Synthetic and Biological Studies of Carbasugar SGLT2 Inhibitors

Wai-Lung Ng1,2,3 and Tony K. M. Shing3,4

1Department of Medicine, Harvard Medical School
Boston, Massachusetts, 02115, U.S.A.
2Department of Cancer Biology, Dana–Farber Cancer Institute
Boston, Massachusetts, 02115, U.S.A.
3*Department of Chemistry, The Chinese University of Hong Kong
Shatin, New Territories, Hong Kong SAR, China
4*Department of Chemistry, Faculty of Science and Technology, Keio University
3–14–1 Hiyoshi, Kohoku-ku, Yokohama 223–8522, Japan

(Received March 6, 2018; E-mail: shing@chem.keio.ac.jp)

Abstract: Type 2 diabetes mellitus (T2DM) is the most common type of diabetes. Unfortunately, current therapeutic agents are not so effective that only less than 36% of the patients have been treated satisfactorily. Thus, we set out to investigate novel small-molecule carbohydrate mimics as potential antidiabetic agents to supplement the existing medication. Selective inhibition of the transporter protein sodium–glucose cotransporter 2 (SGLT2) has emerged as a promising way to control blood glucose level in T2DM patients. We have pioneered the design and synthesis of some novel carbasugars (pseudosugars), readily available from inexpensive α–glycosides, which contains a metabolically stable “pseudo–glycosidic” C–O bond. Their aza–analogues (with a C–N bond) and carbon–analogues (with a C–C bond) have been prepared to provide important insights into the structure–activity relationship (SAR) of these inhibitors, thereby aiding the development of carbasugar SGLT2 inhibitors as potential antidiabetic agents. Our synthetic targets are the carbocyclic analogues of serglicliflozin and dapagliflozin, which are readily accessible via various transition metal–catalyzed cross-coupling reactions. We herein describe our novel synthetic approaches towards carbasugar SGLT2 inhibitors, and discuss their SAR.

1. Introduction

Type 2 diabetes mellitus (T2DM), or non–insulin dependent diabetes mellitus (NIDDM), is the most common type of diabetes.1 T2DM can lead to blindness, renal failure, lower limb amputation and heart attacks.2 It is estimated that the number of people suffering from type 2 diabetes worldwide will rise to 380 million by 2025.3

Some examples of current antihyperglycemic agents, including acarbose, glimepiride, metformin, rosiglitazone and sitagliptine,1 target different organs, such as liver, intestines and pancreas respectively to help lower blood glucose level in diabetes patients. Unfortunately, these therapeutic agents are not effective enough that only less than 36% of diabetes patients have been treated satisfactorily.1 In addition, there are many side effects, such as weight gain and hypoglycemia, that can be associated with the current antidiabetic drugs.1,3 Thus, the quest for novel molecules with different modes of action to supplement the existing medications continues.

Sodium–dependent glucose cotransporter 2 (SGLT2) is a high–capacity, low–affinity transporter protein found mainly in the S1 domain of the proximal tubule of kidneys.4 On the contrary, sodium–dependent glucose cotransporter 1 (SGLT1) is a low–capacity, high–affinity glucose transporter, which is located not only in kidneys, but also in the gut and other tissues.5 SGLT2 is responsible for about 90% of renal glucose reabsorption.6 Inhibition of SGLT2 can therefore help reduce blood glucose level in diabetic patients by promoting urinary glucose excretion,7 whereas inhibition of SGLT1 may lead to delayed absorption of carbohydrates8 or diarrhoea.2 Selective inhibition of SGLT2 over SGLT1 is thus highly desirable.

In recent years, SGLT2 inhibition has emerged as a promising tool to fight against T2DM via an insulin–independent mechanism.4−8 Most of the current SGLT2 inhibitors are carbohydrate–based small molecules in which O–, C– and N–glycosides are dominated.4−8 Some of the marketed SGLT2 inhibitors, such as dapagliflozin and empagliflozin, have shown a reduction in body weight, blood glucose level, major adverse cardiovascular events, as well as lower risk of hypoglycaemia in T2DM patients.5 However, these treatments are also associated with an increased frequency of complications such as diabetic ketoacidosis in a subset of patients.3

Figure 1. Carbohydrate–based SGLT2 inhibitors and carbasugar SGLT2 inhibitors.
In view of the beneficial effects of SGLT2 inhibition, we decided to explore new chemical space to address the drawbacks of the current carbohydrate-based SGLT2 inhibitors. We and Ohtake et al. have pioneered the design and syntheses of carbasugar SGLT2 inhibitors, of which the sugar core rather than the aglycone was modified. We found that some of the carbasugar analogues of sergliflozin (1), such as pseudo O-glycoside 3, pseudo N-glycoside 4, and carbasugar analogue of dapagliflozin (2), such as pseudo C-glycosides 5 and 6, exert a high inhibiting ability and selectivity towards SGLT2, and also exhibit a prolonged blood glucose lowering effect (Figure 1). In this review, we focus our discussion on the design, syntheses, and SAR studies of these carbasugar SGLT2 inhibitors.

2. Design Principles and Construction of the Carbasugar Core

We proposed that pseudo O-, C-, and N-glycosides could be more metabolically stable than the corresponding O-aryl glycoside due to their resistance towards glycosidase degradation/hydrolysis (Figure 2). Thus, in the design of carbasugar SGLT2 inhibitors, the endocyclic oxygen atom was replaced by a methylene group to enhance the stability of the molecule. In our retrosynthetic pathway, all these pseudo glycosides are readily accessible from a common allylic alcohol intermediate that could be synthesized from the commercially available D-glucuronolactone (Figure 2). Pseudo O-glycoside could be obtained by using allylic substitution as the key C=O bond formation step. Pseudo C-glycoside and pseudo N-glycoside could be obtained by using a Suzuki-type coupling reaction and Buchwald–Hartwig amination to form the key C=C and C=N bond, respectively.

The carbasugar core has been effectively constructed from the inexpensive D-glucuronolactone according to a modified synthetic route based on our published results. The key allylic alcohol was obtained in only five steps from D-glucuronolactone (Figure 3). Briefly, D-glucuronolactone was first protected as EOM-ether in the presence of EOMCl and a more hindered, non-nucleophilic base 2,6-lutidine. With the protected lactone 12 in hand, we performed a nucleophilic addition of a lithiated phosphonate at low temperature, yielding phosphonate 13. Sodium borohydride reduction of the lactol moiety in 13 gave diol 14 in a good yield. We found that this protection/nucleophilic addition/reduction sequence could be performed in a telescoping manner over three steps. Not only did this method ease the purification procedures, it also improved the overall yield of the three transformations.

The key carbocyclization sequence involved the Swern oxidation of diol 14 and the concomitant Horner–Wadsworth–Emmons (HWE) olefination. It was done by reacting diol 14 with TFAA and DMSO at −78 °C, followed by using DIPEA as a base to promote both the oxidation step and HWE olefination step. The temperature and time control of this reaction was the key to obtain a good yield of enone 16. We believed that the 1,5-diketone 15 was formed as an intermediate after adding the first portion of DIPEA at −78 °C. The gradual increase in reaction temperature and the addition of the second portion of DIPEA led to the formation of enone 16. With enone 16 in hand, stereoselective hydride reduction by Super-hydride was carried out to afford α-allylic alcohol 17 in 76% yield.
yield. This α-allylic alcohol 17 served as an important common intermediate for the subsequent transformations towards pseudo-O-, C- and N-glycosides.

3. Carbasugar SGLT2 Inhibitors with a "mixed C-O/C-C" Glycosidic Linkage

With the key allylic alcohol in hand, we set out to create the family of pseudo-O-glycosides, our first class of carbasugar SGLT2 inhibitors. The α-allylic alcohol 17 was first mesylated and immediately displaced with chloride anion to give the β-allylic chloride 18 in 81% overall yield. The β-allylic chloride 18 and ortho-substituted phenol 8 were then effectively coupled to give the expected net retention product, β-allylic ether 19 in 94% yield with excellent regio- and stereo-selectivities (Figure 4). Interestingly, we discovered that the ruthenium(II) complex [RuCl₂(cod)]ₙ catalyzed the allylic substitution with a net inversion of configuration at C₁, yielding α-allylic ether 20. It was the first report of a Ru-catalyzed allylic substitution reaction with the inversion of configuration of a chiral allylic electrophile. This Ru-catalyzed reaction thus provided a complementary pathway of the corresponding Pd-catalyzed reaction, enabling easy access to different diastereomeric compounds during diversity-oriented syntheses.

With allylic ether 19 and 20 in hand, reduction of the tri-substituted alkene moiety was carried out by employing different hydrogenation conditions, resulting in the stereoselective formation of four carbasugar analogues of serglirozin (Figure 5). Hydrogenation of 19, catalyzed by Pearlman’s catalyst and followed by hydrolysis, gave pseudo-serglirozin over two steps. Diimide reduction and subsequent hydrolysis furnished an epimeric mixture of tetraols, 3 and 21. On the other hand, Raney-nickel-catalyzed hydrogenation of 20 followed by global deprotection gave tetraol 22, whereas the hydrogenation pathway utilizing diimide stereospecifically gave a D-glucose-configured tetraol 23 in 74% overall yield.

With the use of transition metal-catalyzed allylic substitution and stereodivergent hydrogenation reactions as the key steps, a series of novel carbasugar analogues of serglirozin with diverse stereocenters were synthesized (Figure 6).

We then determined the SGLT2/SGLT1 inhibiting activities of these carbasugars using an in vitro 13C-α-methyl-D-
glucopyranoside (4C-AMG) uptake assay (Figure 6). To our delight, the SAR studies hinted that replacement of the endocyclic oxygen atom by a methylene group in the carbasugar core has a minimal effect on the inhibition towards SGLT2 as well as SGLT2/SGLT1 selectivity. Also, as expected, the β-D-glucose conformation is critical for this class of carbasugar SGLT2 inhibitors. Pseudo-sergli/lozin was found to be a highly potent and selective SGLT2 inhibitor, with an IC50 value of 2.45 nM and > 200 000-fold SGLT2/SGLT1 selectivity.

The combination of its excellent metabolic stability, potency, selectivity, and facile synthetic route makes 3 a promising candidate for future pre-clinical and clinical evaluations as an antidiabetic agent.

### 4. Carbasugar SGLT2 Inhibitors with a mixed C-N/C-C Glycosidic Linkage

Given the success of pseudo-O-glycoside SGLT2 inhibitors, we set out to create their aza-analogues, such as amino-carbasugars 26 and 4, in order to expand our SAR study and to hunt for better SGLT2 inhibitors. We envisioned that the Pd-catalyzed C-N arylation reaction, also known as the Buchwald-Hartwig C-N coupling reaction, is well-suited for synthesizing these amino-carbasugars (Figure 7).

To begin with, the key allylic alcohol intermediate was transformed into an enantiomeric pair of amino-carbasugars 31 and 27 (Figure 8). First, the α-allylic alcohol 17 was mesylated and substituted with LiN3 immediately to give allylic azide 30 in 71% yield. Next, we performed catalytic hydrogenation reaction to reduce the azide and alkene moieties simultaneously. Interestingly, amine 31 was obtained as the sole product in 88% yield. It led us to postulate that an allylic amine was formed as a reaction intermediate, of which the amino group served as a directing group to guide the diastereoselective delivery of hydrogen from the catalyst surface.

In order to obtain the other C-5 diastereoisomer 27, the alkene moiety in allylic alcohol 17 was first reduced diastereoselectively to give alcohol 32, which was then converted to triflate 33. Nucleophilic substitution of the triflate with an azide source then yielded the carbocyclic azide 34. Lastly, hydrogenation of the azide functionality in 34 gave amine 27.

The coupling reaction between 27 and the meta-substituted bromochlorobenzene 28 was accomplished by using the BrettPhos precatalyst system P1/L1 (Figure 9).

Figure 7. Retrosynthetic analysis of amino-carbasugars 26 and 4.

Figure 8. Syntheses of the amino-carbasugars 27 and 31.

Figure 9. Palladium-catalyzed N-arylation using the Buchwald dialkylbiaryl phosphine precatalysts P1 and P2 provided facile access to pseudo-N-glycosides.
chemoselectively (on the bromine instead of chlorine group), and there was no epimerization at C-5 on the carbasugar core (via possible β-hydride elimination of the [L,Pd(NHAlkyl)Ar] intermediate). Thus, the coupled product 35 was isolated in high yield as a single diastereomer. In the meanwhile, the C-5 epimer of 35 was also prepared using the same synthetic steps by using the amino-carbasugars 31 as the starting material instead (not shown in the figure).

The coupling reaction of the ortho-substituted aryl bromide 29 with amino-carbasugar 27 was found very challenging because of the bulkiness of both the ortho-substituted arene and the amino-carbasugar. After extensive optimization, the more recently developed (tBu)PhCPhos precatalyst system P2/L2 can be employed to achieve this challenging transformation. Finally, the N-arylated product 36 was obtained in 88% yield, and acid hydrolysis of 36 yielded 4, the carbasugar azo-analogue of sergli/f_lozin. Its C-5 epimer was also prepared using the same synthetic route.

To gain more comprehensive insight into the SAR of carbasugar SGLT2 inhibitors, the carbocyclic allylic analogues 39 and 41 were also prepared (Figure 10). Staudinger reduction of the β-allylic azide 30 furnished the allylic amine 37 in good yield. The subsequent Pd-catalyzed N-arylation reaction using the BrettPhos (P1/L1) and (tBu)PhCPhos precatalyst system (P2/L2) (with catalytic loading of 5 mol %) gave the coupled amino-carbasugars 38 and 40 in 69 and 43% yield, respectively. Global deprotection under acidic conditions furnished the carbocyclic allylic analogues of sergli/f_lozin and dapagli/f_lozin (39 and 41).

The six newly synthesized amino-carbasugars were then evaluated by the ³⁵Cl-AMG uptake assay (Figure 11). As expected, the amino-carbasugars 4, a carbocyclic azo-analogue of sergli/f_lozin, was a highly selective and potent SGLT2 inhibitor with an IC₅₀ value of 1.9 nM and an over 3000-fold SGLT2/SGLT1 inhibitory selectivity. Surprisingly, the amino-carbasugar 26, a carbocyclic azo-analogue of dapagli/f_lozin, showed almost no inhibitory activities towards either SGLT2 or SGLT1. This result indicated that, for pseudo -N-glycoside-based SGLT2 inhibitors, the amine functionality and the distal aromatic ring should be in ortho-orientation to show a high SGLT2 inhibition.

Consistent with the SAR in pseudo-O-glycoside-based SGLT2 inhibitors, the iodo-configured carbasugar 42 was also inactive, suggesting that the inversion of the stereocenter at C-5 is not tolerated in carbasugar SGLT2 inhibitors. Notably, the allylic amine 41, which presumably adopts a distorted chair conformation, showed an IC₅₀ value of 5.5 nM and > 1,700-fold SGLT2/SGLT1 inhibiting selectivity. Given the conformational similarity between amino-carbasugar 4 and 41, allylic amino-carbasugars could potentially be explored as potent and selective SGLT2 inhibitors.

In short, through a fruitful collaboration with Prof. Stephen L. Buchwald at MIT, we have successfully developed an efficient synthetic route towards pseudo-N-glycosides. The synthesis features a chemo- and dia stereoselective Pd-catalyzed arylation reaction using the Buchwald dialkylbriarylphosphine precatalysts. Further, it has enabled the discovery of two highly selective and potent carbasugar SGLT2 inhibitors 4 and 41.

5. Carbasugar SGLT2 Inhibitors with a "dual C-C" Glycosidic Linkage

Upon the initial success of pseudo-O- and pseudo-N-glycoside-based SGLT2 inhibitors, we sought to further enhance the drug-like properties of carbasugar SGLT2 inhibitors and explore their SAR. We postulated that pseudo-C-glycosides, in which a C (sp2)-C(sp3) bond replace a C-O or C-N bond as in 5 and 6, could be superior car basugar SGLT2 inhibitors due to the presence of the highly stable "dual C-C linkage". Thus, we designed the synthetic pathway of pseudo-C-glycosides again from the β-glucanolactone-derived allylic alcohol (Figure 12). Retrosynthetic analysis

---

**Figure 10.** Preparation of the allylic amine 37 and the subsequent palladium-catalyzed N-arylation.

**Figure 11.** Structures and IC₅₀ values of amino-carbasugars.

**Figure 12.** Retrosynthetic analysis of pseudo-C-glycosides 5 and 6.
showed that the key C–C bond in carbasugar 5 could be constructed by a Suzuki–type cross-coupling reaction between an allylic electrophile 44 and an aryl boronic acid 45. This reaction should be able to couple the two fragments with a net inversion of configuration at C–1 to give β-allylic pseudo-C–aryl glycoside 6. Chemoselective hydrogenation of the olefin moiety in 6 and the subsequent deprotection of the EOM–ethers would harvest the desired pseudo-C–aryl glycoside 5. In short, our synthesis is concise and stereodivergent, featuring an underexploited, stereospecific Pd-catalyzed (Suzuki-type) allyl–aryl coupling reaction.

The key Pd-catalyzed allyl–aryl coupling reaction, which has been underexploited in the literature,21 was carried out for the allylic electrophiles, 47 and 48, and aryl boronic acid 45 (Figure 13). Extensive optimization was carried out for this transformation, as the allylic electrophiles were prone to β-elimination which led to the formation of diene side products. Thus, the proper choice of solvent (1,4-dioxane), the absence of phosphine ligand, and the mild reaction temperature (room temperature) are critical to a high coupling yield. Remarkably, this phosphine-free Pd-catalyzed allyl–aryl coupling reaction is stereospecific and regioselective, yielding the coupled pseudo-C–glycosides 49 and 51 as single products.

Next, the coupled products, cyclohexenes 49 and 51, were subjected to alkene hydrogenation and global deprotection (Figure 14). Pd, Pt or Ni-catalyzed hydrogenation was found to cause dechlorination of the aglycone moiety, and therefore diimide reduction was performed instead. This reaction was highly chemoselective in which the C=C double bond was reduced while the chloride substituent on the proximal aromatic ring was preserved. Our diimide reduction strategy also provided both C–1 and C–5 epimers of the carbasugar analogues of dapagliflozin for SAR study. In short, starting from a single allylic alcohol, all four possible diastereomers (pseudo-C–glycosides 5, 53, 55, and 58) were prepared. To carry out a more comprehensive SAR study, we also synthesized the corresponding pseudo-O–glycosides using the aforementioned Pd-catalyzed allylic substitution reaction.

14C-AMG uptake assay revealed that the β-pseudo-C–glycosides (β-C) were more active than their α-counterparts, suggesting that the β-configuration at C–1 is critical for the SGLT2 inhibition. Surprisingly, within the β-C series, the cyclohexane analogue 5 showed an IC_{50} of 438 nM only and with just ~20-fold SGLT2/SGLT1 selectivity; whereas the allylic analogue 6 showed an IC_{50} of 24 nM and >400-fold SGLT2/SGLT1 selectivity. Nonetheless, unexpectedly, pseudo-O–glycosides 59, 60, and 61 (β-O series) showed very weak inhibition towards both SGLT2 and SGLT1. The poor activity of 59, together with the findings from the SAR of pseudo-N–glycosides, indicates that subtle change in spatial connectivity, e.g. an extra exocyclic

Figure 13. Preparation of pseudo-C–glycosides via palladium-catalyzed allyl–aryl coupling reaction.

Figure 14. Preparation of pseudo-C–glycosides 5, 53, 55, 58.

Figure 15. Structures and IC_{50} values of pseudo-C–glycosides SGLT2 inhibitors.
oxygen atom, or meta– vs. ortho– substitution of the proximal phenyl ring, could be detrimental to the activity of carbasugar analogues of dapagliflozin.

The outstanding performance of allylic analogue 6, together with the unexpected SAR of the other carbasugars, prompted us to use computational modeling to gain a better insight to the ligand space of carbasugar SGLT2 inhibitors. Our in silico docking analysis showed that the distorted chair conformation of 6 favored additional hydrogen bonding interaction between 6 and Ser287 of the SGLT2 protein, leading to a stronger binding affinity when compared with 5 (Figure 16a and 16b). Our computational analysis also revealed that the exocyclic oxygen atom in 59 disrupts π–π interactions between the aglycone and the aromatic residues in the glucose–binding pocket and also prevents the carbasugar core from forming an extensive hydrogen bond network within the binding pocket, resulting in the observed poor SGLT2 inhibition of 59 (Figure 16c).

Thus, we have developed a concise and stereodivergent synthetic route towards pseudo-C-glycosides SGLT2 inhibitors. The key steps involve a mild, regioselective, and stereospecific Pd-catalyzed allyl coupling reaction, and a chemoselective diimide reduction. Notably, we discovered that the metabolically stable allylic carbasugar 6 has an excellent potency and selectivity towards SGLT2, rendering it a highly promising lead compound for further optimization as a clinically useful SGLT2 inhibitor.

6. Conclusion and Prospects

In summary, we have described the design, syntheses, and SAR studies of carbasugar SGLT2 inhibitors. We demonstrated the enormous power of modern transition metal-catalyzed cross-coupling reactions in making medicinally important carbasugars. Further, we showed that our carbasugars are stable, potent, and selective inhibitors towards SGLT2, and that they have a huge potential in being useful drug candidates for the treatment of T2DM. The optimized and modular synthetic routes allowed effective derivatization and scaling up of synthesis, thereby enabling the preparation of analogues and larger scale production for subsequent biological studies. Our SAR studies also provided guidance for future optimization of carbasugar SGLT2 inhibitors, thereby further unlocking their potential for animal studies and subsequent clinical trials. In the future, more mechanistic studies are required in order to elucidate the precise inhibiting mechanism of these SGLT2 inhibitors. Computational studies such as molecular dynamics, when coupled with advanced SAR studies, could effectively guide the development of better carbasugar SGLT2 inhibitors.

Also, the potential of SGLT2 inhibitors in the treatment of diseases beyond T2DM has been indicated, including anticancer activity and reduction in cardiovascular and renal events. Therefore, we believe that carbasugar SGLT2 inhibitors hold the same promises, and their hidden therapeutic potential should be carefully and extensively evaluated.

Acknowledgment

We would like to express our sincere thanks to all of our coworkers involved in the synthetic and biological study. We thank Miss Kuan-Jung Wu (Oxford) for her help during the preparation and revision of this manuscript. We also thank the financial supports from the Strategic Investment Scheme administered by the Center of Novel Functional Molecules of The Chinese University of Hong Kong (CUHK), a Direct Grant from CUHK, and Hong Kong Research Grant Council (HKRGC) GRF grant (ref. no. 2130348). W.L.N. thanks the Lee Hysan Foundation and the Fulbright Program for a visiting scholarship at MIT, and the HKRGC for a Hong Kong PhD Fellowship. The authors declare the following competing financial interest(s): CUHK has filed a patent on the compounds disclosed in this manuscript. If licensed, the authors will receive royalty payments in line with standard university practice.

References

Wai-Lung Ng obtained his PhD in Chemistry from The Chinese University of Hong Kong (CUHK), under the supervision of Prof. Tony K. M. Shing. He was a Fulbright Scholar at MIT, working with Prof. Stephen L. Buchwald. He was also a Croucher Foundation Postdoctoral Fellow at the University of Oxford, researching in protein chemistry with Prof. Benjamin G. Davis, FRS. He is currently a Research Fellow in Prof. Jun Qi's group at Dana–Farber Cancer Institute/Harvard Medical School. With interdisciplinary research training in organic chemistry, protein chemistry, and cancer biology, he aims to understand the molecular mechanism that underlies cancer and thereby creating novel therapeutics.

Tony K. M. Shing is currently a Professor at Keio University, an Adjunct Professor at the Department of Chemistry in CUHK, and Chairman of the Royal Society of Chemistry, Hong Kong Section. His research interests include carbohydrate chemistry, drug discovery, and synthetic organic chemistry.