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PERSPECTIVE

Synthesis of glycosylated 5-hydroxylysine, an important amino acid present in collagen-like proteins such as adiponectin†

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The synthesis of naturally occurring glycosylated (2*S*,5*R*)-hydroxylysine still remains a challenge. This perspective highlights the importance of this post-translationally modified amino acid residue in the observed bioactivity of collagen and related collagen-like proteins such as adiponectin, an important target for the treatment of type II diabetes. Strategies employed to date for the syntheses of (2*S*,5*R*)-hydroxylysine and the methods to effect glycosylation of this modified amino acid are also summarized herein.

1. Introduction

5-Hydroxylysine (5-HL) is an important synthetic target due to its presence, often in a glycosylated form, in collagens and collagen-like proteins. Collagens contain peptide sequences wherein there are repetitions of the triplet Gly-X-Y, where Y is often a prolyl or lysyl residue. During collagen synthesis, certain prolyl and lysyl residues become hydroxylated and subsequently glycosylated, causing the component peptide chains to fold into α-helical chains. Three α-helixes then weave together to form a superhelix.[1]

Different members of the collagen family contain either homotrimeric tertiary structures with three identical α-helixes or heterotrimeric tertiary structures where one or more of the α-helixes have a different peptide sequence. Homotrimeric collagens include types II and III collagen and heterotrimeric collagens include type VI collagen.

The collagen family is then further subdivided according to the protein's quaternary structure which can vary from fibrils, hexagonal networks or beaded filaments depending on the collagen type.[2] Certain collagen-like proteins also contain α-helical chains including complement C1q and the enzyme acetylcholinesterase.[3]

Many collagen and collagen-like proteins have been shown to possess medicinal properties in a wide variety of diseases.[2] Firstly, there has been significant interest in the use of certain collagens as anticancer agents, in particular as angiogenesis inhibitors. Endostatin, a fragment of type XVIII collagen, is currently being developed as an anticancer drug for application in patients with non-small cell lung cancer,[4] and segments of type XV and IV collagens are thought to possess similar anticancer properties.[2] Secondly, type II collagen is being investigated for the treatment of arthritis and osteoporosis.[5,6] Thirdly, adiponectin (a collagen-like protein) is currently being investigated as a drug candidate for the treatment of liver disease, type II diabetes, hypertension and other obesity-related disorders including obesity-related breast cancer.[7–12]

Particular attention has been paid to the use of adiponectin in the treatment of type II diabetes for several reasons. For example, sequence variation in the gene that encodes adiponectin, which is located in the 3q27 chromosomal region, has been linked to alterations in circulating adiponectin levels, and to risk of obesity and type II diabetes.[13] Adiponectin is thought to be a key regulator of fatty acid oxidation and lipid synthesis in the liver and skeletal muscle and an antagonist of tumour necrosis factor-alpha (TNF-α), which is thought to induce metabolic liver injury, as in alcoholic and non-alcoholic steatohepatitis.[14,15] In addition, adiponectin has been shown to decrease the triglyceride concentration in skeletal muscle and liver tissue and thus increase insulin sensitivity.[16]

An adiponectin deficiency state has been identified and characterized in nonclinical models and human disease states. It is strongly linked to the pathogenesis of type II diabetes and related metabolic diseases such as steatohepatitis (that is, to the various members of the so-called 'metabolic syndrome').[12]

Adiponectin treatment, with the aim of restoring its circulating levels in such deficiency states, could be of major therapeutic benefit in these important diseases, wherein adiponectin deficiency appears to be a key aspect of the pathogenetic process.[12] This proposed therapeutic approach is consistent with that employed for numerous other hormone deficiency states, for example in type I diabetes (insulin therapy) and hypothyroidism (thyroxine therapy). However, it has to date proven impractical to make adiponectin suitable for pharmaceutical application by using the required eukaryotic cell-culture approaches, *inter alia*, because of its structural complexity and heterogeneity.[2,4,6]

There nevertheless remains an urgent need to develop a comprehensive understanding of the structure–activity relationship (SAR) in the adiponectin molecule, in order to identify a minimal

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structure capable of eliciting the required pharmacological agonist activities.

However, for reasons identified above, the reliable atomic-level information that will be needed to underpin the development of an efficacious synthetic adiponectin agonist is unlikely to be achieved using recombinant approaches. These considerations have motivated our approach to study adiponectin's SAR *via* its chemical synthesis. As a first step, it will be necessary to synthesize the glycosylated 5-hydroxylysine residues which our group has shown to be necessary for its biological activity, hence the current initiative.[6,24]

2. Chemical structure of 5-HL

The stereochemical configuration of 5-HL has been elucidated to be (2*S*,5*R*) by observation of the optical rotation of naturally-occurring 5-HL.[17] Research presented by Spiro *et al.* on the collagen renal-glomerular basement membrane revealed the chemical structure of glycosylated 5-HL, to be the disaccharide 2-*O*-α-D-glucopyranosyl-D-galactose unit bound to 5-HL by a β-glycosidic bond as shown in Fig. 1.[18–20]

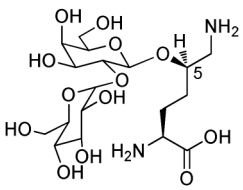


Fig. 1 Proposed structure of hydroxylated, glycosylated (Glu-Gal)-(2*S*,5*R*) lysine residues found in collagen type proteins.

Later research published by Ford *et al.*[21] and Spiro *et al.* [19] demonstrated that the same structure of glycosylated 5-HL is present in other collagens including mammalian skin collagen and bovine tendon collagen. Based on these data, and as adiponectin is also a member of the collagen superfamily,[22] it is reasonable to extrapolate by homology that the structure of the glycosylated 5-HL found in adiponectin is equivalent to that present in these other collagens.

Adiponectin is also a member of the C1q family of collagen-like molecules, and so contains an NH_2-terminal collagen domain[9] and a COOH-terminal globular domain.[23] The crystal structure of the globular domain has been resolved by Shapiro *et al.* but the 3D structure of the collagenous domain remains unavailable at the time of writing.[23]

3. Molecular structure of 5-HL

In 2002, Cooper *et al.* investigated the molecular structure of the 247 amino acid polypeptide chain of mouse adiponectin and found that it had four modified lysine residues, at positions 68, 71, 80 and 104 (Fig. 2).[7] These four lysine residues were found to be hydroxylated and subsequently glycosylated. It is thought that hydroxylated, glycosylated lysyl and prolyl residues in polypeptide chains are necessary for the observed biological activity of these proteins, at least in part.[7,24]

Adiponectin is known to exist as a number of different isoforms in serum: namely trimers, hexamers and higher molecular weight (HMW) oligomers.[9,12,25] Cooper *et al.* have proposed that the hydroxylated, glycosylated lysyl residues play a key role in the folding of the protein to form HMW oligomers.[26] In addition, they have demonstrated the importance of the modified lysyl residues on the observed bioactivity of adiponectin by substituting the lysine (K) residues with arginine (R), which led to decreased glycosylation and progressive impairment of biological activity as successive residues were replaced.

Richards *et al.* have also shown (using MALDI-TOF analysis) that the lysyl residues within adiponectin are hydroxylated and glycosylated.[10] In a similar approach to that of Cooper *et al.*, Richards *et al.* generated mutant adiponectin molecules with various degrees of K-R substitution. These substituted adiponectin variants were analysed by using gel gradient electrophoresis. The results showed that firstly, when all lysine residues were substituted, no HMW oligomers were detected and secondly, that substitution of the second and third lysine residues (as opposed to first and fourth positions) played a larger role in decreasing the proportion of HMW oligomers present in the sample.

Furthermore, Scherer *et al.*[8,12,27] and Cooper *et al.*[7] have shown that bacterially produced full-length adiponectin is biologically inactive, whereas recombinant adiponectin produced from mammalian cell lines does exhibit biological activity in either liver or muscle cells. These observations provide further evidence for the suggestion that the post-translational modified lysine residues are responsible for the observed activity of adiponectin.

Cooper *et al.* have proposed that the reason that bacterially produced full-length adiponectin is inactive, as the protein produced is unable to undergo the necessary post-translational modification steps due to bacterial cells not containing any membrane-bound organelles.[7] Therefore, the lysine residues in the polypeptide chain remain unhydroxylated and subsequently unglycosylated, thus rendering the protein to be biologically inactive.

There is also evidence to suggest that disulfide bonds between cysteine residues in the collagen domains play an important role in the formation and stabilisation of the hexamer and HMW oligomers of adiponectin.[25] Electron microscopy visualisations of

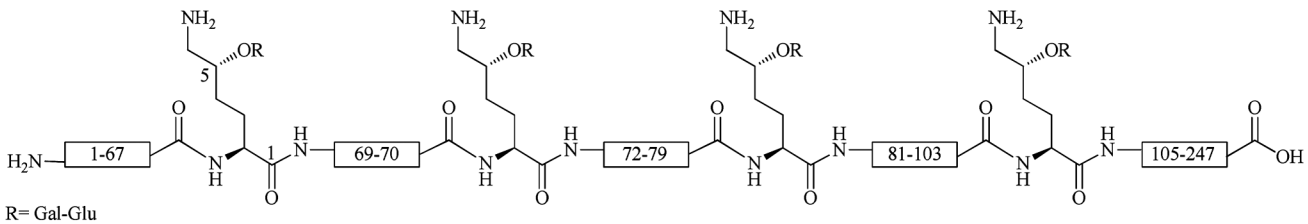


Fig. 2 The molecular structure of adiponectin with modified lysine residues at positions 68, 71, 80 and 104.

the different oligomers as well as experiments whereby the hexamer isoform was reduced to the trimer isoform using dithiothreitol, have illustrated the structural importance of these disulfide bonds. It is likely that both the post-translationally modified lysyl and cysteinyl residues present in the collagen domain of adiponectin are important for the creation and stabilisation of HMW isoforms of adiponectin.

HMW oligomers, hexamers and lower molecular weight trimers are thought to bind to different tissue types within the human body. Cooper *et al.* have shown that the greater the concentration of HMW oligomers, the more pronounced the biological activity of adiponectin and in particular the greater the degree of insulin responsiveness within liver cells.[26] Research presented by Lodish *et al.*[25] and Scherer *et al.*[28] has indicated that trimeric forms of adiponectin may be responsible for the observed bioactivity of adiponectin in skeletal muscle, with HMW oligomers playing a larger role in lipid and glucose metabolism in liver.

4. The need for chemical synthesis of adiponectin

As mentioned above, bacterially produced full-length adiponectin has been shown to be biologically inactive. Therefore, recombinant techniques to produce bioactive adiponectin must employ mammalian cell cultures. Our group has found that this approach is impracticable for large-scale synthesis of adiponectin, due to low yields and inconsistency due to large batch-to-batch variability.

Currently, there is no reported total chemical synthesis of human adiponectin and although synthesis of small glycopeptide fragments of collagens containing glycosylated lysine residues have been achieved, there are no reports to date of the synthesis of glycopeptide fragments of adiponectin.[5,29]

The first step towards a total chemical synthesis of adiponectin is the stereoselective synthesis of (2*S*,5*R*)-hydroxylysine followed by subsequent glycosylation. The glycosylated amino acid may then be incorporated into solid phase peptide synthesis combined with native chemical ligation techniques to afford peptide sequences matching those found in human adiponectin. The synthesis of these peptide chains containing the glycosylated hydroxylysine is required if an atomic-level SAR is to be achieved as the first step in the production of adiponectin agonists suitable for pharmaceutical application.

5. Previous syntheses of 5-HL

Hydroxylation of lysine residues in proteins is an important post-translation modification, and this is well illustrated by the variety of genetic diseases which occur when this modification is perturbed *e.g.* Ehlers-Danlos syndrome whereby the patient exhibits connective tissue disfunctions. It has been suggested that the degree of hydroxylation of lysine resides in bone governs the cross-linking of the α-chains, and thereby the tertiary and quaternary structure of the collagen.[30]

In order to obtain significant quantities of the glycosylated amino acid for use in subsequent peptide synthesis, the synthesis of 5-HL must first be carried out. Although 5-HL is available commercially, the price of the enantiopure (2*S*,5*R*)-HL is expensive for use in academic research ($780, 1 g, Sigma).[31,32] The price for the single enantiomer is expensive, as it must be synthesised chemically *via* the routes discussed in this perspective, or by lengthy physical separation techniques such as fractional crystallisation from the racemic mixture.[33]

As 5-HL is a naturally occurring compound, it can be isolated from naturally occurring collagens. Van Slyke *et al.*[34] first introduced the technique of hydrolysis of collagens with phosphotungstic acid to afford a mixture of diamino acids. The quantity of HL with this diamino acid mixture was then determined by reaction of the mixture with periodic acid and measuring the quantity of liberated ammonia. Although, this method enabled quantification of the HL within the collagen, it did not afford pure HL.

Napoli *et al.*[35] demonstrated the isolation of galactosyl-5HL (GH) and glucosylgalactosyl-5HL (GGH) from human placental collagen and bovine anterior lens collagen. The extraction of the desired glycoproteins was conducted firstly by hydrolysis of the lyophilized collagens followed by gel filtration to isolate GH and GGH. Ion-paired reversed phase HPLC was then employed to isolate GH and GGH separately. However, no yields of GH or GGH are reported in this paper, so the method is assumed to be low yielding.

Due to the high cost of commercial 5-HL and the low yielding isolation techniques employed to date, the task of producing significant quantities of 5-HL presents an opportunity for development of a better chemical synthesis.

There have been six different approaches to the chemical synthesis of 5-HL reported to date which are outlined below. These different methods can be categorized as: a racemic synthesis,[36] a racemic synthesis followed by HPLC resolution of diastereoisomers,[37] a racemic synthesis using a chiral resolving agent,[38] a stereoselective synthesis using a chiral auxiliary,[39] a stereoselective synthesis using a chiral catalyst[40] and a substrate-controlled stereoselective synthesis.[41,42]

Early syntheses of 5-HL were racemic, with the different diastereomers being separated through physical methods such as HPLC. Chiral resolving agents such as thyroxine derivatives have also been used to further enable the physical separation of the resulting diastereomeric pairs.

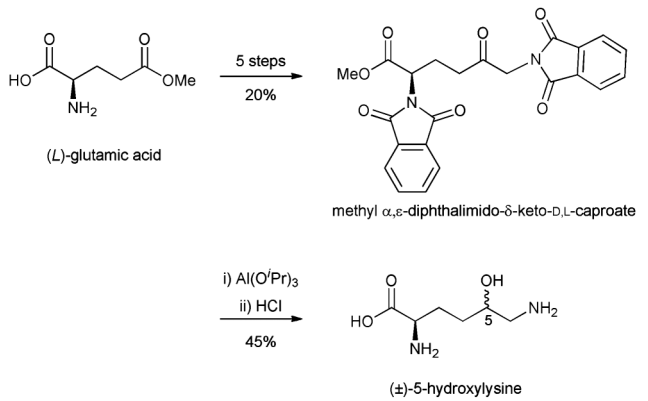
Later, more sophisticated syntheses have made use of asymmetric synthesis techniques to furnish the desired (2*S*,5*R*)-HL. These techniques include: the use of a chiral auxiliary (Williams' glycine template), asymmetric catalysts (Sharpless aminohydroxylation reaction) and substrate controlled stereoselectivity (chelation controlled enolate formation).

Racemic synthesis

The first synthesis of 5-HL was reported by Sheehan *et al.*[36] In this synthesis 5-HL was derived from L-glutamic acid *via* a methyl α,ε-diphthalimido-δ-keto-DL-caproate intermediate (Scheme 1). However, this synthetic pathway was non-stereoselective and found to be low yielding (9% overall).

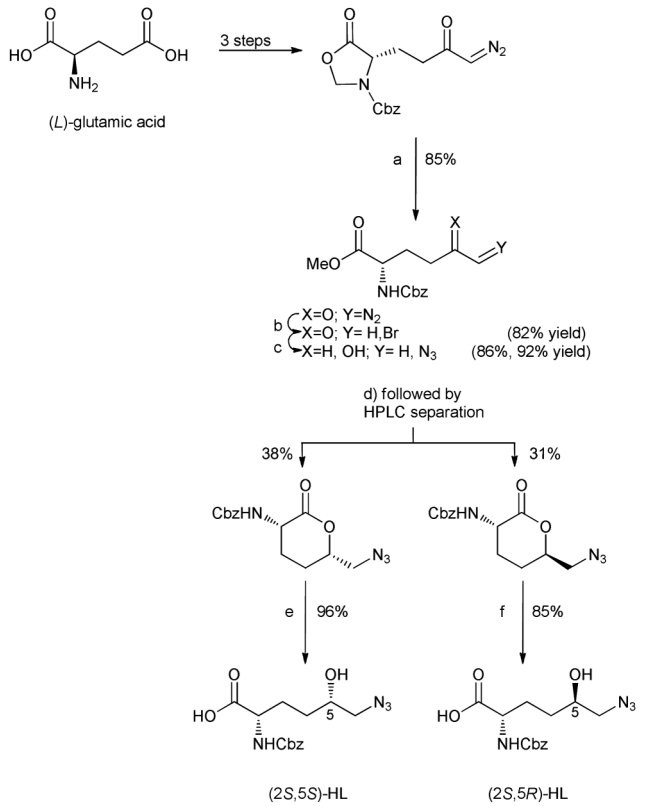
Racemic synthesis using HPLC resolution

Subsequent racemic syntheses made use of HPLC to separate the diastereomers formed at the C-5 stereocentre. The use of HPLC resolution as a purification technique in these syntheses has enabled chemists to produce clean samples of the desired (2*S*,5*R*)-HL stereoisomer.



Scheme 1 Synthesis of racemic 5-HL by Sheehan *et al.*[36]

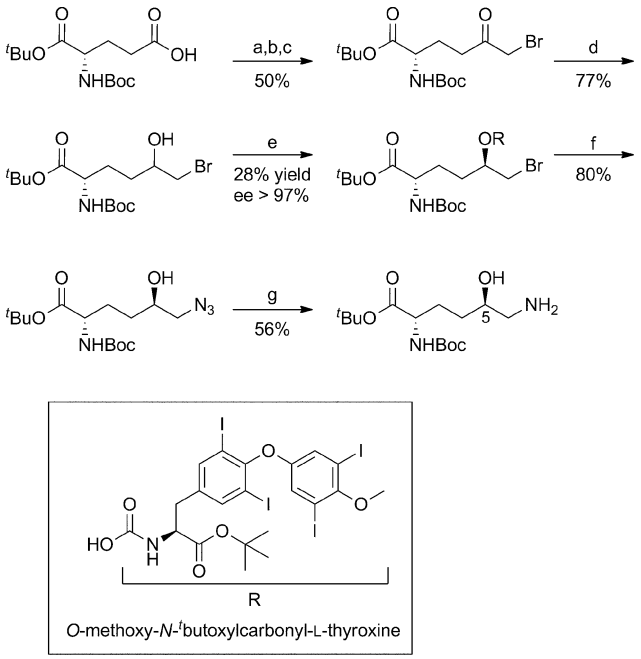
A good example of this strategy was published by Allevi *et al.* who reported the synthesis of both (2*S*,5*R*)-HL and (2*S*,5*S*)-HL in which the key diastereomeric lactone intermediates were synthesised *via* a known diazo ketone[43] derived from glutamic acid.[37] These lactone intermediates were then separated by preparative HPLC to afford the different diastereoisomers (Scheme 2). This route afforded protected derivatives of both isomers of 5-HL but unfortunately was low yielding.



Scheme 2 a) $NaHCO_3$, MeOH, reflux, 10 min; b) HBr 33%, AcOH, THF, 0 °C; c) NaN_3, DMF, rt, 1 h, $NaBH_4$, MeOH, 0 °C, 20 min; d) TFA, rt, 40 min; e) α-chymotrypsin, phosphate buffer/Me_2CO, 25 °C, 12 h; f) Cs_2CO_3, MeOH/H_2O 1 : 1 v/v, rt, 35 min.

Racemic synthesis using a chiral resolving agent

Adamczyk *et al.*[38] utilised a thyroxine-derived chiral resolving agent to enable the separation of the diastereoisomers in their synthesis of (2*S*,5*R*)-HL (Scheme 3). Their approach entailed elongation and conversion of the side-chain of Boc-protected glutamic acid to a 1,2-bromoalcohol, followed by esterification using the thyroxine derivate as a chiral resolving agent.



Scheme 3 a) Isobutyl chloroformate, 4-methylmorpholine, THF, 0 °C; b) CH_2N_2, ether, 0 °C, rt; c) 48% HBr, ether, –20 °C; d) $NaBH_4$, MeOH, 0 °C, 3 h; e) *O*-Methoxy-*N*-ᵗbutoxylcarbonyl-L-thyroxine, DCC, DCM, rt, 24 h then HPLC separation; f) i) NaN_3, DMF, 80 °C, 6h; ii) LiOH, THF:H_2O, rt, 4 h; g) i) Pd/C, H_2, EtOH, 4.5 h; ii) TFA/H_2O, rt, 2 h.

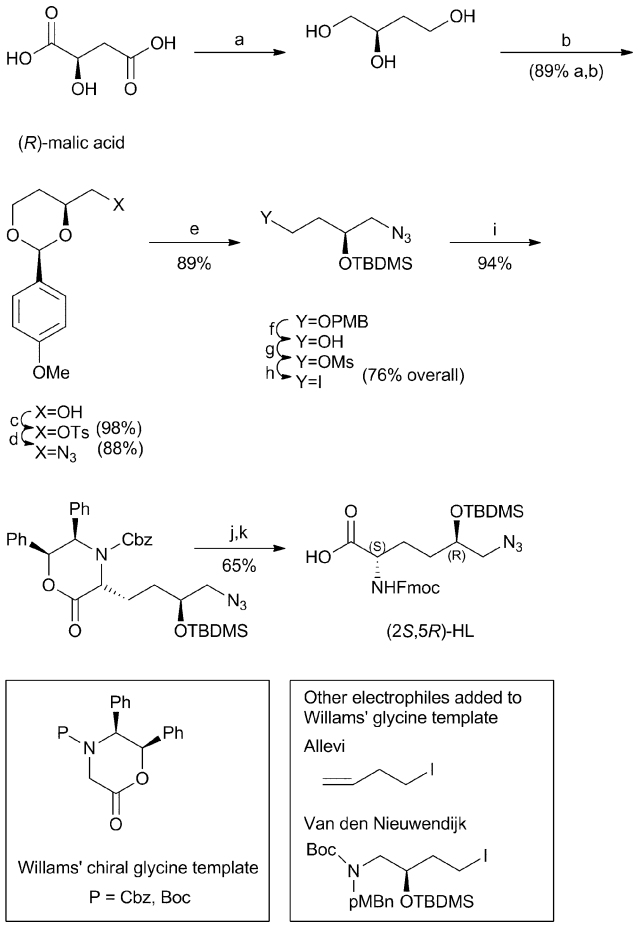
The resulting diastereoisomers were then successfully separated by preparative scale HPLC. The desired (2*S*,5*R*) diastereoisomer was then heated with NaN_3, followed by base-catalysed hydrolysis of the thyroxine ester group. The resulting azide was then reduced over a Pd/C catalyst, H_2 and the Boc protecting group removed by treatment with TFA. Although a good *ee* was obtained, the yield for the HPLC separation was low (28% for the desired diastereoisomer) and the route involved eight linear steps.

Asymmetric synthesis using a chiral auxiliary

Kihlberg *et al.* reported an interesting synthesis of (2*S*,5*R*)-HL from (*R*)-malic acid using a chiral auxiliary for controlled alkylation of an enolate to produce the desired diastereoisomer.[39] They initially attempted to use the chiral auxiliaries developed by Corey *et al.* for the asymmetric enolate alkylation,[44] but when this method proved unsuccessful they switched to the chiral auxiliaries developed by Williams *et al.* (Scheme 4).[45]

Hence, introduction of the stereochemistry at C2 of the final product was controlled using Williams' chiral glycine reagent which furnished the desired (2*S*,5*R*)-HL diastereoisomer. The synthesis required 11 linear steps affording the optically pure (2*S*,5*R*)-HL in 32% overall yield.

Allevi *et al.*[46] have also demonstrated a convenient 4 step synthesis of 5-HL originating from Williams' glycine template, but unfortunately this method was low yielding. This synthesis hinged on the stereoselective alkylation of the chiral template with



Scheme 4 a) $BH_3{\cdot}SMe_2$, $B(OMe)_3$, THF, rt; b) *p*-methoxybenzaldehyde, pyridinium toluene-4-sulfonate, DCM; c) TsCl, Et_3N, DMAP, DCM, rt; d) NaN_3, DMF, 55 °C; e) $NaCNBH_3$, TBDMSCl, CH_3CN, rt; f) DDQ, CH_3CN:H_2O, rt; g) MsCl, Et_3N, DMAP, DCM, 0 °C; h) NaI, acetone; i) Willams' chiral glycine template P = Cbz, NaHMDS, 15-crown-5, THF, −78 to −20 °C; j) i) PPh_3, THF/H_2O, microwave, ii) Boc_2O, Et_3N, THF; k)i) H_2, Pd/C, THF/H_2O, rt, ii) Fmoc-OSu, Na_2CO_3, acetone/H_2O.

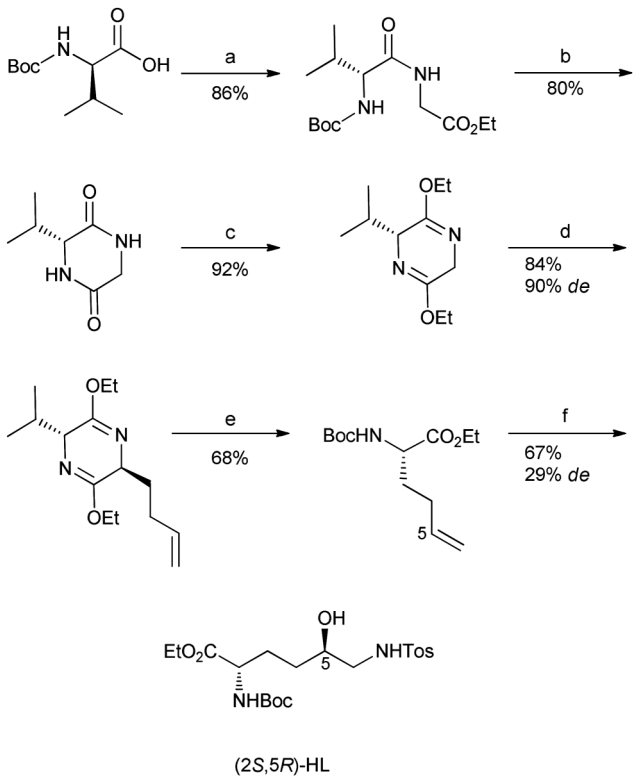
homoallyl iodide followed by subsequent epoxidation of the olefin, azide induced ring opening of the epoxide and hydrogenation to afford 5-HL.

Van den Nieuwendijk *et al.*[47] have also used a stereoselective alkylation of Willams' glycine template to prepare 5-HL using chiral iodides as the electrophiles.

Asymmetric synthesis using chiral catalysis

The first stereoselective synthesis of a 5-hydroxylysine derivative was published by Kunz *et al.*[40] The synthesis started from D-valine and glycine and used the Schöllkopf method[48] to form the α-stereogenic centre. An asymmetric Sharpless aminohydroxylation of the resulting terminal olefin then yielded (2*S*,5*R*)-HL (Scheme 5).

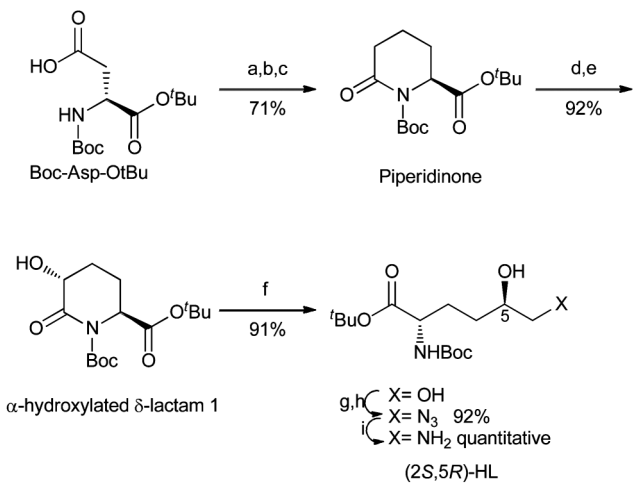
Kunz *et al.*[40] investigated the use of both $(DHQ)_2PHAL$ and $(DHQD)_2PHAL$ as chiral ligands with chloroamine T, chloroamine B and *N*-halocarbamate salts as the nitrogen source for the Sharpless aminohydroxylation reaction. Optimised reaction conditions used $(DHQD)_2PHAL$ and chloroamine T and furnished (2*S*,5*R*)-HL in 67% yield but with a low *de* of only 29%.



Scheme 5 a) Gly-OEt, DIC/HOBt; b) i) TFA, ii) reflux (toluene); c) $(Et_3O)^+(BF_4)^-$; d) 4-bromobutene, ^{n}BuLi; e)i) HCl, ii) Boc_2O; f) Chloroamine T, $(DHQD)_2PHAL$, $K_2OsO_2(OH)_2$.

Substrate directed asymmetric synthesis

Piperidinone derivatives of Meldrum's acid and aspartic acid are also useful intermediates for the synthesis of (2*S*,5*R*)-HL as demonstrated by Guichard *et al.* (Scheme 6).[41,42] The key step is the stereoselective oxidation of a chiral piperidinone derived enolate.



Scheme 6 a) Meldrum's acid, EDC, DMAP, DCM; b) $NaBH_4$, DCM, AcOH; c) toluene, reflux; d) LiHMDS; e) (+)-CSO; f) $NaBH_4$, EtOH; g) MsCl, collidine, CH_2Cl_2; h) NaN_3, DMF; i)HCl, dioxane.

The use of an alkali metal base such as LiHMDS induces chelation control over the substituents at C2 and C3, blocking the upper face of the enolate from electrophilic attack.

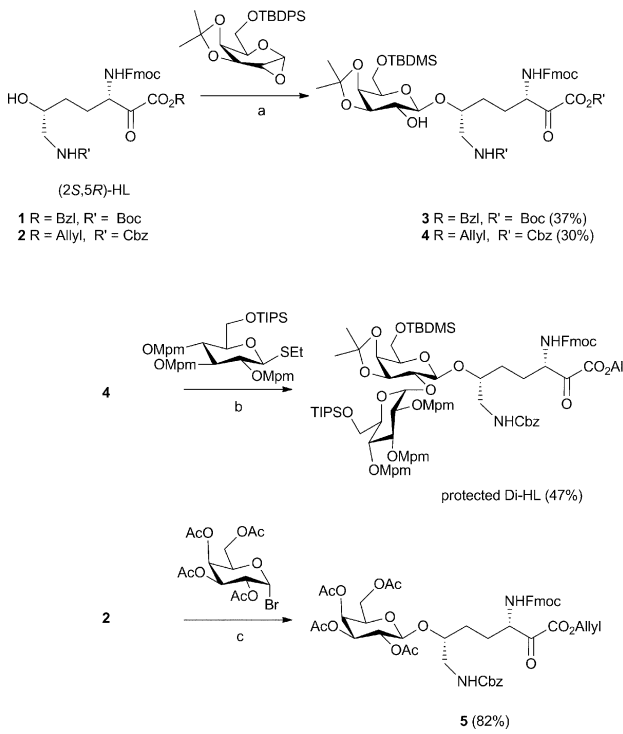
Therefore, upon addition of an electrophilic oxidizing agent such as CSO (10-camphorsulfonyloxaziridine), the lower face of the enolate is attacked preferentially. Guichard *et al.* accordingly reported *de* of >80% with all of the electrophilic oxidizing agents tested, including MoOPH (MoO_5·HMPA·pyridine), PPO (*trans*-2-(phenylsulfonyl)-3-phenyloxaziridine) and CSO, of which (+)-CSO was found to be the most active oxidizing agent for this substrate with a yield of 92% and *de* of 98% reported. Upon addition of (+)-CSO to the piperidinone, and subsequent reductive ring opening using $NaBH_4$, the desired 5*R*-α-hydroxy-δ-lactam can be produced selectively in high yield. This induced stereochemistry translates into the desired (2*S*,5*R*) stereochemistry of the product.

6. Glycosylation of 5-HL

The glycosylation of hydroxylated lysine plays a fundamental role in collagen proteins present in the human body. Kihlberg *et al.*[49,50] have shown that removal of the disaccharide moieties from lysine residues causes the T-cells within the human body to be unable to recognise the protein.

As mentioned previously, Spiro *et al.*[18] elucidated the structure of the disaccharide moiety bound to HL (Di-HL) in many collagens. Since then there has been great interest in the synthesis of the Di-HL building block and its subsequent use in peptide synthesis. One of the challenges of this synthesis is the need to form a β-*O*-galactosyl bond, rather than the anomerically favoured α-link. Different groups have responded to this challenge in a variety of ways, which are outlined below.

Kihlberg *et al.*[5,49,50] have investigated the coupling of protected 5-HL with a α-1,2-anhydro sugar under zinc chloride promoted coupling conditions (a, Scheme 7). Initial investigations were carried out using Boc protection of the ε-amine group and benzyl and Fmoc protection of the amino group.[50] These conditions resulted in production of galactosylated 5-HL in 37% yield. Upon deprotection of the carboxylic acid component of the amino group, this galactosylated building block was then utilised in solid phase peptide synthesis to afford the glycopeptide corresponding to residues 256–270 of type II collagen.

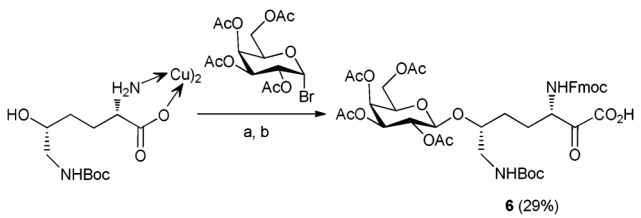


Scheme 7 a) $ZnCl_2$, THF, AW-300MS, −50 °C → rt; b) NIS, AgOTf, CH_2Cl_2, 4 Å MS, −45 °C; c) Silver silicate, 3 Å MS, CH_2Cl_2, 0 °C.

Subsequent work by Kihlberg *et al.*[5] explored the α-glycosylation of the galactosylated-5-HL unit using an activated thioethyl glycoside to afford naturally occurring D-HL. Studies using the Boc protected ε-amine group and benzyl protected amino group resulted in low yields of Di-HL (<10%). However, switching to Cbz protection of the ε-amine group and allyl protection of the amino group resulted in protected Di-HL in an acceptable 47% yield (b, Scheme 7). The Di-HL was then used in solid phase peptide synthesis (SPPS) to produce the glycopeptide corresponding to residues 256–270 of type II collagen in 23% yield.

Later work by Kihlberg *et al.*[51] utilised silver silicate to promote coupling of galactosyl bromide with Cbz protected 5-HL (c, Scheme 7). This synthesis had the advantage of a higher yielding galactosylation step (82% *versus* previous 30%). The galactosylated 5-HL was then used directly in SPPS following deallylation of the amino group.

Fields *et al.*[29] have reported the use of copper complexation of the amino group alongside Boc-protection of the ε-amine group as a useful protecting group strategy for the galactosylation of 5-HL (Scheme 8). In their synthesis protected 5-HL is coupled with acetobromo-galactose using NaH and dry acetonitrile to afford protected galactosyl-HL in 77% yield. Subsequent deprotection of the amino group followed by Fmoc protection of the amino group resulted in galactosyl-HL in overall 29% yield. This building block was then used in SPPS of the glycopeptide corresponding to residues 1263–1277 of type IV collagen.

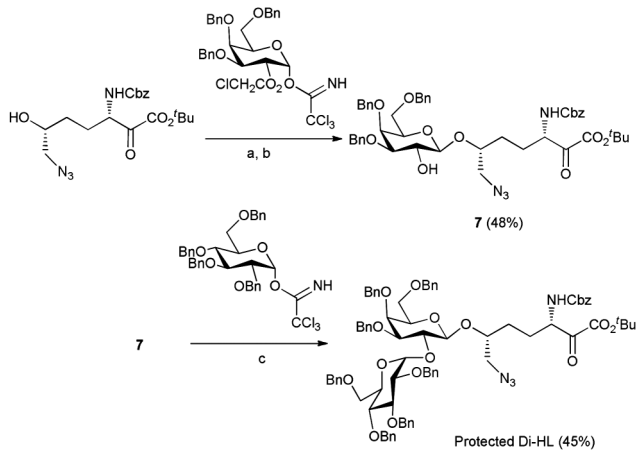


Scheme 8 a) NaH, dry CH_3CN, b) i) Chelex 100 (H^+ form), H_2O:CH_3OH, ii) Fmoc-OSu, $NaHCO_3$, H_2O-acetone.

Adamczyk *et al.*[6] have also coupled Boc- protected 5-HL to acetobromogalactose, but in their report the amino group of 5-HL is Boc and methyl ester protected and $Hg(CN)_2$ in toluene conditions were used to promote the coupling. Aside from being environmentally unfriendly, this method was also low yielding (22%).

Guichard *et al.*[42] have also performed galactosylation of 5-HL using bulkier tetra-pivaloylated galactosyl bromide in preference to tetra-acetylated galactosyl bromide in order to suppress formation of the orthoester. By using this more sterically demanding galactosyl donor excellent yields (89%) of galactosylated 5-HL were obtained, using similar conditions to Kihlberg *et al.*[51] (Guichard: 1.5 eq bulkier tetra-pivaloylated galactosyl bromide, silver silicate, CH_2Cl_2, rt, 8 h). Guichard *et al.*[52] later reported use of their galactosylated-HL in SPPS to afford the glycopeptide corresponding to residues 256–270 of bovine type II collagen.

Allevi *et al.*[24] have reported the coupling of a masked 5-HL to an α- trichloroacetimidate using *tert*-butyldimethylsilyl triflate as a catalyst. The 5-HL is masked by means of an azide functionality in place of a protected ε-amine group. Subsequent regeneration of 2-OH group allows for second coupling reaction with glycoside donor, affording desired Di-HL in 18% overall yield, the best yield reported to date (Scheme 9).



Scheme 9 a) ^{t}BuMe$_2$SiOTf, 3 Å MS, Et$_2$O, 25 °C, 1 h, b) Zn(OAc)$_2$·2H$_2$O, MeOH, reflux, 9 h; c) ^{t}BuMe$_2$SiOTf, 3 Å MS, Et$_2$O, 25 °C, 15 min.

Conclusion

Since Sheehan *et al.* first reported the synthesis of racemic 5-HL, several stereoselective syntheses of the naturally occurring (2*S*,5*R*)-HL have been reported.[36] The use of chiral resolving agents such as thyroxine derivatives reported by Adamczyk *et al.* did allow isolation of the desired diastereoisomer, but with yields as low as 28% for the chiral resolution step.[38]

The use of chiral auxiliaries such as Williams' glycine template to prepare hydroxylysine precursors has been reported by Kihlberg *et al.*[39] and others[46,47] to direct the stereochemistry of the (2*S*)-amino group. However, the highest yielding route reported by Kihlberg *et al.* was low yielding overall (32%).

Stereoselective reactions such as the Sharpless aminohydroxylation reaction presented by Kunz *et al.* have been used to synthesize (2*S*,5*R*)-HL, albeit with low *de*.[40]

The most successful synthesis of (2*S*,5*R*)-HL reported to date is the substrate-directed stereoselective approach published by Guichard *et al.*[41,42] This synthesis produced (2*S*,5*R*)-HL in the highest yield (55%) and *de* (98%) and therefore represents the most efficient stereoselective route to date.

In addition, there has been significant interest in the different methods to effect glycosylation of 5-HL to produce the naturally occurring post-translationally modified Di-HL.[5,6,24,29,51] The key challenge at this stage is to obtain a β-*O*-glycoside link to the galactosyl residue selectively followed by an α-*O*-glycoside link to the subsequent glycosyl residue. The highest yielding method to produce Di-HL is the coupling of masked 5-HL with α-trichloroacetimidates, as reported by Allevi *et al.*[24]

In summary, there still remains a need for a shorter synthetic route for synthesis of Di-HL, especially for use in the total chemical synthesis of the protein adiponectin.

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