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Stereoconservative and stereoselective syntheses of rare and non-natural α-amino acids from (S)-aspartic acid and (S)-malic acid

Review Article

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Summary. A new strategy for stereoconservative and stereoselective syntheses of several types of amino acids starting from α-functional carboxylic acids employing hexafluoroacetone as protecting and activating reagent is described. Outstanding features of this new method are the mild reaction conditions and the high yields for introduction and cleavage of the protective group allowing sensitive functional groups in the side chain to survive. Furthermore, the new concept results in saving of synthetic steps.

Keywords: Amino acids – Rare and non-natural amino acids – Hexafluoroacetone – Aspartic acid – Hydroxyproline – Malic acid – Isoserine – Homoisoserine

Introduction

During the past few years, the field of rare and non-natural amino acids and peptidomimetics has received considerable attention (see Chorev and Goodman, 1993; Gante, 1994; Giannis and Kolter, 1993; Hölzemann, 1991a,b; Kahn, 1993; Morgan and Gainor, 1989; Olson et al., 1993; Wiley, 1993).

We have developed a new synthetic strategy for protection and activation of multifunctional α- and β-amino acids and their incorporation into small peptides using hexafluoroacetone (HFA). HFA reacts with α-functional carboxylic acids like α-amino, α-hydroxy, and α-mercapto acids already at room temperature to give bis(trifluoromethyl) substituted five-membered lactones in high yields.

This heterocyclization process results in a simultaneous protection of the α-functionality and the carboxylic group. However, the protection of the

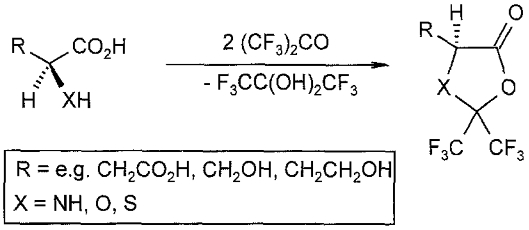


Fig. 1

carboxylic group is limited to electrophilic attack, towards nucleophiles the carboxylic group is activated. Functional groups placed in the side chain remain unaffected or can be regenerated easily and may be transformed further. Consequently, this method can be applied successfully for regioselective functional group transformations in multifunctional α-amino acids. Deprotection of the carboxylic and the α-amino group can be achieved simultaneously on treatment with a mixture of water/2-propanol at room temperature. Nucleophilic ring opening reactions of the lactones are always coupled with the deprotection of the α-functionality.

Protection and deprotection of α-functional carboxylic acids proceed in a stereoconservative manner, as shown by GC/MS and NMR experiments.

The feature of our new concept consists in saving synthetic steps: Protection and deprotection of two adjacent functionalities occur in one step under very mild reaction conditions. When formaldehyde is used, an additional protection of the amino group is necessary. The activation is lower, consequently diluted HCl at elevated temperatures is necessary for deblocking the carboxylic function. Deblocking of the amino group requires another separate step.

Finally, the two trifluoromethyl groups enable the monitoring of the formation and functionalization of the HFA derivatives by NMR.

Syntheses of amino acids starting from aspartic acid and their incorporation into peptides

Aspartic acid (**1**) reacts with HFA to give the oxazolidinone (**2**) in high yields. The carboxylic group in the side chain remains unaffected and may be transformed further. Nucleophilic ring opening with L-phenylalanine methylester gives L-aspartyl-L-phenylalanine methylester ("*aspartame*") (**3**) in 72% overall yield (Burger and Rudolph, 1990). To our knowledge, this is the shortest chemical synthesis of "*aspartame*".

On reaction with thionyl chloride the acid chloride (**4**) is obtained. The centre of highest electrophilicity is now shifted to the ω-position. This acid chloride represents a synthetically highly versatile compound.

Reduction of **4** under Rosenmund conditions (hydrogenolysis in the presence of palladium on barium sulfate) affords the aldehyde (**5**), which can be dihalogenated either with DAST (diethylamino sulfur trifluoride) to give **6** or with phosphorous pentachloride to give 7. Both compounds represent

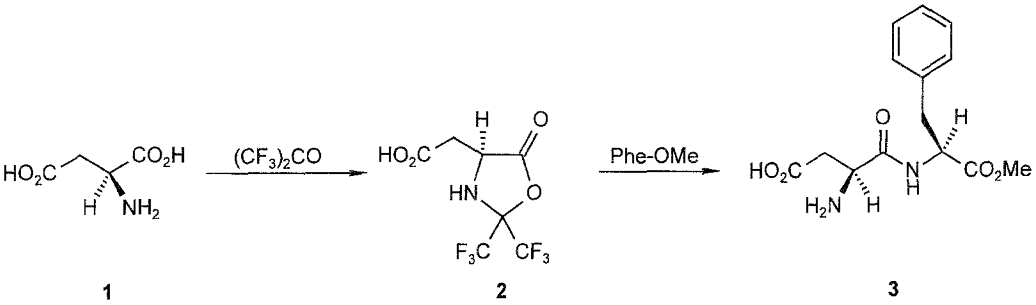


Fig. 2

stable precursors of amino acids and are hydrolyzed with water/2-propanol at room temperature to yield the corresponding free amino acids (Winkler and Burger, 1996). The dichloro compound (**9**) represents the antibiotic *armentomycine* (Argoudelis et al., 1967).

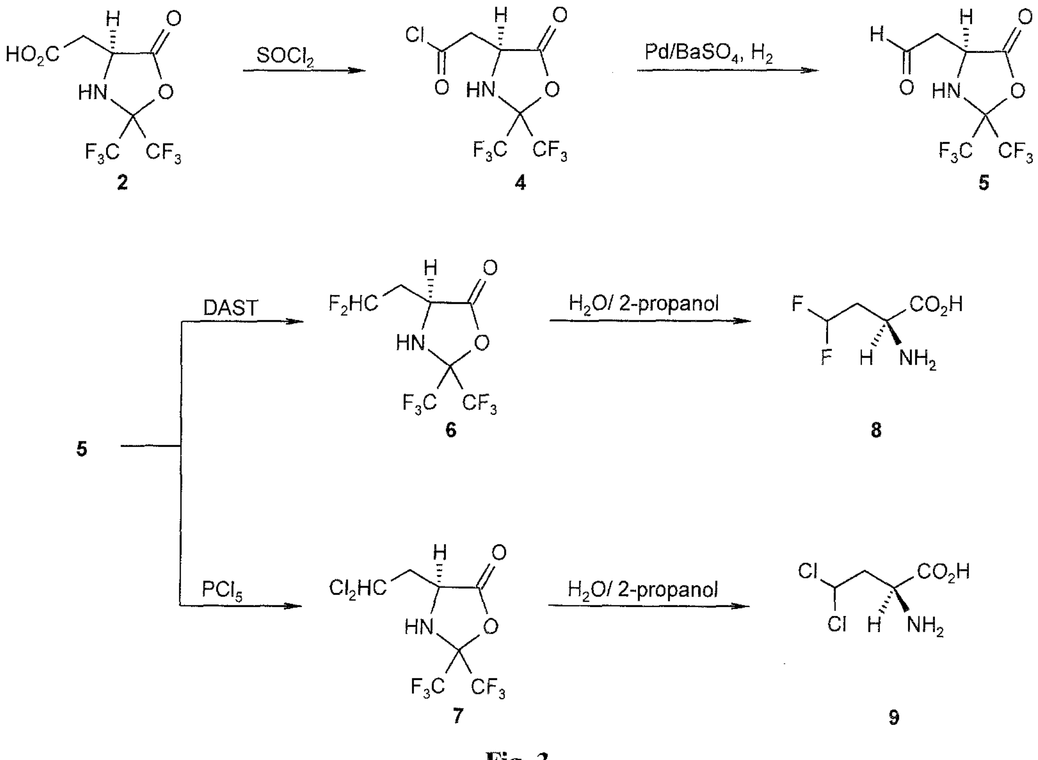


Fig. 3

Treatment of the acid chloride (**4**) with diazomethane yields the diazoketone (**10**), which on reaction with formic acid provides the fully protected 5-hydroxy-4-oxo-L-norvaline ("*HON*" see Kanazawa et al., 1960) precursor (**11**). Simultaneous deprotection of all three functionalities is accomplished with water/2-propanol furnishing the antibiotic "*HON*" (**12**) in 35% overall yield starting from L-aspartic acid (Golubev et al., 1993).

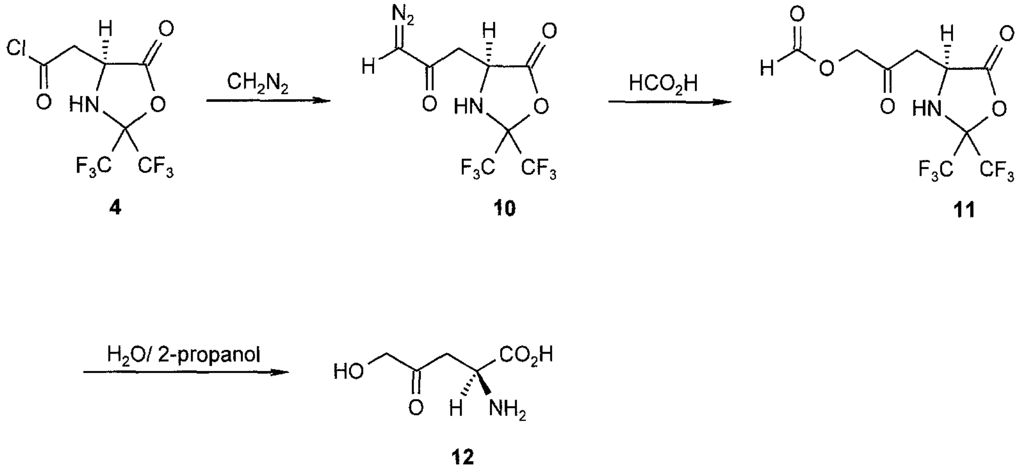


Fig. 4

The acid chloride (**4**) may be coupled with vinyltrimethyltin or vinyltributyltin in the presence of a palladium catalyst according to the Stille protocol to give the enone (**13**). The intramolecular Michael addition of **13** catalyzed by $BF_3 \cdot OEt_2$ results in the formation of the HFA-protected 4-oxo-L-pipecolic acid derivative (**14**). Deblocking of all functionalities is accomplished again in one step by water/2-propanol to give 4-oxo-L-pipecolic acid (**15**), which exists predominantly as the diol (**16**), as demonstrated by NMR-experiments.

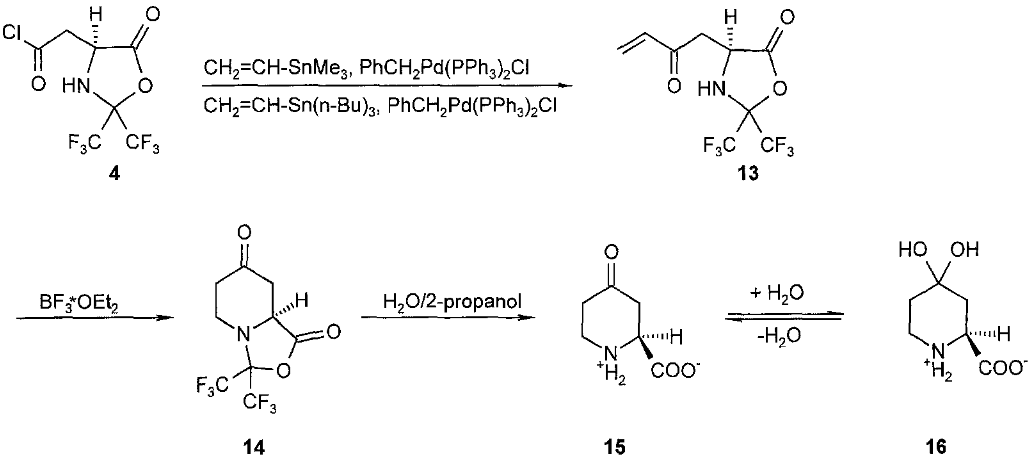


Fig. 5

Due to the concave shape of **14** stereocontrolled addition reactions at the carbonyl group are possible. Stereoselective reduction on treatment with $NaBH_4/C_6F_5OH$ gives **17**. Deprotection of **17** yields *cis*-4-hydroxy-L-pipecolic acid (**19**). Inversion of the configuration at position 4 of compound **18** via Mitsunobu reaction followed by deprotection of all three functionalities leads to *trans*-4-hydroxy-L-pipecolic acid (**20**, see Golubev et al., 1995). 4-Oxo-L-pipecolic acid is a constituent of the *virginiamycins*, a family of cyclopeptides exhibiting antibiotic activity. *Trans*-4-hydroxy-L-pipecolic acid (**20**) is a naturally ocurring amino acid isolated from *Acacia* species (Vanderhaeghe and Parmentier, 1960; Clark-Lewis and Mortimer, 1961).

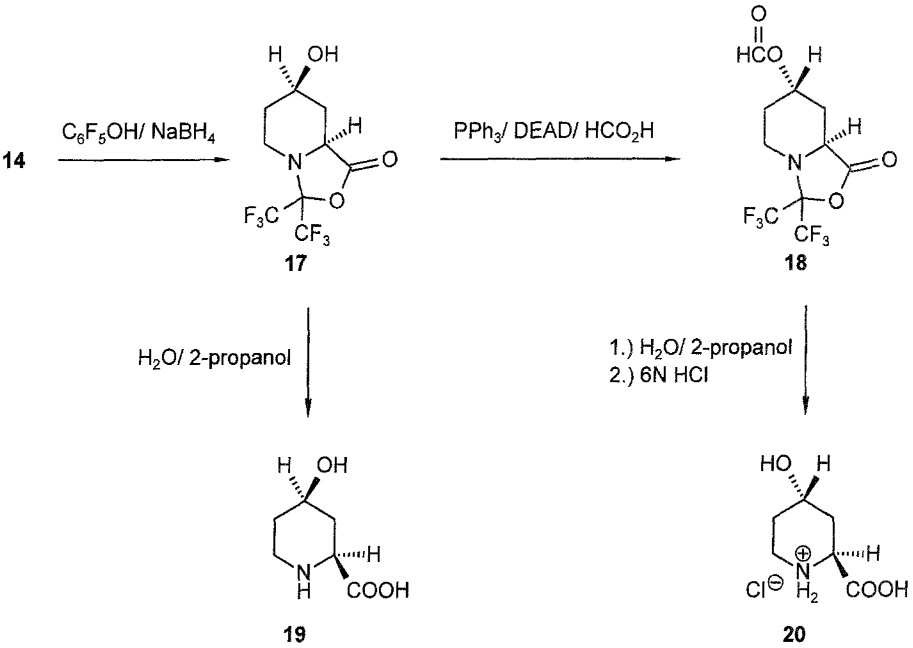


Fig. 6

Bulgecinine (4-hydroxy-5-hydroxymethylproline) is the aglycone of a new class of glycopeptide antibiotics, isolated from *Pseudomonas acidophila* and *Pseudomonas mesoacidophila* (Shinagawa et al., 1984). The bulgecins exhibit a synergistic effect on the antibacterial activity of β-lactames (Imada et al., 1982). Consequently, they are a class of biologically active compounds of current interest.

Our synthesis of bulgecinine (**26**) starts from the acid chloride (**4**). On reaction with diazo acetate compound **21** is formed in high yield. The diazo function of **21** can be decomposed in the presence of dirhodium tetraacetate to give the enol (**22**) which tautomerizes during purification by flash chromatography to give exclusively the *trans* proline derivative (**23**).

Stereoselective reduction of **23** with $NaBH_3CN$ followed by hydrolysis with water/2-propanol provides **25**. Reduction with $LiBHEt_3$ gives (−)-bulgecinine (**26**) in an overall yield of 15% (Fehn and Burger, 1996).

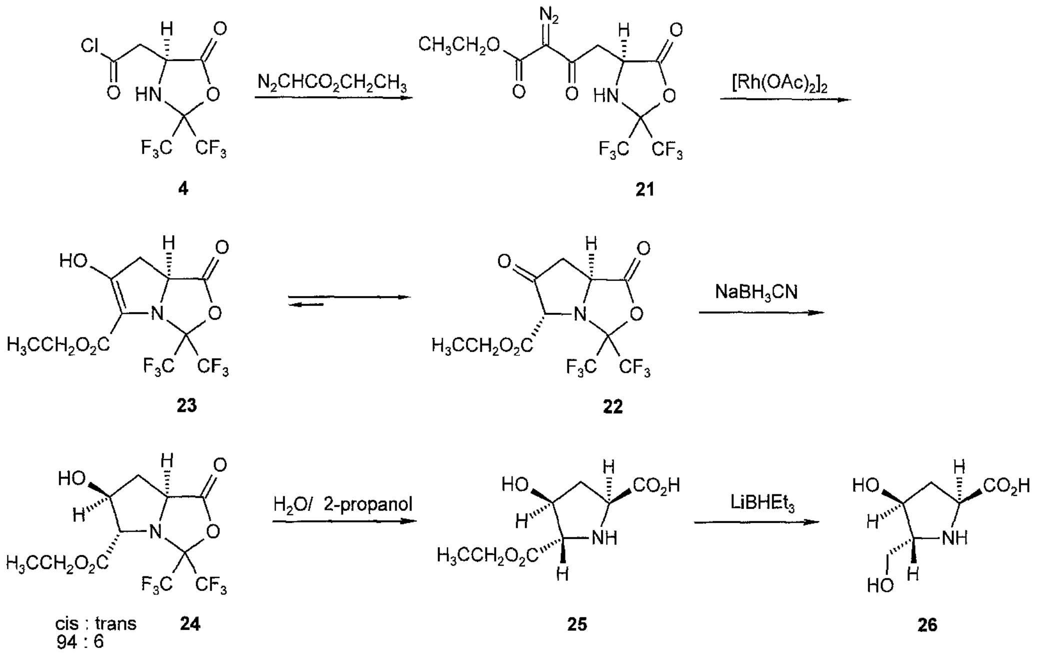


Fig. 7

The relatively cheap *trans*-4-hydroxy-L-proline (**27**) reacts with HFA to give a bicyclic compound (**28**). The keto-proline (**29**) is obtained from **28** upon oxidation with $RuO_2 \cdot H_2O/NaIO_4$ in H_2O/CCl_4. Stereoselective reduction of **29** with $NaBH_3CN$ gives the protected *cis*-4-hydroxy-L-proline (**30**) due to the concave shape of the bicyclic system (de 93:7). On hydrolysis with water/2-propanol **30** is transformed into the rather expensive *cis*-4-hydroxy-L-proline (**31**) (Burger et al., 1993).

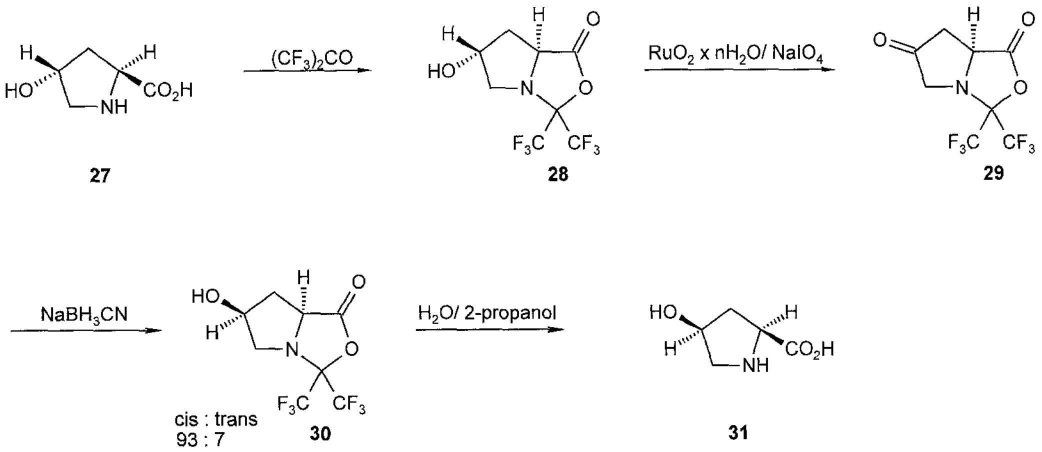


Fig. 8

Compounds **29** and **30** are transformed into the corresponding fluoro derivatives **32** and **35** on fluorination with DAST. Subsequent hydrolysis of **32** and **35** leads to 4,4-difluoro-L-proline (**33**) and *trans*-4-fluoro-L-proline (**36**), respectively. On aminolytic ring opening of **32** with L-phenylalanine *tert.*-butylester the dipeptide **34** with 4,4-difluoro-L-proline in N-terminal position is obtained (Burger et al., 1994).

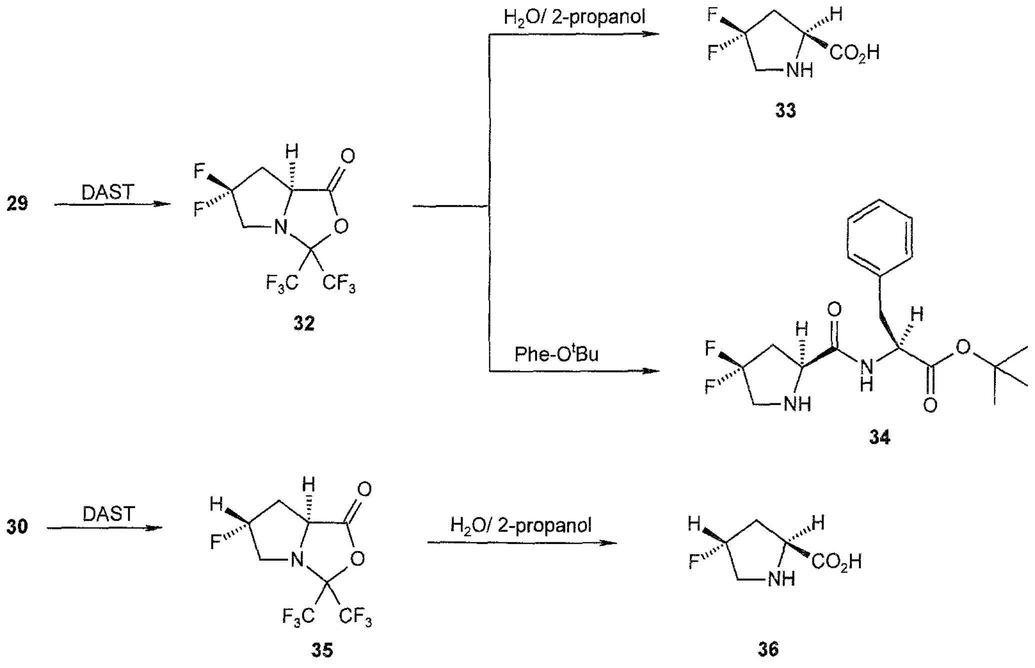


Fig. 9

Syntheses of amino acids starting from malic acid and their incorporation into peptides

Starting from (S)-malic acid, we developed a new strategy for synthesis and incorporation of (S)-isoserine into the N- and C-terminal position of peptides (Burger et al., 1995). (S)-Isoserine is a constituent of antibiotics such as edeine and tatumine, which are produced by the *Bacillus brevis* V_m^4 strains. Furthermore, it is a substructure of keramamide F (Sowinski and Toogood, 1995) and of the antibiotic isepamicin (Morohoshi et al., 1984).

(S)-Malic acid (**37**) is transformed into the bis(trifluoromethyl) substituted dioxolanone (**38**) upon reaction with HFA. Analogously to the amino acids described above, the hydroxyl and the adjacent carboxyl group of α-hydroxy acids are protected simultaneously. Upon reaction with thionyl chloride, **38** is transformed into the acid chloride (**39**). Heating of **39** with trimethylsilyl azide results in the formation of the isocyanate (**40**) via Curtius rearrangement. The isocyanate **40** represents a double activated L-isoserine derivative. It is the key compound for the incorporation of L-isoserine into N- and C-terminal position of peptides.

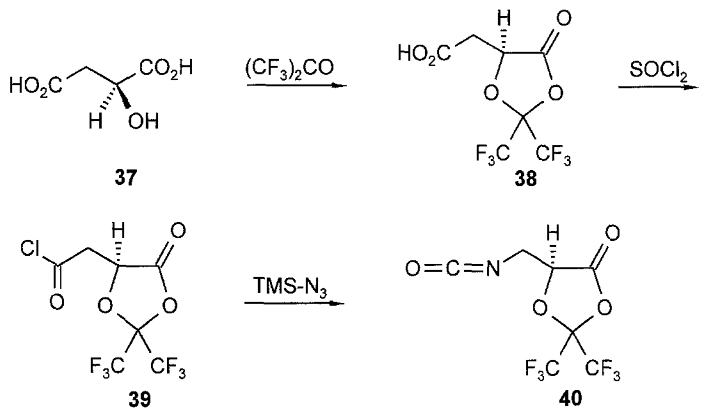


Fig. 10

The addition of equimolar amounts of alcohols results in the formation of urethanes in high yields. Via this route the common N-protection groups of peptide chemistry like Fmoc (**41**), Boc (**42**) or Z (**43**) can be introduced leading to amino protected, carboxyl group activated derivatives of L-isoserine. Ring opening of the lactone with amino acid *tert*-butyl esters provides dipeptides with L-isoserine in N-termal position (**44a–c**, **45**) with concomitant deprotection of the hydroxyl group for further transformations, e.g. with DAST.

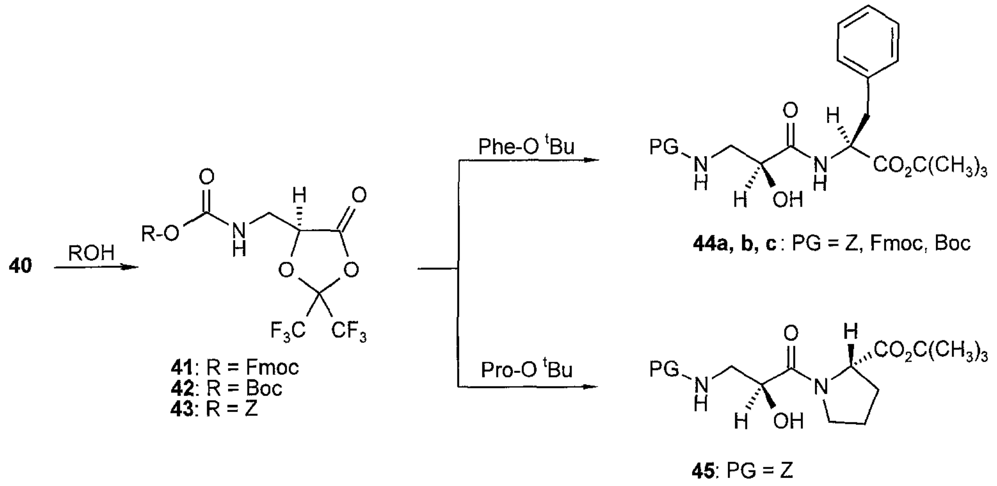
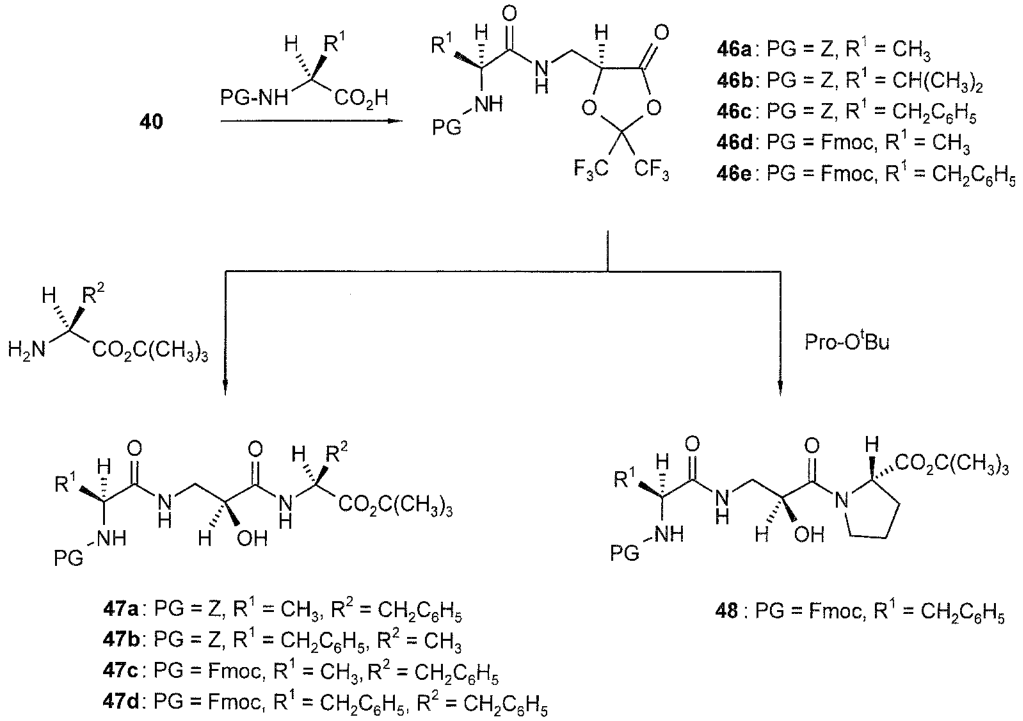


Fig. 11

Acylation of the isocyanate with N-protected amino acids gives N-protected, carboxyl group activated dipeptide derivatives (**46a–e**), which can be stored over longer periods. Nucleophilic cleavage of the lactone ring of compounds **46a–e** offers a preparatively simple, direct access to N-protected tripeptide derivatives (**47a–d**, **48**).



46a: PG = Z, $R^1 = CH_3$
46b: PG = Z, $R^1 = CH(CH_3)_2$
46c: PG = Z, $R^1 = CH_2C_6H_5$
46d: PG = Fmoc, $R^1 = CH_3$
46e: PG = Fmoc, $R^1 = CH_2C_6H_5$

47a: PG = Z, $R^1 = CH_3$, $R^2 = CH_2C_6H_5$
47b: PG = Z, $R^1 = CH_2C_6H_5$, $R^2 = CH_3$
47c: PG = Fmoc, $R^1 = CH_3$, $R^2 = CH_2C_6H_5$
47d: PG = Fmoc, $R^1 = CH_2C_6H_5$, $R^2 = CH_2C_6H_5$

48 : PG = Fmoc, $R^1 = CH_2C_6H_5$

Fig. 12

Furthermore, our methodology offers an elegant entry into the field of γ-amino acids (Pires and Burger, 1996). Starting from the acid chloride (**39**), reaction with diazomethane yields the diazoketone (**49**). Photolytic Wolff rearrangement in the presence of traces of water furnishes the HFA-protected homologic acid (**50**), which can be transformed with thionyl chloride into the corresponding acid chloride (**51**). The latter gives the isocyanate (**52**) on heating with trimethylsilyl azide.

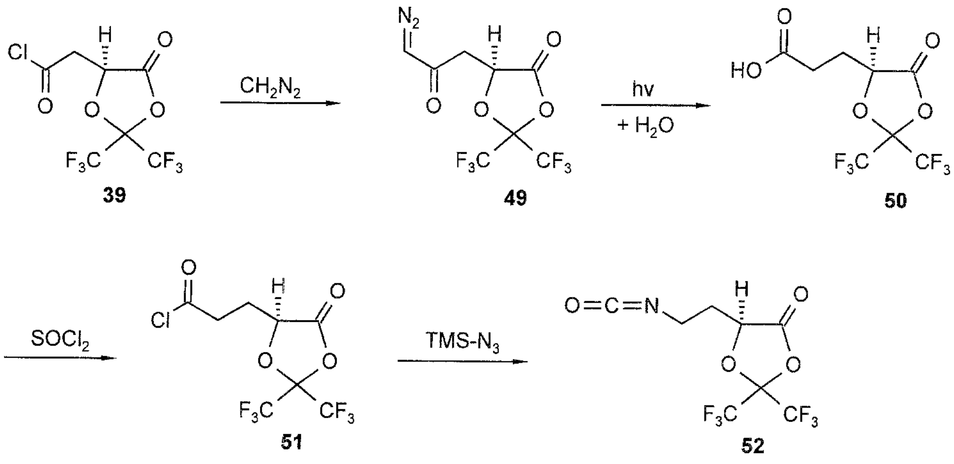
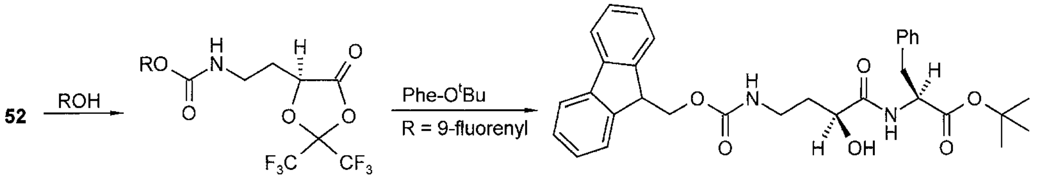


Fig. 13

The chemical reactivity pattern of compound **52** is analogous to that of **40**. Therefore, homoisoserine ((S)-4-amino-2-hydroxybutyric acid, AHB) can be introduced into peptides by this strategy in a preparatively simple way (e.g. **53** → **54**). Since the hydroxy group is deblocked simultaneously during peptide bond formation, it can be transformed further without additional deblocking step. Via this route α-substituted GABA (γ-amino butyric acid) derivatives can be introduced into a peptide chain.



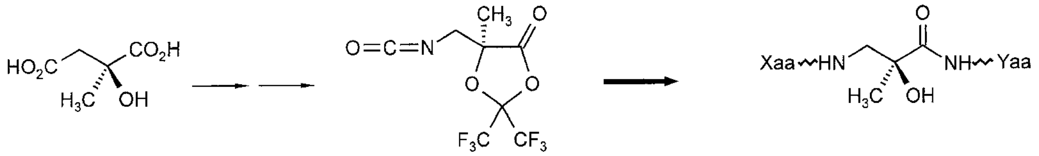
54

53a: R = benzyl
53b: R = 9-fluorenylmethyl
53c: R = tert.butyl

Fig. 14

Attachment of an AHB moiety to the NH_2-1 group of *kanamycins* results in compounds with remarkably enhanced antibacterial activity, e.g. arbekacin or amikacin. Furthermore, homoisoserine was found to be present as substructure in numerous phytosiderophores like distichonic acid, avenic acid, or mugenic acid being responsible for the iron uptake of some plants.

(S)-Citramalic acid (**55**) reacts with HFA in the typical way. The isocyanate (**56**) is obtained by the reaction sequence already described above. Therefore, incorporation of the α-methylisoserine moiety into N- and C-terminal position of peptides is no longer a problem (Pires and Burger, 1996). α-Methylisoserine represents a non-natural amino acid.



55 56

Fig. 15

Syntheses of amino acids starting from thiomalic acid and their incorporation into peptides

The adaptation of the reaction sequence described above for the incorporation of isoserine and homoisoserine into peptides to thiomalic acid (**57**) and homologs provides a preparatively simple access to isocysteine and homoisocysteine containing peptides (Pires and Burger, 1996).

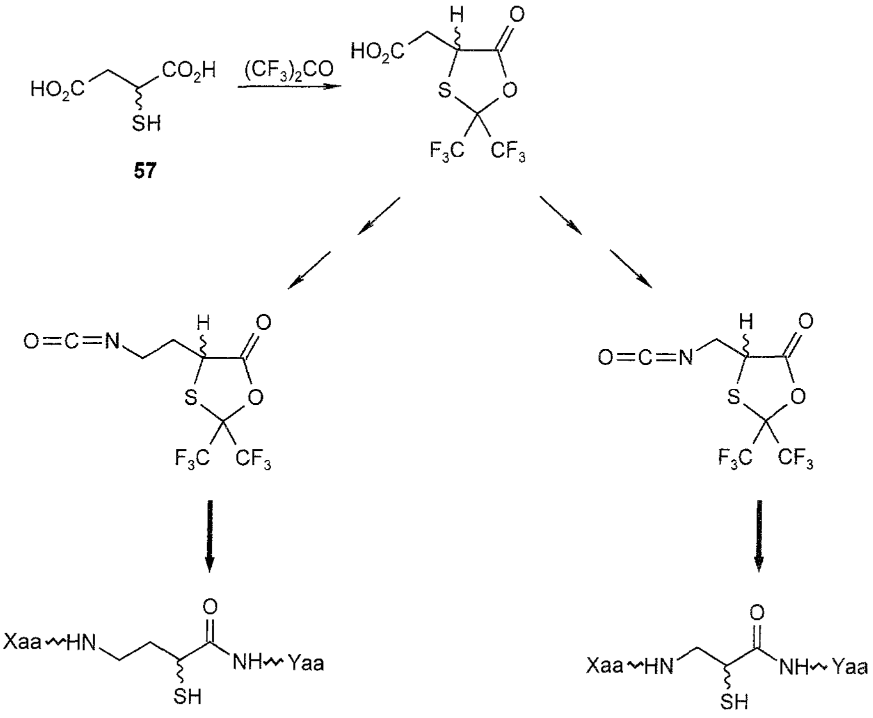


Fig. 16

Acknowledgements

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