A Platinum Open Access Journal for Organic Chemistry

Paper

Free to Authors and Readers | DOAJ Seal | *Arkivoc* **2023**, part v, 30-42

# Pseudo-enantiomeric coupling reagents for predictable incorporation into the peptide chain D and/or L amino acid residue of racemic substrates

**Justyna Fraczyk, Marta Michalska, Beata Kolesinska and Zbigniew J. Kaminski***

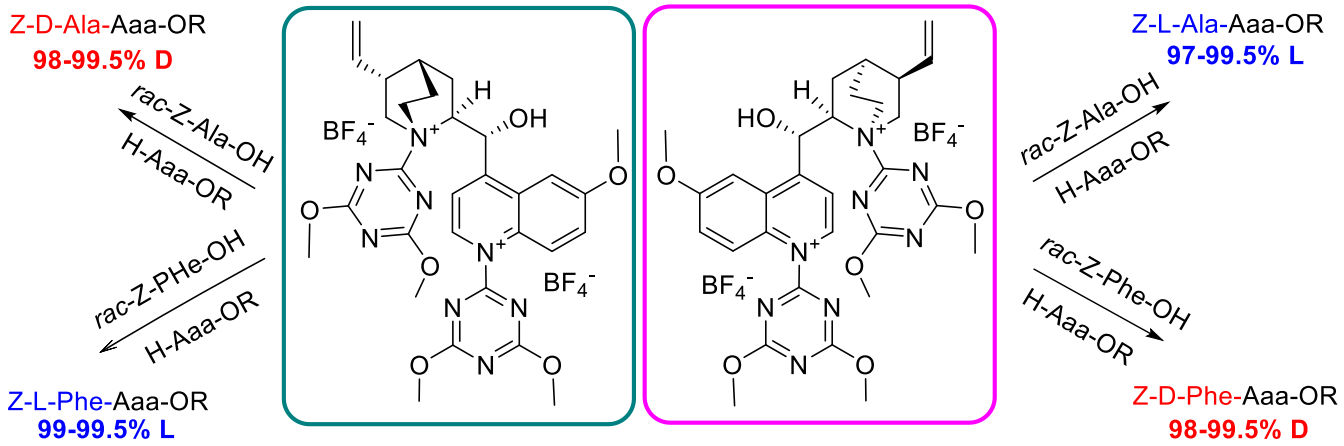
*Institute of Organic Chemistry, Lodz University of Technology, Poland*
*Email: zbigniew.kaminski@p.lodz.pl*

**In Honor of Prof. Józef Drabowicz on the occasion of his 75th birthday**

**Received** 10-12-2022 **Accepted** 11-06-2022 **Published on line** 11-10-2022

## Abstract

Reaction of 2-chloro-4,6-dimethoxy-1,3,5-triazine (CDMT) with bis-tetrafluoroborates of quinine and/or quinidine in the presence of sodium bicarbonate gave a pair of pseudo-enantiomeric coupling reagents: *N*,*N*′-bis-(4,6-dimethoxy-1,3,5-triazin-2-yl)quinine bis-tetrafluoroborate (2DMT/quinine/2$BF_4^-$) and *N*,*N*′-bis-(4,6-dimethoxy-1,3,5-triazin-2-yl)quinidine bis-tetrafluoroborate (2DMT/quinidine/2$BF_4^-$). The reagents activate the opposite enantiomers of racemic *N*-protected amino acids. By their reaction with two equivalents of racemic carboxylic components and diverse esters of amino acids, protected dipeptides were obtained with a predictable configuration and 95–99% enantiomeric homogeneity.

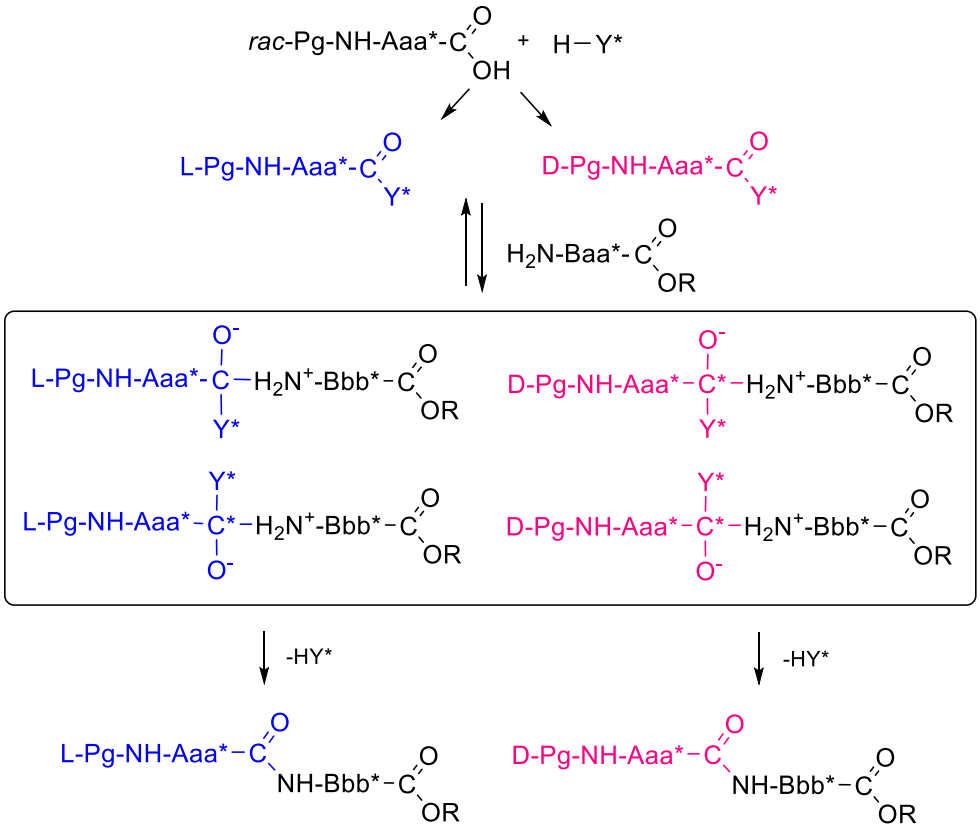



**Keywords**: Chiral coupling reagent, quinine, quinidine, bis-(4,5-dimethoxy-1,3,5-triazin-2-yl) ammonium tetrafluoroborate, peptide synthesis, kinetic resolution, enantioselective peptide synthesis.

## Introduction

Even a single modification of the configuration at numerous chiral centres in a peptide chain can dramatically impact the physicochemical properties, physiological activity, and degradation profiles of a peptide. The variations can be further multiplied by introducing non-proteinogenic building blocks into the peptide. Unfortunately, the search for structures offering the most desirable properties is extremely tedious, due to limited knowledge of structure–activity relationships. The quest for pharmaceutically valuable peptides is in most cases based on systematic synthesis of large numbers of analogues, involving diverse and often "exotic" building blocks. Access to both enantiomers of the chiral amino acid building blocks is therefore a crucial factor in the search. Preliminary attempts described in the literature to design an enantioselective procedure for peptide synthesis from racemic building blocks have been acceptable only for the synthesis of extremely simple targets containing an achiral glycine residue,[1,2] or for diastereoselective synthesis of dipeptides using racemic and optically active components.[3-6] However, there are several excellent procedures and chiral reagents available that enable efficient enantioselective acylation of diverse nucleophiles.[7-10] These include kinetic resolution using chiral 4–dimethylaminopyridine (DMAP) analogues,[11-14] amidine based catalysts,[15] phosphines,[16-18] amines,[19] and others.[20,21]



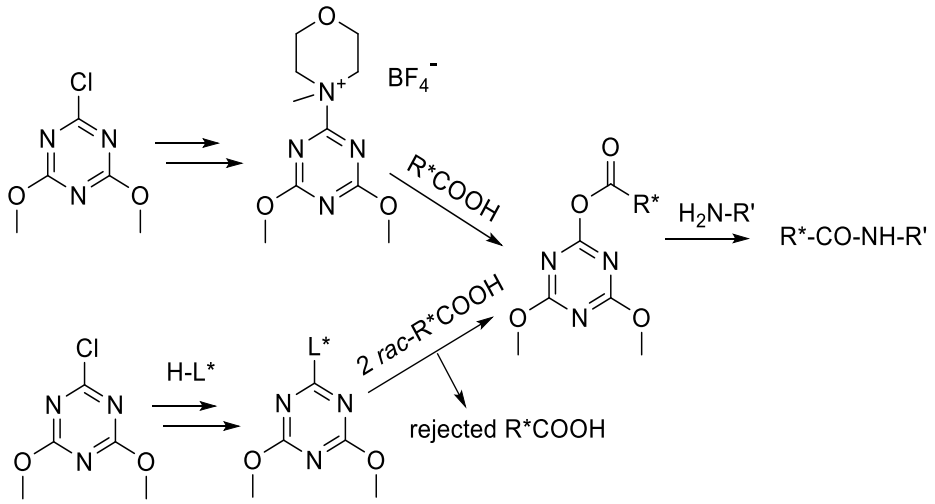

**Scheme 1.** Attachment of *rac*-Pg-NH-Aaa*-COOH to the chiral amino-component $H_2N$-Bbb*-COOR using chiral coupling reagent H-Y* proceeds with diastereoselectivity controlled by the three stereogenic centres already present in the substrates and an additional stereogenic centre in the tetrahedral intermediate.

Due to the specificity of the privileged scheme of peptide synthesis starting from the *C*-terminus, any enantioselective attachment of the subsequent building block *rac*-Pg-Aaa*-OH to the chiral amino-component

$H_2N$-Bbb*-OR using chiral coupling reagent H-Y* proceeds with diastereoselectivity controlled by the three stereogenic centres marked with an asterisk and by the additional stereogenic centre of the temporarily formed tetrahedral intermediate (Scheme 1). Under typical conditions of peptide bond formation, proceeding according to the $A_N$*$D_N^{‡}$ mechanism,[22] the yield, enantiomeric enrichment, and configurational preferences of each enantiomer of *rac*-Pg-NH-Aaa*-COOH for attachment to a distinct, usually complex, amino-component $H_2N$-Bbb*-COOR can be predicted only after precisely optimized selection of the chiral coupling reagent H-Y* acting under strictly defined coupling conditions. Modifying the structure of any single coupling component requires subsequent optimization of all the parameters of the coupling process to obtain satisfactory results. Considering that in the peptide preparation, the amino-component $H_2N$-Bbb*-COOR consists in almost all cases of complex, expensive, and highly diversified products of multistep synthesis, wasting it in an optimization procedure is unacceptable.

To overcome the problem of poor-predictability of peptide synthesis based on kinetic resolution, we designed binary enantioselective reagents for activating carboxylic components. According to the concept, the classic non-chiral triazine reagent with well-documented utility for peptide synthesis[23] was modified by introducing chiral module L*, which is responsible for enantioselective activation of the racemic carboxylic component and then departs after fulfilling its task.[24] All further coupling stages can proceed under conditions optimized for the well-known classic non-chiral reagent. Thus, the preferred configuration and enantiomeric enrichment can be accurately predicted for any set of coupling components, via an experiment involving simple model substrates (Scheme 2).




**Scheme 2.** Classic, non-chiral triazine coupling reagent with documented versatility in peptide synthesis was modified by replacing the *N*-methyl-1,4-morpholinium fragment with chiral leaving group L*. Fragment L*, responsible for enantioselective activation of the racemic carboxylic component, departs after fulfilling its task.

Reagents designed with chiral fragment L* prepared from strychnine or brucine gave fully predictable results of kinetic resolution. This allowed the synthesis of a representative group of enantiomerically enriched dipeptides from the racemic carboxylic component (used in two equivalents only), with 86–99 enantiomeric excess and 85–99% yields. In several cases, the calculated selectivity index *s* exceeded 100, which is comparable to kinetic resolution involving enzymes.[24,25] Moreover, in solid-state peptide synthesis using an excess of the racemic carboxylic component, final peptides were obtained with up to 100% enantiomeric homogeneity.[26]

However, the versatility of the procedure based on the application of strychnine and/or brucine is limited by the fact that both alkaloids are available in only one configuration.

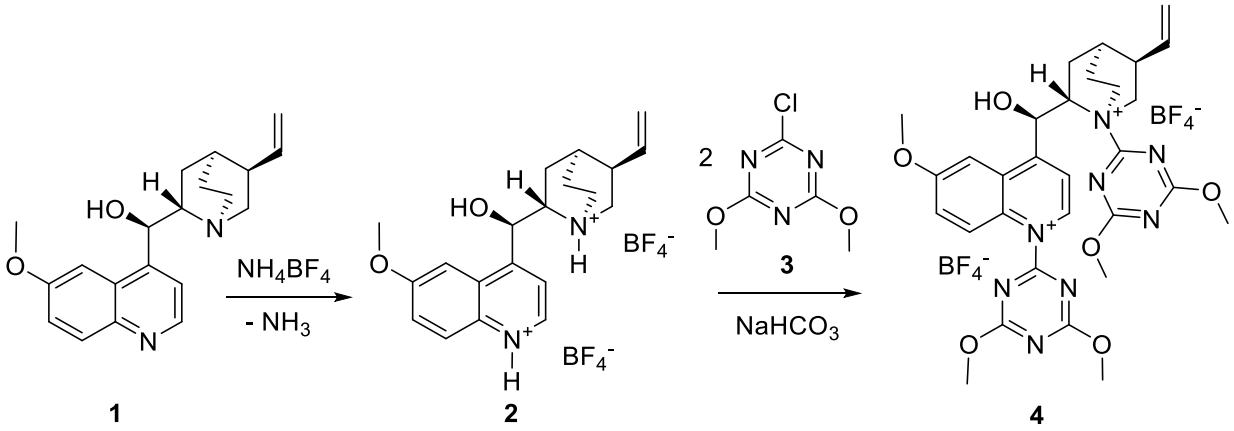
To overcome this limitation, several attempts were made to construct both enantiomeric forms of the chiral component L*. Esters of *N*-methylproline and *N*-allylproline are easily available in both enantiomeric forms. As expected, when applied as L*, these esters opened access to both enantiomeric Z-Ala-Gly-OMe dipeptides, but with only moderate yield (65–67%) and moderate ee of 70–74%.[27] Attempts to improve enantioselective peptide synthesis were also made using reagents prepared from 2-chloro-4,6-dimethoxy-1,3,5-triazine (CDMT, **3**) and (5R) and (5S)-2-(trichloromethyl)-1-aza-3-oxabicyclo[3.3.0]octan-4-one. Kinetic resolution of the *rac*-Z-Ala-OH process gave both expected enantiomeric dipeptides, but with poor yields (13–19%).[28]

Cinchona alkaloids are extremely attractive for use as the chiral component L*, as they are readily available in two pairs of pseudo-enantiomeric forms. However, experiments involving treatment of quinine (**1**) with CDMT (**3**) revealed that the primary reaction product, formed by the reaction of aromatic nitrogen of quinoline fragment with triazine reagent **3**, was not active as a coupling reagent. Moreover, intensive degradation of quinine (**1**) was observed after the transfer of the triazine ring into the quinuclidine fragment followed by the opening the bicyclic system in a reaction with the nucleophilic chloride anion.[29] Replacement of the chloride anion with a poorly nucleophilic tetrafluoroborate anion gave a stable quinine derivative with a triazine ring attached to the aromatic nitrogen of the quinoline fragment, but it was still not active as a coupling reagent.

## Results and Discussion

### Synthesis of coupling reagents

There are two tertiary quinine (**1**) nitrogen atoms prone to quaternization in reaction with CDMT (**3**). To avoid destruction of the quinuclidine bicyclic fragment by the nucleophilic chloride anion,[29] **1** was treated with ammonium tetrafluoroborate obtaining quinine bis-tetrafluoroborate (**2**). The reaction of **2** with two equivalents of CDMT in the presence of sodium bicarbonate proceeded steadily.



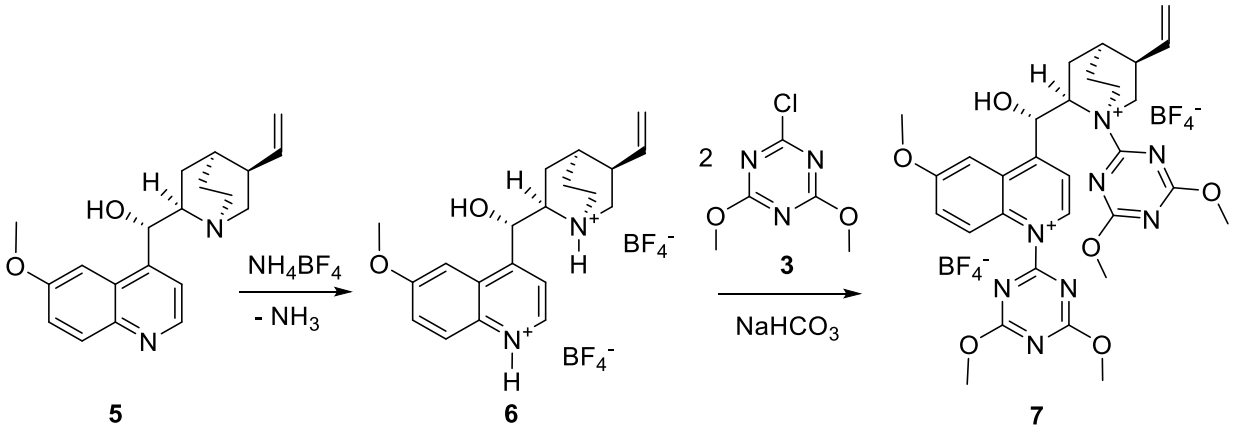

**Scheme 3.** Synthesis of quinine coupling reagent *N*,*N'*-bis-(4,6-dimethoxy-1,3,5-triazin-2-yl)quinine bis-tetrafluoroborate (2DMT/quinine/2$BF_4^-$, **4**) in reaction of quinine bis-tetrafluoroborate (**2**) with two equivalents of CDMT (**3**).

Relatively fast consumption of **3** was observed in the preliminary phase of reaction with quinine bis-tetrafluoroborate (**2**), followed by a slower reaction in the more advanced stage of the process.

In the $^1$H-NMR spectrum of the oily product isolated after treatment of quinine with **3**, the signals of five methoxy groups were detected. The sharp 3H singlet at 3.89 ppm characteristic for the quinoline fragment was accompanied by two downfield shifted 6H singlets at 4.02 ppm and a 6H singlet at 4.05 ppm characteristic of two 2,4-dimethoxy-1,3,5-triazine substituents. The attachment of two 4,6-dimethoxy-1,3,5-triazin-2-yl groups to quinine gives the isolated oily product with the proposed structure *N*,*N'*-bis-(4,6-dimethoxy-1,3,5-triazin-2-yl)quinine bis-tetrafluoroborate (**4**) denoted as 2DMT/quinine/2$BF_4^-$ (Scheme 3).

In an analogous process, quinidine (**5**) was transformed into quinidine bis-tetrafluoroborate (**6**) by treatment with ammonium tetrafluoroborate. In the reaction of **6** with two equivalents of CDMT (**3**), pseudoenantiomeric coupling reagent *N*,*N'*-bis-(4,6-dimethoxy-1,3,5-triazin-2-yl)quinidine bis-tetrafluoroborate (**7**) denoted as 2DMT/quinidine/2$BF_4^-$ was obtained as an oily product.




**Scheme 4.** Synthesis of quinidine coupling reagent 2DMT/quinidine/2$BF_4^-$ (**7**) by the reaction of quinidine bis-tetrafluoroborate (**6**) with two equivalents of CDMT (**3**).

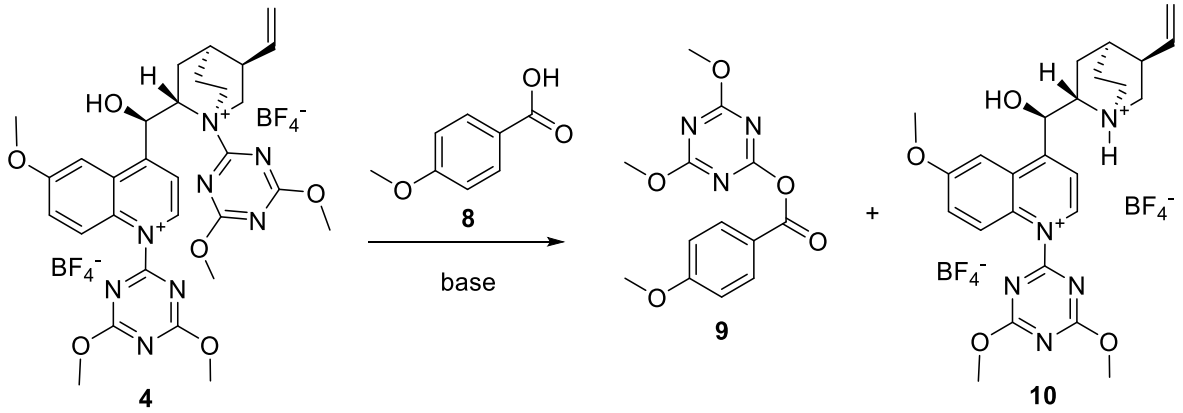
The signals of five methoxy group were observed in the $^1$H-NMR spectrum of the oily product isolated with 70% yield after treatment of **6** with two equivalents of CDMT (**3**). As in the case of quinine (**1**), besides the sharp 3H singlet at 3.89 ppm characteristic of the quinoline fragment of quinidine (**5**) a 6H singlet was observed at 4.02 ppm and a 6H singlet was observed at 4.05 ppm. This strongly suggests attachment of two 4,6-dimethoxy-1,3,5-triazin-2-yl groups to quinidine (**5**) and the formation of quinidine bis-tertrafluoroborate substituted with two 4,6-dimethoxy-1,3,5-triazin-2-yl groups **7**, denoted as 2DMT/quinidine/2$BF_4^-$ (Scheme 4).

**Peptide synthesis**

The kinetic resolution of racemic carboxylic acid by enantioselective activation of the carboxylic function can be severely disturbed by the formation of meso-anhydrides, in a side-reaction of the rejected enantiomer with the activated enantiomer in the opposite configuration.[30] To diminish the risk of this parasitic process, we made model experiments of 4-methoxybenzoic acid (**8**) activation with stoichiometric amounts of chiral coupling reagents **4** and **7** (Scheme 5). The coupling reagents were not completely consumed even after 24 h. Fortunately, the activation rate accelerated substantially after the addition of a tertiary amine to the mixture of 4-methoxybenzoic acid and reagent **4** and/or **7**. The results of optimizing the activation process depicted in Table 1 show that addition of **1**, **5** or diisopropylethylamine (DIPEA) substantially accelerate the reaction rate, reducing

the time necessary for consumption of the substrates to 2 h. In the case of coupling reagents **4** and **7**. the highest coupling yields were obtained using as catalysts 30% of **1** or **5,** respectively.




**Scheme 5.** Reaction of 4-methoxybenzoic acid (**8**) with quinine coupling reagent 2DMT/quinine/2$BF_4^-$ (**4**) affording (4,6-dimethoxy-1,3,5-triazin-2-yl)-4-methoxybenzoate (**9**).

**Table 1**. Reaction of 4-methoxybenzoic acid (**8**) with 2DMT/quinine/2$BF_4^-$ (**4**) and 2DMT/quinidine/2$BF_4^-$ (**7**) leading to 2-(4-methoxybenzoyloxy)-4,6-dimethoxy-1,3,5-triazine (**9**). Optimization of carboxylic group activation

| Entry | Coupling reagent | Amine | Activation time | Yield [%] |
|---|---|---|---|---|
| 1 | 2DMT/quinine/2$BF_4^-$ | 100% DIPEA | 2 | 61 |
| 2 | | 50% DIPEA | 2 | 62 |
| 3 | | 25% DIPEA | 2.5 | 61 |
| 4 | | 30% quinine | 2.5 | 62 |
| 5 | 2DMT/quinidine/2$BF_4^-$ | 100% DIPEA | 2 | 60 |
| 6 | | 50% DIPEA | 2 | 62 |
| 7 | | 25% DIPEA | 2.5 | 60 |
| 8 | | 30% quinidine | 2.5 | 62 |

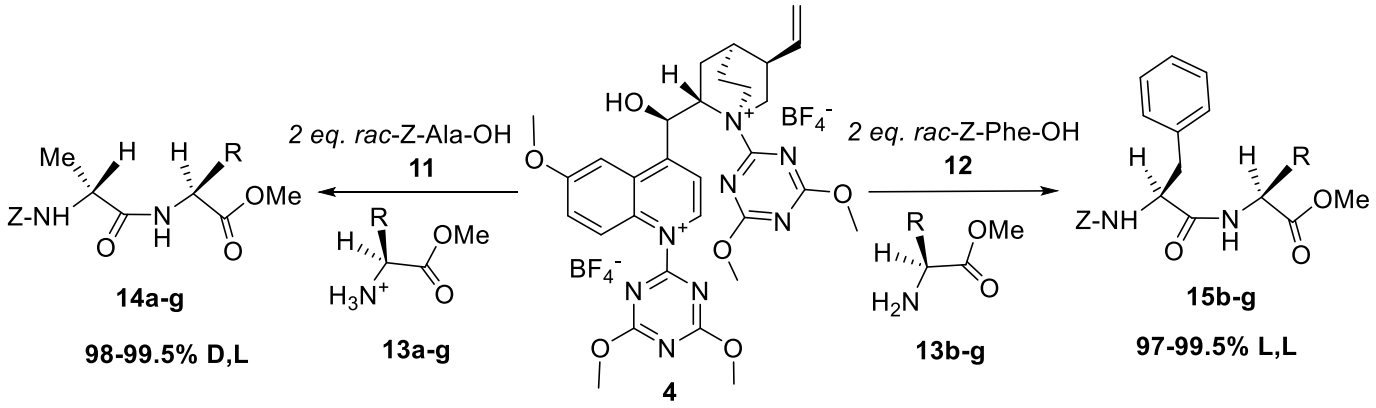
In previous studies using reagents derived from strychnine and brucine, amino acids with aliphatic and aromatic amino acids were activated with opposite configurational preference.[24] Therefore, in the present study on enantioselective activation *rac*-Z-Ala-OH (**11**) with an aliphatic side-chain and *rac*-Z-Phe-OH (**12**) with an aromatic side-chain were used. In both cases, two equivalents of **11** with an aliphatic side-chain and **12** with an aromatic side-chain were coupled with one equivalent of diverse amino components **13a-g** in acetonitrile solution in the presence of **4** and 0.3 equivalents of quinine (**1**) used as catalyst.

Crude dipeptides **14a-g** were isolated after evaporation of the reaction mixture to dryness, extraction of the residue with dichloromethane (DCM), successive washing of the extract according to the standard procedure with 1M HCl and saturated aqueous $NaHCO_3$ solution, drying and evaporation again to dryness. Due to the ionic character of the substrates, by-products, and side-products, this isolation procedure gave more than 90% pure dipeptides while retaining the composition of stereoisomers. All signals expected for dipeptides **14a-g** and **15b-g** were identified in the $^1$H NMR and IR spectra of crude dipeptides prepared. In order to avoid modification of composition of the diastereoisomeric products by excessive purification procedure, the configuration and

enantiomeric composition of the formed dipeptides **14a-m** were determined by gas chromatography on chiral stationary phase after the hydrolysis of crude dipeptides to amino acids.

The results of dipeptide synthesis (Scheme 6) using 2DMT/quinine/2$BF_4^-$ (**4**) are summarized in Table 2.




**Scheme 6.** Enantioselective synthesis of dipeptides by coupling two equivalents of *rac*-Z-Ala-OH (**11**) with methyl esters of amino acids (**13a-g**) and two equivalents of *rac*-Z-Phe-OH (**12**) with methyl esters of amino acids (**13b-g**) in the presence of 2DMT/quinine/2$BF_4$ (**4**) (one equivalent).

In all the couplings of aliphatic *rac*-Z-Ala-OH (two equivalents of **11**) with methyl esters of amino acids **13a-g** using reagent **4**, the formation of D,L-dipeptides **14a-g** was preferred (98–99.5% D,L), isolated with 56–60% yield (Table 2).

**Table 2.** Enantioselective synthesis of dipeptides by coupling two equivalents of *rac*-Z-Ala-OH (**11**) with methyl esters of amino acids (**13a-g**) (one equivalent) in the presence of 2DMT/quinine/2$BF_4$ (**4**) (one equivalent)

| entry | carboxylic component | amino component **13a-g** | dipeptide **14a-g** | yield [%] | L,L/D,L [%] |
|---|---|---|---|---|---|
| 1 | *rac*-Z-Ala-OH (**11**) | H-Phe-OMe*HCl (**13a**) | Z-Ala-Phe-OMe (**14a**) | 60 | 0.5/99.5 |
| 2 | " | H-Leu-OMe*HCl (**13b**) | Z-Ala-Leu-OMe (**14b**) | 61 | 1/99 |
| 3 | " | H-Tyr-OMe*HCl (**13c**) | Z-Ala-Tyr-OMe (**14c**) | 57 | 0.5/99.5 |
| 4 | " | H-Ser-OMe*HCl (**13d**) | Z-Ala-Ser-OMe (**14d**) | 59 | 2/98 |
| 5 | " | H-Val-OMe*HCl (**13e**) | Z-Ala-Val-OMe (**14e**) | 60 | 0.5/99.5 |
| 6 | " | H-Ala-OMe*HCl (**13f**) | Z-Ala-Ala-OMe (**14f**) | 62 | 50.5/49.5 |
| 7 | " | H-Gly-OMe*HCl (**13g**) | Z-Ala-Gly-OMe (**14g**) | 56 | 2/98 |

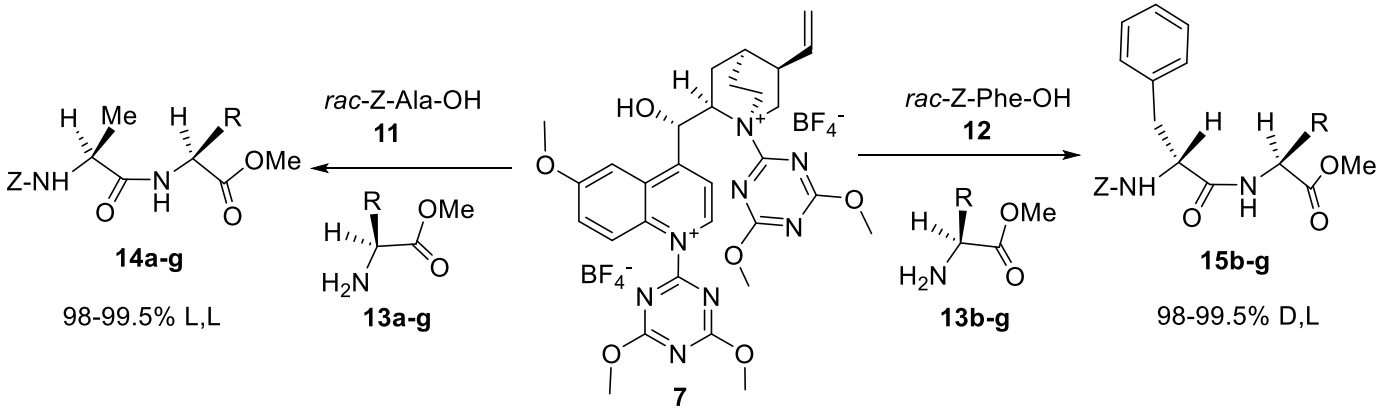
Reversed preferences were found when two equivalents of aromatic *rac*-Z-Phe-OH (**12**) were coupled with methyl esters of L-amino acids **13b-g** in the presence of reagent **4** (Table 3). In all cases, the preferred dipeptides **15b-g** configuration was L,L (97% up to 99.5%), isolated with 56–62% yield.

Dipeptides with opposite configurations were obtained in enantioselective coupling using quinidine reagent 2DMT/quinidine/2$BF_4$ (**7**) (Scheme 7). In all dipeptide syntheses from racemic aliphatic *rac*-Z-Ala-OH (**11**), seven different L-amino components **13a-g** and quinidine reagent **7**, the configuration of the preferred product **14a-g** was L,L (98–99.5%) (Table 4).

**Table 3.** Enantioselective synthesis of dipeptides **15b-g** by coupling two equivalents of *rac*-Z-Phe-OH (**12**) with methyl esters of amino acids (**13b-g**) (one equivalent) in the presence of 2DMT/quinine/2$BF_4$ (**4**)

| entry | carboxylic component | amino component **13b-g** | dipeptide **15b-g** | yield [%] | L,L/D,L [%] |
|---|---|---|---|---|---|
| 1 | *rac*-Z-Phe_OH **12** | H-Leu-OMe*HCl (**13b**) | Z-Phe-Leu-OMe (**15b**) | 61 | 99.5/0.5 |
| 2 | " | H-Tyr-OMe*HCl (**13c**) | Z-Phe-Tyr-OMe (**15c**) | 57 | 99/1 |
| 3 | " | H-Ser-OMe*HCl (**13d**) | Z-Phe-Ser-OMe (**15d**) | 59 | 98/2 |
| 4 | " | H-Val-OMe*HCl (**13e**) | Z-Phe-Val-OMe (**15e**) | 60 | 98/2 |
| 5 | " | H-Ala-OMe*HCl (**13f**) | Z-Phe-Ala-OMe (**15f**) | 62 | 99/1 |
| 6 | " | H-Gly-OMe*HCl (**13g**) | Z-Phe-Gly-OMe (**15g**) | 56 | 97/3 |




**Scheme 7.** Enantioselective synthesis of dipeptides by coupling two equivalents of *rac*-Z-Ala-OH (**11**) with methyl esters of amino acids (**13a-g**) and two equivalents of *rac*-Z-Phe-OH (**12**) with methyl esters of amino acids (**13b-g**) in the presence of 2DMT/quinidine/2$BF_4$ (**7**) (one equivalent).

**Table 4.** Enantioselective synthesis of dipeptides **14a-g** by coupling two equivalents of the racemic carboxylic component *rac*-Z-Ala-OH (**11**) with methyl esters of amino acids **13a-g** in the presence of 2DMT/quinidine/2$BF_4$ (**7**)

| entry | carboxylic component | amino component **13a-g** | dipeptide **14a-g** | yield [%] | L,L/D,L [%] |
|---|---|---|---|---|---|
| 1 | *rac*-Z-Ala-OH (**11**) | H-Phe-OMe*HCl (**13a**) | Z-Ala-Phe-OMe (**14a**) | 55 | 99.5/0.5 |
| 2 | " | H-Leu-OMe*HCl (**13b**) | Z-Ala-Leu-OMe (**14b**) | 60 | 99.5/0.5 |
| 3 | " | H-Tyr-OMe*HCl (**13c**) | Z-Ala-Tyr-OMe (**14c**) | 60 | 99.5/0.5 |
| 4 | " | H-Ser-OMe*HCl (**13d**) | Z-Ala-Ser-OMe (**14d**) | 58 | 99/1 |
| 5 | " | H-Val-OMe*HCl (**13e**) | Z-Ala-Val-OMe (**14e**) | 62 | 99/1 |
| 6 | " | H-Ala-OMe*HCl (**13f**) | Z-Ala-Ala-OMe (**14f**) | 61 | 99.5/0.5 |
| 7 | " | H-Gly-OMe*HCl (**13g**) | Z-Ala-Gly-OMe (**14g**) | 57 | 99.5/0.5 |

Again, reversed configurational preferences were found in the syntheses of dipeptides **15b-g** from aromatic *rac*-Z-Phe-OH (**11**) and methyl esters of L-amino acids **13b-g** (Table 5). In all cases, the configuration of the main products **15b-g** was D,L (98–99.5%). In the cases of both reagents **4** and **7**, excellent enantiomeric enrichment of the isolated dipeptides was accompanied by a relatively moderate yields in the range of 57–65%.

However, this limitation can be efficiently overcome by the application of excess of the acylating component, as is routinely practiced in solid-phase peptide synthesis.

**Table 5.** Enantioselective synthesis of dipeptides **15b-g** by coupling two equivalents of *rac*-Z-Phe-OH (**12**) with methyl esters of amino acids (**13b-g**) (one equivalent) in the presence of 2DMT/quinidine/2$BF_4$ (**7**)

| entry | carboxylic component | amino component **13b-g** | dipeptide **15b-g** | yield [%] | L,L/D,L [%] |
|---|---|---|---|---|---|
| 1 | *rac*-Z-Phe_OH **12** | H-Leu-OMe*HCl (**13b**) | Z-Phe-Leu-OMe (**15b**) | 63 | 2/98 |
| 2 | " | H-Tyr-OMe*HCl (**13c**) | Z-Phe-Tyr-OMe (**15c**) | 65 | 0.5/99.5 |
| 3 | " | H-Ser-OMe*HCl (**13d**) | Z-Phe-Ser-OMe (**15d**) | 39 | 1/99 |
| 4 | " | H-Val-OMe*HCl (**13e**) | Z-Phe-Val-OMe (**15e**) | 60 | 1/99 |
| 5 | " | H-Ala-OMe*HCl (**13f**) | Z-Phe-Ala-OMe (**15f**) | 62 | 0.5/99.5 |
| 6 | " | H-Gly-OMe*HCl (**13g**) | Z-Phe-Gly-OMe (**15g**) | 56 | 2/98 |

The stimulus of the inversion of configurational preferences in the acylation reactions involving Z-Phe-OH (**11**) activated by coupling reagent **4** prepared from quinine as well as coupling reagent **7** derived from quinidine still remain unrecognized. It can be presumed, however, that interactions involving the aromatic ring of the benzyl side-chain in phenylalanine, with quinine and quinidine fragments of coupling reagent, dominate over steric hindrance effects of the aliphatic alanine side-chain.

## Conclusions

The application of pseudo-enantiomeric coupling reagents **4** and **7** derived from quinine (**1**) and quinidine (**5**) respectively, opened a route to the incorporation of an amino acid building block with a predictable favoured configuration using a racemic substrate. The configuration of the reaction product as well the preferred reaction conditions can be accurately predicted after a single model experiment. Enantioselectivity in the range of 97–99.5% was obtained for both enantiomers, creating a brand-new tool for systematic studies of the relationship between the broadly defined properties of peptides and the configuration of the amino acid residues.

## Experimental Section

**General.** TLC plates: Silicagel, Merck 60 Å, 254. Spots were visualized with UV light (254 nm and 366 nm) and with 1% ethanolic 4-(4-nitrobenzyl)pyridine (NBP). Melting points were determined using a Büchi apparatus, model 510. Analytical RP-HPLC was performed with a Waters 600S HPLC system (Waters 2489 UV/Vis detector, Waters 616 pump, Waters 717 plus autosampler, HPLC manager software from Chromax) using a C18 column (25 cm × 4.6 mm, 5 mm, Sigma) with a gradient of 0.1% TFA in H2O (A) and 0.08% TFA in MeCN (B), at a flow rate of 1 mL/min with UV detection at 220 nm, Rt in min.

IR spectra were recorded as KBr pellets or film with a Bruker ALPHA spectrometer or a PerkinElmer Spectrum 100. $^{1}H$ and $^{13}C$ NMR spectra were recorded with a Bruker Avance DPX 250 (250 MHz) spectrometer, with chemical shifts (ppm) relative to TMS used as an internal standard. Multiplicities are marked as s = singlet, d = doublet, t = triplet, q = quartet, quint = quintet, m = multiplet.

Hydrolysis of peptides to amino acids hydrochlorides and derivatization for determination of enantiomeric purity by GC on ChirasilVal capillary column: Peptide (5 mg) in thick-walled test tube was treated with constant boiling hydrochloric acid (5 mL), freezed with dry-ice/ethanol, the pressure was reduced below 10 mbars and the tube was sealed-up. The sealed test tube was heated in boiling water for 24 h, then opened, and hydrochloric acid solution was evaporated to dryness. The residue was dissolved with distilled water (5 mL) and evaporated to dryness. Dissolution and evaporation were repeated three times. Solid residue was dried with $P_2O_5$ in vacuum desiccator, treated with methanol saturated with dry HCl for 24 h, evaporated to dryness, then dissolved in solution of trifluoroacetic acid anhydride (0.2 mL) in DCM (2 mL) for 12 h at room temperature. The solution was analyzed on a Chirasil-Val capillary column. Gas chromatography (GC) – HP 5840 II, FID (H2/air), split 1:30. Column: capilar Chirasil-Val (25 m × 0,32 mm, thickness of film 0.2 μm, carrier gas: hel, pressure 0.45 atm. Temperature program: 4 min at 90 °C, rising 4 °C/min to 190 °C, and then 3 min at 190 °C.

***N,N'*-Bis-(4,6-dimethoxy-1,3,5-triazin-2-yl)quinine bis-tetrafluoroborate (4)** (**2DMT/quinine/2BF$_4^-$**). Quinine (**1**) (32.44 g; 100 mmol) and ammonium tetrafluoroborate (21.00 g; 200 mmol) were dissolved in $CH_3CN$ (400 mL) and heated under reflux until all ammonia evolution ceased. The obtained clear solution was stirred and cooled to 0 °C, then solid $NaHCO_3$ (33.60 g, 400 mmol) was added. The slurry was treated with a solution of CDMT (**3**) (35.00 g, 200 mmol) in $CH_3CN$ (100 mL). Stirring was continued at 0–5 °C until all CDMT was consumed (TLC control, mobile phase 100% DCM, disappearance of spot $R_f$= 0.6, visualization by spraying with 0.5% solution of NBP in ethanol).[31] The slurry was filtered. The filter cake was washed with $CH_3CN$ (3 × 20 mL). The filtrates were combined and evaporated under reduced pressure. The residue was dried in a vacuum desiccator in the presence of $P_2O_5$ and KOH to constant weight yielding *N,N'*-bis-(4,6-dimethoxy-1,3,5-triazin-2-yl)quinine bis-tetrafluoroborate (**4**) (56.18 g, yield 58%) in the form of pale yellow oil.

$^1$H NMR (250 MHz, $CD_3CN$) δ 1.39-2.39 (m, 6H, $CH_2$); 3.58-3.96 (m, 2H, N-$CH_2$); 3.76-4.16 (m, 3H, N-$CH_2$ + N-CH); 3.89 (s, 3H, O-$CH_3$); 4.02 (s, 6H, 2 $OCH_3$); 4.05 (s, 6H, 2 $OCH_3$); 4.97, 5.01 (dd, 2H, $^1J$ 9Hz, $^2J$ 4.5Hz, $CH_2$=C); 5.44 (d, 1H, *J* 7Hz, CH-O); 5.85, 5.92 (dt, 1H, $^1J$ 9Hz $^2J$ 7Hz, C=CH); 7.40-8.03 (m, 4H, CH-arom); 9.25 (d, 1H, *J* 5.5Hz, $N^+$-$CH_{arom}$) [ppm].

***N,N'*-bis-(4,6-dimethoxy-1,3,5-triazin-2-yl)quinidine bis-tetrafluoroborate (7) (2DMT/quinidine/2BF$_4^-$).**

Quinidine (**5**) (32.44 g, 100 mmol) and ammonium tetrafluoroborate (21.00 g, 200 mmol) were dissolved in $CH_3CN$ (400 mL) and heated under reflux until all ammonia evolution ceased. The obtained clear solution was stirred and cooled to 0 °C, then solid $NaHCO_3$ (33.60 g, 400 mmol) was added. The slurry was treated with the solution of CDMT (**3**) (35.00 g; 200 mmol) in $CH_3CN$ (100 mL). Stirring was continued at 0–5°C until all the CDMT was consumed (TLC control, mobile phase 100% DCM, disappearance of spot $R_f$= 0.6, visualization by spraying with 0.5% solution of NBP in ethanol).[31] The slurry was filtered. The filter cake was washed with $CH_3CN$ (3 × 20 mL). The filtrates were combined and evaporated under reduced pressure. The colourless residue was dried in a vacuum desiccator in the presence of $P_2O_5$ and KOH to constant weight, yielding *N,N'*-bis-(4,6-dimethoxy-1,3,5-triazin-2-yl)quinidine bis-tetrafluoroborate (**7**) (54.62 g, yield 56%) in the form of a colourless oil.

$^1$H NMR (250 MHz, $CD_3CN$) δ = 1.39-2.39 (m, 6H, $CH_2$); 3.55-4.12 (m, 3H, N-$CH_2$ + N-H); 3.89 (s, 3H, $OCH_3$); 4.02 (s, 6H, 2 $OCH_3$); 4.05 (s, 6H, 2 $OCH_3$); 4.97, 5.01 (dd, 2H, $^1J$ 9Hz, $^2J$ 4.5Hz, $CH_2$=C); 5.42 (d, 1H, *J* 7Hz, CH-O); 5.85, 5.92 (dt, 1H, $^1J$ 9Hz $^2J$ 7Hz, C=CH); 7.40-8.03 (m, 4H, CH-arom); 9.17 (d, 1H, *J* 5.5Hz, $N^+$-$CH_{arom}$) [ppm].

**Optimization of carboxylic group activation of 4-methoxybenzoic acid (8) by treatment with 2DMT/quinine/2BF$_4^-$ (4) in the presence of quinine (1). Typical procedure 1.** To the intensively stirred solution of 4-methoxybenzoic acid (**8**) (0.152 g; 1 mmol) in $CH_3CN$ (5 mL) cooled to 0 °C (2DMT/quinine/2BF$_4^-$ (**4**) (0.780 g, 1 mmol) and quinine (0101 g, 0.3 mmol) dissolved in $CH_3CN$ (2 mL) were added. The progress of activation was monitored by TLC (mobile phase 100% DCM, TLC plate sprayed with 0.5% solution of NBP for visualization).

Stirring was continued for 2.5 h until all **4** was consumed. Solvent was evaporated under reduced pressure. The residue was dissolved in DCM (10 mL) and washed successively with water (3 mL), 1M aq. $NaHSO_4$, water (3 mL), 1M aq. $NaHCO_3$ (3mL) and again with water (3 mL). Organic phase was dried with $MgSO_4$, filtered, and filtrate concentrated under reduced pressure to dryness. The residue was dried in vacuum desiccator to constant weight. 2-(4-methoxybenzoyloxy)-4,6-dimethoxy-1,3,5-triazine (**9**) (0.180 g, yield 62%) was obtained as solid, mp. 73-75 °C, lit. [32] mp. 73-77 °C. IR film/NaCl: ν = 1788s, 1746s [$cm^{-1}$]. $^1$H-NMR: δ =3.87 (s, 3H), 4.00 (s, 6H), 7.43-8.09 (m, 4H) [ppm].

**Copling of *rac*-Z-Ala-OH (11) with H-Phe-OMe (13a) in the presence of 2DMT/quinine/2$BF_4^-$ (4). Synthesis of 14a. Typical Procedure 2.** To the vigorously stirred solution of 2DMT/quinine/2$BF_4^-$ (**4**) (0.780 g; 1 mmol) in $CH_3CN$ (5 mL), cooled to 0°C, quinine (0.101 g; 0.31 nmol) and *rac*-Z-Ala-OH (0.446 g; 2 mmol) were added. After 10 min. HCl∗H-Phe-OMe (0.216 g; 1 mmol) and NMM (0.110 mL; 1 mmol) were added and stirring was continued for 12 h. Then, solvent was evaporated under reduced pressure and the solid residue was dissolved in DCM (5 mL) and successively washed with water (3 mL), 1M aq. HCl (3 mL), water (3 mL), 1M aq.$NaHCO_3$, again with water (3 mL). organic phase was dried with $MgSO_4$, filtered and filtrate was evaporated to dryness. The solid residue was dried in vacuum desiccator over $P_2O_5$ and KOH to the constant weight affording Z-D-Aola-Phe-OMe (0.231 g; 60% yield), mp = 97-99 °C, lit. mp = 99-101 °C.[24] IR (film): ν 3344, 3055, 3032, 2952; 2729, 2592, 2168, 1960, 1878, 1727, 1671, 1530,1449, 1343, 1248, 1118, 1070, 1033 [$cm^{-1}$]. $^1$H NMR (250 MHz; $CDCl_3$) δ 1.33 (d, 3H, *J* 7 Hz, $CH_3$-CH); 3.05 (d, 2H, *J* 7Hz, $CH_2$-CH); 3.75 (s, 3H, $CH_3O$); 4.44 (qu, 1H, *J* 7Hz, $CH_3$-CH-); 4.84 (q, 1H, *J* 7 Hz, $CH_2$-CH-NH); 5.09 (s, 2H, $CH_2O$); 5.30 (s, 1H, NHCOO); 6.70 (d, 1H, *J* 7 Hz, CONH); 7.08-7.85 (m, 10H, $C_{arom}$) [ppm].

GC: capilar Chirasil-Val (25 m × 0,32 mm, film 0.2 μm), FID ($H_2$/air), split 1:30. carrier gas: helium, pressure 0.45 atm. Temperature program: 4 min at 90 °C, rising 4 °C/min to 200 °C, and then 3 min at 200 °C. $R_t$ 3.35 (D-Ala); $R_t$ 3.56 (L-Ala); $R_t$ 18.74 (L-Phe); L/D (Ala) = 0.5/99.5.

**Coupling of *rac*-Z-Phe-OH (12) with H-Gly-OMe (13g) in the presence of 2DMT/quinidine/2$BF_4^-$ (7). Synthesis of 15g. Typical Procedure 3.** To the vigorously stirred solution of 2DMT/quinidine/2$BF_4^-$ (**7**) (0.780 g; 1 mmol) in $CH_3CN$ (5 mL), cooled to 0°C, quinidine (0.101 g; 0.31 nmol) and *rac*-Z-Phe-OH (**12**) (0.599 g; 2 mmol) were added. After 10 min. HCl∗H-Gly-OMe (**13g**) (0.125 g; 1 mmol) and *N*-methylmorpholine (NMM) (0.110 mL; 1 mM) were added and stirring was continued for 12 h. Then, solvent was evaporated under reduced pressure and the solid residue was dissolved in DCM (5 mL) and successively washed with water (3 mL), 1M aq. HCl (3 mL), water (3 mL), 1M aq. $NaHCO_3$, again with water (3 mL). organic phase was dried with $MgSO_4$, filtered and filtrate was evaporated to dryness. The solid residue was dried in vacuum desiccator over $P_2O_5$ and KOH to the constant weight affording Z-D-Phe-Gly-OMe (**15g**) (0.207 g, yield 56%), mp = 110-111 °C, lit. mp = 111-112 °C.[33]

IR (film): ν 3305, 3064, 2935, 2426, 1881, 1816, 1734, 1689, 1656, 1535, 1441, 1384, 1334, 1294, 1248, 1144, 1079, 1040 [$cm^{-1}$]. $^1$H NMR (250 MHz; $CDCl_3$) δ 3.08 (d, 2H, *J* 6.5 Hz, CH-$CH_2$-$C_6H_5$); 3.92, 4.00 (d AB system, 2H, $^1$*J* 14 Hz, $^2$*J* 8 Hz, -NH-$CH_2$-COO-); 3.98 (s, 3H, $CH_3$-O-); 4.25-4.35 (m, 1H, -CH-$CH_2$-$C_6H_5$); 5.07 (s, 2H, -$CH_2$O-); 5.38 (broad d, 1H, *J* 5 Hz, OCONH); 7.07-7.45 (m, 11H, arom. + NH) [ppm].

GC: capilar Chirasil-Val (25 m × 0,32 mm, film 0.2 μm), FID ($H_2$/air), split 1:30. carrier gas: helium, pressure 0.45 atm. Temperature program: 4 min. at 90 °C, rising 4 °C/min. to 200 °C, and then 3 min at 200 °C. GC: $R_t$ 4.75 (Gly); $R_t$ 18.57 (L-Phe); $R_t$ 18.15 (D-Phe); L-Phe/D-Phe = 2/98.

## Acknowledgements

The authors thank Lodz University of Technology for financial support.

## Supplementary Material

Supplementary material available. Coupling conditions, GC analysis of enantiomeric composition of **14b-g** and **15b-f** are presented.

## References

1. Kaminski, Z. J.; Zajac, K. J.; Jastrzabek, K. *Acta Biochim. Pol.* **2001**, *48*, 1143-1146. https://doi.org/10.18388/abp.2001_3880
2. Kaminski, Z. J.; Kolesinska, B.; Markowicz, S. W.; Pokrzeptowicz; K. *Pol. J. Chem.* **1999**, *73*, 1965-1968.
3. Takero, T.; Mikito, D.; Toshikazu, K.; Makoto, O. *Tetrahedron Lett*. **1981**, *22*, 1109-1112. https://doi.org/10.1016/S0040-4039(01)90249-7
4. Takeda, K.; Tsuboyama, K.; Suzuki, A.; Ogura, H. *Chem. Pharm. Bull*. **1985**, *33*, 2545-2548. https://doi.org/10.1248/cpb.33.2545
5. Teramoto, T.; Kurosaki, T.; Okawara, M. *Tetrahedron Lett.* **1977**, *18*, 1523-1526. https://doi.org/10.1016/S0040-4039(01)93092-8
6. Ötvös, L.; Tömösközi, I.; Mohácsi, T. *Tetrahedron Lett*. **1970**, *11*, 1995-1998. https://doi.org/10.1016/S0040-4039(01)98136-5
7. Frost, A. B.; Greenhalgh, M. D.; Munday, E. S.; Musolino, S. F.; Taylor, J. E.; Smith, A. D. *Org. Reactions* **2020**, *104*, 1–498, Ed. Evans, P. A. ISBN: 978-1-119-65150-5. https://doi.org/10.1002/0471264180.or104.01
8. Krasnov, V. P.; Gruzdev, D. A.; Levit, G. L. *Eur. J. Org. Chem*. **2012**, 1471-1493. https://doi.org/10.1002/ejoc.201101489
9. Bhadra, S.; Yamamoto, H. *Chem. Rev.* **2018**, *118*, 3391-3446. https://doi.org/10.1021/acs.chemrev.7b00514
10. Pellissier, H. *Adv. Synth. Catal.* **2011**, *353*, 1613-1666. https://doi.org/10.1002/adsc.201100111
11. Fu, G. C. *Acc. Chem. Res.* **2000**, *33*, 412–420. https://doi.org/10.1021/ar990077w
12. Fu, G. C. *Acc. Chem. Res.* **2006**, *39*, 853-860. https://doi.org/10.1021/ar068115g
13. Vedejs, E.; Xinhai, C. *J. Am. Chem. Soc*. **1996**, *118*, 1809–1810. https://doi.org/10.1021/ja953631f
14. Murugan, R.; Scriven, E. F. V. *Aldrichimica Acta* **2003**, *36*, 21-27.
15. Birman, V. B. *Aldrichimica Acta*, **2016**, *49*, 23-33.
16. Vedejs, E.; Daugulis, O.; Diver, S. T. *J. Org. Chem*. **1996**, *61*, 430-431. https://doi.org/10.1021/jo951661v
17. Vedejs, E.; Daugulis, O. *J. Am. Chem. Soc.* **1999**, *121*, 5813-5814.

https://doi.org/10.1021/Ja990133O
18. Vedejs, E.; Chen, X. J. *Am. Chem. Soc*. **1997**, *119*, 2584–2585. https://doi:10.1021/JA963666V
19. Oriyama, T.; Imai, K.; Hosoya, T.; Sano, T. *Tetrahedron Lett*. **1998**, *39*, 397-400. https://doi.org/10.1016/S0040-4039(97)10558-5
20. Spivey, A. C.; Maddaford, A.; Redgrave, A. J. *Org. Prep. Proced. Int*. **2000**, 32, 331-365. https://doi.org/10.1080/00304940009355935
21. Song, J.; Zheng, W.-H. *Org. Lett*. **2022**, *24*, 2349-2353.
22. Guthrie, R. D. *Pure Appl. Chem.* **1989**, *61*, 23-56.
23. Kaminski, Z. J.; Kolesinska, B.; Kolesinska, J.; Sabatino, G.; Chelli, M.; Rovero, P.; Blaszczyk, M.; Glowka, M. L.; Papini, A. M. *J. Am. Chem. Soc*. **2005**, *127*, 16912-16920. https://doi.org/10.1021/ja054260y
24. Kolesińska, B.; Kamiński, Z. J. *Org. Lett.* **2009**, *11*, 765-768. https://doi.org/10.1021/ol802691x
25. Kolesińska, B.; Kasperowicz, K.; Sochacki, M.; Mazur, A.; Jankowski, S.; Kamiński, Z J. *Tetrahedron Lett.* **2010**, *51*, 20-22. https://doi,org/10.1016/j.tetlet.2009.06.105
26. Kolesinska, B.; Kasperowicz-Frankowska, K.; Fraczyk, J.; Kaminski, Z. J. *Helv. Chim. Acta*, **2012**, *95*, 2084-2098. https://doi.org/10.1002/hlca.201200426
27. Kasperowicz-Frankowska, K.; Gzik, A.; Dziemidkiewicz, M.; Kolesinska, B.; Kaminski, Z. J. *Acta Pol. Pharm*. **2014**, *71*, 994-1003.
28. Kasperowicz-Frankowska, K.; Kolesinska, B.; Gzik, A.; Jastrzabek, K.; Kaminski, Z. J. *Acta Pol. Pharm*. **2018**, *75*, 921-927. https://doi.org/10.32383/appdr/78726
29. Sukiennik, J.; Kasperowicz-Frankowska, K.; Szczesio, M.; Fraczyk, J.; Kolesinska, B.; Kaminski, Z. J. *Arkivoc*, **2021**, (ii), 85-98. https://doi.org/10.24820/ark.5550190.p011.363
30. Kaminski, Z. J.; Kolesinska, B.; Marcinkowska, M. *Synth. Commun*. **2004**, *34*, 3349-3358. https://doi.org/10.1081/SCC-200030581
31. Epstein, J.; Rosenthal, R. W.; Ess, R. J. *Anal. Chem*. **1955**, *27*, 1435-1439. https://doi.org/10.1021/ac60105a022
32. Kaminski, Z. J. *J. Prakt. Chem*. **1990**, *332*, 579-583.
33. Young, R. W.; Wood, K. H.; Joyce, R. J.; Anderson, G. W. *J. Am. Chem. Soc*. **1956**, *78*, 2126-2131.