Chemical Synthesis and Biological Evaluations of Adiponectin Collagenous Domain Glycoforms

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ABSTRACT: The homogeneously glycosylated 76-amino acid adiponectin collagenous domains (ACDs) with all of the possible 15 glycoforms have been chemically and individually synthesized using stereoselective glycan synthesis and chemical peptide ligation. The following biological and pharmacological studies enabled correlating glycans to function in the inhibition of cancer cell growth as well as the regulation of systemic energy metabolism. In particular, hAdn-WM6877 was tested in detail with different mouse models and it exhibited promising in vivo antitumor, insulin sensitizing, and hepatoprotective activities. Our studies demonstrated the possibility of using synthetic glycopeptides as the adiponectin downsized mimetic for the development of novel therapeutics to treat diseases associated with deficient adiponectin.

INTRODUCTION

Adiponectin is a circulating glycoprotein mainly produced from adipocytes.1−4 It is a key regulator of glucose and lipid metabolism in peripheral organs such as skeletal muscle and liver, thus increasing the systemic insulin sensitivity and energy homeostasis.5−7 A decrease in the production of adiponectin is involved in the pathogenesis of insulin resistance, type 2 diabetes, steatohepatitis, cardiovascular diseases, and certain types of cancers.8−10 In this context, adiponectin supplementation could be a highly beneficial approach with therapeutic potentials for metabolic, cancer, and cardiovascular diseases. Nevertheless, adiponectin-based therapeutics are not possible at present, mainly due to the difficulty in obtaining the full-length glycosylated human adiponectin.11

Human adiponectin is a polypeptide with 244 amino acids and comprises four structurally distinct domains from NH2- to CO2H-terminus: a signal peptide, a variable region, a collagenous domain, and a globular domain (Figure 1). The collagenous domain is glycosylated with 2-O-α-D-glucopyranosyl-D-galactosyl disaccharide via hydroxyslyline.12,13 The glycan structure is very different from the common O-linked or N-linked glycoproteins, which represents a huge challenge to convert the full-length human adiponectin protein into a viable drug via recombinant approaches. Although efforts have been made to identify a minimal structure capable of eliciting the required pharmacological agonist activities, the structure−activity relationships of the individual domain within adiponectin have not been well-defined.14−19

Full-length adiponectin produced from expression in bacteria has been shown to be biologically inactive, due to the lack of glycosylation.15,20 The globular domain forms exclusively trimers and is less active than the full-length adiponectin, especially in its insulin-sensitizing and hepatoprotective functions.21,22 These results indicate the importance of the glycosylation and collagenous domain for adiponectin’s function. Thus, exploration of the glycosylated adiponectin collagenous domain is highly demanded. However, the biofunction of the glycosylated collagenous domain and the role of glycosylation position and degree remain elusive due to its unavailability.

The mammalian adiponectin collagenous domain contains four 5-(2S,3R)-hydroxyl lysine residues (at 65, 68, 77, 101) that are glycosylated with a glucosyl-galactose disaccharide (Figure 1).12,13 The glycosylated domain cannot be accessed using the DNA recombinant process, which hampers the detailed study of this domain’s function. In addition, two-dimensional gels showed multiple adiponectin isoforms,23−25 which are likely resulting from heterogeneous glycoforms, while no information is available on their relative distribution.

In general, heterogeneity issues associated with proteins with post-translational modifications make it difficult to correlate structure to function at a molecular level. Protein chemical synthesis provides a means to generate homogeneous proteins with site-specific and structure-defined natural or unnatural modification(s).24 To fill in this knowledge gap, we undertook a chemical approach to generate the homogeneously glycosylated...
adiponectin collagenous domains with site-specific glycan(s). Herein, we report our efforts to develop a facile strategy to chemically synthesize adiponectin collagenous domains with all possible 15 glycoform combinations. This achievement has allowed us to define the biological role of the glycosylated adiponectin collagenous domains and further to correlate the glycosylation pattern to function.

### RESULTS AND DISCUSSION

**Synthesis of the Hydroxyl Lysine Building Block.**

Prior to the efforts for adiponectin chemical synthesis, the first task was to address the issue associated with a large-scale synthesis of the (2S,5R)-hydroxylysine building block. Although several synthetic routes have been previously reported, none of them could be likely applicable for the large-scale synthesis. Our synthesis is shown in Scheme 1. The key step of our synthetic strategy was the reaction between L-α-vinylglycine and 1-amino-3-butene-2-ol derivative via olefin metathesis. The carboxylic acid of Fmoc-Met-OH was first masked by a Bn group to give 1.1, which was converted to 1.2 by NaIO₄ oxidation. This intermediate underwent pyrolysis to afford the corresponding product 1.3 in overall yields.

**Scheme 1. Synthesis of the (2S,5R)-Hydroxylysine Building Block**

![Scheme 1](image-url)

Reagents and conditions: (a) BnBr, KHCO₃, DMF, 4 h, 75%. (b) NaIO₄, THF/MeOH/H₂O, 0 °C, overnight, 90%. (c) Xylene, reflux, 2 d, 67%. (d) DCC, ESH, DMAP, 90%. (e) 10% Pd/C, Et₃SiH, 3 h, 86%. (f) 1 M vinylmagnesium bromide, THF, −30 °C, 3 h, 55%. (g) Boc-i-Ala-OH, EDCI, DMAP, DCM. (h) 1 M LiOH, THF/H₂O, 1 h, 85%. (i) DIAD, triphenylphosphine, p-nitrobenzoic acid, THF, 0 °C, 1 h. (j) Grubbs II, Cul, Et₂O, overnight, 60%. (k) 10% Pd/C, EA, 5 h; BnBr, DMF, KHCO₃, 3 h, 75%.
Scheme 2. Retrosynthetic Analysis of Glycosylated Hydroxylysine

Scheme 3. Synthesis of Glycosylated Hydroxylysine

Reagents and conditions: (a) See Supporting Information. (b) AgOTf, PhSCI, TTBP, DCM/CH3CN = 1:1, -78 °C, 2 h, 41%, only β. (c) DDQ, DCM/H2O = 18:1, rt. (d) MeSO4NMeOH/DCM = 1:1, 5 h, 90%. (e) AgOTf, PhSCI, DMF, DCM, AW-300 molecular sieves, -15 °C, overnight, 53%, only α. (f) AgOTf, PhSCI, TTBP, DCM/CH3CN = 1:1, -78 °C, 2 h, 43%, only β. (g) LiOH, CaCl2, THF, iPrOH, overnight, 51%.
50% yield. On the other hand, Boc-Gly thioester was subjected to the Fukuyama reduction, and the resultant aldehyde underwent a vinylmagnesium addition to form a racemic alcohol. Subsequently, we used diastereomeric resolution via formation of diastereomeric esters, which could be readily separated and purified by silica gel column. The ester compound was treated with LiOH to regenerate the optical pure product, allowing cross olefination via the Grubbs’ catalyst with compound to produce the precursor. Which underwent hydrogenation and reinstallation of Bn group to obtain (2S,5R)-hydroxylysine building block with orthogonal protecting groups. Moreover, the undesired diastereomer with inverse chiral center could be transformed into the desired one. This synthetic route can be efficiently performed up to 10 g.

Synthesis of the Glycosylated Hydroxylysine. The second challenge is the scalable preparation of the glycosylated hydroxylysine. According to the retrosynthetic analysis (Scheme 2), the designed glycosylated hydroxylysine building block could be synthesized via a stepwise approach (route 1) or convergent approach (route 2). The protecting groups for the disaccharide need to be compatible with Fmoc-solid phase peptide synthesis (SPPS). Benzyl group (Bn) was first used, which, however, was found to be very difficult to remove from the final glycopeptides. In the end, a more acid-labile group, 4-methylbenzyl (MBn), was used. We first tested out the feasibility of the straightforward linear synthesis (route 1) (Scheme 3). Galactosyl donor with 2-Ac protecting group to promote β-selectivity via neighboring group participation effect was employed to glycosylate compound. However, the glycosylation product was not observed due to the formation of the corresponding ortho-ester. Several glycosyl donors and glycosylation conditions were attempted to overcome this issue. Figure 2. Synthesis of glycopeptide hAdn-WM77. (A) Synthetic route for hAdn-WM-77: (a) CH3CN/H2O/diethylamine (4.5/4.5/1, v/v/v), rt, 2 h, lyophilization; (b) hAdn-WM-a, pyridine/acetic acid (1/1, mol/mol), rt, 4 h. (B) UPLC−MS trace of purified hAdn-WM77. (C) ESI-MS spectrum of purified hAdn-WM77. ESI-MS calc for C302H470N92O106S, [M + 4H]4+ m/z = 1780.4, found 1780.4; [M + 5H]5+ m/z = 1424.5, found 1424.6; [M + 6H]6+ m/z = 1187.3, found 1187.3; [M + 7H]7+ m/z = 1017.8, found 1017.7; [M + 8H]8+ m/z = 890.7, found 890.8; [M + 9H]9+ m/z = 791.9, found 792.0; [M + 10H]10+ m/z = 712.8, found 712.6; [M + 11H]11+ m/z = 648.1, found 648.4.

Scheme 4. Synthesis of glyACD Glycoforms

Figure 2. Synthesis of glycopeptide hAdn-WM77. (A) Synthetic route for hAdn-WM-77: (a) CH3CN/H2O/diethylamine (4.5/4.5/1, v/v/v), rt, 2 h, lyophilization; (b) hAdn-WM-a, pyridine/acetic acid (1/1, mol/mol), rt, 4 h. (B) UPLC−MS trace of purified hAdn-WM77. (C) ESI-MS spectrum of purified hAdn-WM77. ESI-MS calc for C302H470N92O106S, [M + 4H]4+ m/z = 1780.4, found 1780.4; [M + 5H]5+ m/z = 1424.5, found 1424.6; [M + 6H]6+ m/z = 1187.3, found 1187.3; [M + 7H]7+ m/z = 1017.8, found 1017.7; [M + 8H]8+ m/z = 890.7, found 890.8; [M + 9H]9+ m/z = 791.9, found 792.0; [M + 10H]10+ m/z = 712.8, found 712.6; [M + 11H]11+ m/z = 648.1, found 648.4.

Scheme 4. Synthesis of glyACD Glycoforms

1: Adn-WM R1 = R2 = R3 = R4 = H 3.0 mg, 37%
2: Adn-WM65 R1 = sugar, R2 = R3 = R4 = H 6.1 mg, 33.6%
3: Adn-WM68 R1 = R2 = R3 = R4 = H 5.3 mg, 36%
4: Adn-WM77 R1 = sugar, R2 = R3 = R4 = H 4.9 mg, 30.8%
5: Adn-WM101 R1 = sugar, R2 = R3 = R4 = H 5.2 mg, 43%
6: Adn-WM6568 R1 = R2 = sugar, R3 = R4 = H 2.1 mg, 24.3%
7: Adn-WM6577 R1 = R2 = sugar, R3 = R4 = H 6.5 mg, 33.4%
8: Adn-WM6510 R1 = R2 = sugar, R3 = R4 = H 4.0 mg, 23%
9: Adn-WM6877 R2 = R3 = sugar, R1 = R4 = H 24.5 mg, 37.8%
10: Adn-WM68101 R2 = R3 = sugar, R1 = R4 = H 6.1 mg, 26.5%
11: Adn-WM77101 R2 = R3 = sugar, R1 = R4 = H 2.1 mg, 17.6%
12: Adn-WM65667 R2 = R3 = R4 = sugar, R1 = H 1.8 mg, 17.1%
13: Adn-WM6566101 R2 = R3 = R4 = sugar, R1 = H 1.9 mg, 17.0%
14: Adn-WM6577101 R2 = R3 = R4 = sugar, R1 = H 2.6 mg, 20.7%
15: Adn-WM6687101 R2 = R3 = R4 = sugar, R1 = H 3.0 mg, 25.3%
16: Adn-WM656677101 R2 = R3 = R4 = sugar 1.2 mg, 16.2%

“WM: The first two amino acids at the N-terminus were used to label compounds. According to the retrosynthetic analysis (Scheme 2), the designed glycosylated hydroxylysine building block could be synthesized via a stepwise approach (route 1) or convergent approach (route 2). The protecting groups for the disaccharide need to be compatible with Fmoc-solid phase peptide synthesis (SPPS). Benzyl group (Bn) was first used, which, however, was found to be very difficult to remove from the final glycopeptides. In the end, a more acid-labile group, 4-methylbenzyl (MBn), was used. We first tested out the feasibility of the straightforward linear synthesis (route 1) (Scheme 3). Galactosyl donor with 2-Ac protecting group to promote β-selectivity via neighboring group participation effect was employed to glycosylate compound. However, the glycosylation product was not observed due to the formation of the corresponding ortho-ester. Several glycosyl donors and glycosylation conditions (see Supporting Information) were attempted to overcome
this synthetic obstacle, yet there was not any improvement to suppress the ortho-ester formation. To overcome this obstacle, p-methoxybenzyl (PMB) was used as in compound 5.2. Without the neighboring group participation, the β-1,2-trans-O-glycosylation was achieved with assistance of the solvent effect. After screening of several glycosylation conditions, the galactosylated hydroxylysine 6.2 was obtained in 42% yield with only the β-glycoside formed. This promising result not only allowed for the next step synthesis but also demonstrated the feasibility of stereoselective glycosylation of the ε-amine Boc protected S-hydroxysine. To continue the second glycosylation, we attempted the PMB deprotection of 6.2 to afford compound 7. Unfortunately, the PMB deprotection reaction was messy. Likely, the MBn group might be sensitive to oxidants and incompatible with PMB groups (Scheme 3).

With this failure, we turned to the convergent approach (route 2) (Scheme 3). The challenge of this route lies in the final β-1,2-trans-glycosylation between the disaccharide and the hydroxysine building block, considering its steric hindrance and lack of β-selectivity driving factor plagued with a glycoside at the C-2 position. To this end, the 5.1 underwent a hydrolysis to generate a 2-OH free product 5.1a in quantitative yield, which was subjected as the glycosyl acceptor to react with thioglycoside 8 for disaccharide moiety construction. The key point was how to selectively introduce an α-1,2-cis glucosyl bond. Fortunately, when the DMF/DCM solvent system 31 and PhSCI/AgOTf initiator 32 were employed, the desired disaccharide thioglycoside with α-O-glycosyl linkage could be obtained in good yield with exclusive α-stereoselectivity. Subsequently, the disaccharide thioglycoside moiety 9 was successfully glycosylated with the HLBB 4 under AgOTf/PhSCI/TTBP conditions, and the usage of DCM/CH3CH2CN solvent system was the key to induce exclusive β-1,2-trans-glycosylation affording the desired product 10. Next, a Fmoc-compatible ester hydrolysis protocol (LiOH, CaCl2)33 was employed to afford the building block 11. This is a convenient and scalable synthetic route, which permitted the synthesis of MBn protected α-O-glucopyranosyl-(1-2)-β-galactopyranosyl (2S,5R)-hydroxylysine building block for the glycopeptide fragment preparation (Scheme 3).

Synthesis of Glycosylated Adiponectin Collagenous Domain (glyACD). With all the building blocks in hand, we continued with the total synthesis of glyACD. The attempt with direct SPPS failed to generate any desired product. After several trials, we finalized the synthesis via ligating two peptide fragments via Ser/Thr ligation (Figure 2). The 47-amino acid-glycopeptide hAdn-WM77-b was prepared using SPPS, followed by TMSOTf treatment to remove the MBn groups present on the glycans, affording hAdn-WM77-b after HPLC isolation (8.9% yield based on the resin loading). It is worth noting that the replacement of Bn protecting groups of the disaccharide to MBn protecting groups was critical, as all MBn groups could be cleanly removed without cleaving the glycosidic linkage. The resultant glycopeptide underwent deFmoc conditions, which then was, without HPLC purification, directly ligated with peptide salicylaldehyde ester hAdn-WM-a under Ser/Thr ligation conditions in 30.8% yield over two steps after HPLC isolation. With the same strategy, the syntheses of glyACD with all possible glycoforms (15 in total) have been successfully completed (Scheme 4).

Antiproliferative Activity of the glyACD. The human breast cancer MDA-MB-231 cells were used to evaluate the antiproliferative activities of adiponectin, the 15 glyACD, and nonglycosylated hAdn-WM peptides (see Supporting Information for details of the assay). After 24 h of incubation, the cell number was manually counted to calculate the maximum inhibition rate (E_{max}). Half-maximal effective concentration. This peptide contains a tiny amount of small molecular weight impurity. hAdn: human adiponectin.

<table>
<thead>
<tr>
<th>Peptides</th>
<th>E_{max} (%)</th>
<th>EC_{50} (μg/mL)</th>
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<tr>
<td>hAdn-WM</td>
<td>23.0±4.23</td>
<td>6.27±0.62</td>
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<tr>
<td>hAdn-WM 65</td>
<td>39.3±5.05</td>
<td>2.00±0.89</td>
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<td>hAdn-WM 68</td>
<td>38.3±2.40</td>
<td>1.84±0.13</td>
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<td>hAdn-WM 77</td>
<td>33.9±2.59</td>
<td>1.26±0.12</td>
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<tr>
<td>hAdn-WM 101</td>
<td>32.1±5.05</td>
<td>1.78±0.76</td>
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<tr>
<td>hAdn-WM6568</td>
<td>31.0±4.66</td>
<td>1.46±0.81</td>
</tr>
<tr>
<td>hAdn-WM6577</td>
<td>35.5±1.95</td>
<td>1.19±0.68</td>
</tr>
<tr>
<td>hAdn-WM65101</td>
<td>36.3±3.81</td>
<td>1.86±0.58</td>
</tr>
<tr>
<td>hAdn-WM6877</td>
<td>38.5±3.75</td>
<td>1.33±0.10</td>
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<tr>
<td>hAdn-WM68101</td>
<td>35.2±2.63</td>
<td>1.55±0.72</td>
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<tr>
<td>hAdn-WM77101</td>
<td>34.2±3.72</td>
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<td>hAdn-WM656877</td>
<td>37.7±6.2</td>
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<td>45.0±3.46</td>
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</tr>
<tr>
<td>hAdn</td>
<td>69.3±3.34</td>
<td>0.33±0.11</td>
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</table>

*After 24 h of incubation, the cell number was manually counted to calculate the maximum inhibition rate (E_{max}). Half-maximal effective concentration.
mimic the in vivo conditions, in which the adiponectin levels vary among different individuals. This result provides important information on the optimal dose for the best synergistic effects. Moreover, the nonglycosylated peptide hAdn-WM inhibits the antiproliferative activity of the hAdn, suggesting the essentiality of the glycan.

Anti-Breast-Cancer Activity of the hAdn-WM68 glyACD. Among all variants, the tetraglycan WM656877101 demonstrates the highest inhibition rate and the lowest EC\textsubscript{50}, but the overall yield of its chemical synthesis is the lowest, requiring a large amount of the glycosylated hydroxylysine building block. Thus, considering the synthetic efficiency and overall bioactive performances of \( E\text{\textsubscript{max}} \) and EC\textsubscript{50} of all the variants, the diglycan peptide hAdn-WM6877 was selected and synthesized to a larger amount (20 mg) for examining the anti-breast cancer activity (see Supporting Information for details). As the tumor can be generated with subcutaneous injection of tumor cell into immunocompromised mice, Nude and Nod/Scid mice were adopted for in vivo study. First, the MDA-MB-231 cells treated with phosphate buffered saline (PBS), hAdn-WM, or hAdn-WM6877 for 24 h were implanted orthotopically into the third right mammary fat pad of athymic nude (Figure 4A) or NOD/Scid mice (Figure 4B). The development of mammary tumors was monitored on a regular basis. Compared to the vehicle group, tumor development was significantly attenuated in mice implanted with MDA-MB-231 cells pretreated with hAdn-WM6877 (Figure 4A,B). Body weight did not show significant differences between the vehicle and treatment groups. However, the percentage tumor weights were significantly decreased in both nude and NOD/Scid mice implanted with MDA-MB-231 cells pretreated with hAdn-WM6877 (Figure 4A,B).

The transgenic MMTV PyVT mice lacking the Adipoq alleles (PyVT-AKO) exhibit spontaneous mammary tumor development starting from the age of 7 or 8 weeks. The PBS, hAdn-WM, or hAdn-WM6877 (40 \( \mu \)g/mouse)/day) was intraperitoneally injected into PyVT-AKO mice from the age of 8 weeks. Tumor development was monitored on a weekly basis. Treatment with hAdn-WM6877 significantly inhibited mammary tumor development in PyVT-AKO mice (Figure 4C). After 5 weeks of treatment, tumors were collected for examination. When compared to the vehicle group, the tumor-to-body weight ratios were significantly decreased (by over 2-fold) in PyVT-AKO mice treated with hAdn-WM6877 (Figure 4C). Collectively, the above results demonstrate that the diglycan peptide hAdn-WM6877 elicited potent anti-breast-cancer activity in mouse models.

Metabolic Functions of the hAdn-WM6877 glyACD. The adiponectin knockout (AKO) mice of the C57BL/6j...
background was used to study the metabolic functions of hAdn-WM6877. AKO mice were given a high-fat diet (HFD) for 12 weeks, starting from the age of 4 weeks. The PBS or hAdn-WM6877 peptide (40 (μg/mouse)/day) was administered intraperitoneally during the last 4 weeks of HFD treatment. Compared to the vehicle control group, daily injection with hAdn-WM6877 significantly attenuated the gain of body weight and body fat mass (Figure 5A). After 4 weeks of injection, mice treated with hAdn-WM6877 exhibited significantly improved glucose and insulin tolerance (Figure 5B).

The metabolic performance was evaluated by the Comprehensive Laboratory Animal Monitoring System (CLAMS; see Supporting Information for details). Compared to those of the vehicle controls, treatment with hAdn-WM6877 significantly increased oxygen consumption (VO2) during both dark (2345.0 ± 102.9 vs 2689.1 ± 142.2 (mL/kg)/h, P < 0.05) and light (2070.7 ± 33.6 vs 2416.9 ± 142.7 (mL/kg)/h, P < 0.05) cycles (Figure 5C, left) and increased carbon dioxide release (VCO2) during dark (2077.1 ± 68.0 vs 2320.2 ± 74.1 (mL/kg)/h, *, P < 0.05) cycles (Figure 5C, middle).
As a result, the respiratory exchange ratio (RER) during the light cycle was significantly lower in mice treated with hAdn-WM6877 than those of the vehicle controls (0.84 ± 0.04 vs 0.92 ± 0.02, P < 0.01). The fasting serum levels of triglyceride and total cholesterol were also significantly decreased in mice treated with hAdn-WM6877 (Figure 5D).

H&E and Oil Red O staining revealed that daily treatment with hAdn-WM6877 significantly reduced the lipid accumulation in livers of HFD-fed AKO mice (Figure 6A). The triglycerides and cholesterol contents were decreased by ~51.9% and ~35.4% in livers of mice treated with WM6877 when compared to the vehicle control animals (Figure 6B, left).

The circulating levels of liver injury markers, alanine transaminase (ALT) and aspartate aminotransferase (AST), were both significantly decreased by treatment with hAdn-WM6877 (Figure 6B, right). When compared to the vehicle control animals, the mRNA expression levels of steatohepatitis-associated genes, including those encoding tumor necrosis factor α (TNFA), monocyte chemoattractant protein-1 (CCL2), low-density lipoprotein receptor (LDLR), and collagen types I (COL1) and VI (COL6), were significantly decreased in livers of mice treated with hAdn-WM6877 (Figure 6C). In summary, the results suggest that hAdn-WM6877 mimicked adiponectin to elicit insulin-sensitizing, anti-inflammatory, and hepatoprotective functions in AKO mice challenged with HFD.

**CONCLUSION**

Adiponectin is a circulating hormone produced abundantly from adipose tissue and possesses many therapeutic potentials for metabolic, cancer, and cardiovascular diseases. However,
biochemical and pharmacological studies of the human adiponectin correlating the domain structure to function have been restricted by protein heterogeneity and difficulty in obtaining the homogeneous collagenous domain with site-specific modification(s). In this study we have developed an accessible and scalable synthetic route of the glycosylated adiponectin collagenous domain by using stereoselective glycan synthesis and chemical peptide ligation, which led to the achievement of 15 homogeneously glycosylated variants of ACD for the first time. Subsequently, the biological activities and pharmacological properties of the glycosylated adiponectin collagenous domain by using stereoselective glycan synthesis and chemical peptide ligation, which led to the achievement of 15 homogeneously glycosylated variants of ACD for the first time. Subsequently, the biological activities and pharmacological properties of the glycosylated adiponectin collagenous domain by using stereoselective glycan synthesis and chemical peptide ligation, which led to the achievement of 15 homogeneously glycosylated variants of ACD for the first time. Subsequently, the biological activities and pharmacological properties of the glycosylated adiponectin collagenous domain by using stereoselective glycan synthesis and chemical peptide ligation, which led to the achievement of 15 homogeneously glycosylated variants of ACD for the first time. Subsequently, the biological activities and pharmacological properties of the glycosylated adiponectin collagenous domain by using stereoselective glycan synthesis and chemical peptide ligation, which led to the achievement of 15 homogeneously glycosylated variants of ACD for the first time. Subsequently, the biological activities and pharmacological properties of the glycosylated adiponectin collagenous domain by using stereoselective glycan synthesis and chemical peptide ligation, which led to the achievement of 15 homogeneously glycosylated variants of ACD for the first time. Subsequently, the biological activities and pharmacological properties of the glycosylated adiponectin collagenous domain by using stereoselective glycan synthesis and chemical peptide ligation, which led to the achievement of 15 homogeneously glycosylated variants of ACD for the first time. The results demonstrate that the glycan plays an important role of the collagenous domain of adiponectin in the inhibition of cancer cell growth as well as the regulation of systemic energy metabolism.

The key feature of this work is to demonstrate the power of chemical synthesis of glycosylated adiponectin collagen domain in systematically addressing the role of glycosylation on activity and specificity. This study not only shows how chemistry facilitates the solution to the unsolved biological issues associated with posttranslational glycosylation at a molecular level but also paves the way to the total synthesis of glycosylated full-length human adiponectin for further therapeutic study. More importantly, our in vivo studies showed that hAdn-WM6877 exhibited impressive antitumor activity, enhanced fatty acid consumption, and improved glucose tolerance and insulin sensitivity, which opens the door to explore the opportunity of using the synthetic glycopeptide as a potential adiponectin downsized mimic supplementary in metabolic diseases treatment, and the extensive knowledge in adiponectin glycosylation might trigger the development of new drug and mechanism studies.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/jacs.1c02382.

Full details of chemical synthesis, protocols of biological experiments, NMR spectra, and UPLC−MS traces (PDF)

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Figure 6. HAdn−WM6877 glyACD alleviates HFD-induced fatty liver injuries. The adiponectin deficient (AKO) mice of the C57BL/6J background were fed a high fat diet (HFD) starting from the age of 4 weeks. After 8 weeks of HFD, equal volume of PBS or hAdn-WM6877 (40 (μg/mouse)/day) was intraperitoneally injected into AKO mice for another 4 weeks. At the end of treatment, mice were sacrificed after fasting with food removal for 16 h. (A) Liver tissue sections were prepared for hematoxylin and eosin [H&E] and Oil Red O (left) staining to evaluate the accumulation and distribution of lipid droplets. (B) Triglyceride and cholesterol contents in liver samples were examined by biochemical assays (left). ALT/AST in blood circulation was examined by biochemical assays (right). (C) Quantitative PCR (QPCR) was performed to measure the gene expression levels of TNFA, CCL2, LDLR, COL1, and COL6 in liver tissue. Data are presented as the mean ± SEM (n = 6): *, P < 0.05 vs corresponding vehicle controls (n = 6). Differences between mean values were determined using the unpaired Student’s t tests.
Complete contact information is available at: https://pubs.acs.org/10.1021/jacs.1c02382

Author Contributions
§H.W., Y.Z., and Y.L. contributed equally.

Notes
The authors declare no competing financial interest.

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ABBREVIATIONS
hAdn, human adiponectin; AKO, adiponectin knockout

REFERENCES


