H), 1.4 (s, 9H); ^{13}C NMR (300 MHz, $CDCl_3$) δ 156.0, 133.5, 133.2, 129.6, 128.6, 128.5, 128.1, 127.9, 126.3, 126.1, 125.9, 79.4, 73.1, 71.4, 71.1, 70.4, 70.1, 69.5, 68.2, 63.1, 62.8, 55.4, 53.2, 39.0, 35.7, 35.2, 32.7, 29.7, 28.7; HRFAB (positive) m/e 520.3063 calculated for $C_{32}H_{42}NO_5$ $(M+H)^+$, found 520.3056.

{1*R*-[6*S*-(3-(bis-Boc)Guanidino-propyl)-4-(naphthalen-2-ylmethoxy)-tetrahydro-pyran-2*R*-yl]-2-phenyl-ethyl}-carbamic acid *tert*-butyl ester 17 (Scheme 4d). Diisopropyl-azodicaboxylate (0.04 mmol) was added dropwise at 0 °C to a solution of alcohol **16** (0.015 g, 0.03 mmol), 1,3-bis(*tert*-butoxycarbonyl)-guanidine (0.012 g, 0.045 mmol) and triphenylphosphine (0.011 g, 0.04 mmol) in anhydrous THF (0.5 mL) over 2 h. The reaction mixture was concentrated under reduced pressure. The residue was purified on a silica column (hexane/EtOAc 8:2) to give 0.017 g (yield 75%) of **17**. ^{1}H NMR (300 MHz; $CDCl_3$) δ 7.94–7.72 (m, 3H), 7.56–7.44 (m, 2H), 7.38–7.19 (m, 7H), 5.10–4.96 (m, 1H), 4.84–4.52 (m, 2H), 4.26–4.10 (m, 1H), 4.09–3.68 (m, 4H), 3.64–3.49 (m, 1H), 2.10–1.56 (m, 8H), 1.5 (bs, 27H); ^{13}C NMR (300 MHz, $CDCl_3$) δ 161.1, 158.6, 156.1, 137.4, 136.3, 134.1, 134.2, 129.7, 128.6, 128.1, 128.0, 126.4, 126.1, 125.9, 84.0, 81.7, 79.3, 72.9, 71.2, 53.4, 44.5, 39.2, 35.5, 30.0, 28.6, 28.4, 25.8.

***N*-{3-[6*S*-(1*R*-Amino-2-phenyl-ethyl)-4-(naphthalen-2-yl-methoxy)-tetrahydro-pyran-2*R*-yl]-propyl}-guanidine 18.** This was obtained from **17** by a standard Boc cleavage using trifluoroacetic/dichlomethane/water 1/2/0.1 at room temperature and purification on a preparative HPLC. HPLC retention time time 8.77 min; HRFAB (positive) m/e 461.2917 calculated for $C_{28}H_{37}N_4O_2$ $(M+H)^+$, found 461.2912.

[1*R*-(4-Cyanomethylene-6*R*-naphthalen-2-ylethynyl-tetrahydro-pyran-2*R*-yl)-2-phenyl-ethyl]-carbamic acid *tert*-butyl ester 19 (Scheme 5a). Diethylcyanomethylphosphate (0.028 mL, 0.17 mmol) was added dropwise into a mixture of NaH (0.007 g, 0.17 mmol, 60% in mineral oil) in THF (1 mL) (mineral oil was removed with hexane) at 0 °C and the reaction mixture was stirred for 1 h. Then mixture was cooled to −40 °C and ketone **5b** (0.064 g, 0.14 mmol) dissolved in THF (1 mL) was added. After stirring for 1 h, the reaction was quenched with 10% aq NH_4Cl, extracted with Et_2O. Purification on a silica column (hexanes/EtOAc 2:1) afforded **19** (0.06 g, 87%). HPLC retention time 21.59 min; ^{1}H NMR (300 MHz; $CDCl_3$) δ 7.98–7.79 (m, 4H), 7.62–7.52 (m, 2H), 7.50–7.23 (m, 3H), 7.20–7.06 (m, 3H), 5.38–5.22 (m, 2H), 5.06–4.91 (m, 1H), 4.14–4.01 (m, 1H0, 3.99–3.78 (m, 1H), 3.12 (d, 0.59H, J = 13.8 Hz), 3.00–2.88 (m, 2H), 2.85–2.67 (m, 1.41H), 2.58–2.40 (m, 1H), 2.26–2.16 (m, 1H), 1.4 (s, 9H); ^{13}C NMR (300 MHz, $CDCl_3$) δ 159.9, 156.1, 138.0, 133.3, 133.1, 132.3, 130.0, 128.7, 128.3, 128.1, 127.2, 127.0, 126.9, 126.7, 119.4, 116.5, 96.7, 96.5, 89.4, 85.3, 85.2, 80.0, 71.3, 70.9, 66.5, 55.4, 40.7, 39.0, 38.7, 38.2, 35.8, 28.7; HRFAB (positive) m/e 493.2491 calculated for $C_{32}H_{33}N_2O_3$ $(M+H)^+$, found 493.2490.

{1*R*-[4-Cyanomethyl-6*S*-(2-naphthalen-2-yl-ethyl)-tetrahydro-pyran-2*R*-yl]-2-phenyl-ethyl}-carbamic acid tert-butyl ester 20 (Scheme 5b). Nitrile **19** (0.05 g, 0.1 mmol) was hydrogenated in toluene (4 mL) in a presence of Wilkinson catalyst at 40PSI for 2 days. The reaction mixture was filtered, evaporated and chromatographed on a silica column (hexane/EtOAc, 8:1), affording 0.04 g (yield 80%) of **20**. (two diastereoisomers in ratio 4.5:1) ^{1}H NMR (300 MHz; $CDCl_3$) δ 7.9–7.75 (m, 3H), 7.63 (s, 1H), 7.54–7.40 (m, 2H), 7.39–7.19 (m, 6H), 5.02 (d, 0.18H, J = 9.6 Hz), 4.75 (d, 0.72H, J = 8.7 Hz), 4.38–

4.06 (m, 1H), 3.95–3.62 (m, 2H), 3.10–2.91 (m, 2H), 2.90–2.68 (m, 2H), 2.36–2.19 (m, 2H), 2.18–2.01 (m, 1H), 2.00–1.74 (m, 4H), 1.73–1.49 (m, 2H), 1.4 (m, 9H); ^{13}C NMR (300 MHz, $CDCl_3$) δ 156.0, 139.9, 137.7, 134.0, 133.9, 132.2, 132.3, 129.8, 129.6, 128.7, 128.3, 127.9, 127.5, 126.8, 126.4, 126.2, 125.4, 118.1, 79.6, 72.5, 72.3, 70.7, 67.8, 55.6, 50.9, 39.2, 38.8, 38.2, 36.6, 34.7, 34.6, 32.8, 32.6, 32.1, 31.8, 29.1, 28.7, 28.2, 27.9, 24.8, 24.6; HRFAB (positive) *m/e* 499.2961 calculated for $C_{32}H_{39}N_2O_3$ $(M+H)^+$, found 499.2980.

The mixture of diastereoisomers **20** was separated on a preparative HPLC.

Major isomer of **20** HPLC retention time 20.74 min. ^{1}H NMR (300 MHz; $CDCl_3$) δ 7.87–7.76 (m, 3H), 7.64 (s, 1H), 7.52–741 (m, 2H), 7.38–7.22 (m, 6H), 4.76 (d, $J=7.5$ Hz, 1H), 4.36–4.17 (m, 1H), 3.86–3.69 (m, 2H), 3.09–2.93 (m, 2H), 2.86–2.70 (m, 2H), 2.32–2.22 (m, 2H), 2.20–2.01 (m, 1H), 2.00–1.74 (m, 4H), 1.54 (td, $J_1=6$ Hz, $J_2=12.9$ Hz, 1H), 1.44 (s, 9H), 1.25–1.10 (m, 1H); ^{13}C NMR (300 MHz, $CDCl_3$) δ 156.0, 140.0, 137.7, 133.9, 132.2, 129.8, 128.7, 128.2, 127.9, 127.7, 127.5, 126.8, 126.4, 126.2, 124.3, 118.1, 79.6, 75.5, 70.7, 50.9, 38.8, 38.2, 36.6, 32.6, 31.8, 28.7, 28.2, 24.6.

Minor isomer of **20** HPLC retention time 21.75 min; ^{1}H NMR (300 MHz; $CDCl_3$) δ: 7.88–7.76 (m, 3H), 7.64 (s, 1H), 7.55–7.44 (m, 2H), 7.39–7.22 (m, 6H), 5.02 (d, $J=9.6$ Hz, 1H), 4.20–4.04 (m, 1H), 3.94–3.80 (m, 1H), 3.68 (d, $J=11.4$ Hz, 1H), 3.08–2.75 (m, 4H), 2.36–2.00 (m, 4H), 1.78–1.50 (m, 4H), 1.4 (s, 9H), 1.38–1.23 (m, 1H); ^{13}C NMR (300 MHz, $CDCl_3$) δ 156.1, 139.2, 138.7, 133.9, 132.3, 1129.6, 128.8, 128.3, 128.0, 127.6, 127.5, 126.7, 126.3, 125.6, 118.1, 79.6, 72.3, 67.8, 55.6, 39.2, 34.8, 34.6, 32.7, 32.1, 28.7, 27.9, 24.8.

***N*-{2-[2*R*-(1*R*-BocAmino-2-phenyl-ethyl)-6*S*-(2-naphthalen-2-yl-ethyl)-tetrahydro-pyran-4*S*-yl]-ethyl}-bis(Boc)-guanidine 21 and *N*-{2-[2*R*-(1*R*-Bocamino-2-phenyl-ethyl)-6*S*-(2-naphthalen-2-yl-ethyl)-tetrahydro-pyran-4*R*-yl]-ethyl}-bis(Boc)guanidine 22 (Scheme 5c).** Nitrile **20** (0.06 g, 0.07 mmol) was converted to **21** and **22** by hydrogenation and guanidylation using the same procedure as those applied for transformations of **10** affording 0.06 g (87%) of diastereoisomers **21** and **22** in a ratio 2.2:1 which were separated on a preparative HPLC. Major isomer **22**: HPLC retention time 23.58 min; ^{1}H NMR (300 MHz; $CDCl_3$) δ 11.5 (s, 1H), 8.36 (t, 1H, $J=9.6$ Hz), 7.90–7.70 (m, 3H), 7.63 (s, 1H), 7.54–7.37 (m, 2H), 7.36–7.15 (m, 6H), 4.76 (d, 1H, $J=7.2$ Hz), 4.46–4.19 (m, 1H), 3.86–3.57 (m, 2H), 3.56–3.28 (m, 1H), 3.20–2.88 (m, 2H), 2.87–2.60 (m, 2H), 1.98–1.62 (m, 8H), 1.54 (s, 9H), 1.51 (s, 9H), 1.43 (s, 9H), 1.10–0.90 (m, 1H); ^{13}C NMR (300 MHz, $CDCl_3$) δ 163.5, 156.5, 156.0, 153.6, 140.3, 137.9, 133.9, 132.2, 130.0, 128.6, 128.1, 127.8, 127.7, 127.6, 126.7, 126.4, 126.1, 125.3, 83.6, 80.0, 79.4, 73.0, 70.8, 50.5, 38.6, 37.8, 36.6, 32.7, 32.3, 28.7, 28.5, 28.3.

Minor isomer **21** HPLC retention time 24.34 min; ^{1}H NMR (300 MHz; $CDCl_3$) δ 11.41 (s, 1H), 8.48 (bs, 1H), 7.91–7.72 (M, 3H), 7.69–7.59 (m, 1H), 7.55–7.41 (m, 2H), 7.40–7.08 (m, 6H), 5.03 (d, 1H, $J=9.6$ Hz), 4.15–4.02 (m, 1H), 3.93–3.79 (m, 1H), 3.78–3.57 (m, 1H), 3.53–3.31(m, 2H), 3.08–2.67 (m, 4H), 1.98–1.62 (m, 9H), 1.53 (s, 9H), 1.46 (bs, 18H).

^{13}C NMR (300 MHz, $CDCl_3$) δ 162.9, 1565, 156.1, 153.5, 139.6, 139.0, 133.9, 132.3, 130.0, 129.7, 128.7, 128.2, 127.9, 127.7, 127.6, 126.7, 126.5, 126.2, 125.4, 83.8, 80.8, 79.3, 72.7, 68.2, 55.8, 39.0, 36.4, 35.6, 35.2, 32.9, 32.3, 30.0, 28.7, 28.5, 28.2, 27.7.

***N*-{2-[2*R*-(1*R*-Amino-2-phenyl-ethyl)-6*S*-(2-naphthalen-2-yl-ethyl)-tetrahydro-pyran-4*S*-yl]-ethyl}-guanidine 23 and *N*-{2-[2*R*-(1*R*-Amino-2-phenyl-ethyl)-6*S*-(2-naphthalen-2-yl-ethyl)-tetrahydro-pyran-4*R*-yl]-ethyl}-guanidine 24 (Scheme 5d).** This was obtained by cleavage of the Boc groups from **21** and **22** (diastereoisomers mixture) with a solution of dichloromethane, triflouroacetic acid and water 2:1:0.1 for 3 h at room temperature. The diastereoisomers **23** and **24** were separated on a preparative HPLC. Major isomer **24** retention time 8.91 min; ^{1}H NMR (300 MHz; $CDCl_3$) δ 7.86–7.76 (m, 3H), 7.68 (s, 1H), 7.50–7.30 (m, 8H), 4.20–3.95 (m, 2H), 3.88–3.70 (m, 1H), 3.11–3.00 (m, 3H), 2.95–2.83 (m, 3H), 2.14–2.00 (m, 1H), 1.97–1.80 (m, 3H), 1.75–1.58 (m, 1H), 1.55–1.38 (m, 3H), 1.17–1.05 (m, 1H); ^{13}C NMR (300 MHz, $CDCl_3$) δ 157.4, 139.9, 135.7, 134.0, 132.4, 129.2, 129.0, 127.8, 127.4, 127.2, 127.0, 126.0, 125.8, 125.1, 73.1, 71.0, 51.9, 38.5, 37.6, 36.3, 35.9, 35.3, 31.4, 31.3, 27.5; HRFAB (positive) *m/e* 445.2967 calculated for $C_{28}H_{37}N_4O_1$ $(M+H)^+$, found 445.297.

Minor isomer **23** retention time 9.91 min; ^{1}H NMR (300 MHz; $CDCl_3$) δ 7.86–7.77 (m, 3H0, 7.68 (s, 1H), 7.52–7.30 (m, 8H), 4.27–4.16 (m, 2H), 3.79 (d, 1H, $J=9.6$ Hz), 3.52–3.42 (m, 1H), 3.27–3.12 (m, 2H), 3.10–2.82 (m, 4H), 1.96–1.80 (m, 2H), 1.79–1.66 (m, 2H), 1.60–1.25 (m, 3H), 1.00–0.91 (m, 1H); ^{13}C NMR (300 MHz, $CDCl_3$) δ 168.2, 157.5, 139.4, 135.9, 134.0, 132.5, 131.2, 129.2, 129.0, 128.7, 127.9, 127.4, 127.2, 126.2, 125.9, 125.1, 73.67.9, 67.2, 56.8, 39.0, 38.5, 35.8, 34.7, 32.5, 32.0, 30.5, 29.0, 27.0.

References and Notes

1. Hirschmann, R.; Nicolaou, K. C.; Pietranico, S.; Salvino, J.; Leahy, E. M.; Sprengeler, P. A.; Furst, G.; Strader, C. D.; Smith, A. B., III *J. Am. Chem. Soc.* **1992**, *114*, 9217.
2. Recent comprehensive review on the application of carbohydrate-based scaffolds in drug design: Gruner, S. A. W.; Locardi, E.; Kessler, H. *Chem. Rev.* **2002**, *102*, 491.
3. Review of peptidomimetics based on glucose and 5-memebered fused oxacyclic scaffolds: Peri, F.; Cipolla, L.; Forni, E.; Nicotra, F. *Monatsh. Chem.* **2002**, *133*, 369.
4. Review on the application of glycosamino acids as building blocks for combinatorial synthesis of peptidomimetics: Schweizer, F. *Angew. Chem.* **2002**, *41*, 230.
5. Chakraborty, T. K.; Jayaprakash, S.; Ghosh, S. *Comb. Chem. High T. Scr.* **2002**, *5*, 373.
6. Review on the application of sugar amino acids in designing bioactive molecules: Chakraborty, T. K.; Ghosh, S.; Jayaprakash, S. *Curr. Med. Chem.* **2002**, *9*, 421.
7. Recent application of glucal cycloadducts for NK-2

peptidomimetic ligands: Capozzi, G.; Giannini, S.; Menichetti, S.; Nativi, C.; Giolitti, A.; Patacchini, R.; Perrotta, E.; Altamura, M.; Alberto Maggi, C. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 2263.
8. Review on the design and activity of sugar amino acids based peptidomimetics: Lohof, E.; Burkhart, F.; Born, M. A.; Planker, E.; Kessler, H. *Advances in Amino Acid Mimetics and Peptidomimetics* **1999**, *2*, 263.
9. Synthetic protocols concerning azasugars: Le Merrer, Y.; Poitout, L.; Depezay, J. C. *Method. Mol. Med.* **1999**, *23*, 227.
10. Nicolaou, K. C.; Trujillo, J. I.; Chibale, K. *Tetrahedron* **1997**, *53*, 8751.
11. Han, Y.; Mierke, D. F.; Chorev, M. *Biopolymers* **2002**, *64*, 1.
12. Freidinger, R. M.; Perlow, D. S.; Veber, D. F. *J. Org. Chem.* **1982**, *47*, 104.
13. Aube, J. *Adv. Amino Acid Mimetics and Peptidomimetics* **1997**, *1*, 193.
14. Toniolo, C. *Int. J. Pept. Protein Res.* **1990**, *35*, 287.
15. Hruby, V. J.; Balse, P. M. *Curr. Med. Chem.* **2000**, *7*, 945.
16. Mazur, A. W.; Tian, X.; Hu, X. E.; Ebetino, F. H. WO 0285925, 2002.
17. Ebetino, F. H.; Mazur, A. W.; Hayes, J. C.; Wang, F.; Solinsky, M. G.; Colson, A. O.; Lin, Q. WO 0226774, 2001.
18. Mazur, A. W.; Wang, F.; Sheldon, R. J.; Ebetino, F. H. WO 0058361, 2000.
19. Abdel-Malek, Z.; Suzuki, I.; Tada, A.; Im, S.; Akcali, C. *Ann. N. Y. Acad. Sci. 885*, 117.
20. Ollmann, M. M.; Wilson, B. D.; Yang, Y. K.; Kerns, J. A.; Chen, Y.; Gantz, I.; Barsh, G. S. *Science* **1997**, *278*, 135.
21. Lu, D.; Willard, D.; Patel, I. R.; Kadwell, S.; Overton, L.; Kost, T.; Luther, M.; Chen, W.; Woychik, R. P.; Wilkison, W. O. *Nature* **1994**, *371*, 799.
22. Wessells, H.; Levine, N.; Hadley, M. E.; Dorr, R.; Hruby, V. *Int. J. Impot. Res.* **2000**, *12* (Suppl. 4), S74.
23. Haskell-Luevano, C.; Hendrata, S.; North, C.; Sawyer, T. K.; Hadley, M. E.; Hruby, V. J.; Dickinson, C.; Gantz, I. *J. Med. Chem.* **1997**, *40*, 2133.
24. Holder, J. R.; Xiang, Z.; Bauzo, R. M.; Haskell-Luevano, C. *J. Med. Chem.* **2002**, *45*, 5736.
25. Grieco, P. A.; Moher, E. D. *Tetrahedron Lett.* **1993**, *34*, 5567.
26. Willson, T. M.; Kocienski, P.; Jarowicki, K.; Isaac, K.; Hitchcock, P. M.; Faller, A.; Campbell, S. F. *Tetrahedron* **1990**, *46*, 1767.
27. Eriksson, M.; Iliefski, T.; Nilsson, M.; Olsson, T. *J. Org. Chem.* **1997**, *62*, 182.
28. Konieczny, M. T.; Toma, P. H.; Cushman, M. *J. Org. Chem.* **1993**, *58*, 4619.
29. Kim, K. S.; Qian, L. *Tetrahedron Lett.* **1993**, *34*, 7677.
30. Wadsworth, W. S., Jr. *Org. React.* **1977**, *25*, 73.
31. Hudlicky, M. *Reductions in Organic Chemistry (ACS monograph 188)*; American Chemical Society: Washington, DC. 1996; p 298.
32. Russell, T. W.; Duncan, D. M.; Hansen, S. C. *J. Org. Chem.* **1976**, *42*, 551.
33. Sebhat, I. K.; Martin, W. J.; Ye, Z.; Barakat, K.; Mosley, R. T.; Johnston, D. B. R.; Bakshi, R.; Palucki, B.; Weinberg, D. H.; MacNeil, T.; Kalyani, R. N.; Tang, R.; Stearns, R. A.; Miller, R. R.; Tamvakopoulos, C.; Strack, A. M.; McGowan, E.; Cashen, D. E.; Drisko, J. E.; Hom, G. J.; Howard, A. D.; MacIntyre, D. E.; van der Ploeg, L. H. T.; Patchett, A. A.; Nargund, R. P. *J. Med. Chem.* **2002**, *45*, 4589.