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Design and synthesis of functionalized trisaccharides as p53-peptide mimics

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ABSTRACT

Oligosaccharides represent potentially useful scaffolds for the development of peptidomimetics. We report here the design and synthesis of functionalized trisaccharides modeled after an α-helical 15-mer peptide region of p53 which binds to its cellular regulator MDM2. The trisaccharide scaffold was obtained efficiently by applying the sulfoxide glycosylation reaction as a key methodology.

Oligosaccharides offer attractive scaffolds for the development of peptdomimetics.[1–5] Monosaccharides possess polyhydroxyl groups which can be readily functionalized and their well-defined conformation allows the presentation of the functionalities to be designed in a predictable fashion. There are a variety of monosaccharides, which could be built into various architectures of polymers by employing different types of glycosidic linkages. However, oligosaccharides had not been extensively exploited to mimic relatively large protein secondary structures. We previously demonstrated the use of a pentasaccharide as a scaffold for an α-helical peptide mimic.[5] A functionalized pentasaccharide was designed based on an α-helical DNA-binding peptide region of a bZIP protein and was shown to indeed bind DNA. α-Helical peptides are also common recognition elements in protein-protein interactions such as in p53-MDM2 and BAD-BcLxL, which are important chemotherapeutic targets. Because of the potential utility as therapeutics and biological probes, development of synthetic α-helix mimics as inhibitors of protein–protein interactions has been an active area of research.[6] To expand the scope of oligosaccharides as suitable scaffolds for the α-helix mimics, we next explored the design of oligosaccharides with protein-binding functionalities. Here, we report the design and synthesis of a trisaccharide scaffold bearing the MDM2-binding functionalities of an α-helical peptide region of p53.

An X-ray structure of the N-terminal domain of MDM2 bound to a p53-derived 15-residued peptide fragment has shown that Phe19, Trp23, and Leu26, corresponding to *i*, *i* + 4, *i* + 7 residues of the helix, make hydrophobic contacts to the MDM2 pocket (Fig. 1a and b).[7] It suggested that the side chain functionalities of the three residues represent the minimal structural elements critical for binding MDM2. To test this notion, we designed a p53-peptide mimic based on a trisaccharide scaffold as shown in Figure 1c. Based on the design strategy developed in our prior studies,[5] an α-1,4-linked tri-2-deoxygalactose was employed as a suitable scaffold to match the molecular dimension of the MDM2-binding region of the p53 peptide.[10] It has been proposed earlier that overall conformation of oligosaccharides could be predicted qualitatively through conformational analysis of glycosidic bonds, which are primarily governed by an exo-anomeric effect and non-bonded steric effects between the adjacent sugars.[4,8] It was thus predicted that the glycosidic bonds in the α-1,4-linked oligogalactose system would favor an arrangement where monomer sugars are stacked on top of each other, resulting in an overall rod-shaped molecule.[5,9] In this proposed conformation, C3 and C6 hydroxyl groups of the adjacent monosaccharide units would emerge from the same face of the rod-shaped trisaccharide (Fig. 1c). By comparing a computer-generated model for an α-1,4-linked tri-2-deoxygalactose scaffold and the crystal structure of the MDM2-bound p53 peptide (Fig. 1b and d), C6–OH of A ring, C3–OH of B ring, and C6–OH of C ring were chosen to bear the side chain analogues for Phe, Trp, and Leu residues (Fig. 1c). To endow the molecule with water solubility, amino groups were incorporated to the A and C ring monomers.[11] The MDM2-bound p53 peptide has distinct amphiphilic faces with hydrophobic residues lined up on one side of the helix and hydrophilic residues on the opposite side. It was anticipated that mimicking such an amphiphilic property may also help enhance the MDM2 binding ability of the trisaccharide.

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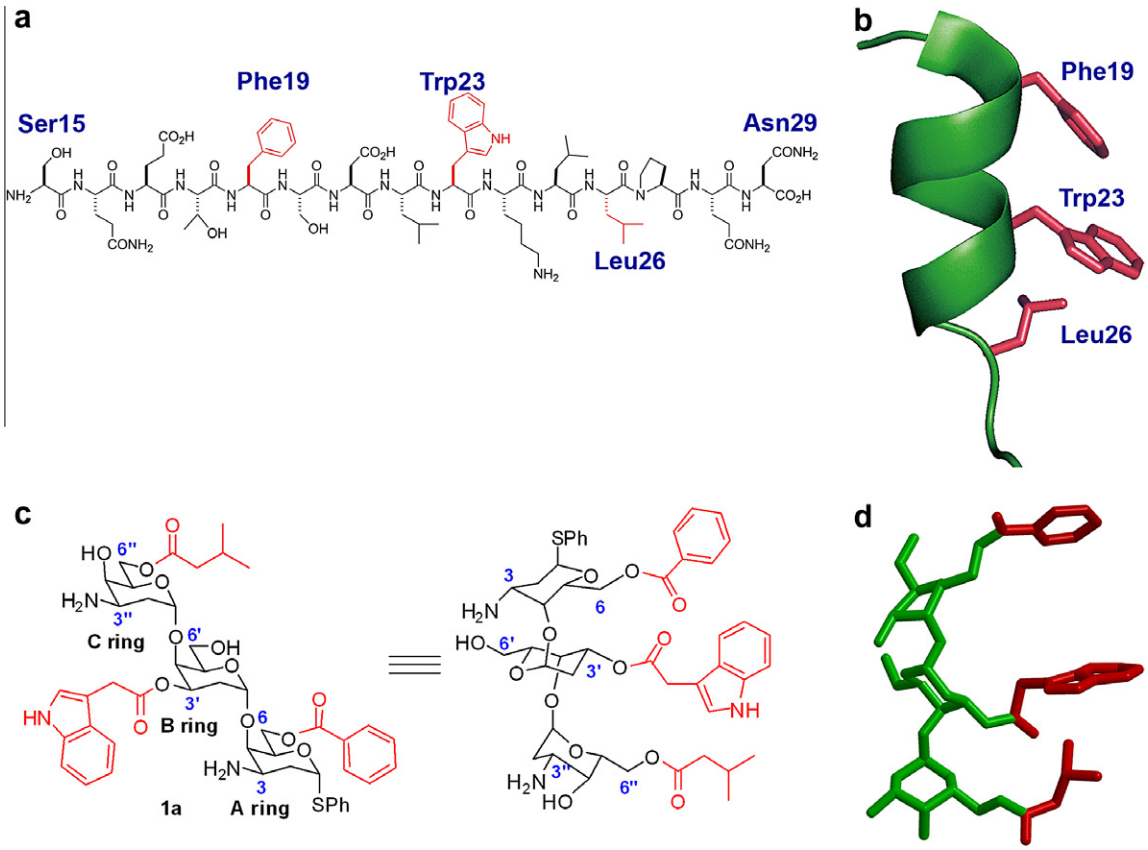


Figure 1. (a) N-terminal p53 peptide (15–29), (b) MDM2-bound conformation of 15-mer p53 peptide (side chains other than Phe, Trp, and Leu are omitted for clarity),[7] (c) functionalized trisaccharide as a p53-peptide mimic, (d) predicted conformation for the functionalized trisaccharide **1a** (anomeric phenylsulfide group is omitted for simplicity).

The synthesis of the target trisaccharides **1a–d** was planned as illustrated in Figure 2. As a preliminary effort to pursue a synthetic route which provides access to several analogues of **1a**, we also designed derivatives **1b–d**, which bear different Trp side chain analogues. For the stepwise assembly of the monomer sugars (**3**–**5**; A, B and C rings, respectively), the sulfoxide glycosylation reaction was employed as an efficient coupling methodology.[5,12–14]

Each monomer (**3**–**5**) was first prepared with appropriate hydrophobic side chain functionalities and protecting groups (Scheme 1). The A ring **3** and the C ring **5** required site-specific derivatization of the C6 hydroxyl groups, whereas the B ring **4** required derivatization

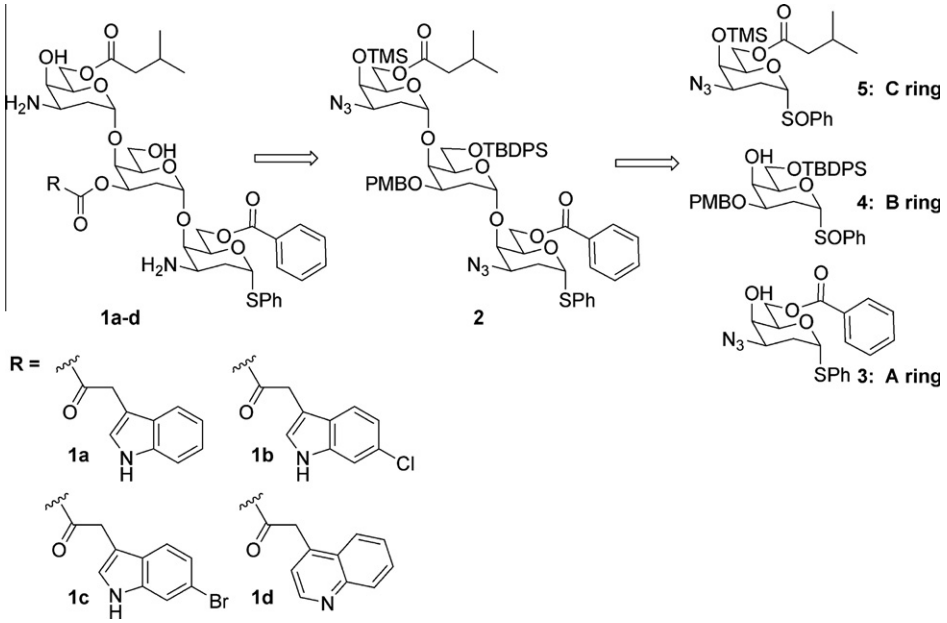
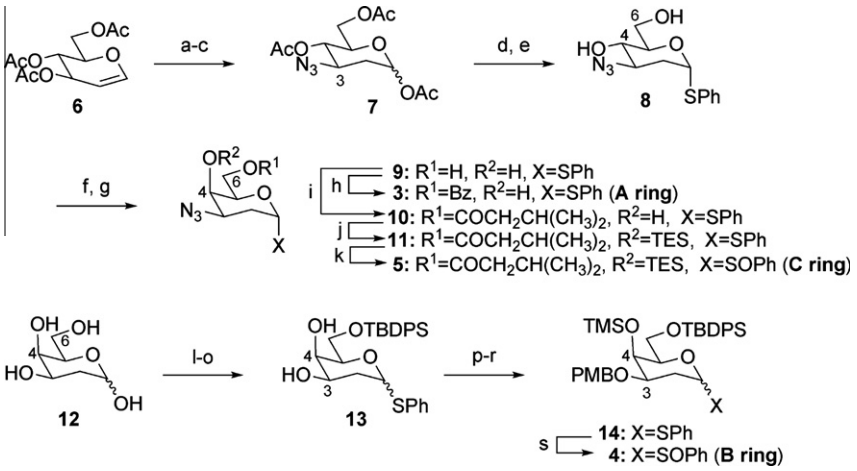


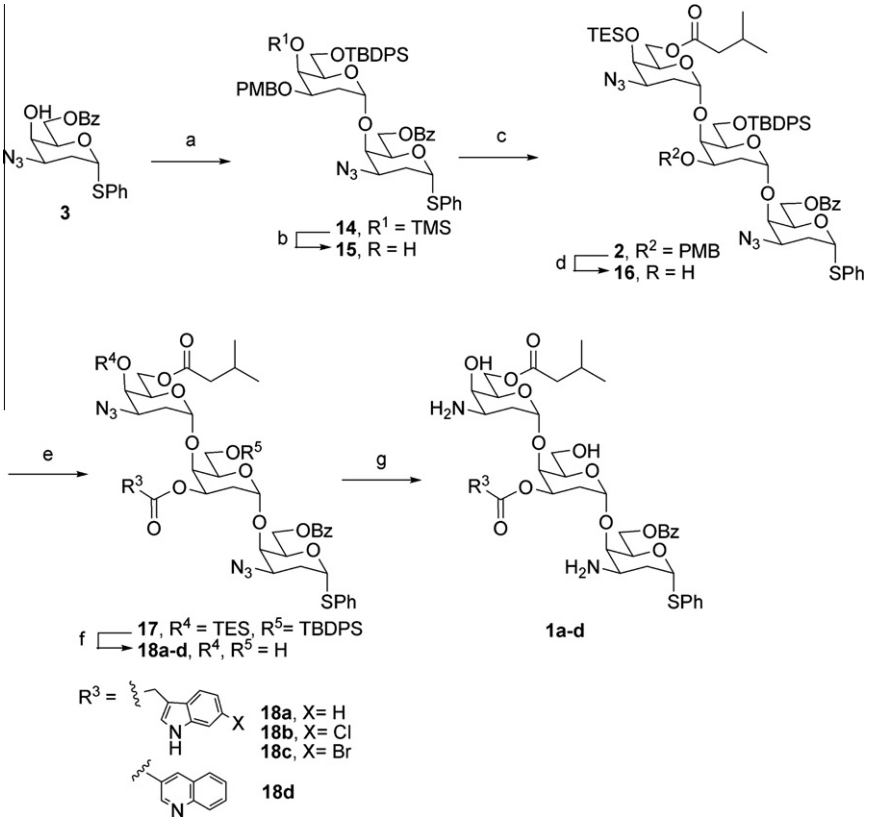
Figure 2. Retrosynthesis of the trisaccharide-based p53 peptide mimic (**1a–d**).



Scheme 1. Synthesis of the monomers. Reagents and conditions: (a) H_2O, reflux; (b) NaN_3, 60% AcOH–H_2O; (c) Ac_2O, Py, (d) PhSH, $BF_3{\cdot}Et_2O$, CH_2Cl_2, 60%; (e) NaOMe, MeOH; (f) Ph_3P, DEAD, *p*-nitrobenzoic acid, toluene; (g) NaOMe, MeOH, 80%; (h) BzCl, Py, CH_2Cl_2, −30 °C to −5 °C, 84%; (i) $(CH_3)_2CHCH_2COCl$, Py, CH_2Cl_2, 73%; (j) TESOTf, 2,6-lutidine, CH_2Cl_2, −20 °C, 96%; (k) *m*CPBA, CH_2Cl_2; (l) Ac_2O, Py; (m) PhSH, $BF_3{\cdot}Et_2O$, CH_2Cl_2; (n) NaOAc, MeOH, (α/β = 6:1), 96%; (o) TBDPSCl, imidazole, DMF, 78%; (p) $(Bu_2Sn)_2O$, benzene; (q) PMBCl, Bu_4NI, benzene, 80%; (r) TMSOTf, Et_3N, CH_2Cl_2, −78 °C to −20 °C, 89%; (s) *m*CPBA, CH_2Cl_2, −78 °C.

of the C3 hydroxyl group. Synthesis of the common precursor **9** for the A and C rings started with peracetylated D-glucal **6**. Ferrier rearrangement of **6** followed by 1,4-addition of azide group and acetylation generated 3-azido-2,3-dideoxy-glucose **7**.[15,16] Installation of anomeric phenylsulfide followed by deacetylation provided **8**. The C4 and C6 hydroxyl groups of **8** simultaneously reacted under the Mitsunobu condition to afford galactose derivative **9** after hydrolysis of the resulting *p*-nitrophenyl groups. Selective benzoylation of C6–OH of **9** then provided the A ring **3**. The C ring **5** was obtained in three steps from **9** with selective C6–OH acylation with isovaleryl group (–$COCH_2CH(CH_3)_2$), and protection of C4–OH as a TES ether and *m*CPBA oxidation.

The B ring **4** was prepared from commercially available 2-deoxy D-galactose **12**. Compound **12** was first peracetylated and then converted into phenyl thioglycoside**,** which was followed by deacetylation and selective protection of the C6–OH as TBDPS ether to give diol **13**. The C3 hydroxyl group of **13** was selectively protected with *p*-methoxy benzyl group via 3,4-stannylate intermediate.



Scheme 2. Assembly of functionalized trisaccharides. Reagents and conditions: (a) **4**, Tf_2O, DTBMP, CH_2Cl_2, 4-allyl-1,2-dimethylbenzene, −78 °C, 75%; (b) PPTS, THF–MeOH, 96%; (c) **5**, Tf_2O, DTBMP, CH_2Cl_2, 4-allyl 1,2-dimethylbenzene, −78 °C, 78%; (d) DDQ, CH_2Cl_2–H_2O, 0 °C to rt, 80%; (e) R_3CO_2H, DIC, DMAP, CH_2Cl_2 (**17a**, 80%, **17b**, 99%, **17c**, 79%, **17d**, 95%); (f) TASF, DMF (**18a**, 70%, **18b**, 77%, **18c**, 83%, **18d**, 92%); (g) polymer-supported Ph_3P, THF–H_2O, 50 °C (**1a**, 22%, **1b**, 14%, **1c**, 55%, **1d**, 26%).

Protection of the C4 hydroxyl group as TMS ether and then oxidation with *m*CPBA provided the B ring **4**.

The A–C rings (**3**–**5**) were then assembled stepwise from the reducing end to the non-reducing end using the sulfoxide-mediated glycosylation reaction (Scheme 2).[5,12–14] Both steps of the glycosylation were achieved in good yields with corresponding desired products as a single stereoisomer (78% for the A–B disaccharide **15** and 75% for the A–B–C trisaccharide **2**). For the glycosylation reaction between the A ring **3** and the B ring **4**, small amounts of the A–B–B trisaccharide was also isolated. This observation was consistent with the earlier finding in our laboratory that the TMS group could be cleaved during the sulfoxide glycosylation reaction.[5,13,14] Oxidative removal of the PMB group provided the trisaccharide precursor **16**. The alcohol **16** was furnished with four types of Trp side chain analogues (**17a–d**) via esterification, which were synthesized with good yields (79–99%). The desired trisaccharides were to be achieved simply by a removal of a TBDPS group and the reduction of azide groups. These final two steps, however, were met with unexpected difficulties. First, the standard deprotecting conditions for a TBDPS group using HF or TBAF failed to give the desired diol (**18a–d**) without significant decomposition. The presence of bulky groups at the B ring presumably prohibited access of the reagents to TBDPS group for desilylation. TAS-F in DMF was thus used to deprotect the TBDPS group, which was successfully achieved with good yields (70–92%).[17] The final step for the reduction of the azide groups was performed in the presence of polymer-supported triphenyl phosphine in the aqueous THF. The resulting crude product was purified by reverse-phase HPLC as TFA salts. While the use of resin-bound reducing agent greatly facilitated the subsequent purification step of the products, the overall yields were modest (14–55%). This is presumably due to either inefficient hydrolysis of the iminophosphorane intermediates on resin or absorption of diamine products to the resin.

The inhibitory activity of the functionalized trisaccharides against the p53-MDM2 interaction was evaluated by an inhibition ELISA. In this assay, the wt-p53 15-mer peptide inhibited the p53-MDM2 interaction at an IC_{50} value of 4 μM, which was consistent with the literature precedence.[18] Two of the functionalized trisaccharides (**1c**–**d**) showed weak inhibitory activity against the p53-MDM2 interaction, while compounds **1a** and **1b** were inactive. The quinoline-substituted trisaccharide **1d** inhibited with an IC_{50} of 550 μM whereas the 3-bromoindole-bearing trisaccharide **1c** gave IC_{50} of 850 μM. This finding was rather unexpected since **1a** and **1b** display the same side chain functionalities as those of the p53 peptide or its known high-affinity analogue.[19] Our results suggested that the presentation of the hydrophobic side chains on the trisaccharide scaffold was not optimal for the interaction with MDM2. This may be due to not only the scaffold conformation but also the side chain conformation and positioning. The interaction between the p53 peptide and MDM2 occurs through a double induced-fit mechanism, which requires a highly complementary interface to overcome the entropic penalty incurred. Recent structural studies on the p53 analogs complexed with MDM2 showed that the inhibitor molecules fulfill the strict steric complementarity to the hydrophobic pockets of MDM2 with rigid backbone structures and precisely positioned hydrophobic side chains.[19–23] Therefore, additional structure-activity studies on the side chain functionalities for the trisaccharide scaffold would be desired to achieve the steric requirements for binding the MDM2 pocket. Detailed structural analysis on the present trisaccharide scaffold would also facilitate further rational optimization of the design of a helical p53-peptide mimic.

In conclusion, we designed and synthesized functionalized α-1,4-linked trisaccharides modeled after a 15-residued helical region of p53. The synthesis of the target trisaccharides was achieved by using the sulfoxide glycosylation reaction as the key methodology. Two of the functionalized trisaccharides were found to weakly inhibit the p53-MDM2 interaction. Our synthesis demonstrated an efficient assembly of a trisaccharide scaffold, which should be useful for further development of trisaccharide-based α-helix mimics for MDM2 binding.

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References and notes

1. Meutermans, W.; Le, G. T.; Becker, B. *ChemMedChem* **2006**, *1*, 1164. See references therein.
2. Jensen, K. J.; Brask, J. *Biopolymers* **2005**, *80*, 747. See references therein.
3. Suhara, Y.; Izumi, M.; Ichikawa, M.; Penno, M.; Ichikawa, Y. *Tetrahedron Lett.* **1997**, *38*, 7167.
4. Hoffmann, R. W. *Angew. Chem., Int. Ed.* **2000**, *39*, 2054.
5. Xuereb, H.; Maletic, M.; Gildersleeve, J.; Pelczer, I.; Kahne, D. *J. Am. Soc. Chem.* **2000**, *122*, 1883.
6. Davis, J. M.; Tsou, L. K.; Hamilton, A. D. *Chem. Soc. Rev.* **2007**, *36*, 326.
7. Kussie, P. H.; Gorina, S.; Marechal, V.; Elenbaas, B.; Moreau, J.; Levine, A. J.; Pavletich, N. P. *Science* **1996**, *274*, 948.
8. Limieux, R. U.; Koto, S. *Tetrahedron* **1974**, *30*, 1933.
9. A crystal structure of galabiose (*O*-α-D-galactopyranosyl-1,4-D-galactopyranose) shows an overall structure in support of our conformational analysis. See: Svensson, G.; Albertsson, J.; Svensson, C.; Magnusson, G.; Dahmen, J. *Carbohydr. Res.* **1986**, *146*, 29.
10. 2-Deoxy sugar was used for synthetic ease and for removal of potential steric hinderance at the trisaccharide-MDM2 interface.
11. In our preliminary studies, related trisaccharides functionalized with hydrophobic side chains exhibited solubility problems in aqueous solution.
12. Kahne, D.; Walker, S.; Cheng, Y.; Vanengen, D. *J. Am. Chem. Soc.* **1989**, *111*, 6881.
13. Gildersleeve, J.; Pascal, R. A., Jr.; Kahne, D. *J. Am. Chem. Soc.* **1998**, *120*, 5961.
14. Gildersleeve, J.; Smith, A.; Sakurai, K.; Rhagavan, S.; Kahne, D. *J. Am. Chem. Soc.* **1999**, *121*, 6176.
15. Florent, J. C.; Monneret, C. *J. Chem. Soc., Chem. Commun.* **1987**, 1171.
16. Daley, L.; Roger, P.; Monneret, C. *J. Carbhydr. Chem.* **1997**, *16*, 25.
17. Scheidt, K. A.; Chen, H.; Follows, B. C.; Chemler, S. R.; Coffey, D. S.; Roush, W. R. *J. Org. Chem.* **1998**, *63*, 6436.
18. Sakurai, K.; Chung, H.-S.; Kahne, D. *J. Am. Chem. Soc.* **2004**, *126*, 16288.
19. Garcia-Echeverria, C.; Chène, P.; Blommers, M. J. J.; Furet, P. *J. Med. Chem.* **2000**, *43*, 3205.
20. Vassilev, L. T.; Vu, B. T.; Graves, B.; Carvajal, D.; Podlaski, F.; Filipovic, Z.; Kong, N.; Kammlott, U.; Lukacs, C.; Klein, C.; Fotouhi, N.; Liu, E. A. *Science* **2004**, *303*, 844.
21. Grasberger, B. L. et al *J. Med. Chem.* **2005**, *48*, 909.
22. Sakurai, K.; Schubert, C.; Kahne, D. *J. Am. Chem. Soc.* **2006**, *128*, 11000.
23. Fasan, R.; Dias, R. L.; Moehle, K.; Zerbe, O.; Obrecht, D.; Mittl, P. R.; Grütter, M. G.; Robinson, J. A. *ChemBioChem* **2006**, *7*, 515.