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Review

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Recent advances in (di-)hydroxyproline synthesis

Sebastian Dominik Graf

Gertraud-Kaltenecker Straße 24, Regensburg, Germany (independent researcher)
Email: s.graf.reg@gmail.com

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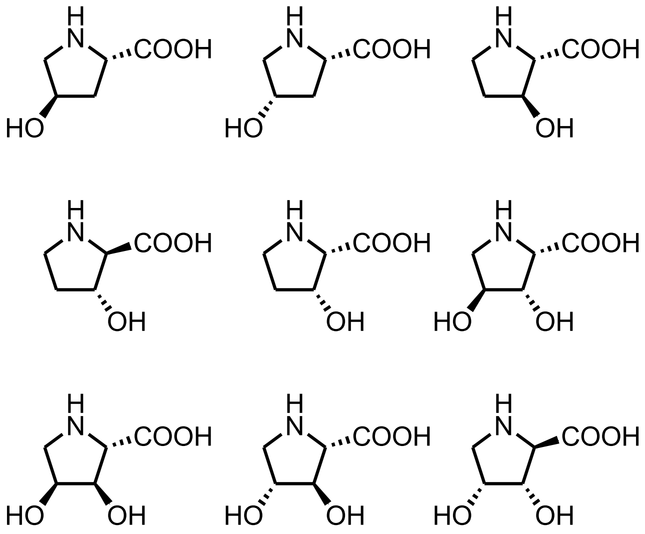
Abstract

Hydroxylated prolines constitute a group of natural or synthetic compounds that possess biological activity, catalytic utility or are implemented in pharmaceutically relevant peptides. Over the decades different approaches for the assembly of this compound class were elucidated. Herein, a review about modern examples of stereoselective syntheses towards the most prominent representatives, namely 3-hydroxyprolines, 4-hydroxyprolines and 3,4-dihydroxyprolines, is given.



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1. Introduction

Hydroxyprolines are hydroxylated derivatives of the essential aminoacid L-proline or its enantiomer, D-proline, which are wide-spread in nature and possess manifold biological and pharmaceutical relevance (Figure 1, left).[1-6] The most occurring and prominent representative is (2*S*,4*R*)-4-hydroxyproline (**1**), which is incorporated in mammalian collagens with a content of up to 13%. It is synthesized post translationally by the enzyme prolyl hydroxylase and ascorbic acid, and is indispensable for the rigidity of collagens.[7] Notably, this structural feature arises from a gauche conformation between the hydroxy group and the amine motif of **1**, which allows for a hyperconjugative stabilization. In this way, a *trans*-conformation between the adjacent carbonyl moieties is enabled creating an additional $n_O \rightarrow \pi^*_{CO}$ overlap. Both electronic effects together with intra- and intermolecular H-bonding via H_2O bridges lead to the unique triple helix structure of collagens (Figure 1, right).[8] Therefore, the content of **1** in collagens is indicative for the structural integrity of human tissue and bones. A deficit of incorporation or secretion into the urine or blood is often symptomatic for diseases like osteoporosis, sclerosis or fibrosis.[9-11]

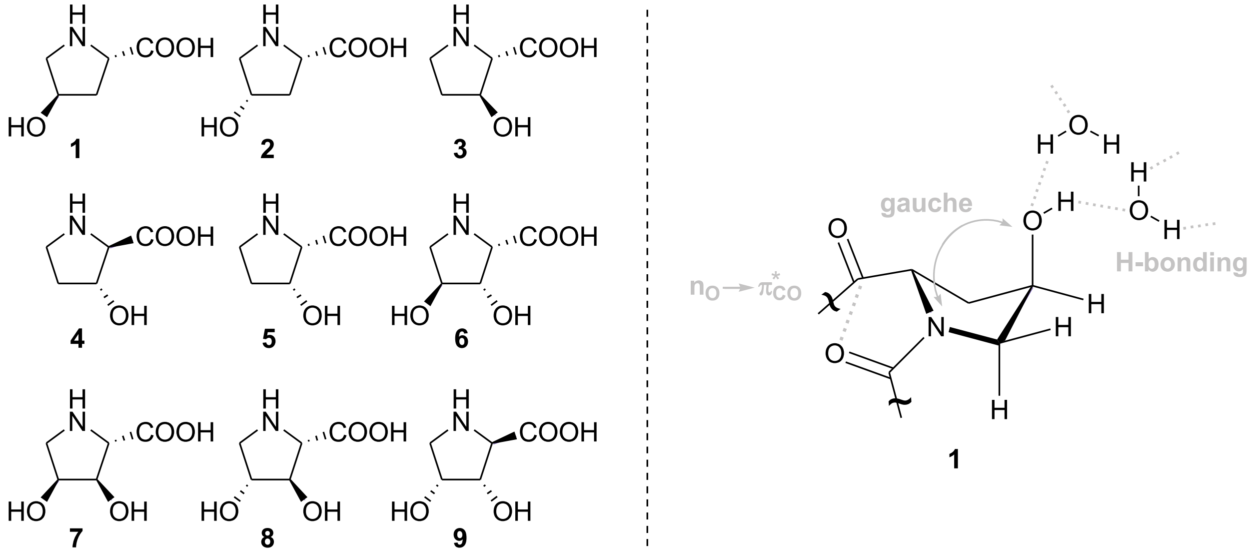


Figure 1. Left: Naturally occurring 3-, 4- and 3,4-(di)hydroxyprolines. Right: Conformational stabilization of **1** in collagens via electrostatic interactions and H-bonding.

While other hydroxyprolines are also present within structural proteins of living organisms and entail similar stabilizing features, they can also be found incorporated into smaller motifs, especially cyclic peptides

(Figure 2). Both, (2*S*,3*R*)-3-hydroxyproline (**5**) and (2*S*,3*S*)-3-hydroxyproline (**3**) were isolated originally from a *Streptomyces* species, where they are present within the cyclopeptide telomycin.[12] Additionally, **3** could be isolated from different sponges, plants and fungi and is incorporated in alkaloids, such as zizyphine Q[13] and paliurine E,[14] and **5** is present within the DNA gyrase inhibitor cyclothialdine.[15] Danoprevir is a potent inhibitor against hepatitis C virus proteases and includes **1** as a key unit.[16] The same motif is part of oxaceprol, an anti-inflammatory drug for the treatment of osteoarthritis[17] and the marine collagen peptide APHCP from *Gadus Chalcogrammus*, which was found to be an HIV-1 infection inhibitor.[18] The fungal toxins phalloidine,[19] a derivative of the heptapeptidic phallotoxins apparent in *Amanita phalloides*, and viroidine,[20] a member of the virotoxin family, contain (2*S*,4*S*)-4-hydroxyproline (**2**) and (2*S*,3*R*,4*R*)-2,3-dihydroxyproline (**8**), respectively.

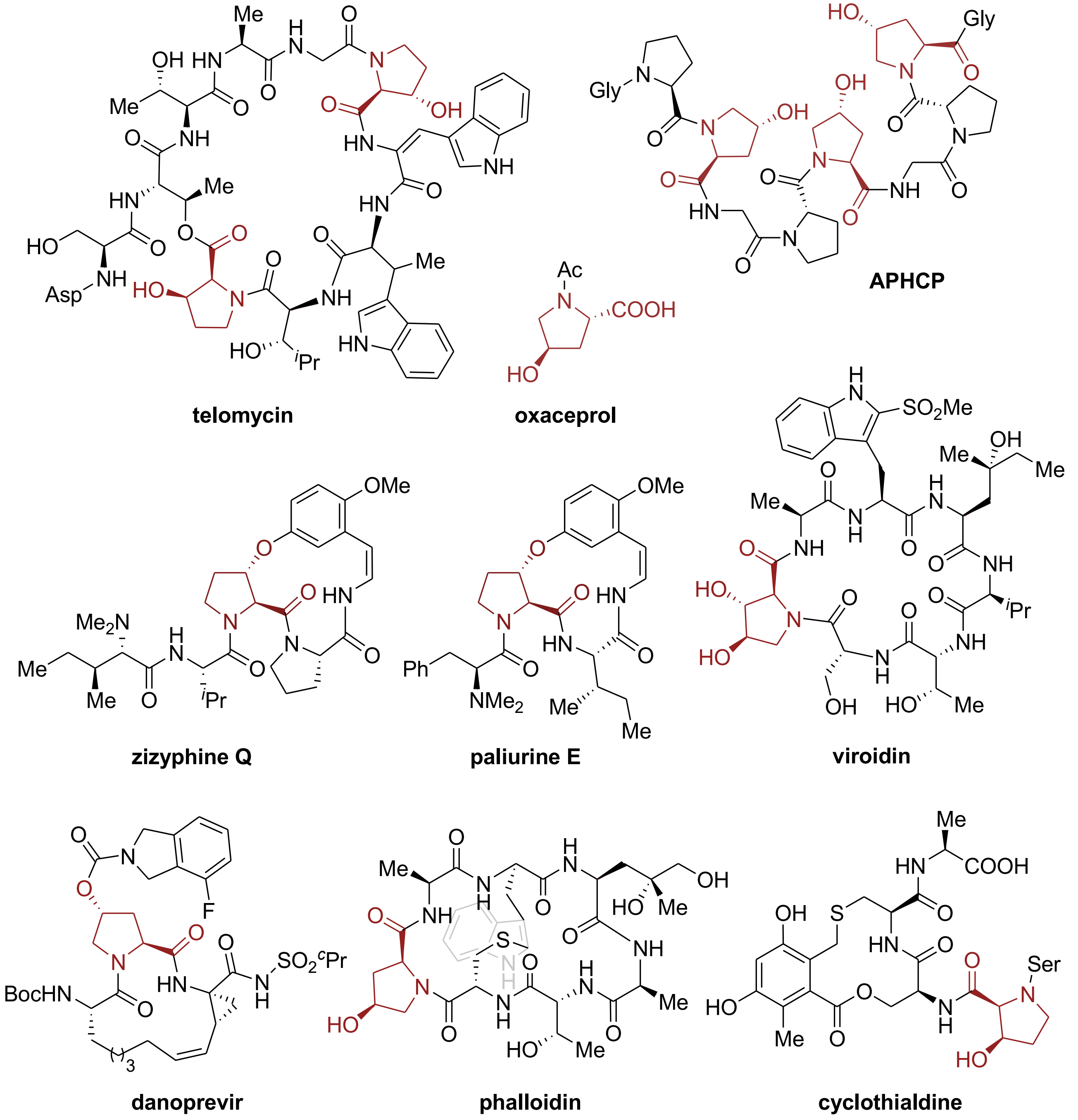


Figure 2. Exemplary scope of natural products or pharmaceuticals containing (di-)hydroxyproline(s). Asp: L-asparagine, Gly: glycine, Ser: L-serine.

Besides their appearance in natural products, recently, hydroxyprolines have also gained attention in the realm of asymmetric proline catalysis (Figure 3) to conduct e.g. stereoselective Aldol reactions, Michael reactions, Mannich reactions or Strecker reactions. Here, the additional hydroxy group enables secondary H-bonding (**10** and **11**), the attachment of ionic moieties (**12**), sterically repelling groups (**13** and **14**), polymers (**15**) or fullerenes (**16**). A good overview of the reactions conducted via **1**-directed catalysis was given by Agirre *et al.* in 2019.[21]

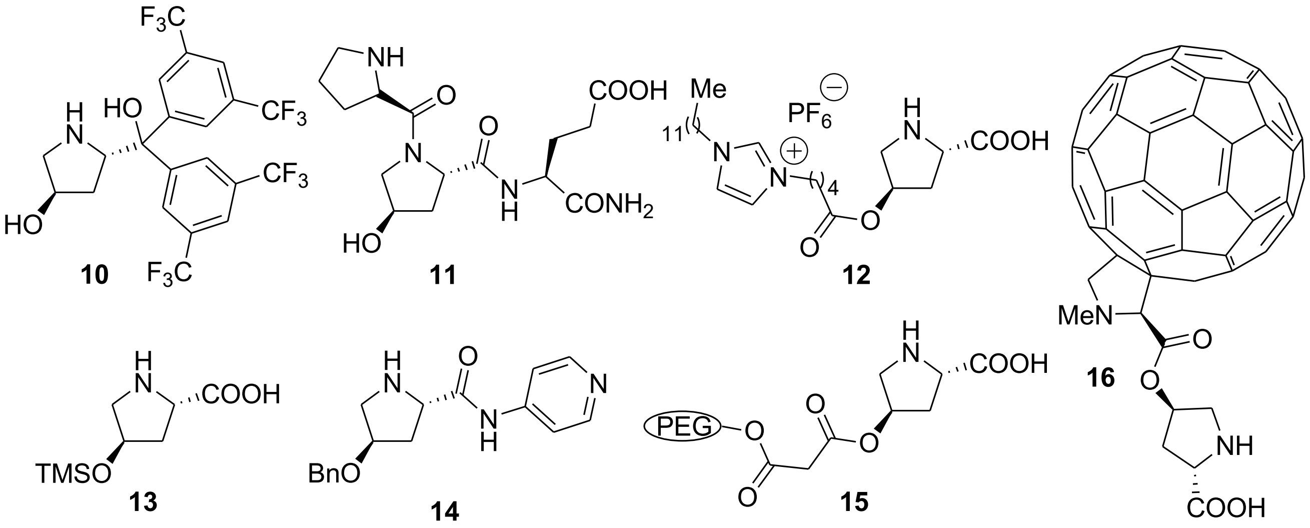


Figure 3. Overview of **1**-based catalysts used in asymmetric transformations. PEG: polyethylene glycol.

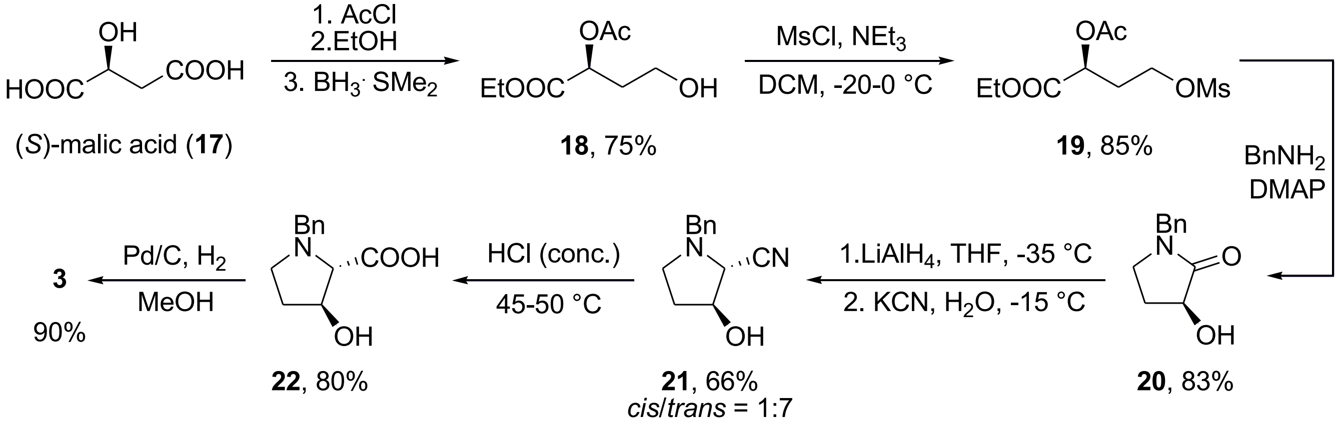
Given the importance of hydroxyprolines within biological systems, pharmaceutical compounds and asymmetric catalysis, it is not surprising that multiple stereoselective syntheses towards this compound class have been developed over the years. A comprehensive summary of synthetic methods from the 1960s to the early 2000s has already been elaborated by El-Ashry and Nemr in 2003.[22] However, as the amount of hydroxyproline containing compounds has been continuously growing in the last decades,[4,23,24] modern methods to access these have emerged. This review should cover new achievements in the stereoselective assembly of the most common mono- and dihydroxylated proline derivatives, namely 3-hydroxyproline, 4-hydroxyproline and 3,4-dihydroxyproline. Here, the focal point is set on prolines, which bear only additional hydroxy or protected hydroxy moieties, but no further functional pattern, such as methyl- or amine moieties.[2,25]

2. Review

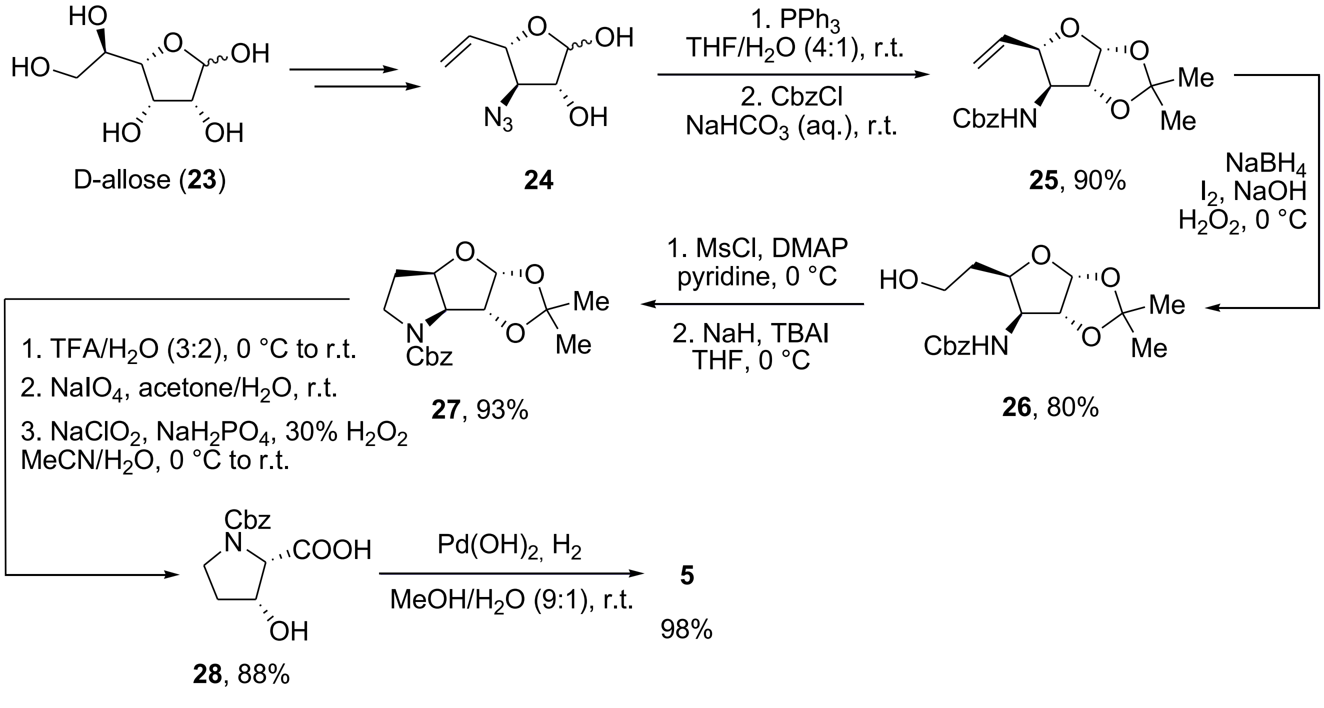
Multiple synthetic approaches towards hydroxylated prolines have been investigated in recent years. Generally, all techniques can be categorized in 3 different approaches: (1) stereospecific transformation of natural carbohydrates, (2) stereoselective manipulation of achiral motifs via chiral auxiliaries or catalysts and (3) enzymatic conversion of specific precursors. In the following sections these synthetic procedures are described for the respective hydroxyproline moieties.

2.1. Synthesis of 3-hydroxyprolines

In 2004, Huang *et al*. could use natural (*S*)-malic acid (**17**) for the synthesis of **3** (Scheme 1).[26] According to a previous protocol from the same group **17** was converted to diester **18** in 75%.[27] Next, mesylation of the primary alcohol from **18** and subsequent addition of $BnNH_2$ led to the cyclization of **19** to afford 2-pyrrolidinone **20** in 83%. Reduction with $LiAlH_4$ and stereospecific cyanation yielded a 7:1 diastereomeric mixture of **21** with preference of the *trans*-isomer. Acidic treatment to afford the carboxylic acid and deprotection eventually led to free hydroxyproline **3**.

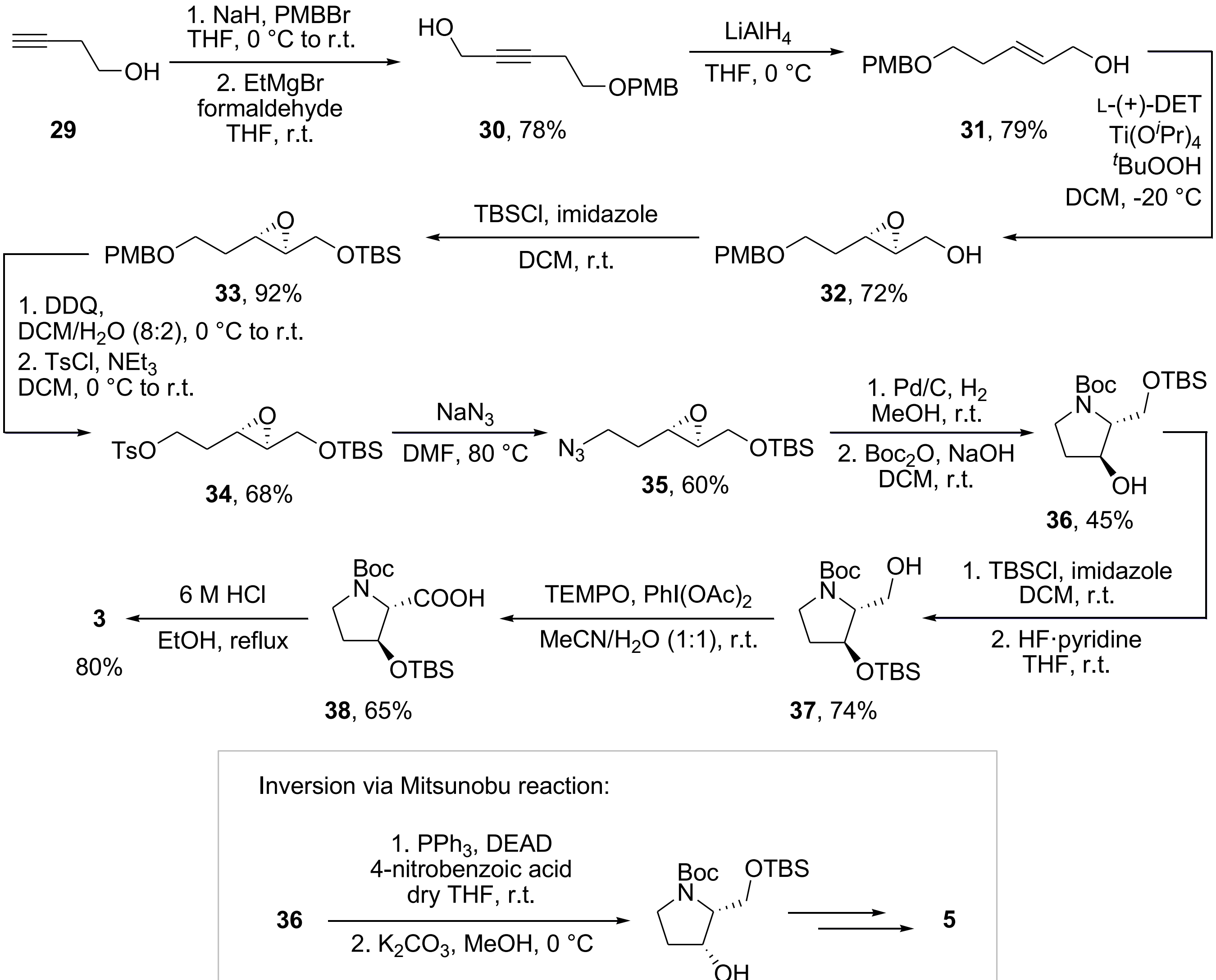


Scheme 1. Synthesis of **3** by Huang *et al.* via stereospecific cyanation.



Scheme 2. Synthesis of **5** by Kalamkar *et al.* from D-allose (**23**).

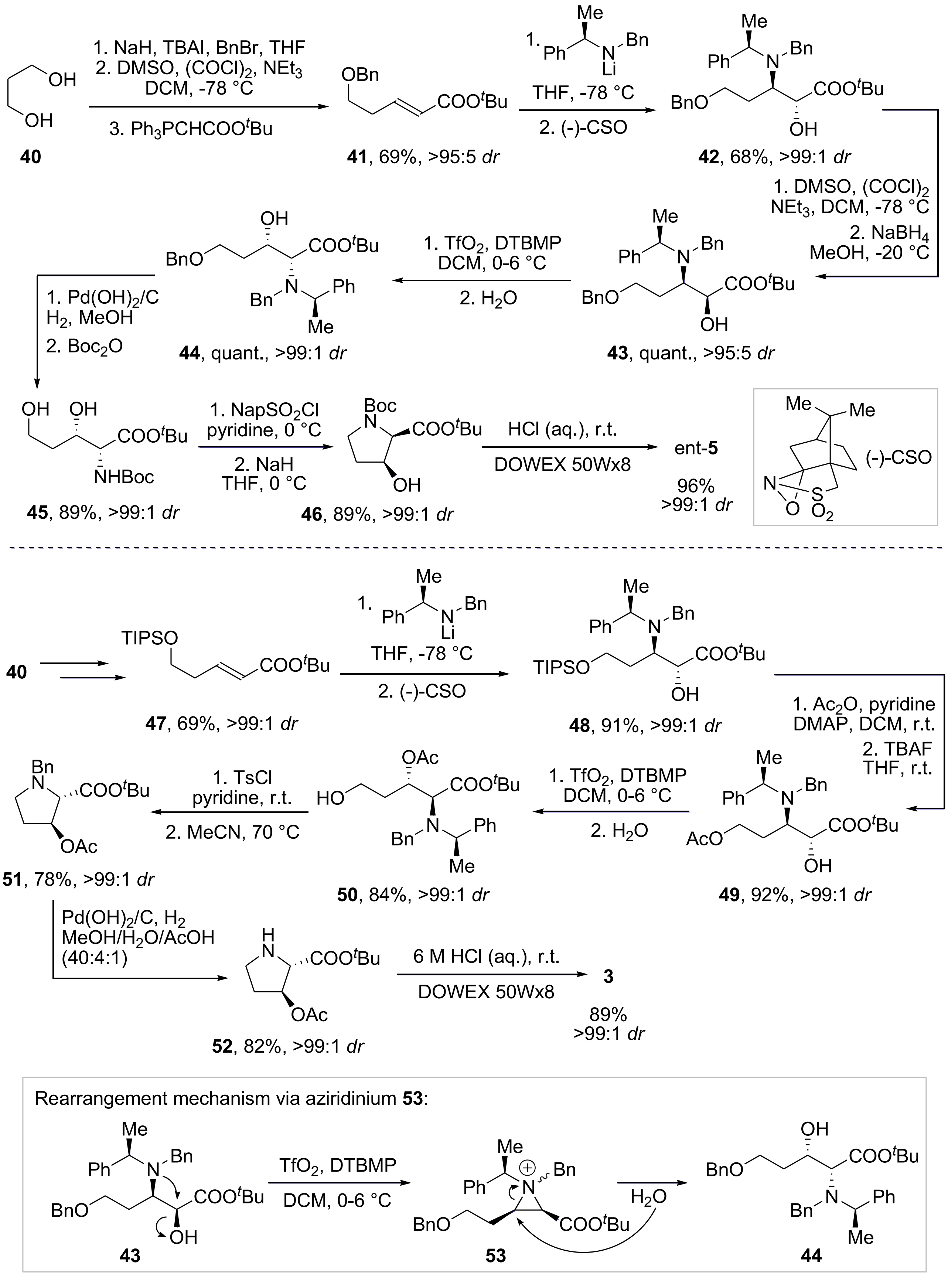
Kalamkar *et al.* accomplished the synthesis of 3-hydroxyproline **5** from D-allose (**23**, Scheme 2).[28] By a former procedure from Chaudhari *et al.* **23** was converted to azide **24** within 5 steps, which involve an inversive azide substitution on C3 while the remaining hyroxy groups were protected.[29] **24** was reduced with PPh_3 within a Staudinger reaction to form an intermediate primary amine, which was Cbz-protected thereafter. From **25**, an I_2-catalyzed hydroxylation of the olefinic moiety led to alcohol **26**. Mesylation of the alcohol and deprotection of the amine afforded pyrrolidine **27**, which was then deprotected under acidic conditions and oxidized to form **28**. Eventually, the amine moiety was deprotected and **5** was obtained within 9 steps in total from **24**.



Scheme 3. Synthesis of all stereoisomers of 3-hydroxyproline (only **3** and **5** are shown) by Kumar *et al*. via Sharpless epoxidation and Mitsunobu inversion.

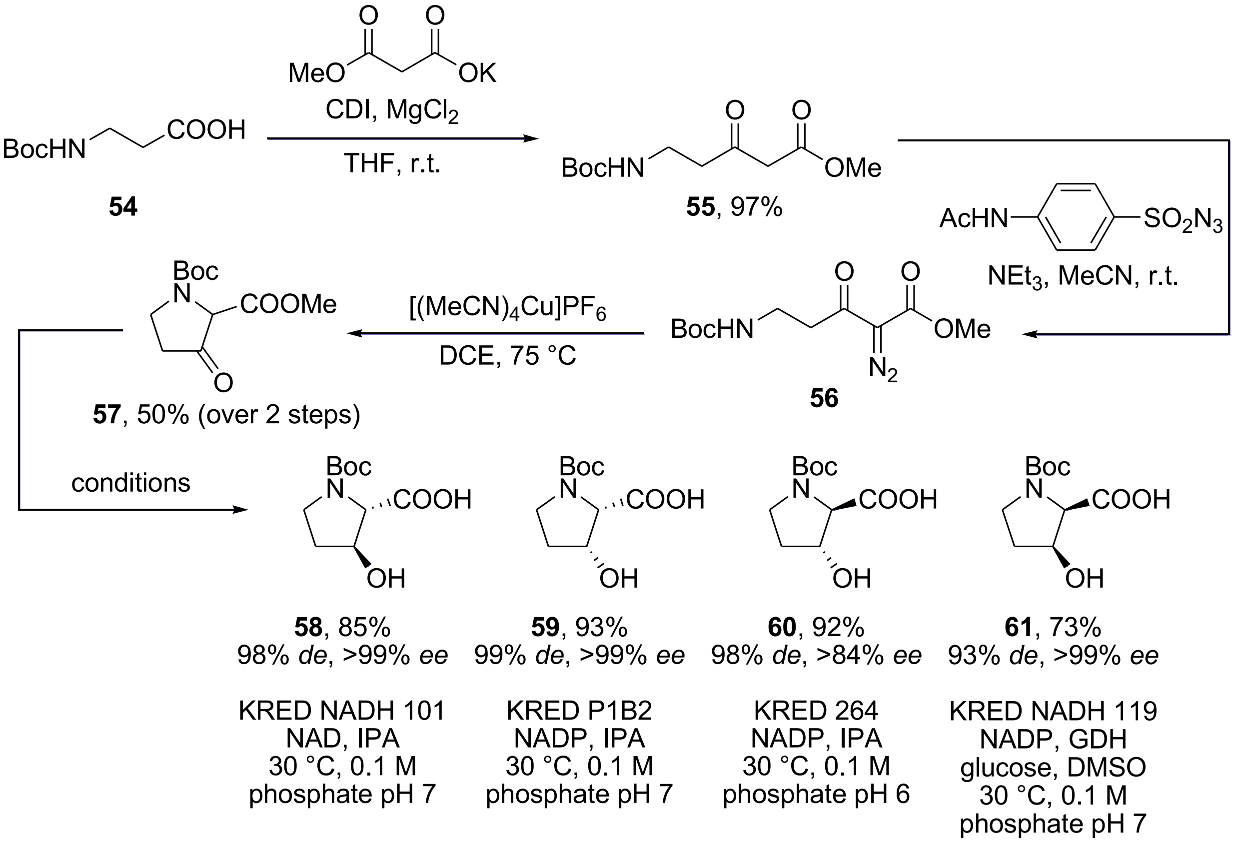
To access all four stereoisomers of 3-hydroxyproline Chandrasekhar *et al.* used an interplay between stereoselective Sharpless epoxidation and inversive Mitsunobu substitution of hydroxy groups (Scheme 3).[30] This synthesis also includes the utilization of common precursors, **36** or ent-**36**, which allow for the divergent synthesis of all stereoisomers. The synthesis starts with the protection of the alcohol moiety of alkyne **29** and subsequent nucleophilic addition to formaldehyde. **30** was reduced to alkene **31**, which was stereoselectively epoxidized via Sharpless epoxidation. The primary alcohol of **32** was TBS-protected and the PMB moiety on the other alcohol group was interchanged for a Ts-protecting group. Nucleophilic substitution with NaN_3 could afford **35**, which underwent a cyclization upon reduction to the amine. A sequence of TBS-protection on the secondary and deprotection on the primary alcohol of **36** yielded **37**. Eventually TEMPO-induced oxidation and deprotection led to **3**. By the use of D-(-)-DET instead of D-(+)-DET in the fourth step, the authors could also synthesize the enantiomer, **4**. Notably, intermediate **36** could also undergo an isomerization reaction to change the alignment of its secondary hydroxy moiety via Mitsunobu reaction to afford **39**, which was converted to **5** by the same reaction sequence. Also, ent-**5** was synthesized from ent-**39**, which was accessed by the inversion of ent-**36** obtained from the D-(-)-DET sequence. Despite the utility of **36** and ent-**36** as common precursors within this method, both compounds have to be prepared in a laborious 10 step synthesis (with a yield of only 7.5% for **36**). Therefore, this pathway should not be considered as a straight-forward and high yielding synthesis towards one specific 3-hydroxyproline, but as a versatile tool to access different targets.

In 2018, Davies *et al.* developed a synthetic pathway towards ent-**5** and **3** (Scheme 4, above).[31] The key step of this transformation involves an aziridinium intermediate that opens upon a stereoselective rearrangement. Alkene **41** could be synthesized from 1,3-propanediol (**40**) via benzylation, oxidation and esterification. A stereoselective hydroxyamination of the olefinic moiety led to **42** with perfect *dr*. To access **43**, the hydroxy group of **42** was inverted via oxidation and stereospecific reduction. Rearrangement via aziridinium intermediate **53** could afford **44** in quantitative yield and >99:1 *dr* (Scheme 4, below). After the amine moiety was deprotected under reductive conditions, Boc-protection of the free amine and activation of the primary alcohol with $NapSO_2Cl$ led to cyclized product **46**, which upon acidic treatment released ent-**5**. For the synthesis of **3**, **40** was converted to alkene **47** within 3 steps (Scheme 4, above). Again, hydroxyamination led to **48**. The TIPS protecting group was changed to acetate to afford **49**. Then, a similar rearrangement as described before yielded **50** in perfect *dr*. Tosylation of the alcohol and stirring in hot MeCN initiated the cyclization to afford protected 3-hydroxyproline **51**. Eventual deprotection and acidic treatment formed **3**. As can easily be seen, both routes involve several, but consistently high yielding steps. Remarkably, both stereoselective steps, the addition from **41** to **42** and the following rearrangement towards **43**, performed perfect selectivities. It also needs to be mentioned that Davies *et al.* could use this technique for the assembly of more azasugars than the ones shown here.



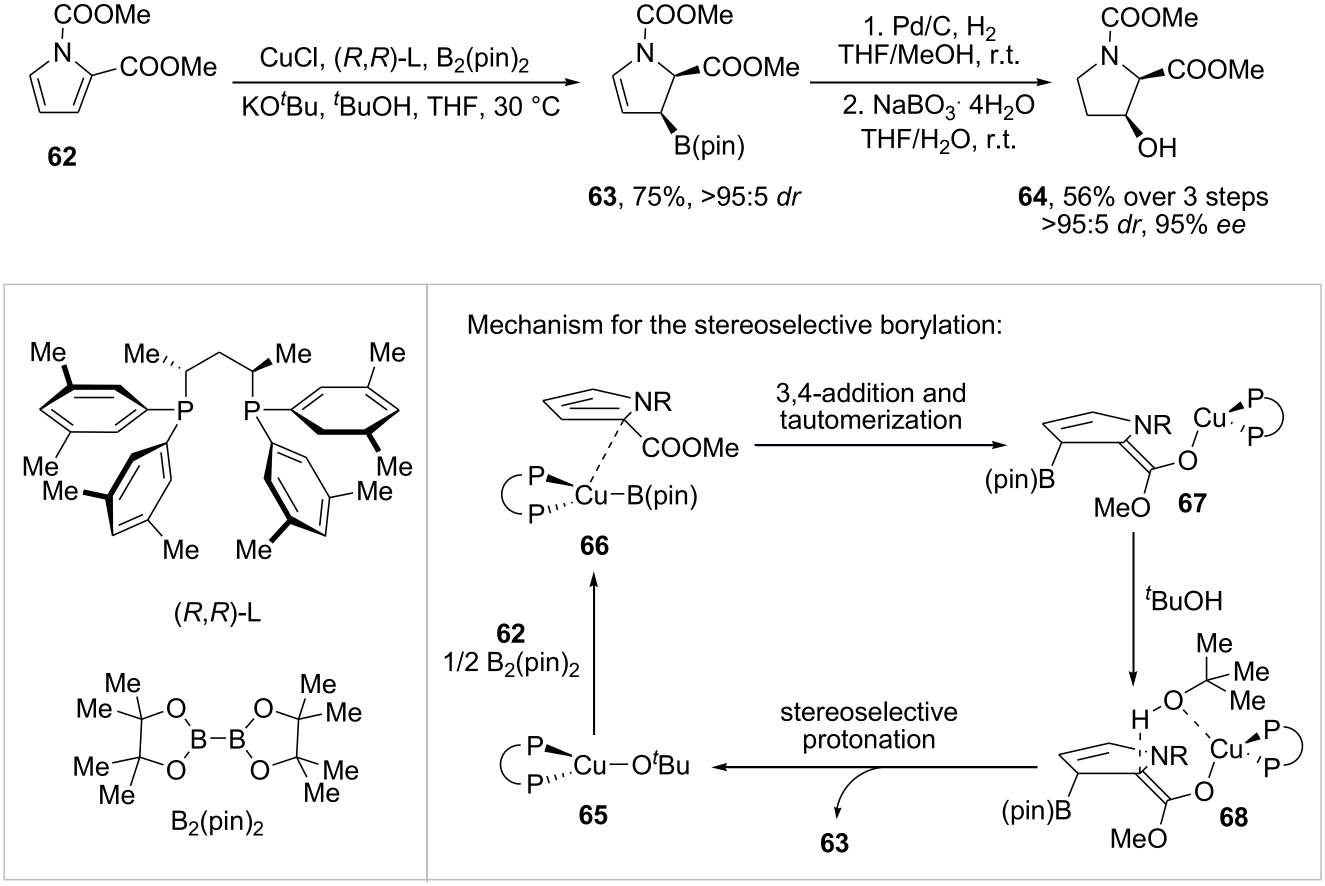
Scheme 4. Above: Synthesis of ent-**5** and **3** by Davies *et al.* using a stereoselective rearrangement of aziridinium intermediates. Below: Rearrangement mechanism via aziridinium ion **53**. DTBMP = 2,6-di-*tert*-butyl-4-methyl-pyridine.

One year later, Prier *et al.* made use of a set of ketoreductases (KREDs) to synthesize the 4 different stereoisomers of *N*-Boc-3-hydroxyproline (Scheme 5).[32] The synthesis starts with **54**, which was converted to ketoester **55** and diazotized subsequently to afford **56**. A Cu-catalyzed N-H insertion of **56** gave cyclic ketoester **57**. From here, different KREDs were used for the stereoselective synthesis of 3-hydroxyprolines **58**-**61** in good yields and diastereoselectivities and excellent enantioselectivities. Thereby, this strategy pronounces the advantages of enzymes in organic synthesis as a mild and straight-forward technique for stereodivergent synthesis.



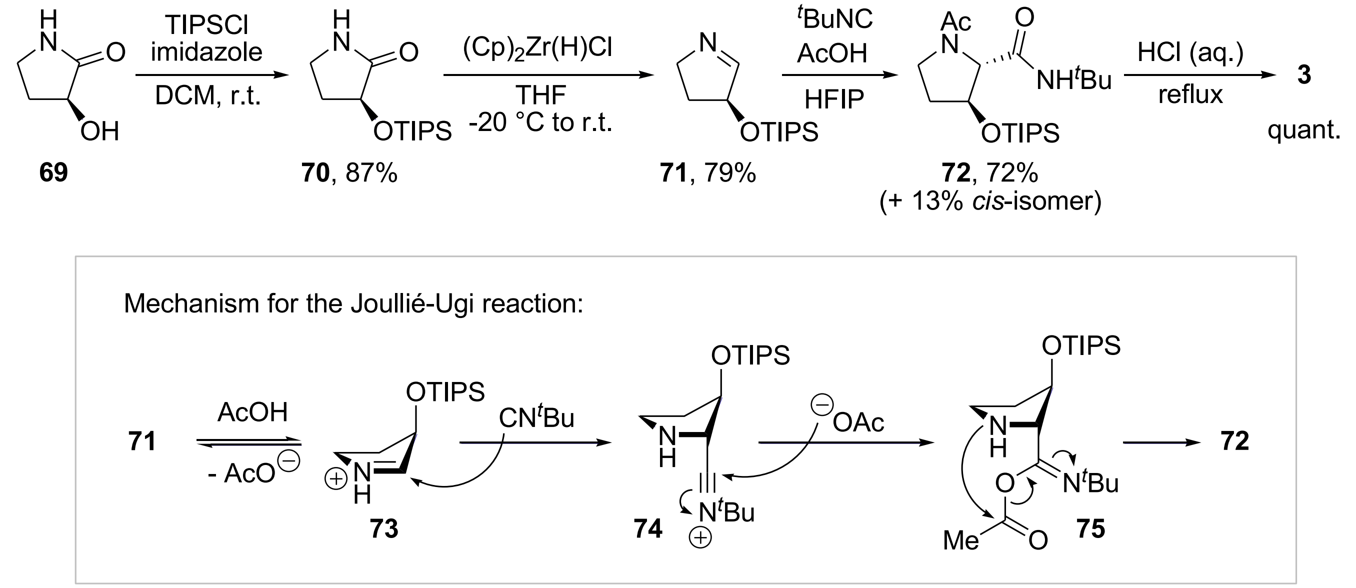
Scheme 5. Synthesis of all stereoisomers of *N*-Boc-3-hydroxyproline by Prier *et al.* via enzymatic catalysis. CDI = 1,1`-carbonyldiimidazole. KRED = ketoreductase.

Within the research on enantioselective borylations on pyrroles, Hayama *et al.* found a rapid and effortless technique to construct 3-hydroxyproline derivative **64** (Scheme 6, above).[33] Starting with the enantioselective borylation of **62**, which gave **63** with good preference of the *cis*-isomer, a Pd/C-catalyzed hydrogenation gave the saturated pyrrolidine moiety, which was hydroxylated without purification of the intermediate to form **64** in high diastereo- and enantioselectivity. Notably, only one chromatographical step was necessary during the whole synthesis. For the stereoselective borylation the authors propose the following mechanism (Scheme 6, below). Cu(I)-species **65** forms in situ from CuCl and the diphosphine ligand in ^{t}BuOH, reacts with $B_2(pin)_2$ and coordinates to the pyrrole moiety of **62**. After enantioselective 3,4-addition controlled by the diphosphine ligand **67** emerges. Then, tautomerization and ^{t}BuOH coordination in *anti*-periplanar position to the B-pin substituent is followed by a stereoselective protonation to afford **63**. Concomitantly, the authors could show within deuterium-labelling experiments that the hydrogen from ^{t}BuOH is transferred in the last protonation step. This short synthesis emphasizes the advantage of stereoselective catalytic reactions over multi-step routes starting from carbohydrates as shown before, because of the significant time saving and less consumption of chemicals. Moreover, the synthesis of ent-**64** could also be achieved only by using the enantiomer of the phosphine ligand within the first step.



Scheme 6. Above: Synthesis of **64** by Hayama *et al.* using a stereoselective borylation. Below: Mechanism for the stereoselective borylation.

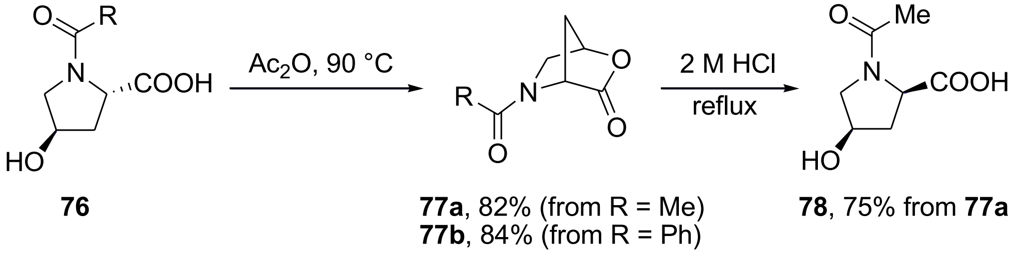
Recently, Takashina *et al.* were able to assemble **3** from commercial **69** in only 4 steps using a modified technique that the same research group had developed in 2016 (Scheme 7, above).[34,35] TIPS-protection of **69** afforded **70** with 87% yield, which could be converted to cyclic imine **71** via zirconium catalysis. From here, the authors applied their previous protocol of a Joullié-Ugi reaction involving ^{t}BuNC and AcOH in HFIP to build up **72** with preference of the *trans*-isomer. Acidic deprotection revealed hydroxyproline **3**, which was obtained with a remarkable yield of 49% over the whole reaction sequence. The key step of this synthesis, from **71** to **72**, is shown in detail in Scheme 7 below. Here, after protonation of **71**, the stereoselective attack of ^{t}BuNC yields iminium ion **74**. Nucleophilic attack of acetate leads to intermediate **75**, which eventually undergoes a rearrangement to afford **72**.



Scheme 7. Above: Synthesis of **3** by Takashima *et al.* via Joullié-Ugi three component reaction. Below: Mechanism of the Joullié-Ugi reaction.

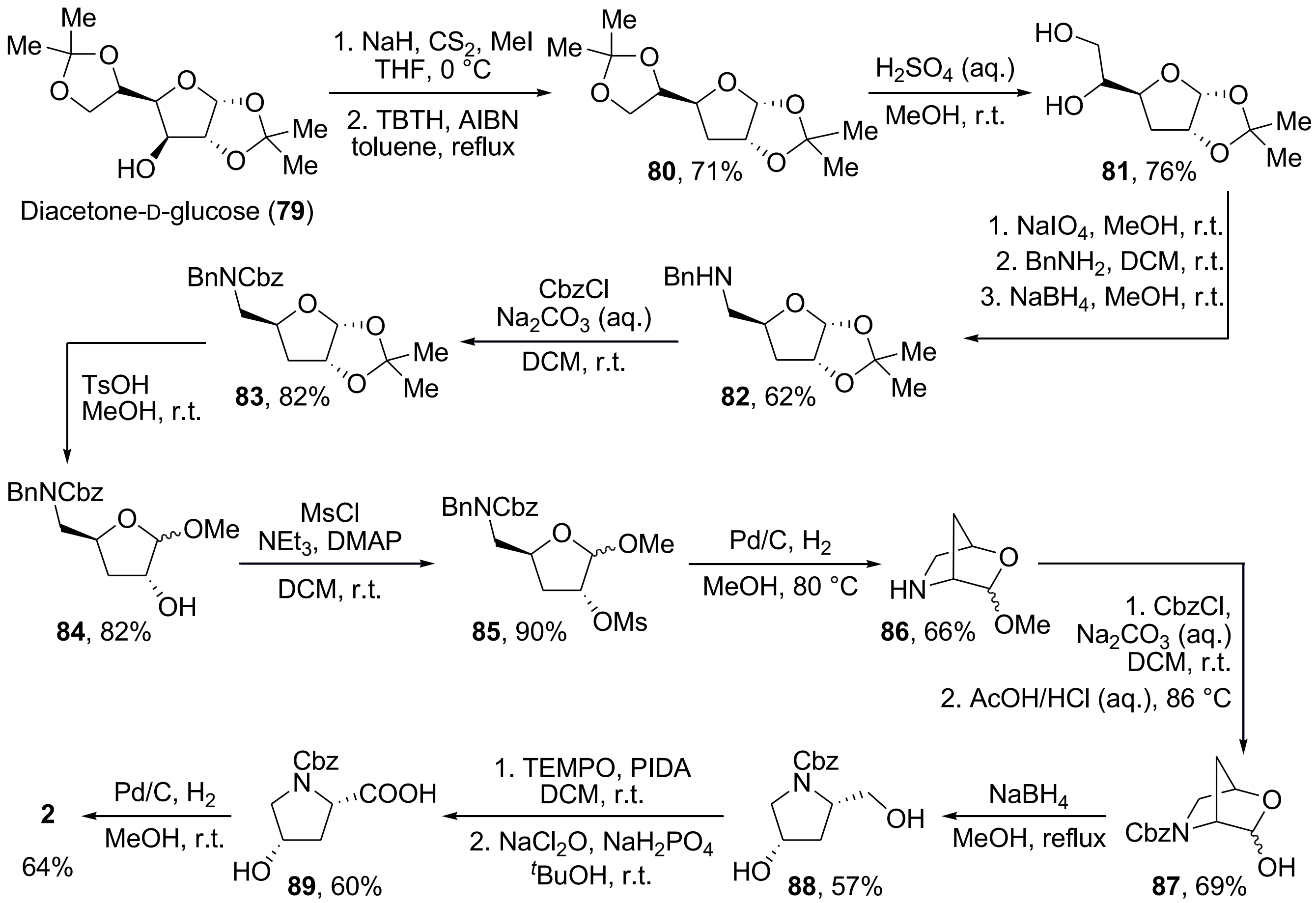
2.2. Synthesis of 4-hydroxyprolines

(2*S*,4*R*)-4-hydroxyproline (**1**) is the most abundant member of the hydroxyproline family and its stereoselective synthesis is therefore of minor interest. However, for the assembly of its enantiomer and diastereomers few techniques were discovered. A quick transformation from *trans*-isomer **76** of 4-hydroxyproline into *cis*-isomer **78** was reported by Dalla Croce *et al.* in 2002 (Scheme 8).[36] Here, merely the heating in Ac_2O afforded bicyclic lactones **77a** or **77b**. **77a** was then reopened by acidic treatment and yielded *cis*-4-hydroxyproline **78** in a good yield of 75%.



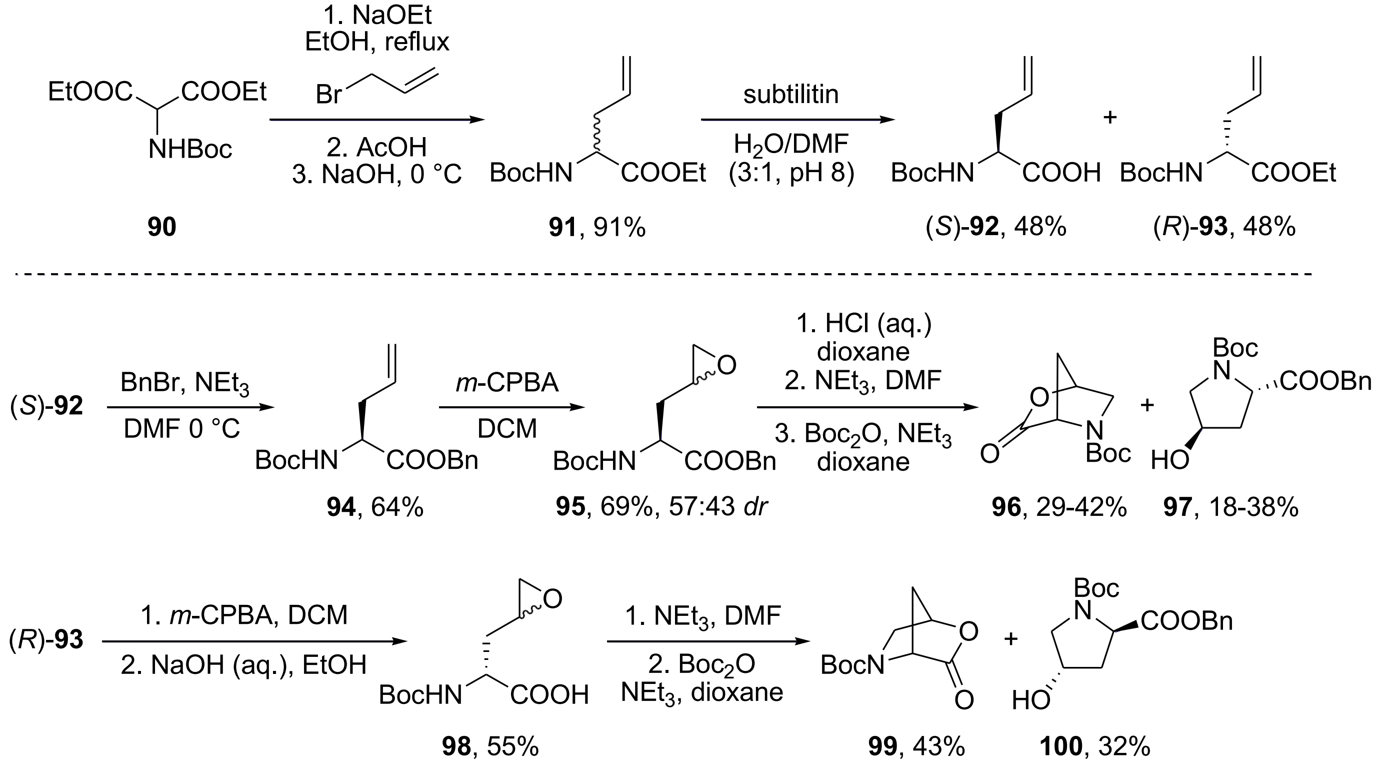
Scheme 8. Synthesis of **78** by Croce *et al.* via acidic *trans-cis* isomerization.

Mereyela *et al.* used diacetone-D-glucose (**79**) for the synthesis of hydroxyproline **2** (Scheme 9).[37] After removal of the unprotected alcohol moiety via defunctionalization **80** was obtained in 71% yield. Regioselective acidic cleavage of the ketal, subsequent oxidation and reductive amination afforded **82** in consistently high yields. Cbz-protection and acidic cleavage of the second full acetale unit in MeOH led to methylated intermediate **84**, whose free alcohol group was mesylated subsequently. Removal of both amine protecting groups led to an internal cyclization. Another *N*-protection, acidic cleavage of the lactol and reduction yielded the pyrrolidine core structure of **88**. Oxidation of the primary alcohol moiety of **88** and reductive deprotection of the Cbz unit afforded hydroxyproline **2** in a total yield of 2% over 16 steps. Despite the large synthetic effort of Mereyela *et al.* several steps led to only moderate yields (**81**→**82**, **85**→**86**→**87**→**88**→**89**→**2**).



Scheme 9. Synthesis of **2** by Mereyela *et al.* from diacetone-D-glucose (**79**). TBTH = tributyltin hydride. PIDA = (diacetoxyiodo)benzene.

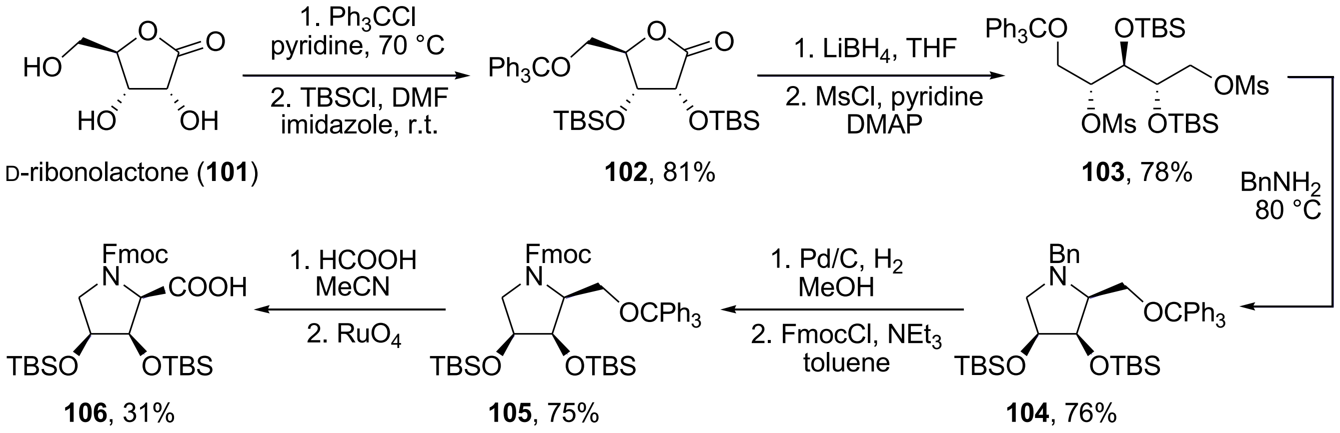
In 2014, Krishnamurthy *et al.* made use of the enzyme subtilisin, which was able to perform a kinetic enzymatic resolution of **91** separating free acid (*S*)-**92** and ester (*R*)-**93** (Scheme 10).[38] The compounds could be separated and were individually taken for the respective 4-hydroxyproline synthesis. At the beginning of this synthesis, malonate **91** was received from **90** in 91%, before the resolution with the enzyme subtilisin took place. (*S*)-**92** was esterified and epoxidized to afford **95** in 69% as a diastereomeric mixture of 57:43. Then, acidic treatment to yield an intermediate pyrrolidinium salt and subsequent basification followed by Boc-protection led to **96**, the internal ester of *N*-Boc-**2**, and **97**. Similarly, (*R*)-**93** was epoxidized, basified and Boc-protected to generate **99**, the internal ester of *N*-protected ent-**2**, and **100**. This technique clearly demonstrates the synthetic applicability of subtilisin but is less oriented towards the targeted synthesis of one motif, since two different products are obtained in both synthetic pathways. This results from the unselective epoxidaton in both cases.



Scheme 10. Synthesis of **96**, **97**, **99** and **100** by Krishnamurthy *et al.* via kinetic enzymatic resolution.

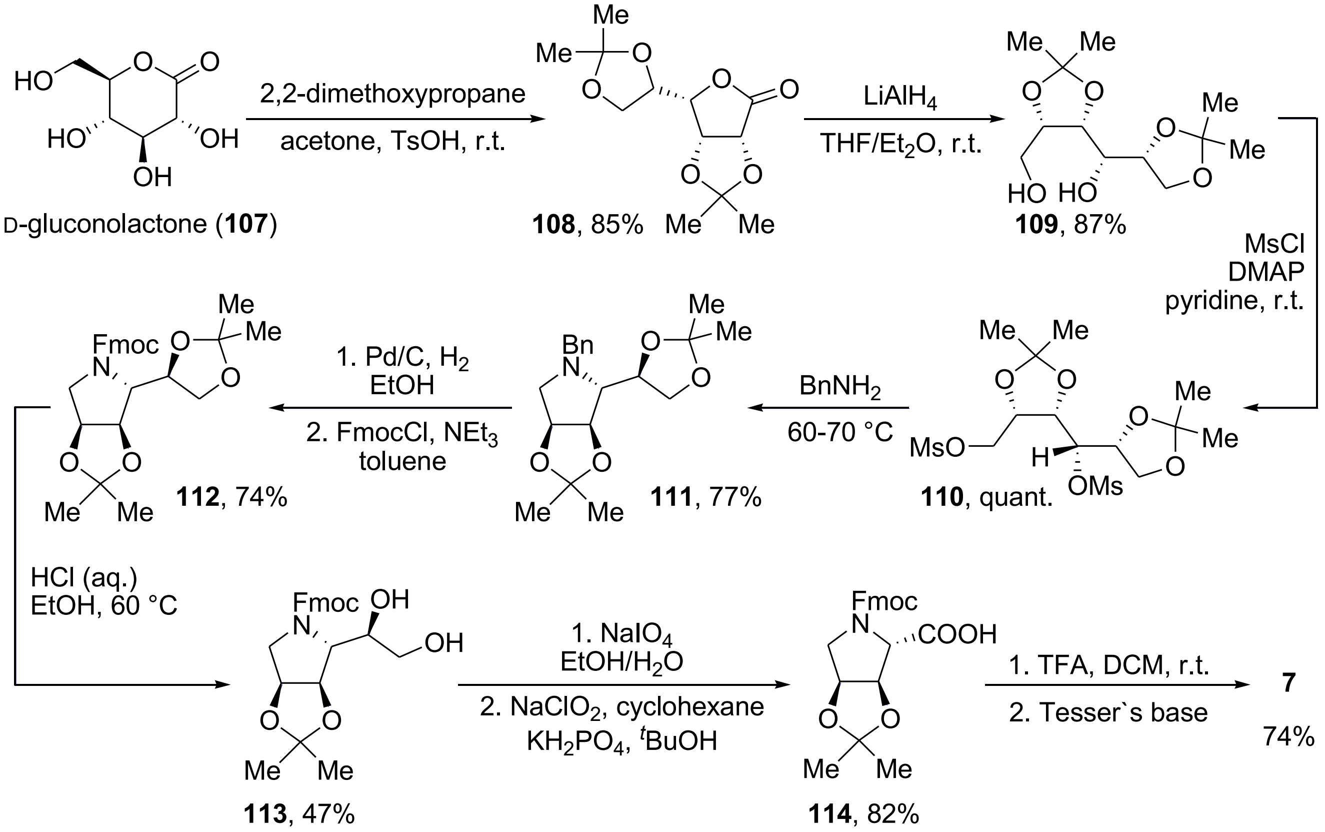
2.3. Synthesis of 3,4-dihydroxyprolines

In 1999, Weir *et al.* used natural D-ribonolactone (**101**) for the assembly of 3,4-dihydroxyproline **106** within 9 steps (Scheme 11).[39] After regioselective protections of the alcohol moieties, reductive ring opening and mesylation of the generated alcohol were performed to yield **103**. The addition of $BnNH_2$ led to formation of pyrrolidine **104**, whose Bn moiety was changed for an Fmoc protecting group. Finally, RuO_4-induced oxidation of **105** generated **106**, which corresponds to a protected version of (2*R*,3*R*,4*S*)-3,4-dihydroxyproline, an unnatural dihydroxyproline motif.



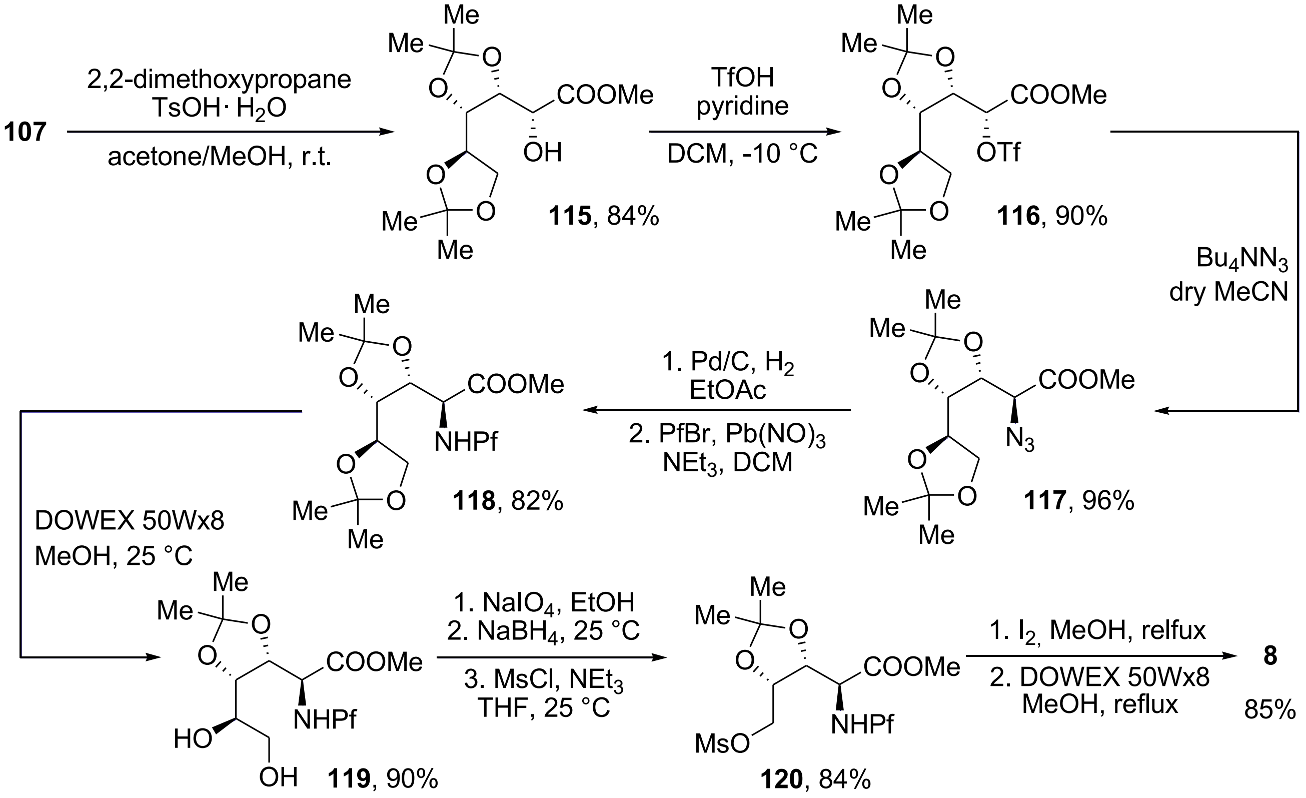
Scheme 11. Synthesis of **106** by Weir *et al.* from D-ribonolactone (**101**).

In the same year Weir *et al.* also developed a synthesis of *syn*-3,4-dihydroxyproline **7**, whose precursor, **114**, was shown to act as a suitable building block in peptide synthesis (Scheme 12).[40] Starting from D-gluconolactone (**107**), double protection with 2,2-dimethoxypropane and reduction led to intermediate **109**. After dimesylation of the free alcohols, $BnNH_2$ was used for the stereospecific cyclization building up the pyrrolidine ring of **111**. A switch of the amine protecting group and acidic cleavage of one of the acetonides enabled the oxidation of the diol moiety to carboxylic acid **114**. Notably, during the acidic deprotection step of **112**, also considerable amounts of the completely deprotected unit were observed. Finally, full deprotection with TFA and subsequent addition of Tesser`s base yielded **7**. As seen, in this synthesis a change of protecting groups from **111** to **112** is required. This is most likely because $FmocNH_2$ would not lead to the cyclization of **110** right away, and therefore $BnNH_2$ was chosen as a better nucleophile in step 4. Unfortunately, the authors did not comment on that. It is also apparent that the unselective deprotection from **112** decreases the yield of desired product **113**. Regardless, all other steps proceed with consistently high yields and involve only simple transformations.



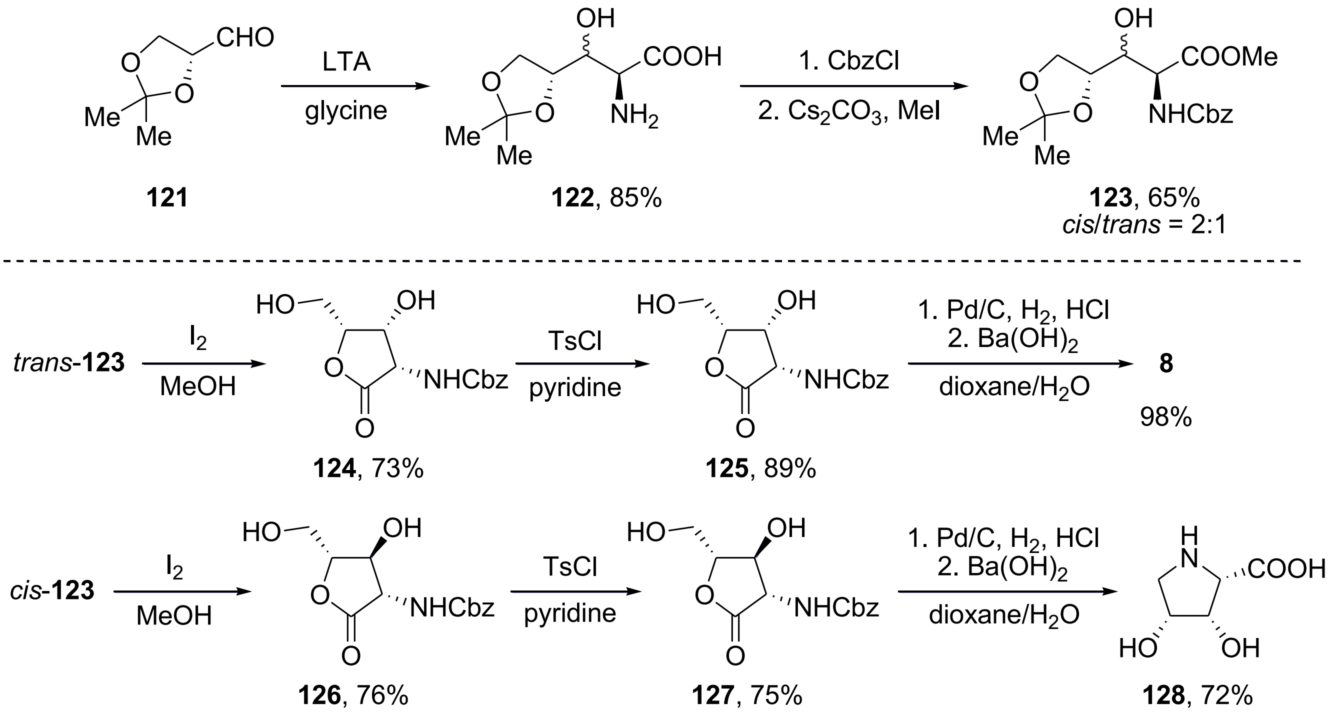
Scheme 12. Synthesis of **7** by Weir *et al.* from D-gluconolactone (**107**). Tesser`s base = 4 M NaOH in MeOH/dioxane and 5 M NH_3 in MeOH/DCM.

Kim *et al.* discovered a technique for the deprotection of *N*-9-phenylfluoren-9-yl (Pf) group using catalytic I_2 in MeOH under reflux (Scheme 13).[41] Amongst other heterocycles, also *trans*-3,4-dihydroxyproline **8** could be assembled using this deprotection. As a starting point, again, **107** was chosen and protected with 2,2-dimethoxypropane and MeOH to afford diketal **115**. After activation of the free alcohol moiety with TfOH, an inversive substitution using azide nucleophile Bu_4NN_3 led to **117**. The reduction of **117** and subsequent Pf-protection yielded **118**. Regioselective deprotection and a sequence involving the oxidation of the diol moiety of **119**, reduction and mesylation afforded **120**. Finally, the I_2-catalyzed deprotection of **120** triggered an aminocyclization and after eventual deprotection **8** was formed. During the synthesis the Pf-protecting group plays an important role. It withstands acidic, oxidative and reductive conditions from **118** to **120** and thereby enables the installment of the primary mesylate group of **120**.



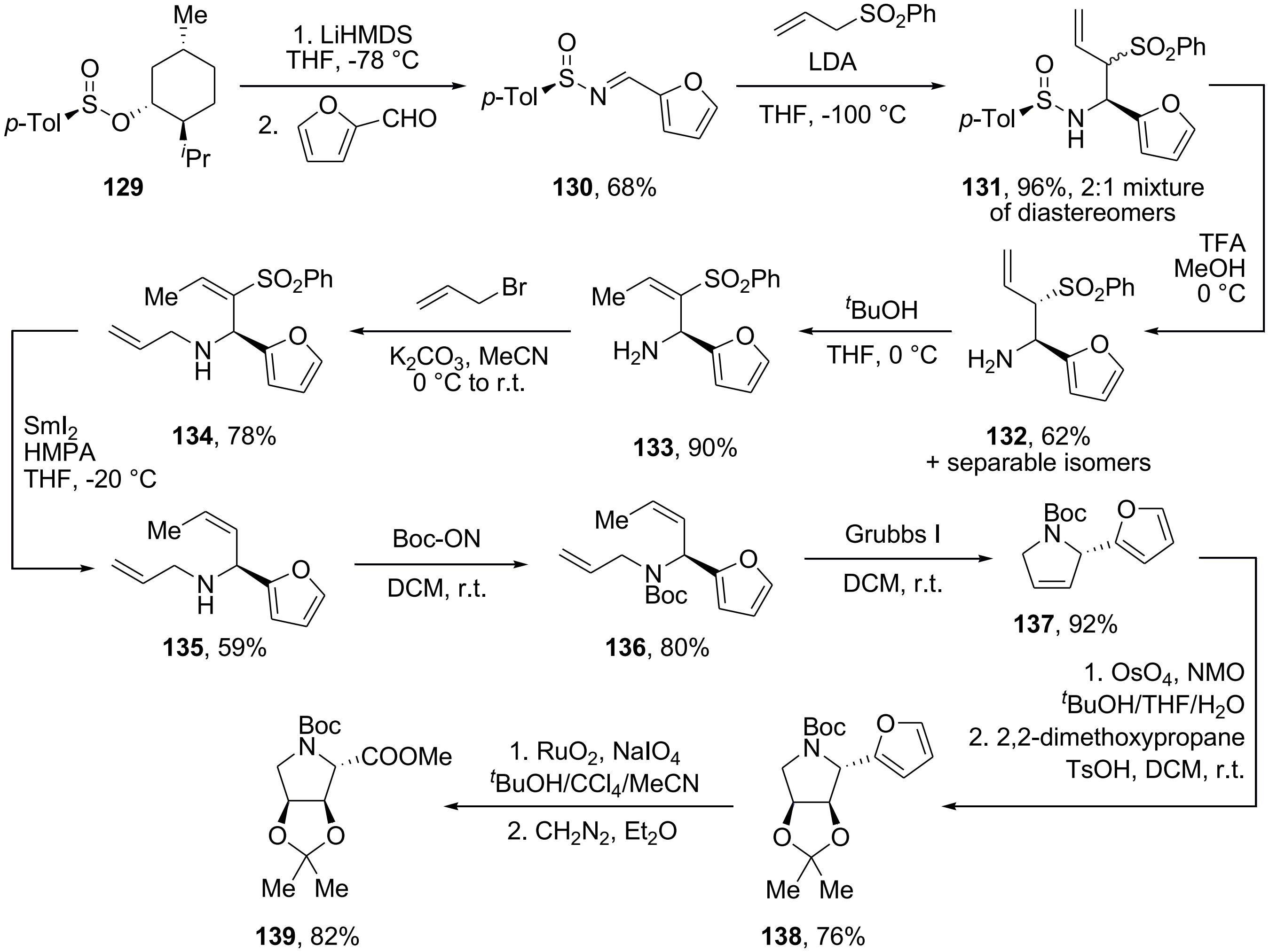
Scheme 13. Synthesis of **8** by Kim *et al.* from D-gluconolactone (**107**) involving the I_2-catalyzed deprotection of the *N*-9-phenylfluoren-9-yl group (Pf).

In 2000, Fujii *et al.* developed a synthesis of *cis*-3,4-dihydroxyproline **128** and *trans*-3,4-dihydroxyproline **8** starting from **121**, a precursor derived from abundantly available glycerol (Scheme 14).[42] Here, **121** could undergo a stereoselective Aldol reaction with respect to the α-C of aminoacid **122** by the help of the enzyme L-threonine aldolase (LTA). Cbz-protection yielded a mixture of *cis*- and *trans*-isomer of **123**, which could be separated via chromatography. Both isomers could be treated with iodine individually leading to lactones **124** and **126**, respectively. Tosylation of the primary alcohol and subsequent ester hydrolysis, ring closure and deprotection yielded the respective free dihydroxylated prolines **8** and **128**. Although this pathway does not represent a straight-forward synthesis towards one specific stereoisomer as the step from **122** to **123** only shows mediocre selectivity, the practical application of LTA in the context of natural product synthesis is well demonstrated here.



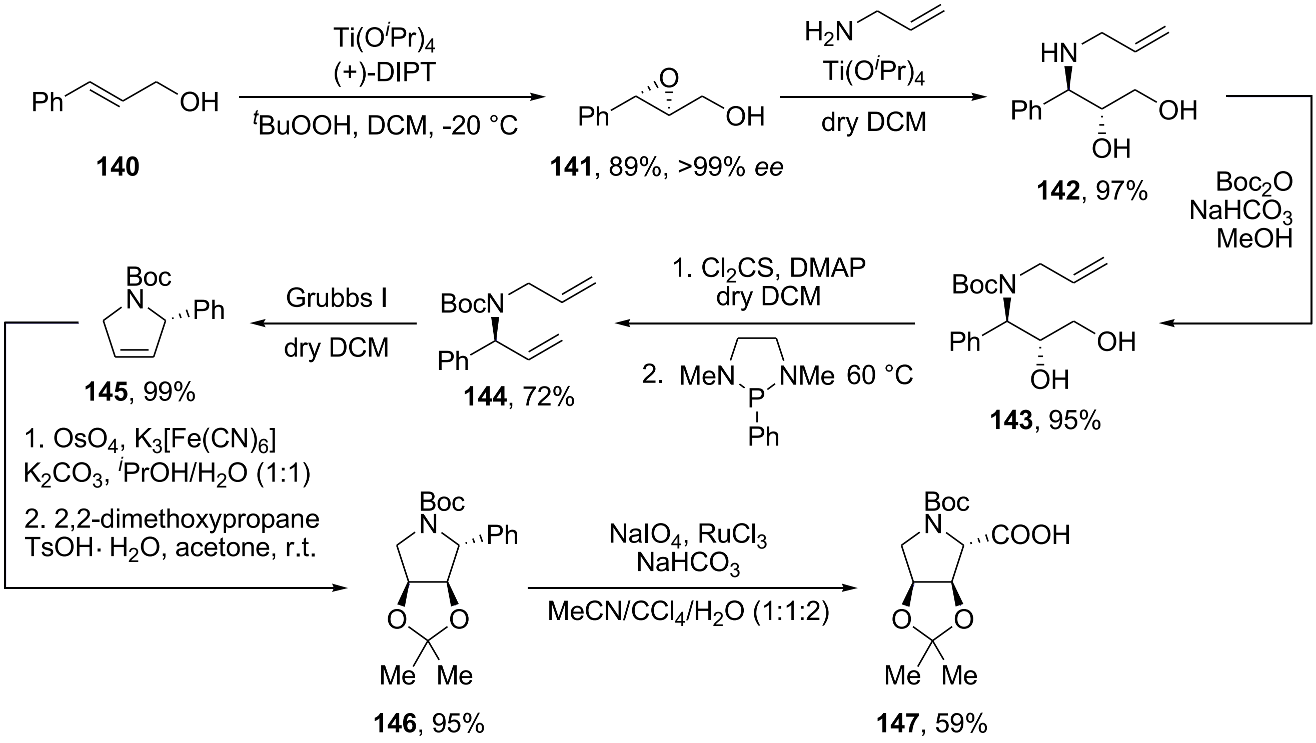
Scheme 14. Synthesis of **8** and **128** by Fujii *et al.* using L-threonine aldolase (LTA).

Shortly thereafter, Kumareswaran *et al.* achieved a synthesis of protected dihydroxyproline **139** starting from (-)-menthol derived chiral auxiliary **129** (Scheme 15).[43] Here, the stereocontrol was achieved by the (-)-menthol scaffold and the 5-membered pyrrolidine unit was obtained via Grubbs methathesis as the key step. After imination of **129** and stereoselective addition of allylphenylsulfone, amine **131** was received as a mixture of diastereomers. *Trans*-diastereomer **132** could be isolated in 62% after acidic cleavage of the sulfinamide unit and was isomerized in ^{t}BuOH to afford internal alkene **133**. Allylation of the amine and SmI_2-induced cleavage of the sulfone moiety yielded **135**. Secondary amine **135** was Boc-protected and cyclized via Grubbs metathesis to form 3-pyrrolin **137**. Subsequent dihydroxylation, protection and eventual oxidation of the 2-furfuryl unit gave rise to protected 3,4-dihydroxyproline **139**. As mentioned, during step 4 considerable amounts of isomers, which were formed due to a rather unselective sulfonylation and protic rearrangements, were separated and formally discarded leading to a decrease of yield. Nevertheless, Kumareswaran *et al.* demonstrate here the advantageous application of cheap and readily available chiral auxiliaries.



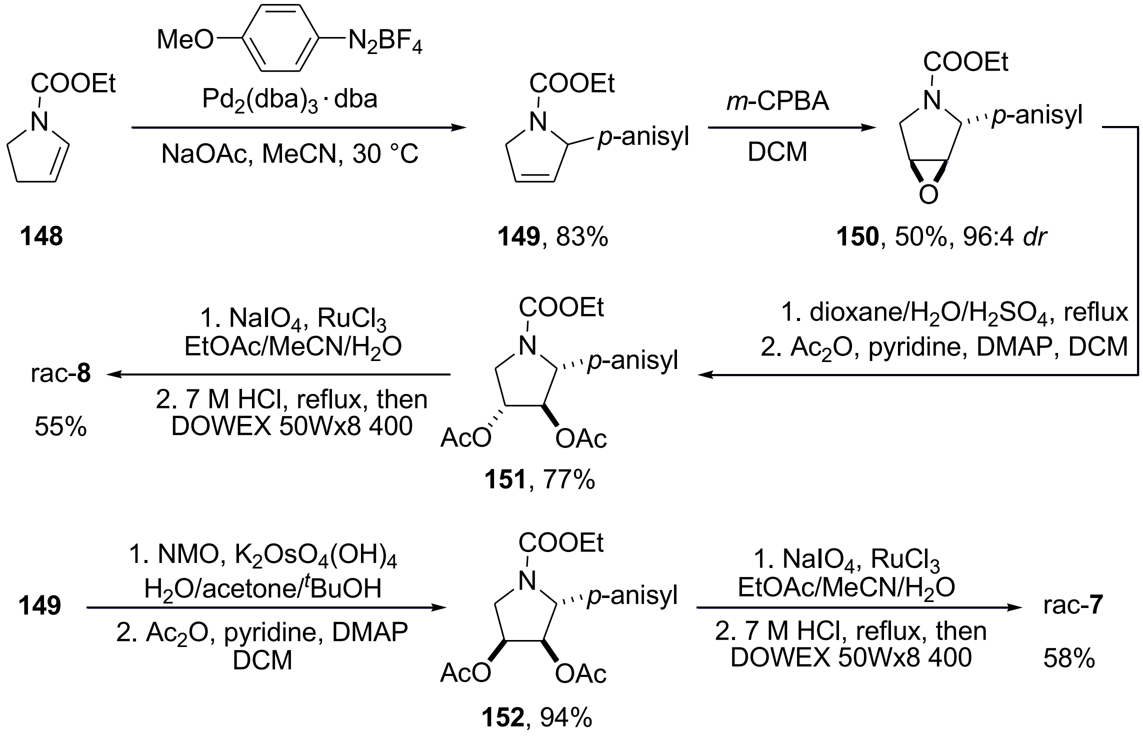
Scheme 15. Synthesis of **139** by Kumareswaran *et al.* from **129** as chiral auxiliary. Boc-ON = 2-(*tert*-butoxycarbonyloxyimino)-2-phenyl-acetonitril.

Martin *et al.* designed a route that also involved Grubbs metathesis to build up the same structural motif as a free carboxylic acid (Scheme 16).[44] After a Sharpless epoxidation from allylic alcohol **140** leading to epoxide **141** in perfect enantioselectivity, ring opening of the epoxide with allylamine yielded **142**. Boc-protection, thiocarbonate formation and pyrolysis using 1,3-dimethyl-2-phenylphosphazolidine could afford bisallylamine **144**. 3-Pyrroline **145** was built up via Grubbs metathesis, then dihydroxylated and protected with 2,2-dimethoxypropane. Final oxidation using a protocol from Carlsen *et al.* led to protected 3,4-dihydroxyproline **147**.[45] Hence, the assembly of **147** was achieved with 9 steps in total, which was the shortest route towards this motif at the time.



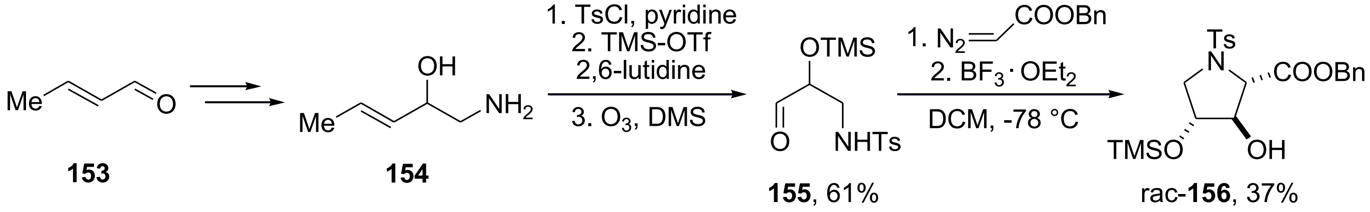
Scheme 16. Synthesis of **147** by Martin *et al.* via Sharpless epoxidation and Grubbs metathesis. DIPT = diisopropyl tartrate.

In 2003, Garcia *et al.* developed a protocol for the synthesis of *cis*-3,4-dihydroxyproline rac-**7** and *trans*-3,4-dihydroxyproline rac-**8** starting from commercially available enecarbamate **148** (Scheme 17).[46] After a Heck reaction with *p*-methoxybenzenediazonium tetrafluoroborate, 3-pyrroline moiety **149** was obtained, which could be epoxidized with *m*-CPBA. Opening of epoxide **150** and protection enabled the selective oxidation of the *p*-methoxy moiety to carboxylic acid. Acidic cleavage of the protection groups afforded free *trans*-3,4-dihydroxyproline rac-**8**. Besides, 3-pyrroline **149** could also be *syn*-dihydroxylated and protected with Ac$_2$O. Again, oxidative degradation of the *p*-methoxyphenyl group could yield free *cis*-3,4-dihydroxyproline rac-**7**. For future work, the advancement of this racemic protocol to a stereoselective one could be explored. According to current literature in stereoselective Heck reactions, such an improvement would be conceivable.[47]



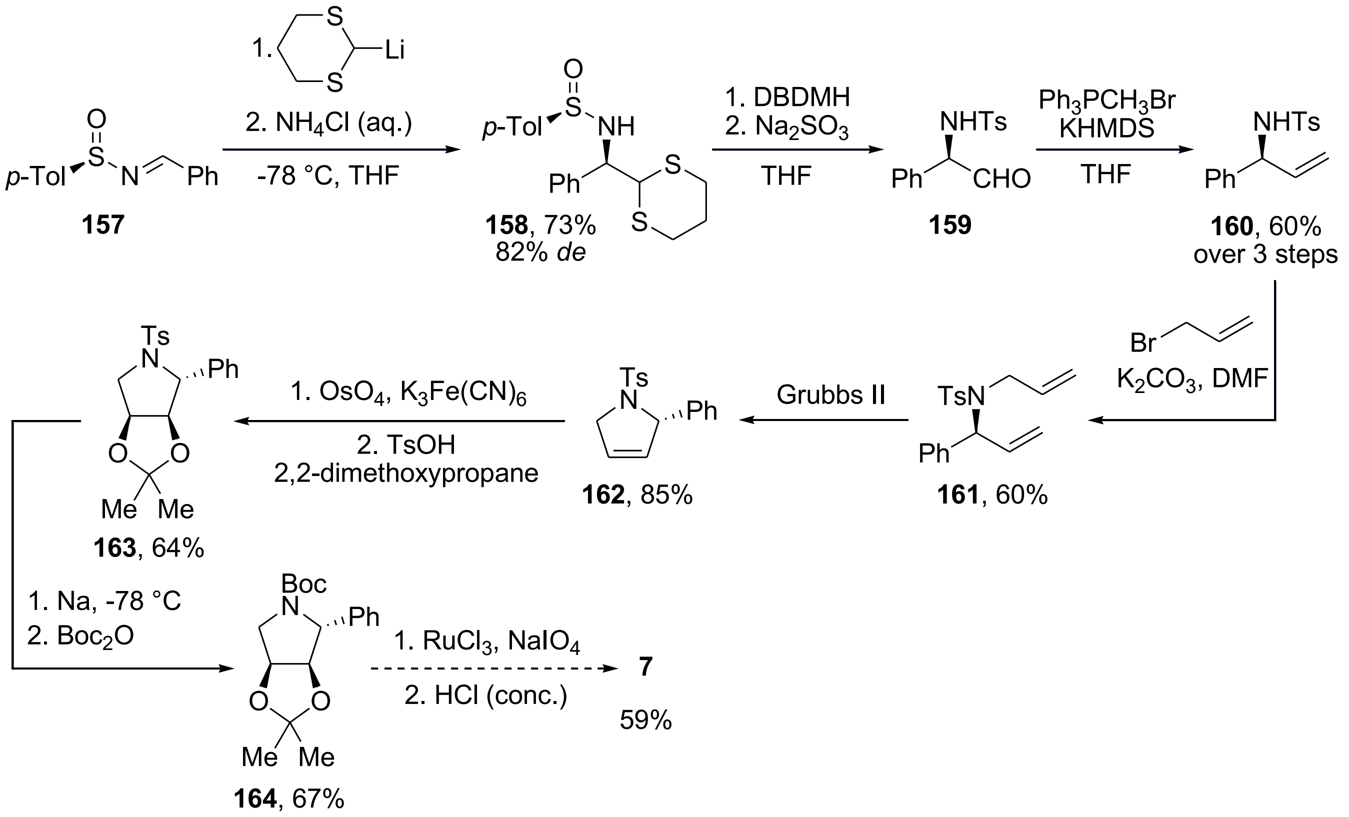
Scheme 17. Synthesis of rac-**7** and rac-**8** by Garcia *et al.* from enecarbamate **148**.

In the following year Angle *et al.* could use crotylaldehyde (**153**) as a cheap substrate for their synthesis of dihydroxyproline rac-**156** (Scheme 18).[48] According to a procedure from Evans *et al.* **153** was converted to amino alcohol **154** within 2 steps.[49] Tosylation of the primary amine, TMS-protection of the alcohol and subsequent ozonolysis were used to synthesize intermediate **155**. The addition of diazoester in the presence lewis acidic BF_3 led to *trans*-dihydroxyproline rac-**156** in rather moderate yield, which- according to the authors- proceeds either via 1,3-dipolar addition or nucleophilic addition of the diazonium species.



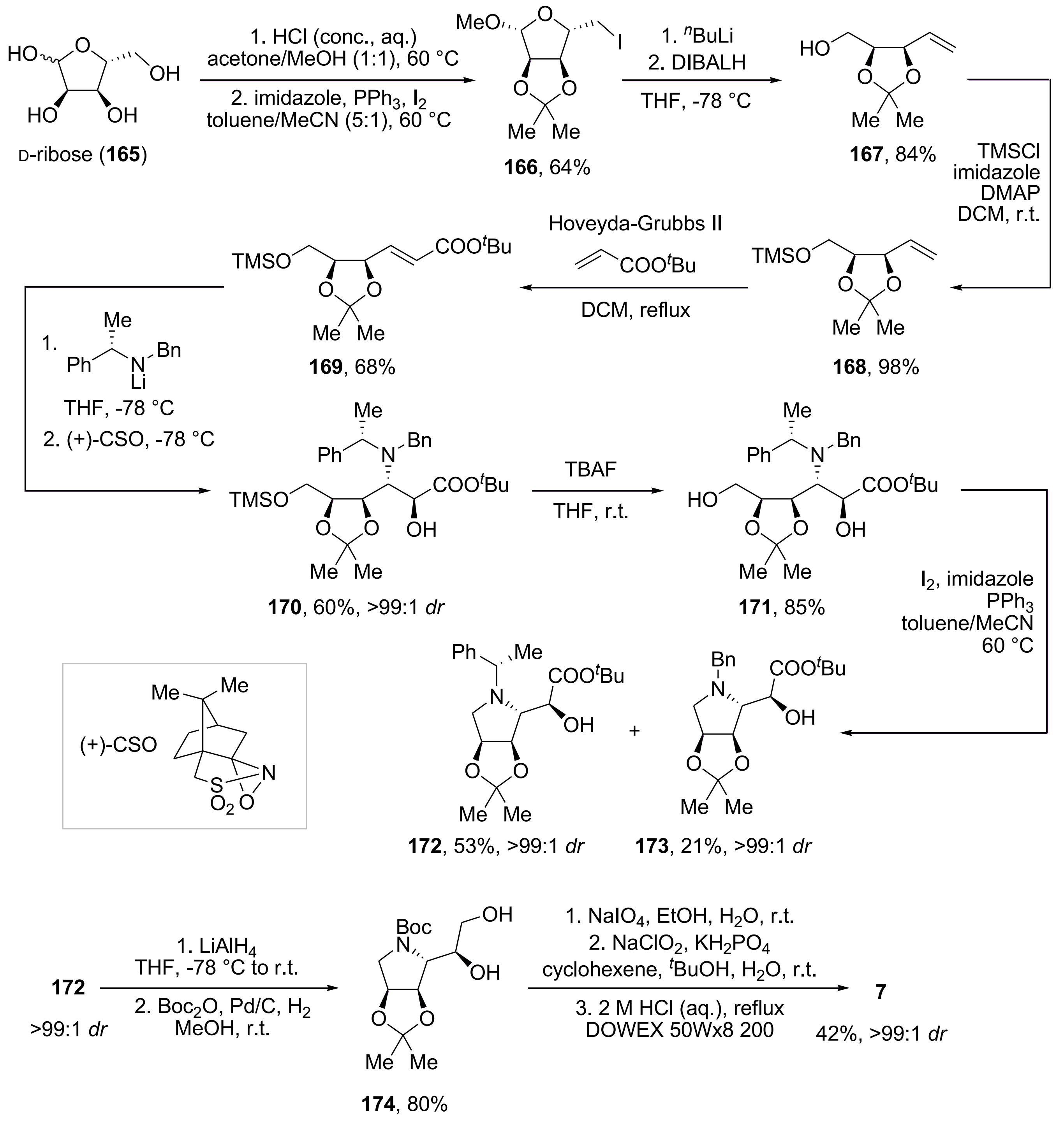
Scheme 18. Synthesis of rac-**156** by Angle *et al.* via formal stereoselective [3+2] cycloaddition.

Davis *et al.* used a chiral sulfinamide as stereoinducing auxiliary for the formal synthesis of **7** (Scheme 19).[50] Here, **157**, derived from benzaldehyde and (*R*)-*p*-toluenesulfinamide, underwent a stereospecific addition with a 2-lithio-1,3-dithiane to form **158**. Cleavage of the 1,3-dithiane, Wittig reaction and subsequent allylic substitution led to **161**, which was cyclized via Grubbs metathesis. **162** was dihydroxylated and protected with 2,2-dimethoxypropane, before the Ts-protecting group was interchanged with a Boc moiety. The last step towards **7** corresponds to an oxidation reaction conducted by Carlsen *et al.* as described before.[45] Similar to Kumareswaran *et al.*,[43] Davis *et al.* show here the sophisticated use of chiral auxiliaries. Overall, moderate to good yields were obtained by this route.



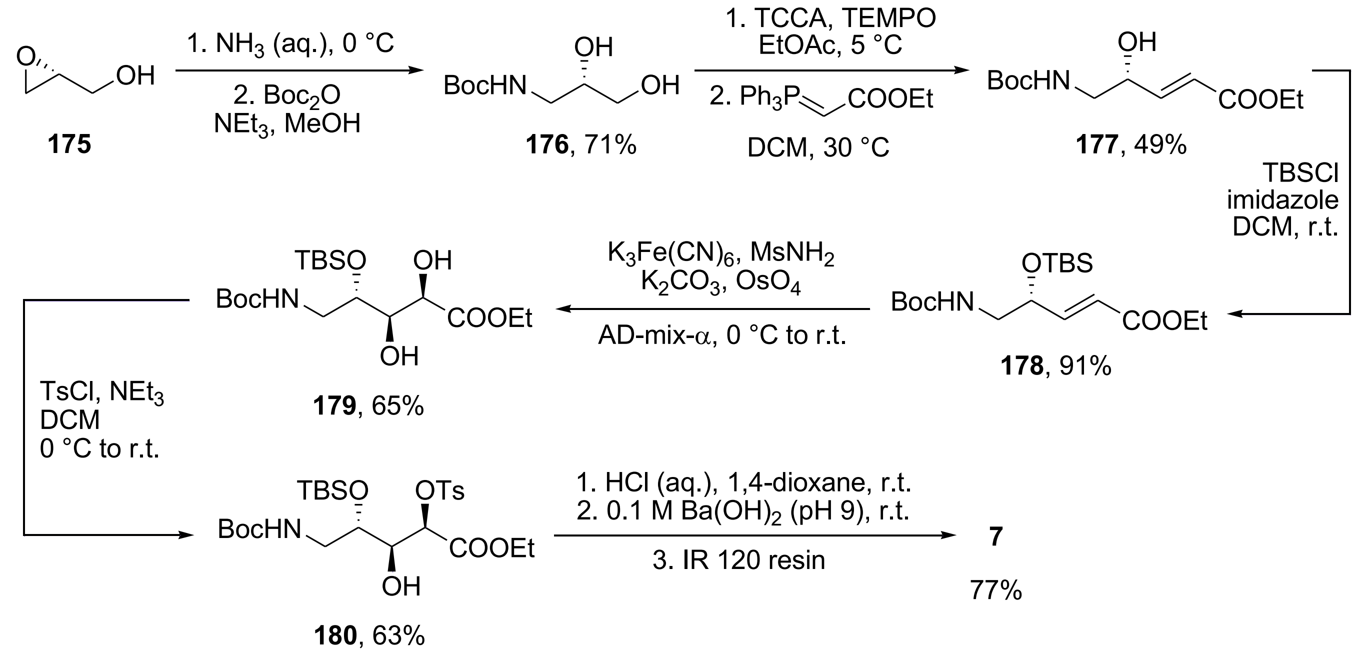
Scheme 19. Formal synthesis of **7** by Davis *et al.* using (*R*)-*p*-toluenesulfinamide (**157**) as chiral auxiliary and Grubbs metathesis. DBDMH = 1,3-dibromo-5,5-dimethylhydantoin.

Several years later, Davies *et al.* also contributed to the total synthesis of dihydroxylated proline motifs by researching the conversion of D-ribose (**165**) to an array of azasugars including **7** (Scheme 20).[51] Therefore, the primary alcohol moiety of **165** was iodinated, before elimination and cleavage of a ketal moiety led to **167**. Protection of the alcohol and Grubbs metathesis yielded **169**, which underwent an aminohydroxylation similar to the one described in Scheme 4 towards **170**.[31] Cleavage of protecting groups led to a mixture of pyrrolidines **172** and **173**, which could be separated via chromatography. Intermediate **172** was further reduced and the protecting group was changed yielding diol **174** in 80%. Oxidation and acidic treatment generated **7** in consistently high *dr* values over the whole path. Despite the long synthetic pathway, the stereoselective addition in step 7 could also be used as a platform for the synthesis of other azasugars, as already mentioned for Scheme 4.[31,51]



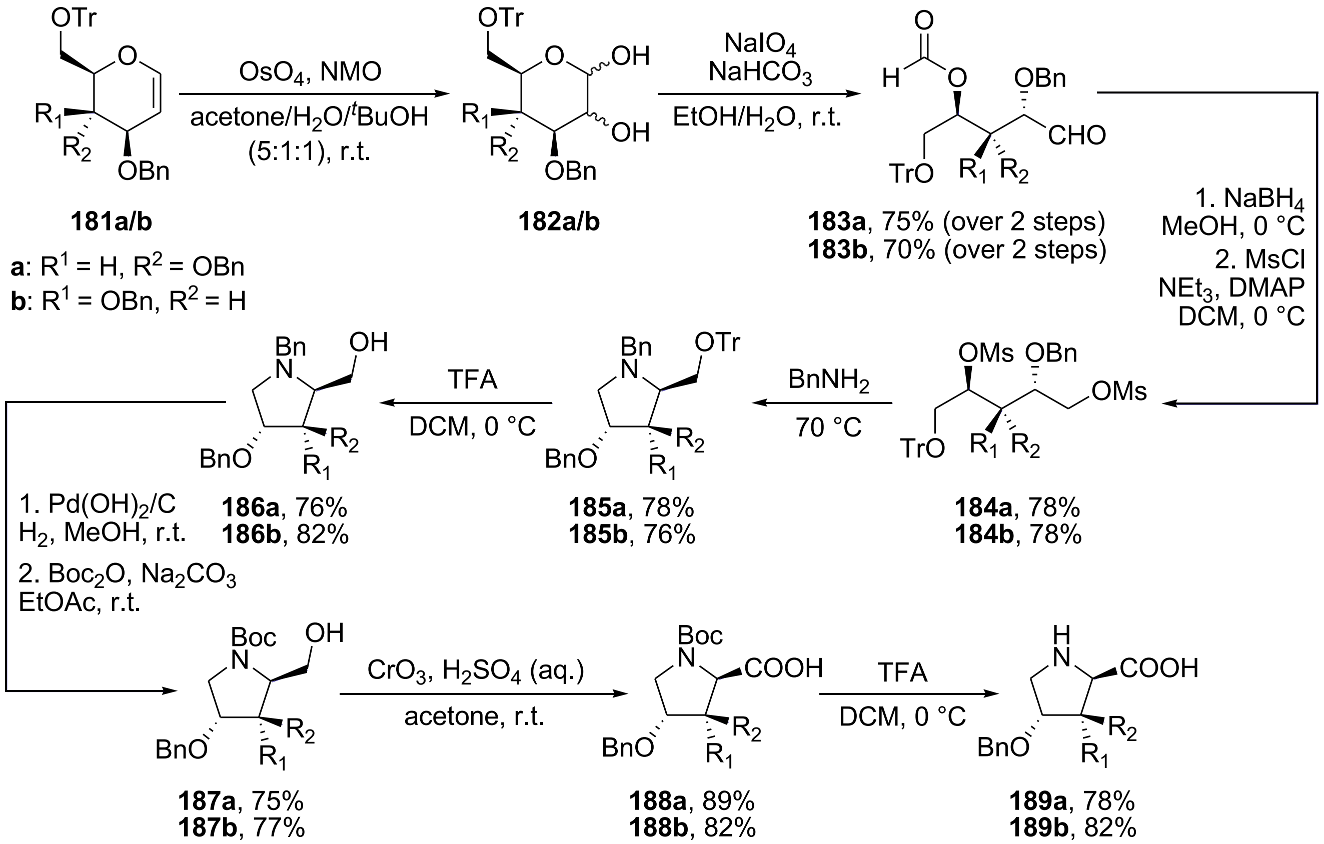
Scheme 20. Synthesis of **7** by Davies *et al.* from D-ribose (**165**).

In 2015, Gajare *et al.* could use enantiopure epoxide **175** for the assembly of 3,4-dihydroxyproline **7** (Scheme 21).[52] Treatment of **175** with ammonia and subsequent Boc-protection of the primary amine led to **176**. The oxidation and consecutive Wittig reaction afforded **177** in 49% yield. The secondary alcohol moiety could be protected with TMBCl and the alkene was *syn*-dihydroxylated using AD-mix-α. Regioselective tosylation could afford intermediate **180** in 63%. Consecutive acidic and basic treatment led to the cyclization and deprotonation of **180**. Eventual purification over IR 120 resin yielded free 3,4-dihydroxyproline **7**.



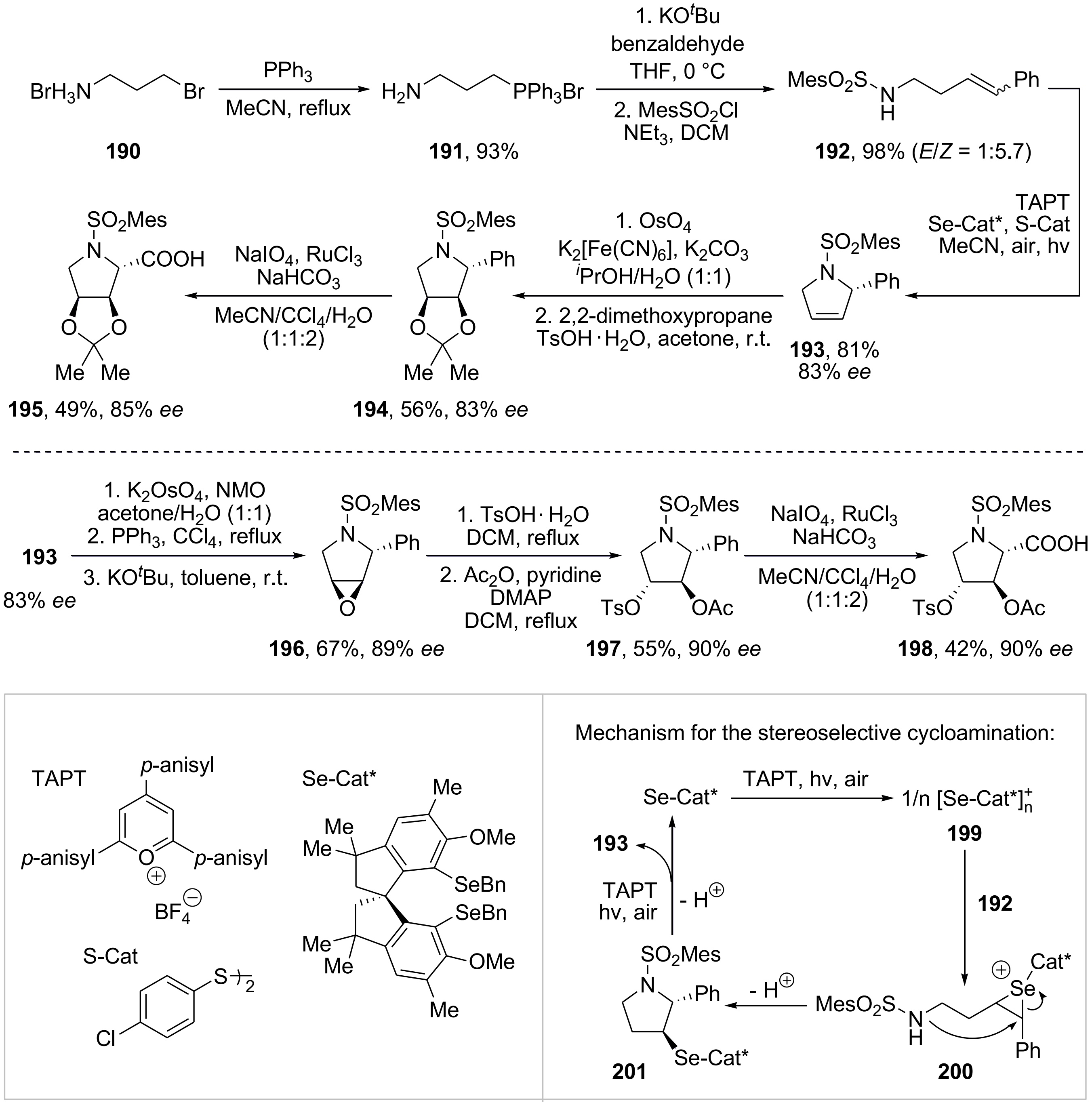
Scheme 21. Synthesis of **7** by Gajare *et al.* from epoxide **175**. TCCA = trichloroisocyanuric acid.

In 2019, Verma *et al.* developed a synthetic pathway towards two diastereomers of dihydroxyproline, **189a** and **189b**, starting from 6-O-trityl-3,4-dibenzyl-glycals **181a** and **181b**, respectively (Scheme 22).[53] After a dihydroxylation and oxidative ring-opening reaction to **183a** and **183b**, reduction and mesylation of the free alcohols led to **184a** and **184b**. The pyrrolidine core could be built up by the addition of $BnNH_2$ and the trityl group was cleaved by TFA. Change of the Bn- with a Boc-protecting group enabled the Jones oxidation towards **188a** and **188b**, and eventual deprotection yielded **189a** and **189b** in consistently good yields and perfect stereospecifity. Despite the consistently good yields towards both target compounds, the synthesis is similarly lengthy as other carbohydrate-based routes, especially considering that prior steps for **181a** and **181b** are required.



Scheme 22. Synthesis of **189a** and **189b** by Cumar *et al.* from **181a** or **181b**.

Very recently, Lei *et al.* found a facile and quick avenue towards enantioenriched 3-pyrrolines, which can be utilized- as shown before- for the assembly of hydroxylated prolines (Scheme 23, above).[54] This route starts with the Wittig salt formation from **190** and a subsequent Wittig reaction to yield **192** as an isomeric mixture (*E*/*Z* = 1:5.7). By the help of a protocol involving chiral selenium-π-acid- and photocatalysis, the authors were able to construct versatile 3-pyrroline moiety **193** in 83% *ee*. From here, dihydroxylation, diol protection and terminal oxidation of the phenyl ring led to **195**, which includes the core structure of **7**, in a total of 7 steps. Also **193** could be used for the assembly of a protected derivative of **8**.[55] Here, **193** was epoxidized within a sequence of dihydroxylation, an Appel reaction and internal substitution. The obtained epoxide, **196**, was opened by acidic treatment with TsOH and the free alcohol moiety protected with Ac_2O. Terminal oxidation of the phenyl moiety yielded **198**. A simplistic overview of the key reaction mechanism from **192** to **193** is shown in Scheme 23 below.[56] After single electron transfer from the excited photocatalyst (TAPT) to the chiral selenium catalyst (Se-Cat*), alkene **192** adds to the so obtained oligomeric selenonium moiety, **199**. Seleniranium ion **200** undergoes an intramolecular cyclization towards **201**. Another photooxidation initiates the elimination of Se-Cat*. Meanwhile TAPT is continuously reformed by oxidation upon ambient air. Notably, the authors could show that the additional disulfide acts as a cocatalyst and has twofold impact on this reaction. On the one hand, it acts as an electron-shuttle between photoexcited TAPT and Se-Cat*, on the other hand it adds onto oxidized **201** and thereby facilitates the terminal elimination step of the selenium catalyst. In comparison to the techniques shown before, the step from **192** to **192** represents a clear innovation. Herein, the construction of the 3-pyrrolin motif as well as the stereocenter are achieved at once.



Scheme 23. Above: Synthesis of **195** and **198** via selenium/photoredox catalysis. Below: Reaction mechanism of the photo-aerobic selenium-π-acid catalyzed cycloamination. Mes = 2,4,6-trimethylphenyl.

3. Conclusions

In summary, the last two decades have brought a variety of techniques to assemble hydroxyprolines. While syntheses based on carbohydrates from the available chiral pool are still numerically superior over stereoselective techniques starting from achiral compounds, the trend slowly moves towards the latter. These are especially advantageous over the others since stereodivergent syntheses accessing both enantiomers can be accomplished by the choice of the respective chiral catalyst or auxiliary. Furthermore, all carbohydrate-based syntheses typically involve the use of multiple protecting/deprotecting strategies and are therefore more laborious and less step-economic. Another trend, which has gained increasing attention is the use of common precursors, such as 3-pyrrolines, to access several stereoisomers of (di-)hydroxyprolines at once. Likewise, the use of enzymes enables the rapid assembly of different members. As shown, significant progress in hydroxyproline synthesis has been progressing over the years. Nevertheless, due to the increasing number of hydroxyproline containing peptides this research field will undoubtably continue to grow.

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Author's Biography

Sebastian Graf was born in 1995 in Landshut and received his B. Sc. (2017) and M. Sc. (2019) at the University of Regensburg. Within his masters studies he carried out research in the field of photopharmacology. During his PhD (2024) under the guidance of Prof. Dr. Alexander Breder his research was focused on stereoselective alkene functionalizatons via dual photo-/organocatalysis. Since 2023 he has worked for a supplier of chemical devices in the field of chromatography and mass spectrometry.