Published in final edited form as:

Angew Chem Int Ed Engl. 2012 October 1; 51(40): 10185–10189. doi:10.1002/anie.201205601.

Chemical Synthesis of Homogeneous Syndecan-1 Heparan Sulfate Glycopeptide

Dr. Bo Yang, Dr. Keisuke Yoshida, Dr. Zhaojun Yin, Hang Dai, Herbert Kavunja, Dr. Mohammad H. El-Dakdouki, Suttipun Sungsuwan, Steven B. Dulaney, and Prof. Dr. Xuefei Huang

Department of Chemistry, Michigan State University, 578 S. Shaw Lane, East Lansing, Michigan 48824 (USA), Fax: (+1) 517-353-1793

Xuefei Huang: xuefei@chemistry.msu.edu

First Synthesis

The highly complex structure of syndecan-1 heparan sulfate glycopeptide was successfully assembled. The protective groups utilized and sequences for glycosyl linkage formation and protective group removal are critical for the success of synthesis. This is the first time this type of sulfated glycopeptides has been prepared, which lays the foundation to access other members of this important class of molecules.

Keywords

glycopeptides; glycosylation; synthetic design; sulfation; total synthesis

Heparan sulfate proteoglycans (HSPGs), which are composed of three parts: heparan sulfate (HS), a tetrasaccharide linker and the core protein, are ubiquitous components of mammalian cell surface and the extracellular matrix.^[1] This family of molecules can serve as receptors for many adhesion molecules and growth factors, such as fibroblast growth factor, integrin, Wnt proteins and herpes simplex virus glycoprotein D.^[2] HSPGs play important roles in a variety of biological processes including cancer development, angiogenesis, viral infection, and wound repair,^[3] which render them interesting targets for the development of novel therapeutic interventions.^[4] Although the biological activities of HSPGs have been largely attributed to the HS chains, there are increasing evidences that both the HS chain and the core protein can be important for directing the biological functions.^[3a, 5] Naturally existing HSPGs are highly heterogeneous mixtures, where a single core protein contains diverse HS structures. This renders it extremely challenging to isolate HSPGs bearing a homogeneous HS chain, hindering the thorough understanding of the structure-function relationship. Chemical synthesis of HSPGs can reduce the structural heterogeneity and provide sufficient quantity for biological evaluations. Although much work has been done on synthesis of HS^[6–7] and the tetrasaccharide linker,^[8] no synthetic methods are available to date to prepare HSPGs. Herein, we report our studies in synthesis of HS glycopeptides containing homogenous HS glycans.

We are interested in human syndecan-1, an important HSPG on cell membrane.^[4, 9] Our synthetic target is HS glycopeptide **1** from the extracellular domain of syndecan-1, which contains the binding sites for many receptors.^[9d] The octasaccharide in **1** incorporates

Correspondence to: Xuefei Huang, xuefei@chemistry.msu.edu.

Supporting information for this article is available on the WWW under <http://www.angewandte.org> or from the author

typical structural features of HSPGs, including the full tetrasaccharide linker, glucosamine (GlcN) α linked to both glucuronic acid (GlcA) and iduronic acid (IdoA), 2-*O* sulfation, 6-*O* sulfation and *N*-acetylation. Successful synthesis of this molecule will lay the groundwork for accessing other HS glycopeptides.

To prepare the octasaccharide of glycopeptide **1**, our original strategy adapted a 2+5+1 glycosylation approach, where the linkages between units B (GlcN) and C (IdoA), and units G and H were to be formed by coupling disaccharide **2** with pentasaccharide **3** and pentasaccharide donor **4** with xyloside serine derivative **5** respectively. Although the α glycosyl linkage between GlcN and IdoA was formed stereospecifically using monosaccharide building blocks in our previous study,^[10] the coupling between disaccharide donor **2** and pentasaccharide **3** failed to yield the desired heptasaccharide with donor decomposition products isolated as the major side products. Varying the protective groups on **2** and **3** did not improve the situation. The failure was attributed to the reduced glycosylation power of these building blocks towards unreactive acceptors compared to their monosaccharide counterparts. This was presumably due to their larger sizes and the electron withdrawing properties of additional glycan units, which can reduce their reactivities towards acceptors.^[11] Besides the difficulty encountered in the 2+5 coupling, the 5+1 glycosylation of pentasaccharide donor **4** with the xyloside serine acceptor **5** was not productive either. These setbacks led us to consider a new 3+2+3 synthetic route, where the difficult linkages between B and C, G and H were to be formed earlier in the synthesis.

The “3 + 2 + 3” strategy called for the preparation of ABC trisaccharide **7**, DE disaccharide **8** and FGH trisaccharide **9** (Figure 1). The synthesis of ABC trisaccharide **7** started from the coupling of donor **10** with acceptor **11** using the pre-activation protocol^[12] with the promoter system of *p*-TolSCI/AgOTf.^[13] This afforded α linked BC disaccharide **12** in 80% yield with complete stereoselectivity (Scheme 1a). All the pre-activation based glycosylation reactions were performed with a slight excess (1.1 eq) of glycosyl donor compared to the acceptor. Previously, when the *p*-methoxybenzyl (PMB) group was used to mask the 6-*O* position of glycosyl donors, the electron rich 6-OPMB moiety participated in the reaction forming an 1,6-anhydro sugar shutting down the desired glycosylation.^[10] To avoid this side reaction, the PMB in **12** was exchanged with the *tert*-butyldiphenylsilyl group (TBDPS) followed by protective group adjustment generating disaccharide acceptor **13**. Coupling of the idosyl thioglycoside **14** with disaccharide **13** produced ABC trisaccharide **7** (Scheme 1b).

The middle DE disaccharide **8** was formed in 75% yield by reaction of the glucosamine donor **15** with glucoside acceptor **16** under the pre-activation condition (Scheme 1c). Removal of the *tert*butyldimethylsilyl (TBS) moiety with subsequent exchange of PMB with TBDPS produced disaccharide acceptor **8**.

With the ABC and DE modules in hand, we turned our attention to the preparation of FGH trisaccharide acceptor **9**. TBS protected xyloside donor **18** reacted with serine **19** followed by TBS removal leading to acceptor **5** (Scheme 2a). The formation of the challenging GH linkage was then tested using galactoside donor **20**, which successfully glycosylated acceptor **5** in 81% yield (Scheme 2b). This suggested that the difficulty in pentasaccharide **4** glycosylation of **5** was indeed a result of the additional glycans on the pentasaccharide donor.

To prepare trisaccharide **9**, diLev protected galactoside donor **22** glycosylated acceptor **23** forming digalactoside **24** in 85% yield (Scheme 2c). **24** was then subjected to reaction with acceptor **5**. The desired trisaccharide **25** was obtained in 43% yield in addition to the α anomer **25a** (45%). We tested various glycosylation conditions (donor/acceptor ratio, solvent, temperature, etc). However, none of these trials improved the stereoselectivity. Considering the high stereoselectivity in formation of **21** and disaccharide donor **24** bearing 2-OBz moiety at the reducing end, which can function as a participating neighbouring group, the loss of stereoselectivity in reaction of **24** with **5** was intriguing and the exact reason is unknown. Several other groups have reported the loss of 1,2-*trans* selectivities using 2-*O* acyl protected galactosyl donors.^[14] As an alternative, the Lev group in disaccharide **21** was removed and the resulting acceptor was glycosylated with galactoside **22** to generate trisaccharide **25** in 62% yield (Scheme 2b). The two Lev groups in trisaccharide **25** were removed by hydrazine acetate to afford FGH trisaccharide acceptor **9** (Scheme 2c).

Having prepared the three required modules, we investigated the union of these building blocks. The 3+2 coupling between trisaccharide donor **7** and disaccharide acceptor **8** proceeded smoothly producing pentasaccharide **26** in an excellent 93% yield. Subsequent “5 + 3” coupling between **26** and trisaccharide **9** gave octasaccharide **27** in 83% yield, thus completing the oligosaccharide backbone of the glycopeptide (Scheme 3). Octasaccharide **27** was treated with HF in pyridine to cleave the TBDPS protective groups and expose three primary hydroxyl groups, which were oxidized^[15] followed by methyl ester formation^[16] to afford octasaccharide amino ester **6** ready for peptide chain elongation (Scheme 3).

To avoid the undesired reaction on the 2-OH of unit F in **6** during *O*-sulfation, we blocked this 2-OH with acetyl (Ac), followed by piperidine treatment exposing the amino group (Scheme 4a). The free amine **28** was then subject to coupling with dipeptide **29** to afford glycopeptide **30**. Selective hydrogenation of **30** in the presence of NH₄OAc^[17] generated the free carboxylic acid **31** without affecting the benzyl ethers in the molecule.

Parallel to oligosaccharide synthesis, solid phase peptide synthesis was performed to prepare the C-terminal peptide **33** (Scheme 4b). As the final product will contain acid sensitive *O*-sulfates, the traditional strong acid promoted peptide deprotection needs to be avoided. Due to this consideration, the side chains of glutamic acid and threonine were protected as benzyl ester and benzyl ether respectively. The peptide was prepared on chloro-trityl resin and cleaved from solid phase by acid treatment (TFA/H₂O/Phenol/TIPS) (Scheme 4b). The free carboxylic acid terminal was subsequently protected as benzyl ester and the N-terminal Fmoc was removed to generate the nonapeptide **33** with a free amino terminal. Coupling of octasaccharide carboxylic acid **31** with peptide **33** without any base additive^[18] successfully

produced glycopeptide **34** in 75% yield (Scheme 4b). It was important to omit the base during this step to avoid the β -elimination of the glycan chain from the peptide backbone.

To accomplish the deprotection of fully protected glycopeptide **34**, the right reaction sequence is critical. Despite the fact that simultaneous hydrogenolysis of the benzyl ethers and the azido groups in HS oligosaccharide synthesis were reported before,^[7], 10, 19] treatment of **34** under catalytic hydrogenation condition led to a complex reaction mixture. To overcome this, we resorted to selective reduction of azide by zinc and copper sulfate with simultaneous acetylation of the newly liberated free amines (Scheme 4c).^[20] The acetylated glycopeptides were then subjected to selective removal of the levulinoyl (Lev) groups by hydrazine acetate, followed by *O*-sulfation of the free hydroxyl groups and hydrogenation producing glycopeptide **35** (Scheme 4c). Although hydrolysis of the acetate and benzoate esters was the only step remaining to the final glycopeptides, it proved to be extremely challenging. We investigated a variety of reagents including LiOOH, NaOH and LiOH with pH values ranging from 8 to 11. Unfortunately, with pH around 8.5, Bz could not be removed while under higher pH, we observed significant degree of β -elimination of the glycan, which is a common problem in glycopeptide synthesis.^[21]

The failure to deprotect **35** prompted us to redesign our deprotection sequence. Since β -elimination is presumably caused by deprotonation of the α -proton of the glycosylated serine (H^a marked in **35**), we envision that if the serine carboxylic acid is kept in its free form, its deprotonation should raise the pKa value of the α -proton on the neighbouring carbon due to electrostatic repulsion, thus reducing the potential for β -elimination. Furthermore, Kihlberg and coworkers reported that partially deprotected glycopeptide was more stable against β -elimination compared to the fully protected form.^[21] Thus, an alternative route was designed to first remove all the protecting groups on compound **30** except the three methyl esters, and to couple this partially deprotected glycopeptide to peptide **33**. The assembled glycopeptide can be transformed to **1** via hydrogenation and mild base treatment since methyl esters are more sensitive to base hydrolysis. To test this, we converted **30** to its partially protected form **36** (azide reduction/acetylation, Lev removal, *O*-sulfation, hydrogenation and transesterification). For the transesterification step, pH was carefully maintained at 9.5 in order to minimize β -elimination while retaining the methyl esters by NaOMe treatment. All Ac and Bz groups were cleaved under this condition in 40 hours. With compound **36** in hand, we tested its coupling with peptide **33**. It was shown that sulfates did not affect peptide coupling reactions.^[18] Indeed, reaction of **36** with **33** produced glycopeptide **37** in 60% yield. Subsequent hydrogenation and methyl ester hydrolysis afforded the final glycopeptide **1** (Scheme 5).

In conclusion, we have established a strategy for the first synthesis of the highly complex structure of syndecan-1 HS glycopeptide. The protective groups utilized and the sequences for glycosyl linkage formation and protective group removal are critical for the successful synthesis. We believe the approach established and knowledge gained can be applied to the synthesis of other HS glycopeptides. Work in this regard as well as investigation of the exciting biological functions of the prepared HSPG bearing homogenous HS glycans is currently ongoing.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

This work was supported by the National Science Foundation (CHE 1111550) and the National Institute of General Medical Sciences, NIH (R01GM072667).

References

- Bernfield M, Götte M, Park PW, Reizes O, Fitzgerald ML, Lincecum J, Zako M. *Annu Rev Biochem.* 1999; 68:729–777. [PubMed: 10872465]
- Tumova S, Woods A, Couchman JR. *Int J Biochem Cell Biol.* 2000; 32:269–288. [PubMed: 10716625]
- a) Choi Y, Chung H, Jung H, Couchman JR, Oh ES. *Matrix Biol.* 2011; 30:93–99. [PubMed: 21062643] b) Lambaerts K, Wilcox-Adelman SA, Zimmermann P. *Curr Opin Cell Biol.* 2009; 21:662–669. [PubMed: 19535238] c) Khotskaya YB, Dai Y, Ritchie JP, MacLeod V, Yang Y, Zinn K, Sanderson RD. *J Biol Chem.* 2009; 284:26085–26095. [PubMed: 19596856] d) Bass MD, Morgan MR, Humphries MJ. *Sci Signal.* 2009; 2:pe18. [PubMed: 19336838] e) Lin X. *Development.* 2004; 131:6009–6021. [PubMed: 15563523]
- Yang Y, MacLeod V, Dai Y, Khotskaya-Sample Y, Shriver Z, Venkataraman G, Sasisekharan R, Naggi A, Torri G, Casu B, Vlodavsky I, Suva LJ, Epstein J, Yaccoby S, Shaughnessy JD, Barlogie B, Sanderson RD. *Blood.* 2007; 110:2041–2048. [PubMed: 17536013]
- a) De Cat B, Muyldermans SY, Coomans C, Degeest G, Vanderschueren B, Creemers J, Biemar F, Peers B, David G. *J Cell Biol.* 2003; 163:625–635. [PubMed: 14610063] b) Iozzo RV. *J Clin Invest.* 2001; 108:165–167. [PubMed: 11457866]
- For representative reviews on HS synthesis, see: Dulaney SB, Huang X. *Adv Carbohydr Chem Biochem.* 2012; 67:95–136. [PubMed: 22794183] and references cited therein; Noti C, Seeberger PH, Garg HG, Linhardt RJ, Hales CA. *Chemistry and Biology of Heparin and Heparan Sulfate.* ElsevierOxford2005:79–142. Petitou M, van Boeckel CAA. *Angew Chem Int Ed.* 2004; 43:3118–3133. and references cited therein; Codee JDC, Overkleeft HS, van der Marel GA, van Boeckel CAA. *Drug Discovery Today: Technol.* 2004; 1:317–326. Poletti L, Lay L. *Eur J Org Chem.* 2003:2999–3024. Karst NA, Linhardt RJ. *Curr Med Chem.* 2003; 10:1993–2031. [PubMed: 12871100]
- For representative publications on HS synthesis, see: Zulueta MML, Lin SY, Lin YT, Huang CJ, Wang CC, Ku CC, Shi Z, Chyan CL, Irene D, Lim LH, Tsai TI, Hu YP, Arco SD, Wong CH, Hung SC. *J Am Chem Soc.* 2012; 134:8988–8995. [PubMed: 22587381] and references cited therein; Xu Y, Wang Z, Liu R, Bridges AS, Huang X, Liu J. *Glycobiology.* 2012; 22:96–106. [PubMed: 21835782] and references cited therein; Czechura P, Guedes N, Kopitzki S, Vazquez N, Martin-Lomas M, Reichardt NC. *Chem Commun.* 2011; 47:2390–2392. Tiruchinapally G, Yin Z, El-Dakdouki M, Wang Z, Huang X. *Chem Eur J.* 2011; 17:10106–10112. [PubMed: 21786352] Arungundram S, Al-Mafraji K, Asong J, Leach FE, Amster IJ, Venot A, Turnbull JE, Boons GJ. *J Am Chem Soc.* 2009; 131:17394–17405. [PubMed: 19904943] Baleux F, Loureiro-Morais L, Hersant Y, Clayette P, Arenzana-Seisdedos F, Bonnaffé D, Lortat-Jacob H. *Nat Chem Biol.* 2009; 5:743–748. [PubMed: 19734912] and references cited therein; Tatai J, Fügedi P. *Tetrahedron.* 2008; 64:9865–9873. Chen J, Zhou Y, Chen C, Xu W, Yu B. *Carbohydr Res.* 2008; 343:2853–2862. [PubMed: 18619582] Polat T, Wong CH. *J Am Chem Soc.* 2007; 129:12795–12800. [PubMed: 17914818] Noti C, de Paz JL, Polito L, Seeberger PH. *Chem Eur J.* 2006; 12:8664–8686. [PubMed: 17066397] Codee JDC, Stubba B, Schiattarella M, Overkleeft HS, van Boeckel CAA, van Boom JH, van der Marel GA. *J Am Chem Soc.* 2005; 127:3767–3773. [PubMed: 15771511] Fan RH, Achkar J, Hernandez-Torres JM, Wei A. *Org Lett.* 2005; 7:5095–5098. [PubMed: 16235966] Poletti L, Fleischer M, Vogel C, Guerrini M, Torri G, Lay L. *Eur J Org Chem.* 2001:2727–2734. Petitou M, Herauld JP, Bernat A, Driguez PA, Duchaussoy P, Lormeau JC, Herbert JM. *Nature.* 1999; 398:417–422. [PubMed: 10201371] Sinay P, Jacquinet JC, Petitou M, Duchaussoy P, Lederman I, Choay J, Torri G. *Carbohydr Res.* 1984; 132:C5–C9.
- For representative publications on tetrasaccharide linker synthesis, see: Huang TY, Zulueta MML, Hung SC. *Org Lett.* 2011; 13:1506–1509. [PubMed: 21332152] Tamura, J-i; Nakamura-Yamamoto, T.; Nishimura, Y.; Mizumoto, S.; Takahashi, J.; Sugahara, K. *Carbohydr Res.* 2010; 345:2115–2123. [PubMed: 20813352] Shimawaki K, Fujisawa Y, Sato F, Fujitani N, Kuroguchi M, Hoshi H,

- Hinou H, Nishimura SI. *Angew Chem, Int Ed.* 2007; 46:3074–3079. Thollas B, Jacquinet JC. *Org Biomol Chem.* 2004; 2:434–442. [PubMed: 14747874] Tamura J, Yamaguchi A, Tanaka J. *Bioorg Med Chem Lett.* 2002; 12:1901–1903. [PubMed: 12113804] Allen JG, Fraser-Reid B. *J Am Chem Soc.* 1999; 121:468–469. Yasukochi T, Fukase K, Suda Y, Takagaki K, Endo M, Kusumoto S. *Bull Chem Soc Jpn.* 1997; 70:2719–2725. Neumann KW, Tamura J, Ogawa T. *Glycoconjugate J.* 1996; 13:933–936. Neumann KW, Tamura JI, Ogawa T. *Bioorg Med Chem.* 1995; 3:1637–1650. [PubMed: 8770388] Rio S, Beau JM, Jacquinet JC. *Carbohydr Res.* 1993; 244:295–313. [PubMed: 8348554]
9. a) Seidel C, Sundan A, Hjorth M, Turesson I, Dahl IMS, Abildgaard N, Waage A, Børset M. *Blood.* 2000; 95:388–392. [PubMed: 10627439] b) Kato M, Wang H, Kainulainen V, Fitzgerald ML, Ledbetter S, Ornitz DM, Bernfield M. *Nature Med.* 1998; 4:691–697. [PubMed: 9623978] c) Kainulainen V, Wang H, Schick C, Bernfield M. *J Biol Chem.* 1998; 273:11563–11569. [PubMed: 9565572] d) Mali M, Jaakkola P, Arvilommi AM, Jalkanen M. *J Biol Chem.* 1990; 265:6884–6889. [PubMed: 2324102]
10. Wang Z, Xu Y, Yang B, Tiruchinapally G, Sun B, Liu R, Dulaney S, Liu J, Huang X. *Chem Eur J.* 2010; 16:8365–8375. [PubMed: 20623566]
11. Koeller KM, Wong CH. *Chem Rev.* 2000; 100:4465–4493. [PubMed: 11749355] and references cited therein; Zeng Y, Wang Z, Whitfield D, Huang X. *J Org Chem.* 2008; 73:7952–7962. [PubMed: 18808187]
12. a) Sun B, Srinivasan B, Huang X. *Chem Eur J.* 2008; 14:7072–7081. [PubMed: 18601234] b) Miermont A, Zeng Y, Jing Y, Ye XS, Huang X. *J Org Chem.* 2007; 72:8958–8961. [PubMed: 17939723] c) Huang L, Wang Z, Li X, Ye XS, Huang X. *Carbohydr Res.* 2006; 341:1669–1679. [PubMed: 16442505]
13. Huang X, Huang L, Wang H, Ye XS. *Angew Chem Int Ed.* 2004; 43:5221–5224.
14. a) Kato M, Hirai G, Sodeoka M. *Chem Lett.* 2011; 40:877–879. b) Jacquinet JC. *Carbohydr Res.* 2004; 339:349–359. [PubMed: 14698893] c) Imamura A, Ando H, Korogi S, Tanabe G, Muraoka O, Ishida H, Kiso M. *Tetrahedron Lett.* 2003; 44:6725–6728. d) Chen L, Kong F. *Tetrahedron Lett.* 2003; 44:3691–3695.
15. Van den Bos LJ, Codee JDC, Van dTJC, Boltje TJ, Van BJH, Overkleeft HS, Van der Marel GA. *Org Lett.* 2004; 6:2165–2168. [PubMed: 15200311]
16. a) Barbier M, Grand E, Kovensky J. *Carbohydr Res.* 2007; 342:2635–2640. [PubMed: 17868662] b) Xie J. *Eur J Org Chem.* 2002:3411–3418.
17. Kim KS, Kim JH, Lee YJ, Lee YJ, Park J. *J Am Chem Soc.* 2001; 123:8477–8481. [PubMed: 11525654]
18. Kaczmarek P, Tocci GM, Keay SK, Adams KM, Zhang CO, Koch KR, Grkovic D, Guo L, Michejda CJ, Barchi JJ Jr. *ACS Med Chem Lett.* 2010; 1:390–394.
19. Lee JC, Lu XA, Kulkarni SS, Wen YS, Hung SC. *J Am Chem Soc.* 2004; 126:476–477. [PubMed: 14719939]
20. Vohra Y, Buskas T, Boons GJ. *J Org Chem.* 2009; 74:6064–6071. [PubMed: 19606831]
21. Sjoelin P, Kihlberg J. *J Org Chem.* 2001; 66:2957–2965. [PubMed: 11325260]

Figure 1.
Retrosynthetic analysis of octasaccharide amino ester **6**.

Scheme 1.

Reagents and conditions: a) AgOTf, *p*-TolSCl, MS 4Å, -78°C, then **11**, TTBP, -78°C-0°C; b) (1) Mg(OMe)₂, DCM, -20°C-0°C; (2) LevOH, EDC-HCl, DMAP, DCM; (3) DDQ, DCM/H₂O; (4) HF/Pyridine; (5) TBDPSCl, imidazole, DCM; c) AgOTf, *p*-TolSCl, MS 4Å, -78°C, then **13**, TTBP, -78°C-0°C; d) AgOTf, *p*-TolSCl, MS 4Å, -78°C, then **16**, TTBP, -78°C-0°C; e) (1) DDQ, DCM/H₂O; (2) HF/Pyridine; (3) TBDPSCl, imidazole, DCM.

Scheme 2.

Reagents and conditions: a) (1) AgOTf, *p*-TolSCl, MS 4Å, -78°C , then **19**, TTBP, -78°C – 0°C ; (2) Tf₂O, THF, H₂O; b) AgOTf, *p*-TolSCl, MS 4Å, -78°C , then **5**, TTBP, -78°C – 0°C ; c) (1) AcOH, NH₂NH₂, DCM/MeOH; (2) **22**, AgOTf, *p*-TolSCl, MS 4Å, TTBP, -78°C – 0°C ; d) AgOTf, *p*-TolSCl, MS 4Å, -78°C , then **23**, TTBP, -78°C – 0°C ; e) AgOTf, *p*-TolSCl, MS 4Å, -78°C , then **5**, TTBP, -78°C – 0°C ; f) AcOH, NH₂NH₂, DCM/MeOH.

Scheme 3.

Reagents and conditions: a) AgOTf, *p*-TolSCl, MS 4 Å, -78°C , then **8**, TTBP, -78°C – 0°C ; b) AgOTf, *p*-TolSCl, MS 4 Å, -78°C , then **9**, TTBP, -78°C – 0°C ; c) (1) HF/pyridine; (2) TEMPO, BAIB, DCM/H₂O/*t*-BuOH; (3) MeI, K₂CO₃, DMF.

Scheme 4.

Reagents and conditions: a) (1) Ac_2O , pyridine, 50 °C; (2) piperidine, DCM; b) HATU, DIPEA, DMF; c) Pd/C, H_2 , NH_4OAc , DCM/MeOH; d) multiple rounds of automated solid phase peptide synthesis; e) TFA/ H_2O /Phenol/TIPS; f) (1) BnBr, DIPEA, DMF; (2) piperidine, DMF; g) HATU, DMF; h) (1) Zn, CuSO_4 (sat.), Ac_2O /THF/ HOAc ; (2) $\text{NH}_2\text{NH}_2 \cdot \text{H}_2\text{O}$, HOAc , DCM/MeOH; (3) $\text{SO}_3 \cdot \text{Et}_3\text{N}$, DMF, 55 °C; (4) H_2 , Pd/C, DCM/MeOH; i) base catalyzed hydrolysis