Pergamon

Tetrahedron Letters, Vol. 38, No. 41, pp. 7167-7170, 1997

0040-4039/97 $17.00 + 0.00

PII: S0040-4039(97)01764-4

PEPTIDE-SUGAR HYBRIDS: LIKE PEPTIDE, LIKE OLIGOSACCHARIDE

Yoshitomo Suhara,¶ Masayuki Izumi,¶ Mie Ichikawa,¶ Margaret B. Penno,§ and Yoshitaka Ichikawa*¶

¶Department of Pharmacology and Molecular Sciences, §Department of Medicine
Johns Hopkins University School of Medicine, Baltimore, MD 21205

Abstract: A novel peptide-sugar hybrid with a repeating sequence of glycamino acid and aspartic acid residues was synthesized and was found to inhibit tumor cell migration with an IC_{50} of 10 μM.

β-Peptides are composed of β-amino acid residues[1] and have been found to adopt α-helical conformations similar to those of their counterparts consisting of the naturally occurring α-amino acids.[2] These β-peptides are expected to be metabolically stable alternatives to the biologically active natural peptides, and therefore a variety of such amino acid and peptide analogs have been developed.[3] Glycamino acids are a unique class of carbohydrate-derived amino acid analogs that possess a carboxylate group and an amino group replacing one of the OH groups of the carbohydrate. Thus, they exhibit some of the structure and properties of amino acids as well as carbohydrates.[4] Kessler et al. have demonstrated that incorporation of a glycamino acid into a peptide sequence creates a conformationally rigid peptide-like structure, indicating that these derivatives can be utilized as peptide mimetics.[5] Their homo-oligomers have also been constructed,[6-9] and shown to exhibit a potent anti-HIV activity by us.[9] Herein, we report the synthesis and in vitro assay of a new class of peptide analogs, peptide-sugar hybrids **1** that have an alternating sequence of glycamino acid and β-amino acid residues. Our objective was to create another new class of non-natural peptide molecules and screen these molecules in an in vitro assay system for the biological activity that is relevant to cancer treatment. In addition, we sought to design relatively simple and small molecules that are easy to assemble.

In our general synthetic strategy (Fig. 1) the amino group of all each amino acid derivative is protected with a Boc group in order to allow this strategy to extend to be a solid-phase type of synthesis. The elongation reaction is carried out by BOP chemistry, and each building block is chosen to be either an amino acid or a glycamino acid, thereby increasing the diversity of the molecular structures and the number of potential peptide analogs.

For the first hybrid molecules we chose a glucose-derived glycamino acid, 4-NH_2-D-Glc-1-CO_2H **2** as the δ-amino acid and aspartic acid **3** as

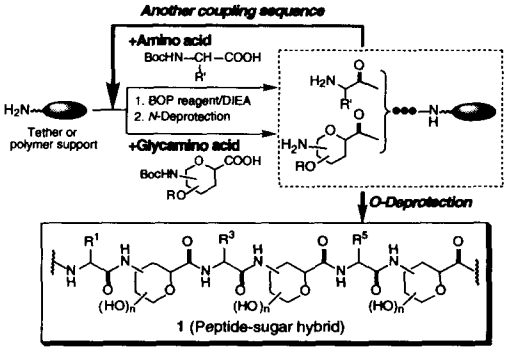


Fig. 1. General synthetic strategy for the hybrid molecules.

the β-amino acid component. We conjugated the glycamino acid through the *C*-4 NH_2 group to the β-carboxylic acid moiety of aspartic acid (Fig. 2). We also employed both D- and L-aspartic acids in order to examine how the enantiomeric stereochemistry would affect the overall conformation and biological activity of the hybrid molecules.

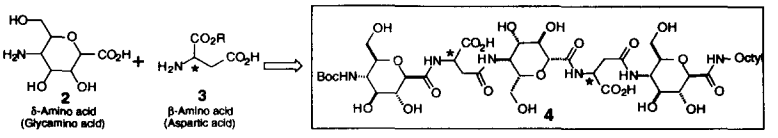
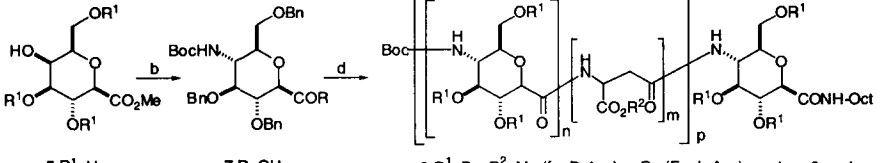


Fig. 2. The designed hybrid molecule and its amino acid components.

The two hybrid molecules, **4D** with the D-form of aspartic acid and **4L** with the L-form, were synthesized as shown in Scheme 1. A known galactose derivative **5**[10] was converted to the glycamino acid building block **7** via **6**. n-Octylamine was first coupled with **7** in the presence of BOP reagent and diisopropylethylamine (DIEA)[11] in DMF to give **8** in 82% yield. The *N*-Boc derivative **8** was treated with 2N HCl/EtOAc, and the resulting amino derivative was coupled with *N*-Boc-L-aspartic acid α-methyl ester[12] in the presence of BOP reagent and DIEA in DMF to give a dimeric compound **9(D)** in 62% overall yield. By the same synthetic manipulation, **9(D)** was coupled with **7** to give the trimeric compound **10(D)** in 54% yield. The second aspartic acid residue was introduced into **10(D)** to afford the tetrameric compound **11(D)** in 64% yield. Another addition of **7** to **11(D)** gave the pentameric compound **12(D)** (90% yield), which was then deprotected to give **4(D)**.[13] Employing L-aspartic acid α-benzyl ester, the pentameric compound **4(L)**[13] was also synthesized in a similar synthetic procedure.

To assess the conformational properties of the hybrids we used MacroModel (v. 5.5) for molecular dynamics calculations: conformational energy minimization of the hybrid structures was carried out with the AMBER* force field, the GB/SA water model, and a Monte Carlo approach.[14] Although these calculations may tend to give heavy weight to intramolecular hydrogen bonding to the nearby functional groups, we identified two factors that were critical in determining the conformations shown in **I** (Fig. 3). First, because of the electrostatic repulsion with the ring oxygen and intramolecular hydrogen-bonding stabilization with the *C*-2 OH group, the *C*-1 amido carbonyl groups of the glycamino acid residues were found to be positioned away from the sugar ring oxygen atom, an observation consistent with that reported by the Kessler group.[5b] Second, the α-carboxylic acid moieties of the

Scheme 1[a]



[a]Key: (a) i) $PhCHO/HCO_2H$, ii) NaH/BnBr, iii) $NaBH_3CN/HCl/THF$; (b) $Tf_2O/pyr/CH_2Cl_2$, ii) NaN_3/DMF, iii) $H_2S/pyr\text{-}H_2O$, iv) $Boc_2O/Et_3N/MeOH$, v) $LiOH/THF\text{-}MeOH\text{-}H_2O$; (c) n-Octyl-$NH_2$/BOP/DIEA/DMF; (d) i) HCl/EtOAc, ii) *N*-Boc-D-aspartic acid α-methyl ester or *N*-Boc-L-aspartic acid α-benzyl ester/BOP/DIEA/DMF; (e) i) HCl/EtOAc, ii) **7**/BOP/DIEA/DMF; (f) i) $H_2/Pd(OH)_2/THF\text{-}MeOH\text{-}H_2O$ (for D-Asp derivative: ii) $LiOH/THF\text{-}MeOH\text{-}H_2O$)

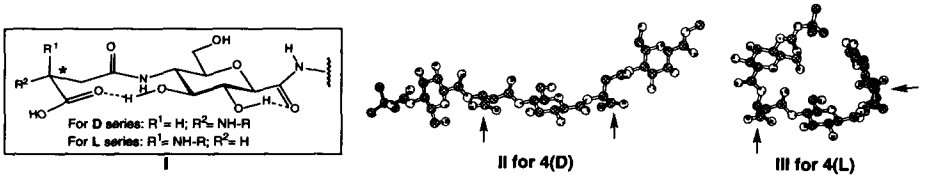


Fig. 3. Intramolecular hydrogen-bonding obtained through molecular dynamics calculation for a dipeptide moiety of aspartic acid and the glycamino acid (**I**). Proposed stable conformations: **II** for **4(D)** and **III** for **4(L)** on the basis of the partial conformation of **I**. Arrows indicate the carboxylic acid moieties, and the octyl groups are replaced with the methyl groups.

aspartic acid residues were found to play a role in determining the conformation of these hybrid molecules by forming a hydrogen bond to the *C*-3 OH group. Therefore, we assumed that **4(D)** and **4(L)** possess quite different stable conformations because of the stereochemistry of D- and L-aspartic acid residues incorporated, and we propose the stable conformers **II** for **4(D)** and **III** for **4(L)**.

We examined the biological activity of the hybrid molecules in two *in vitro* systems involving highly metastatic tumor cell lines. These assays measured the ability of the hybrid molecules to inhibit cell adhesion (chemotaxis)[15] and invasion,[16] two processes that are critical to cancer metastasis. In a chemotactic assay in which CD44-expressing lung carcinoma cells (A549) adhere to vitronectin, the deprotected form of the D-aspartic acid-containing trimer **10(D)** inhibited adhesion by 46% at 10 μM, whereas the L-isomer **10(L)** showed no inhibition at this concentration (Fig. 4). In the assay measuring tumor cell invasion (Fig. 5), a 10 μM solution of the pentamer **4(L)** inhibited by 64% the invasion of A549 cells through a polycarbonate membrane coated with basement membrane components (Matrigel®),[17] whereas **4(D)** inhibited the invasion by 38% at the same concentration. Although we were not able to determine the precise mechanism by which the compound prevented lung tumor cell adhesion and invasion, these data suggest that these simple hybrid molecules can inhibit critical steps in metastasis.

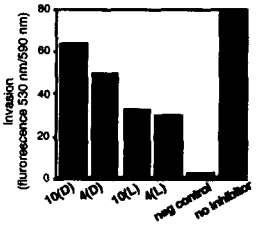


Fig. 5. Inhibition of tumor cell invasion through Matrigel® at 10 μM.

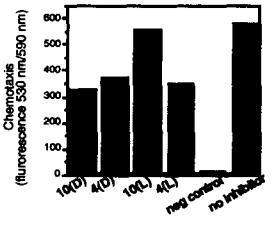


Fig. 4. Inhibition of tumor cell chemotaxis to vitronectin at 10 μM.

In summary, we have designed and synthesized a new class of peptide analogs, peptide-sugar hybrids with an alternating sequence of glycamino acid and β-amino acid. The assembly of all the oligomers could be carried out in the same sequence: i) *N*-deprotection with HCl/EtOAc and ii) coupling with BOP reagent and DIEA in DMF. Showing a potent inhibition of lung tumor cell invasion in a preliminary biological evaluation, these peptide-sugar hybrids may provide a new class of molecules with a significant biological activity.

Acknowledgments:
We are grateful to Dr. K. Ikeda (University of Shizuoka, Japan) for performing the NMR experiments. Some of the NMR studies were performed in the Biochemistry NMR Facility at Johns Hopkins University, which was established by a grant from the National Institutes of Health (GM 27512) and a Biomedical Shared Instrumentation Grant (1S10-RR06262-0). A fellowship from Uehara Memorial Foundation (to Y.S.) is gratefully acknowledged.

References and notes

1. (a) Iverson, B.L. *Nature* **1997**, *385*, 113-114. (b) Borman, S. *Chem. & Eng.. News* **1997**, 32-35.
2. (a) Appella, D.H.; Christianson, L.A.; Karle, I.L.; Powell, D.R.; Gellman, S.H. *J. Am. Chem. Soc.* **1996**, *118*, 13071-13072. (b) Seebach, D.; Overhand, M.; Kühnle, F.N.M.; Martinoni, B.; Oberer, L.; Hommel, U.; Wildmer, H. *Helv. Chim. Acta* **1996**, *79*, 913. (c) Seebach, D.; Ciceri, P.E.; Overhand, M.; Jaun, B.; Rigo, D.; Oberer, L.; Hommel, U.; Amstutz, R.; Wilmer, H. *Helv. Chim. Acta* **1996**, *79*, 2043-2066. (d) Appella, D.H.; Christianson, L.A.; Klein, D.A.; Powell, D.R.; Huang, X.; Barchi. J.J., Jr.; Gellman, S.H. *Nature* **1997**, *387*, 381-384.
3. (a) Simon, R.J.; Kania, R.S.; Zuckermann, R.N.; Huebner, V.D.; Jewell, D.A.; Banville, S.; Ng, S.; Wang, L.; Rosenberg, S.; Marlowe, C.K.; Spellmeyer, D.C.; Tan, R.; Frankel, A.D.; Santi, D.V.; Cohen, F.E.; Bartlett, *P.A. Proc. Natl. Acad. Sci. USA* **1992**, *89*, 9367-9371. (b) Hagihara, M.; Anthony, N.J.; Stout, T.J.; Clardy, J.; Schreiber, S.L. *J. Am. Chem. Soc.* **1992**, *114*, 6568-6570. (c) Cho, C.Y.; Moran, E.J.; Cherry, S.R.; Stephans, J.C.; Fodor, S.P.A.; Adams, C.L.; Sundaram, A.; Jacobs, J.W.; Schultz, P.G. *Science* **1993**, *261*, 1303-1305. (d) Smith, A.B., III; Guzman, M.C.; Sprengeler, P.A.; Keenan, T.P.; Holcomb, R.C.; Wood, J.L.; Carroll, P.J.; Hirschman, R. *J. Am. Chem. Soc.* **1994**, *116*, 9947-9962. (e) Gennari, C.; Salom, B.; Potenza, D.; Williams, A. *Angew. Chem. Int. Ed. Engl.* **1994**, *33*, 2067-2069. (f) Burgess, K.; Linthicum, D.S.; Shin, H. *Angew. Chem. Int. Ed. Engl.* **1995**, *34*, 907-909. (g) Nowick, J.S.; Mahrus, S.; Smith, E.M.; Ziller, J.W. *J. Am. Chem. Soc.* **1996**, *118*, 1066-1072. (h) Han, H.; Janda, K.D. *J. Am. Chem. Soc.* **1996**, *118*, 2539-2544.
4. (a) Heyns, K.; Paulsen, H. *Chem. Ber.* **1955**, *88*, 188-195. (b) Fuchs, E.-F.; Lehmann, J. *Chem. Ber.* **1975**, *108*, 2254-2260.
5. (a) von Rodern, E.G.; Kessler, H. *Angew. Chem. Int. Ed. Engl.* **1994**, *33*, 687-689. (b) von Roedern, E.G.; Lohof, E.; Hessler, G.; Hoffman, M.; Kessler, H. *J. Am. Chem. Soc.* **1996**, *118*, 10156-10167.
6. Yoshimura, J.; Ando, H.; Sata, T.; Tsuchida, S.; Hashimoto, H. *Bull. Chem. Soc. Jpn.* **1976**, *49*, 2511-2514.
7. (a) Müller, C.; Kitas, E.; Wessel, H.P. *J. Chem. Soc., Chem. Commun.* **1995**, 2425-2426. (b) Wessel, H.P.; Mitchell, C.M.; Lobato, C.M.; Schmid, G. *Angew. Chem. Int. Ed. Engl.* **1995**, *34*, 2712-2713.
8. Suhara, Y.; Hildreth, J.E.K.; Ichikawa, Y. *Tetrahedron Lett.* **1996**, *37*, 1575-1578.
9. Suhara, Y.; Ichikawa, M.; Hildreth, J.E.K.; Ichikawa, Y. *Tetrahedron Lett.* **1996**, *37*, 2549-2552.
10. BeMiller, J.N.; Yadav, M.P.; Kalabokis, V.N.; Myers, R.W. *Carbohydr. Res.* **1990**, *200*, 111-126.
11. Anisfeld, S. T.; Lansbury, P. Jr. *J. Org. Chem.* **1990**, *55*, 5560-5562.
12. It was prepared from a commercially available D-aspartic acid α-benzyl ester by careful treatment with NaOMe in MeOH.
13. **4(D)**: white powder; ^{1}H NMR (300 MHz, D_2O) δ 4.63 (t, 1H, *J*= 6.4 Hz), 3.79 (dd, 1H, *J*= 7.2, 12.0 Hz), 3.73-3.36 (m, 20H), 3.14 (t, 2H, *J*= 7.2 Hz, $NHC\underline{H}_2$), 2.82 (dd, 2H, *J*= 7.2, 15.6 Hz), 2.74-2.69 (m, 2H), 1.44 (2H, CH_2), 1.35 (s, 9H, tBu), 1.21–1.15 (10H, 5×CH_2), 0.77 (t, 3H, *J*= 6.6 Hz, CH_3); ^{13}C NMR (72.5 MHz, D_2O) δ 215.9 (CO_2H), 173.5, 171.2 (CONH), 158.1 ($NH\underline{C}O_2tBu$), 81.5, 79.3, 78.85, 78.78, 78.6, 78.3, 78.2, 75.1, 74.8, 72.4, 72.22, 72.17, 71.9, 61.4, 61.3, 60.8, 51.6, 39.6, 37.9, 31.4, 28.7, 27.9, 26.4, 22.3, 13.8 ($NH(CH_2)_7\underline{C}H_3$); mass spectrum (FAB) *m/e* 1049.5 ((M^++Na), calcd for $C_{42}H_{70}N_6O_{23}Na$: 1049.5), 1027.5 ((M^++H), calcd for $C_{42}H_{71}N_6O_{23}$: 1027.5), 926.5 ((M^+–Boc+2H), calcd for $C_{37}H_{62}N_6O_{21}$: 926.4). **4(L)**: white powder; ^{1}H NMR (300 MHz, D_2O) δ 4.63 (2H, 2×$C\underline{H}(CO_2H)$), 3.87-3.45 (m, 21H), 3.08 (t, 2H, *J*= 6.9 Hz, $NHC\underline{H}_2$), 2.45 (m, 4H, 2×$(CO_2H)C\underline{H}_2$), 1.44 (2H, $NHCH_2C\underline{H}_2(CH_2)_5CH_3$), 1.34 (br s, 9H, $NHCO_2t\underline{Bu}$), 1.18 (br s, 10H, $NHCH_2CH_2(C\underline{H}_2)_5CH_3$), 0.77 (t, 3H, *J*= 7.1 Hz, $NH(CH_2)_7C\underline{H}_3$); mass spectrum (FAB) m/e 927.4 ((M^+–Boc+2H), calcd for $C_{37}H_{63}N_6O_{21}$: 927.5).
14. All calculations were performed on a Silicon Graphics Indy R5000 workstation using MacroModel (v. 5.5) software. Initial structures, built within MacroModel, were subjected to conjugate gradient energy minimization with the AMBER* force field and the GB/SA water model. A Monte Carlo (MC) approach was used for global conformational search. Sugar pyran rings were left at their stable $^4C_1$ chair conformation and the torsional angles of the amide bond: C-C(=O)-N(H)-C were left at 180°, and all other torsion angles were randomly modified at each MC step. For both **4(D)** and **4(L)**, 1,000 MC steps were carried out, and after each MC step the resulting geometry was minimized with 2,000 gradient conjugate steps. Four conformations were found within 50kJ/mol for **4(D)** and two for **4(L)** structures. These conformers were all subjected to further conjugate gradient energy minimization to obtain a global minimum geometry.
15. Carnaggio, J.A.; Smith, J.A.; Penno, M.B. *Methods in Cell Sci.* **1995**, *17*, 263-270.
16. Penno, M.B.; Mousa, S.; Bozarth, J.; Hart, J.C. submitted.
17. Matrigel has been used as a model of the extracellular matrix in tumor cell invasion assays. See examples: (a) Baatout, S. *Anticancer Res.* **1997**, *17*, 451-455. (b) Navab, R.; Mort, J.S.; Brodt, P. *Clin. Exp. Metastasis* **1997**, *15*, 121-129.

(*Received in USA* 8 *August* 1997; *revised* 20 *August* 1997; *accepted* 21 *August* 1997)