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COMMUNICATION

Bicyclic amino acid-carbohydrate-conjugates as conformationally restricted hydroxyethylamine (HEA) transition-state isosteres†‡

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This communication describes a general synthetic route to bicyclic amino acid-carbohydrate-conjugates, which would be useful as conformationally restricted hydroxyethylamine (HEA) transition-state isosteres. The synthesis was achieved in 12 steps starting from D-glucose. The striking features of this system are the bicyclic rigid core displaying an α-amino acid side chain and hydroxyethylamine moiety – both of which would be potentially important for receptor interactions, leading to various biomedical responses, as described in the literature. Crystal structure investigation suggested extensive intermolecular hydrogen-bonding interactions in this system, involving the backbone amide and hydroxyl groups.

Proteases, a class of peptide-bond-cleaving enzymes that control protein synthesis and function, offer a highly promising target for therapeutic intervention, in several severe pathological conditions such as AIDS, cancer, Alzheimer's disease, malaria *etc.*[1–3] Reactions mediated by proteases proceed *via* a tetrahedral transition-state,[4] which results from nucleophilic attack by a water molecule on the scissile peptide bond carbonyl group. Among the transition-state isosteres that have been successfully used in the design of protease inhibitors, hydroxy-based isosteres, in particular HEA isosteres,[5] assumes special importance (Fig. 1). A transition-state isostere is defined as a functional group that can mimic the tetrahedral transition-state of amide bond hydrolysis, but is stable and non hydrolyzable. Phosphinates,[6] hydroxyethylcarbonyls (statins),[7] hydroxyethylamines,[5] hydroxymethylcarbonyls (norstatins),[8] hydroxyethylenes,[9] and dihydroxyethylenes[10] have proven to be potent inhibitors of various proteases.

A strategy that has proved very successful for the design of efficient protease inhibitors is based on the incorporation of a transition-state analog into a peptide/peptidomimetic structure,

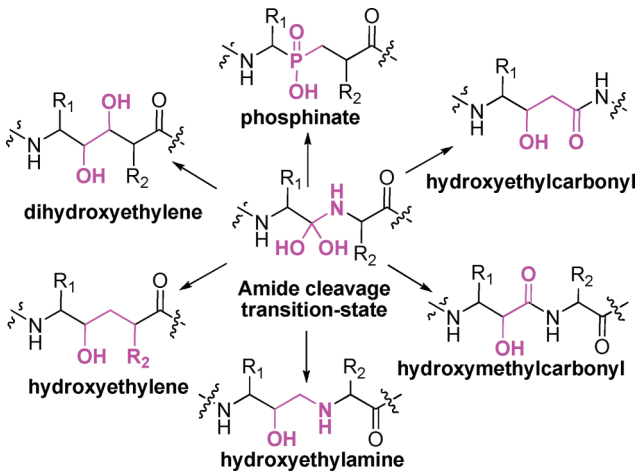

Fig. 1 Transition-state mimics of scissile peptide bond.

carrying an amino acid side chain as evident from a myriad of examples reported in the recent literature.[11]

The peptide backbone that carries amino acid side chains or their surrogates facilitates receptor recognition, leading to efficient reversible binding of the ligand with the receptor (Fig. 2).[12] However, one issue that still remains to be addressed is the conformational flexibility of the ligand that may adversely affects receptor selectivity. In order to address this intriguing problem, conformationally constrained peptide/mimetics carrying transition-state isosteres have been designed, with varying success.[13] It is noteworthy that conformational restriction has been proven to be an effective and simple tool to improve the

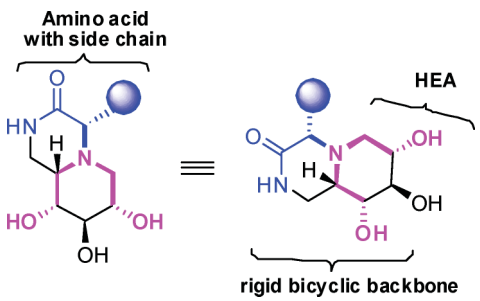

Fig. 2 Proposed conformationally constrained carbohydrate-derived bicyclic HEA isosteres featuring amino acid side chain.

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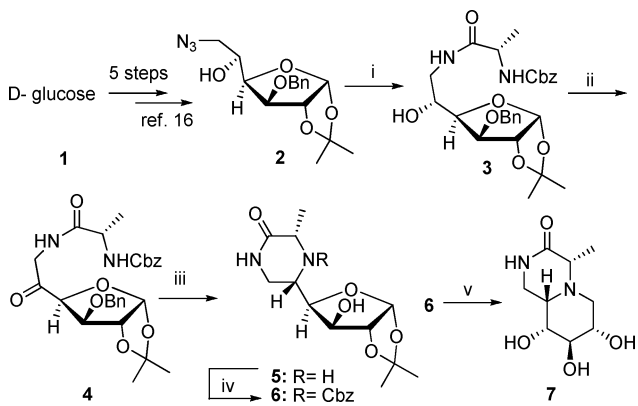
† Dedicated to Prof. George W. J. Fleet, University of Oxford, UK, in recognition of his exemplary contribution in the area of carbohydrates.

‡ Electronic supplementary information (ESI) available: General experimental procedures, ^{1}H, ^{13}C, DEPT-135 NMR spectra and ESI mass spectra of all new compounds are included. See DOI: 10.1039/c1ob06215h

druggability, particularly the receptor selectivity, of acyclic/flexible counterparts.[13e] Restricting conformational freedom of flexible molecules can lead to substantial changes in assay performance, as was demonstrated in a recent study using multi-dimensional screening.[13e]

It is noteworthy that several bioactive natural products and their synthetic analogs feature the bicyclic core of varying sizes, although without amino acid side chains, as in the present case. Some of the prominent examples would be castanospermine, swainsonine, their ring expanded derivatives[14] and a glycine-tethered deoxynojirimycin analogue.[15] Intriguingly, many of these natural products and their synthetic analogues mentioned above share a common bioactivity profile as glycosidase inhibitors.

The synthetic methodology disclosed herein is a short and straightforward one starting from the readily available glucose as the starting material. The synthetic route (Scheme 1) was initially planned such that the amino acid-furanose-conjugated intermediate **4** could be subjected to a two-step acetonide deprotection-*cum*-reductive amination protocol to straight away furnish the required target bicyclic system **7**. However, this strategy did not yield a tangible result, despite best efforts. Therefore, we opted to isolate the partially reduced advanced intermediate **6**, followed by successive acetonide deprotection and reductive amination to yield **7**. To begin with the synthesis, we started from D-glucose and converted it to the acetonide protected azide derivative **2**, in five steps, following literature protocols.[16] The known furanoside derivative **2** was subjected to selective azide reduction followed by coupling with N-terminal protected (as benzyloxy carbonyl, Cbz) L-alanine to yield **3**. The secondary alcohol in **3** could be efficiently oxidized to the ketone **4** using PCC in DCM, which was ready for the first reductive amination sequence aided by $Pd(OH)_2/H_2$ to yield **5**. It is noteworthy that this reductive amination proved to be troublesome when $Pd/C/H_2$, a standard condition, was attempted. The free amine **5** was isolated and characterized as its Cbz protected derivative **6**. Next, **6** was subjected to acetonide deprotection followed by reductive amination to successfully furnish **7**.



Scheme 1 Regent and conditions: (i) a. TPP, THF-H_2O, rt, 3 h; b. Z–Ala–OH, EDC.HCl, CH_3CN, rt, 12 h, 73%; (ii) PCC, NaOAc, MS, DCM, rt, 5 h, 72%; (iii) $Pd(OH)_2/H_2$, MeOH, 120 psi, 24 h; (iv) Cbz–Cl, $NaHCO_3$, MeOH–H_2O, rt, 4 h, 60%; (v) a. TFA–H_2O (3 : 1); b. $Pd(OH)_2/H_2$, MeOH, 120 psi, 24 h, 79%.

The relative stereochemistry in **7** could be unambiguously assigned from its crystal structure (Fig. 3). Single crystals of good quality could be obtained by slow evaporation of an aq. methanolic solution of **7**. Analysis of the crystal structure of **7** clearly reveals the relative stereochemistry of the backbone at C4, C7, C8, and C9, as *S*, *S*, *R*, and *R*, respectively, and the newly formed stereo junction at C9A as *S*. The amide group is locked in *cis*-conformation, as found in the biologically important diketopiperazines.[17]

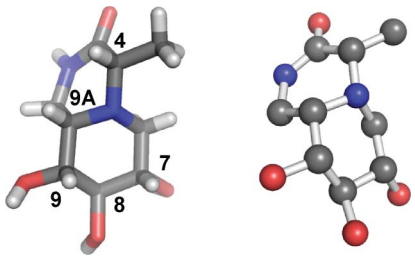


Fig. 3 Different views of the crystal structure of **7**, clearly revealing the relative stereochemistry. Stick PyMOL rendering (left) and ball and stick (right). H-atoms have been deleted for clarity in the ball and stick representation.

It is noteworthy that **7** has been found to undergo extensive H-bond-mediated self-assembly, as evident from its crystal structure (Fig. 4). Three molecules of **7** self-assemble using a collection of hydrogen bonding interactions primarily using the *cis*-amide group of one molecule, and hydroxyl groups of two other molecules forming a 14-membered H-bonded network. Intriguingly, two hydroxyl groups of one molecule in the H-bonded network act as hydrogen bonding donors, and two hydroxyl groups (OH) of another molecule act as hydrogen bonding acceptors.[18]

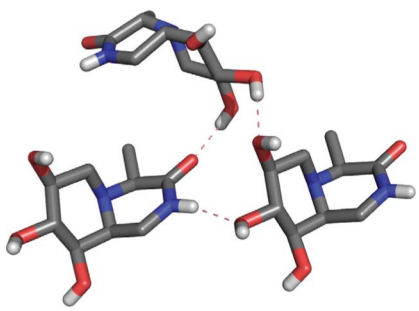


Fig. 4 Self-assembled H-bonded network involving three molecules of **7**, as revealed from its crystal structure.

A preliminary investigation of the biological activity of the constrained bicyclic amino acid-carbohydrate-conjugated HEA isostere **7** showed 15% trypsin inhibitory activity, while no inhibition was observed against chymotrypsin. Intriguingly, the compound **7** did not show any inhibitory activity against any of the glycosidases tested, under the assay conditions (ESI, S15–S16‡).

In conclusion, the present work details an efficient protocol for the synthesis of bicyclic amino acid-carbohydrate-conjugates, which would be of use as conformationally restricted hydroxyethylamine (HEA) transition-state isosteres. The synthetic methodology disclosed herein is noteworthy since it is a straightforward one utilizing the readily available glucose as the starting material and delivering the target conformationally constrained bicyclic HEA isostere in high yielding synthetic sequences. The salient features of this reported system are the bicyclic rigid core displaying an

α-amino acid side chain and hydroxyethylamine moiety – both of which would be potentially important for receptor interactions, leading to various biomedical responses, as described in the literature.[5,11]

Experimental section

Single crystal X-ray crystallographic studies of 7

Colorless crystals of approximately 0.43 × 0.09 × 0.02 mm dimensions, were used for data collection. Crystal to detector distance 6.05 cm, 512 × 512 pixels/frame, quadrant data acquisition. Total frames = 2424, Oscillation/frame –0.3°, exposure/frame = 5.0 s/frame, maximum detector swing angle = –30.0°, beam center = (260.2, 252.5), in plane spot width = 1.24, SAINT integration, θ range = 2.66 to 25.00°, completeness to θ of 25.0° is 99.8%. SADABS correction applied $C_9H_{16}N_2O_4$, M = 216.24. Crystals belong to monoclinic, space group $P2_1$, a = 7.8831(7), b = 8.2422(7), c = 7.9473(7) Å, β = 105.506(1)°, V = 497.57(8) Å^3, Z = 2, D_c = 1.443 g/cc, μ(Mo-Kα) = 0.114 mm^{-1}, T = 296(2)K, 4835 reflections measured, 1742 unique [$I > 2\sigma(I)$], R value 0.0302, wR_2 = 0.0673.

(4*S*,7*S*,8*R*,9*R*,9a*S*)-7,8,9-Trihydroxy-4-methylhexahydro-1*H*-pyrido[1,2-a]pyrazin-3(2H)-one 7. A solution of **6** (0.19 g, 0.47 mmol) in TFA–H_2O (6 mL, 3 : 1) was stirred at r.t for 4 h. The reaction mixture was stripped of TFA completely by co-evaporation with toluene to furnish a thick liquid, which was taken up in methanol (20 mL) and hydrogenated with 20% $Pd(OH)_2$ (0.07 g) at 120 psi for 24 h. The catalyst was filtered through celite, washed with methanol, and the filtrate was concentrated to furnish thick liquid which was purified by column chromatography to afford **7** (0.10 g, 79%); R_f 0.35 (MeOH/$CHCl_3$: 30/70); mp: 233.2–235.8 °C; $[\alpha]_D^{22}$ –12.0 (*c* 0.5, $CHCl_3$); IR (ν) $CHCl_3$, (cm^{-1}) 3385, 3275, 1659, 1460; ^{1}H NMR (400 MHz, DMSO-*d6*) δ: 7.58 (s, 1H), 4.99–4.95 (m, 2H), 4.76–4.74 (d, J = 6.0 Hz, 1H), 3.47–3.44 (m, 4H), 3.01–2.93 (m, 3H), 2.60–2.55 (m, 2H), 1.20–1.18 (d, J = 6.8 Hz, 3H); ^{13}C NMR (50 MHz, DMSO-*d6*) δ: 171.5, 71.3, 71.0, 70.2, 59.6, 56.1, 49.6, 39.9; HRMS calcd. for $C_9H_{16}N_2O_4Na$, 239.1008; Found, 239.1008. Elemental analysis calcd. for $C_9H_{16}N_2O_4$: Anal. C, 49.99; H, 7.46; N, 12.96; Found: C, 49.57, H, 7.69, N, 13.21.

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Notes and references

1 (*a*) B. E. Maryanoff and M. J. Costanzo, *Bioorg. Med. Chem.*, 2008, **16**, 1562–1595; (*b*) G. Fear, S. Komarnytsky and I. Raskin, *Pharmacol. Ther.*, 2007, **113**, 354–368; (*c*) C. J. Scott and C. C. Taggart, *Biochimie*, 2010, **92**, 1681–1688.
2 (*a*) G. Abbenante and D. P. Fairlie, *Med. Chem.*, 2005, **1**, 71–104; (*b*) G. Ruiz-Go'mez, J. D. A. Tyndall, B. Pfeiffer, G. Abbenante and D. P. Fairlie, *Chem. Rev.*, 2010, **110**, PR1–PR41.
3 (*a*) D. L. Buckley, T. W. Corson, N. Aberle and C. M. Crews, *J. Am. Chem. Soc.*, 2011, **133**, 698–700; (*b*) A. K. Ghosh, B. D. Chapsal, A. Baldridge, M. P. Steffey, D. E. Walters, Y. Koh, M. Amano and H. Mitsuya, *J. Med. Chem.*, 2011, **54**, 622–634; (*c*) J. Nguyen, K. Kato, K. Hidaka, H. Kumada, T. Kimura and Y. Kiso, *Bioorg. Med. Chem. Lett.*, 2011, **21**, 2425–2429.
4 (*a*) F. WÅngsell, P. Nordeman, J. Sävmarker, R. Emanuelsson, K. Jansson, J. Lindberg, A. Rosenquist, B. Samuelsson and M. Larhed, *Bioorg. Med. Chem.*, 2011, **19**, 145–155; (*b*) A. Brik and C. Wong, *Org. Biomol. Chem.*, 2003, **1**, 5–14.
5 (*a*) Marcin, *Bioorg. Med. Chem. Lett.*, 2011, **21**, 537–541; (*b*) M. Sova, G. Cadez, S. Turk, V. Majce, S. Polanc, S. Batson, A. J. Lloyd, D. I. Roper, C. W. G. Fishwick and S. Gobec, *Bioorg. Med. Chem. Lett.*, 2009, **19**, 1376–1379; (*c*) A. Gautier, D. Pitrat and J. Hasserodt, *Bioorg. Med. Chem.*, 2006, **14**, 3835–3847; (*d*) K. Akaji, K. Teruya and S. Aimoto, *J. Org. Chem.*, 2003, **68**, 4755–4763.
6 (*a*) T. Yamagishi, H. Ichikawa, T. Haruki and T. Yokomatsu, *Org. Lett.*, 2008, **10**, 4347–4350; (*b*) R. Grzywa and J. Oleksyszyn, *Bioorg. Med. Chem. Lett.*, 2008, **18**, 3734–3736; (*c*) M. Drag, R. Grzywa and J. Oleksyszyn, *Bioorg. Med. Chem. Lett.*, 2007, **17**, 1516–1519.
7 (*a*) D. Gupta, R. S. Yedidi, S. Varghese, L. C. Kovari and P. M. Woster, *J. Med. Chem.*, 2010, **53**, 4234–4247; (*b*) M. Muller, *Angew. Chem., Int. Ed.*, 2005, **44**, 362–365.
8 (*a*) S. Nakatani, K. Hidaka, E. Ami, K. Nakahara, A. Sato, J. Nguyen, Y. Hamada, Y. Hori, N. Ohnishi, A. Nagai, T. Kimura, Y. Hayashi and Y. Kiso, *J. Med. Chem.*, 2008, **51**, 2992–3004; (*b*) A. Stoeckel-Maschek, B. Stiebitz, R. Koelsch and K. Neubert, *Bioorg. Med. Chem.*, 2005, **13**, 4806–4820; (*c*) S. Weik, T. Luksch, A. Evers, J. Bçttcher, C. A. Sotriffer, A. Hasilik, H. Lçffler, G. Klebe and J. Rademann, *Chem. Med. Chem.*, 2006, **1**, 445–457.
9 (*a*) C. Björklund, H. Adolfsson, K. Jansson, J. Lindberg, L. Vrang, A. Hallberg, A. Rosenquist and B. Samuelsson, *Bioorg. Med. Chem.*, 2010, **18**, 1711–1723; (*b*) Y. Yamaguchi, K. Menear, N. Cohen, R. Maha, F. Cumin, C. Schnell, J. M. Wood and J. Maibaum, *Bioorg. Med. Chem. Lett.*, 2009, **19**, 4863–4867; (*c*) S. Hanessian, G. Yang, J. Rondeau, U. Neumann, C. Betschart and M. Tintelnot-Blomley, *J. Med. Chem.*, 2006, **49**, 4544–4567.
10 (*a*) J. Wannberg, Y. A. Sabnis, L. Vrang, B. Samuelsson, A. Karlen, A. Hallberga and M. Larhed, *Bioorg. Med. Chem.*, 2006, **14**, 5303–5315; (*b*) M. S. Plummer, A. Shahripour, J. S. Kaltenbronn, E. A. Lunney, B. A. Steinbaugh, J. M. Hamby, H. W. Hamilton, T. K. Sawyer, C. Humblet, A. M. Doherty, M. D. Taylor, G. Hingorani, B. L. Batley and S. T. Rapundalo, *J. Med. Chem.*, 1995, **38**, 2893–2905; (*c*) T. I. Oprea, C. L. Waller and G. R. Marshall, *J. Med. Chem.*, 1994, **37**, 2206–2215.
11 (*a*) C. Sund, O. Belda, D. Wiktelius, C. Sahlberg, L. Vrang, S. Sedig, E. Hamelink, I. Henderson, T. Agback, K. Jansson, N. Borkakoti, D. Derbyshire, A. Eneroth and B. Samuelsson, *Bioorg. Med. Chem. Lett.*, 2011, **21**, 358–362; (*b*) Maillard, *J. Med. Chem.*, 2007, **50**, 776–781.
12 (*a*) R. E. Dolle, C. V. C. Prasad, C. P. Prouty, J. M. Salvino, M. M. A. Awad, S. J. Schmidt, D. Hoyer, T. M. Ross, T. L. Graybill, G. J. Speier, J. Uhl, B. E. Miller, C. T. Helaszek and M. A. Ator, *J. Med. Chem.*, 1997, **40**, 1941–1946; (*b*) Olson, *J. Med. Chem.*, 1993, **36**, 3039–3049.
13 (*a*) A. Guarna, A. Guidi, F. Machetti, G. Menchi, E. G. Occhiato, D. Scarpi, S. Sisi and A. Trabocchi, *J. Org. Chem.*, 1999, **64**, 7347–7364; (*b*) D. Scholz, P. Hecht, H. Schmidt and A. Billich, *Monatshefte fur Chemie*, 1999, **130**, 1283–1300; (*c*) J. A. Robl, M. P. Cimarusti, L. M. Simpkins, B. Brown, D. E. Ryono, J. E. Bird, M. M. Asaad, T. R. Schaeffer and N. C. Trippodo, *J. Med. Chem.*, 1996, **39**, 494–502; (*d*) E. Marsault and M. L. Peterson, *J. Med. Chem.*, 2011, **54**, 1961–2004; (*e*) Y.-K. Kim, M. A. Arai, T. Arai, J. O. Lamenzo, E. F. Dean, N. Patterson, N. Patterson, P. A. Clemons and S. L. Schreiber, *J. Am. Chem. Soc.*, 2004, **126**, 14740–14745.
14 (*a*) N. Wang, L. Zhang and X. Ye, *Org. Biomol. Chem.*, 2010, **8**, 2639–2649; (*b*) N. Kumari and Y. D. Vankar, *Org. Biomol. Chem.*, 2009, **7**, 2104–2109; (*c*) K. S. Ajish Kumar, V. D. Chaudhari and D. D. Dhavale, *Org. Biomol. Chem.*, 2008, **6**, 703–711; (*d*) C. Schaller and P. Vogel, *Helv. Chim. Acta*, 2000, **83**, 193–232; (*e*) W. H. Pearson and E. J. Hembre, *J. Org. Chem.*, 1996, **61**, 5537–5545; (*f*) D. D. Dhavale, S. M. Jachak, N. P. Karchea and C. Trombini, *Tetrahedron*, 2004, **60**, 3009–3016; (*g*) G. W. J. Fleet, N. M. Carpenter, S. Petursson and N. G. Ramsdena, *Tetrahedron Lett.*, 1990, **31**, 409–412.
15 A. Kilonda, F. Compernolle, K. Peeters, G. J. Joly, S. Toppet and G. J. Hoornaert, *Tetrahedron*, 2000, **56**, 1005–1012.
16 J. Wang, J. Li, D. Tuttle, J. Y. Takemoto and C. T. Chang, *Org. Lett.*, 2002, **4**, 3997–4000.
17 Gondry, *Nat. Chem. Biol.*, 2009, **5**, 414–420.
18 Crystallographic data of **7** has been deposited with the Cambridge Crystallographic Data Centre: CCDC - 826859.

