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A simple route towards peptide analogues containing substituted (*S*)- or (*R*)-tryptophans

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ABSTRACT

We investigated the Lewis acid-promoted Friedel–Crafts alkylation of indole and substituted indoles with dehydroalanine-containing dipeptides *R*-Xaa-Dha-OR[1]. The reaction proceeded with modest to sufficient diastereoselectivity, and yields strongly varied depending on the Lewis acid selected. The substituent R^1 of the ester group revealed some impact on the preferential formation of (*S*)-Trp or (*R*)-Trp. We exploited the reaction to prepare different peptides containing substituted tryptophans. To test the efficacy of this method for preparing biologically relevant compounds, we synthesized two unprecedented analogues of endomorphin-1, the endogenous agonist of the μ-opioid receptor, having either (*S*)- or (*R*)-2-methyltryptophan in position 3.

1. Introduction

Peptides based on natural amino acids are widely used as therapeutic agents.[1] However, their efficacy is hampered by several problems, in particular high conformational flexibility, low in vivo stability against proteolysis and scarce ability to cross biological barriers, resulting in poor receptor selectivity, short duration of action and poor bioavailability.

The preparation of unusual amino acids and their introduction in peptide sequences have attracted considerable interest to overcome the pharmacological limitations of bioactive peptides.[1,2] Modification of individual amino acids has been shown to be responsible for changes in peptide conformation and for increased enzymatic stability.[3] Besides, the introduction of modified amino acids has been utilized for the elucidation of an individual residue's biological function.[3,4]

In connection with ongoing projects[5] on the synthesis, conformational analysis and pharmacological behaviour of endomorphin-1[6] (H-Tyr-Pro-Trp-Phe-NH_2) analogues as potential drugs for pain management,[7] we have been interested in the preparation of new peptide derivatives containing modified tryptophans. Several examples of tryptophans bearing substituent on the indole ring can be found in complex structures. For instance, halotryptophans are present in peptides of microbial[8] or marine origins.[9]

A few preparations of (*S*)- or (*R*)-Trp analogues have been reported in the literature.[10] Halotryptophans can be obtained by palladium-mediated heteroannulation of a chiral auxiliary;[11] by electrochemical oxidation of proline followed by Fischer indole synthesis;[12] from serine, by means of the lysate of a commercially available microorganism containing tryptophan synthase.[13] (*S*)- and (*R*)-Br-tryptophans have been obtained with L-aminoacylase or D-aminoacylase.[14] More often, reactions lead to the preparation of racemates. Very recently, the regioselective electrophilic substitution of indoles with *N*-acetyl dehydroalanine (Dha), promoted by different transition metal salts, provided an efficient protocol towards racemic-functionalized tryptophans.[15]

In this context, we envisaged the opportunity to develop a simple procedure to synthesize modified (*S*)- or (*R*)-tryptophans within a peptide sequence. Herein, we report our results on the Friedel–Crafts alkylation of indoles[15,16] with dipeptides containing dehydroalanine (Xaa-Dha) in the presence of different Lewis acids.

Further, to endorse the methodology, we describe the preliminary synthesis in a few steps of two novel endomorphin-1[6] analogues, [(*S*)-2-MeTrp]-endomorphin-1 and [(*R*)-2-MeTrp]-endomorphin-1. Among the different endogenous opioid peptides,[1] endomophin-1 is unique for high receptor affinity and selectivity, being considered the endogenous agonist of the μ-opioid receptor (MOR). Since none of the other naturally occurring opioid peptides contain a Trp in the sequence, the preparation and pharmacological assay of endomorphin-1 analogues containing modified Trp in position 3 could be of help to investigate the role of this pharmacophore in ligand-receptor recognition.[17] Besides, the presence of substituted Trp can also enhance lipophilicity and blood–brain barrier (BBB) permeability of peptide active towards the CNS.[18]

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2. Results and discussion

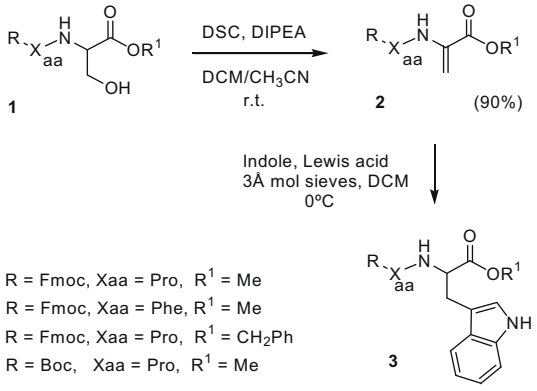
As anticipated in the introduction, indoles undergo Friedel-Crafts (F-C.) alkylation with *N*-Ac-Dha methyl ester in the presence of Lewis acids, giving racemic 3-indolyl-α-amino acids.[15] Since we were interested in optically pure amino acids, we thought to carry out a diastereoselective version of this reaction with dipeptides of type Xaa-Dha, taking advantage of the asymmetric induction exerted by Xaa. The protected dipeptides Xaa-Dha **2** were easily obtained from dipeptides Xaa-serine **1**, prepared in turn by standard in-solution peptide synthesis, using 1-ethyl-3-[3-dimethylaminopropyl]carbodiimide hydrochloride (EDC-HCl) and 1-hydroxybenzotriazole hydrate (HOBt) as activating agents (Supplementary data). The dehydration of **1** with *N*,*N*′-disuccinimidyl carbonate (DSC) and *N*,*N*-diisopropylethylamine (DIPEA) gave **2** in very good yield,[19] isolated by flash chromatography over silica gel (Scheme 1).

Initially, the reaction was tested with indole. The treatment of Pro-Dha **2a** with a Lewis acid and indole, in the presence of 3 Å molecular sieves, afforded the protected dipeptide Pro-Trp **3a** as a mixture of diastereoisomers (Scheme 1). Yields and diastereomeric ratios strongly varied depending on the Lewis acid selected (Table 1). In the absence of molecular sieves, the reaction gave variable quantities of by-products arising from peptide bond and/or ester hydrolysis, as revealed by reversed phase (RP)-HPLC and electron-spray (ES)-MS analyses. On the other hand, molecular sieves lead to better yields, while the unreacted starting material was recovered unaltered.

The diastereomeric ratios of the reaction mixtures (Table 1) were measured by normal phase-HPLC, with an analytical Kromasil Diol column. The separation of the diastereoisomers by analytical RP-HPLC under different conditions was unfeasible. Racemization was excluded on the basis of chiral HPLC by using a CHIRALPAK IC column. The configuration of the newly created stereocentre on Trp was determined by comparison with that of the authentic samples of (*S*,*S*)-**3a** and (*S*,*R*)-**3a**, prepared by standard peptide synthesis in solution from the commercially available amino acids. Yields (Table 1) were determined after isolation by flash chromatography over silica gel.

The use of 1 equiv of $ZnOTf_2$, $FeCl_3$, InF_3, $CeCl_3$, $RuCl_3$ and $Yb(OTf)_3$ gave no reaction or traces of the dipeptide **3a**. Other Lewis acids, $MgBr_2$, BBu_2OTf, $TiCl_4$, $Cu(OTf)_2$, $Sc(OTf)_3$, $ZrCl_4$, $AlEtCl_2$ and $AlEt_2Cl$ (1 equiv), gave the product **3a** in low yields (<5%, data not shown). All reactions were carried out at 0 °C for 24 h in dichloromethane (DCM).

Increasing time and temperature gave negligible improvements. The only exception was $Yb(OTf)_3$; indeed, performing the reaction with 1 equiv of $Yb(OTf)_3$ at 80 °C in dichloroethane



Scheme 1. Synthesis of the protected dipeptides Xaa-Dha **2a–d** and Lewis acid-induced F-C alkylation of indole.

Table 1
Synthesis of dipeptides Xaa-Trp **3a–d** by F-C reaction of indole with dipeptides Xaa-Dha **2a–d** promoted by different Lewis acids, at 0 °C for 24 h in DCM (with the exception of entries 1 and 2)

Entry	Lewis acid	Equiv	**3**	Yield[c] (%)	*S*,*S*/*S*,*R*
1	$Yb(OTf)_3$[a]	1	**a**	20	—
2	$Yb(OTf)_3$[b]	1	**a**	55	22/78
3	$MgBr_2$	4	**a**	5	—
4	BBu_2OTf	2	**a**	5	—
5	$TiCl_4$	4	**a**	15	—
6	$CuOTf_2$	2	**a**	10	—
7	$ScOTf_3$	2	**a**	10	—
8	$ZrCl_4$	2	**a**	25	—
9	$AlEtCl_2$	3.5	**a**	70	50/50
10	$AlEt_2Cl$	3.5	**a**	60	24/76
11	$AlEtCl_2$	3.5	**b**	65	45/55
12	$AlEt_2Cl$	3.5	**b**	55	35/65
13	$AlEtCl_2$	3.5	**c**	65	55/45
14	$AlEt_2Cl$	3.5	**c**	55	62/38
15	$AlEtCl_2$	3.5	**d**	55	50/50
16	$AlEt_2Cl$	3.5	**d**	35	30/70

[a] DCE, reflux.
[b] DCE, MW, 400 W.
[c] After isolation by flash chromatography over silica gel.

(DCE) for 24 h, **3a** was obtained in 20% yield (entry 1) and when the reaction was conducted under microwave (MW) irradiation (400 W) for 3.5 h, yield increased up to 55%, with a satisfactory 22/78 diastereomeric ratio in favour of (*S*,*R*)-**3a** (entry 2).

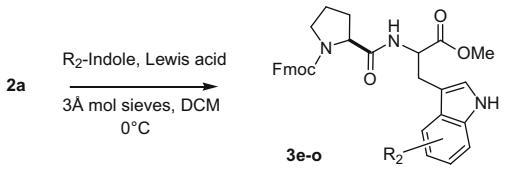
Making use of a catalytic amount of $Yb(OTf)_3$ led to a drop of the yield, while the use of a excess seemed inexpedient, for the high molecular weight of such Lewis acid. Reactions with $MgBr_2$, BBu_2OTf, $TiCl_4$, $Cu(OTf)_2$, $Sc(OTf)_3$ and $ZrCl_4$ were slightly improved by increasing the amount of Lewis acid (yields 5–25%, Table 1, entries 3–8).

Finally, in the presence of an excess of $AlEtCl_2$ (3.5 equiv)[15] the reaction gave the desired dipeptide **3a** in reasonable yield, after isolation by flash chromatography over silica gel, albeit with a disappointing 50/50 diastereomeric ratio (entry 9). On the other hand, in the presence of $AlEt_2Cl$ (3.5 equiv) the reaction gave lesser amount of **3a**, but with a reasonable 24/76 diastereomeric ratio, in favour of the (*S*,*R*)-**3a** stereoisomer (entry 10).

The eventual effect of the residue Xaa preceding Dha and the protecting groups R, R^1 on yield and stereoselectivity was examined. The reaction of indole with the dipeptide Phe-Dha **2b** occurred with comparable yield, but with lower stereoselectivity (entries 11 and 12). Changing the methyl ester with a benzyl ester (**2c**) lead to a moderate stereoselectivity, in favour of the isomer (*S*,*S*)-**3c** (entries 13 and 14). The presence of a Boc-protecting group (**2d**) instead of Fmoc gave lower yield and selectivity (entries 15 and 16).

The configuration of Trp in the dipeptides **3b–d** was determined by comparison with authentic samples, prepared by standard peptide synthesis in solution. Interestingly, apart from the slightly different yields, changing the ester allowed preparing preferentially the dipeptides containing either (*S*)-Trp or (*R*)-Trp. Besides, the analysis of the crude reaction mixtures revealed no trace of concurrent α-amidoalkylation reaction.[15,20]

The reaction of **2a** with substituted indoles in the presence of 3.5 equiv of $AlEt_2Cl$ or $AlEtCl_2$ (Scheme 2) was performed at 0 °C for 24 h in DCM and in the presence of 3 Å molecular sieves (Supplementary data). The reaction afforded the dipeptides **3e–o** (Table 2). For the moment, we were mainly interested in verifying the applicability of the procedure to diverse kinds of indoles, therefore the diastereoselectivity issue was not faced in detail. The absolute configurations of the diastereoisomers were determined by comparison of the chiral-HPLC analyses with that of **3a**.



Scheme 2. Synthesis of the dipeptides **3e–o** by Lewis acid-induced F-C alkylation of substituted indoles.

The reaction of **2a** with 5′-fluoroindole promoted by $AlEtCl_2$ (entry 1) or $AlEt_2Cl$ (entry 2) gave **3e** in sufficient yield, albeit only $AlEt_2Cl$ ensured acceptable stereoselectivity. The reaction of **2a** and 5′-chloro indole or 5′-bromoindole with $AlEtCl_2$ proceeded in lower yields of **3f** and **3g**, respectively (entries 3 and 4), while $AlEt_2Cl$ was almost ineffective.

In a similar way, **2a** reacted with 1-methylindole in the presence of $AlEtCl_2$ affording **3h** (entry 5) with scarce selectivity. The reaction with 2-methylindole in the presence of $AlEtCl_2$ (entry 6) or $AlEt_2Cl$ (entry 7) gave **3i** in excellent yields. 7-Methylindole, 2′-methyl-7′-bromoindole, 2′-methyl-5′-bromoindole and 2-aminophenylindole gave **3l**, **3m**, **3n** and **3o**, respectively (entries 8–11), in sufficient to good yields. Finally, 5-nitroindole scarcely reacted with **2a** giving **3p** (entry 12), while 2-benzylsulfonylindole gave only traces of the corresponding product (not shown).

In addition, we confirmed that changing the methyl ester with a benzyl ester gave a moderate switch of stereoselectivity, from (*S*,*R*) to (*S*,*S*), also with some substituted indoles. Indeed, the reaction of 5′F-indole with 2c in the presence of $AlEt_2Cl$, under the same conditions reported for 2a, gave the 5′F-Trp-containing dipeptide in 55% yield and 65/35 d.r., while 2′Me-indole gave the corresponding product in 70% yield with the same d.r.

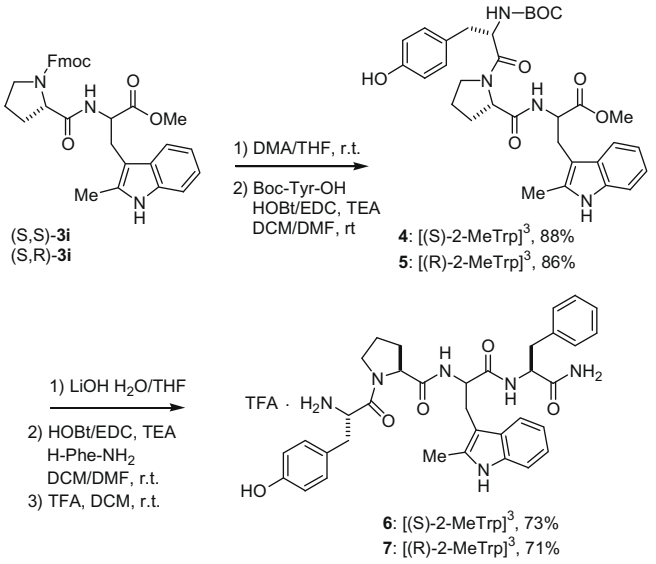
In order to validate the procedure as a facile access to analogues containing modified Trp in either (*S*) or (*R*) configuration, we synthesized in a few steps the unprecedented analogues of endomorphin-1 **6** and **7**, containing (*S*)-2-MeTrp and (*R*)-2-MeTrp, respectively (Scheme 3).

The endomorphin analogues **6** and **7** were obtained from the dipeptides (*S*,*S*)-**3i** and (*S*,*R*)-**3i**, obtained in turn by alkylation of 2-methylindole with **2a** (Table 2, entry 6). The identities of all the intermediates were confirmed by ES-MS analyses. Purities were determined by RP-HPLC and by normal phase HPLC. After separation by flash chromatography over silica gel, (*S*,*S*)-**3i** (41%) and (*S*,*R*)-**3i** (49%) were quantitatively deprotected by treatment with 2 M DMA in THF and reacted without prior purification with Boc-Tyr-OH in the presence of HOBt/EDC. The tripeptides Boc-Tyr-Pro-[(*S*)-2-MeTrp]-OMe, or Boc-Tyr-Pro-[(*R*)-2-MeTrp]-OMe, were isolated in high yield by flash chromatography over silica gel. The tripeptides were treated with LiOH in H_2O/THF and the resulting tripeptide acids were utilized without further purification.

Table 2
Synthesis of dipeptides **3e–p** by F-C. reaction of substituted indoles with **2a**, promoted by 3.5 equiv of Lewis acids, at 0 °C for 24 h in DCM.

Entry	R_2	Lewis acid	**3**	Yield[a] (%)	*S*,*S*/*S*,*R*
1	5′-F	$AlEtCl_2$	**e**	65	45/55
2	5′-F	$AlEt_2Cl$	**e**	55	25/75
3	5′-Cl	$AlEtCl_2$	**f**	30	45/55
4	5′-Br	$AlEtCl_2$	**g**	20	—
5	1′-Me	$AlEtCl_2$	**h**	45	45/55
6	2′-Me	$AlEtCl_2$	**i**	90	45/55
7	2′-Me	$AlEt_2Cl$	**i**	75	28/72
8	7′-Me	$AlEtCl_2$	**l**	60	40/60
9	2′-Me-7′-Br	$AlEtCl_2$	**m**	50	40/60
10	2′-Me-5′-Br	$AlEtCl_2$	**n**	40	40/60
11	2-amino Ph	$AlEtCl_2$	**o**	50	—
12	5′-NO_2	$AlEtCl_2$	**p**	15	—

[a] After isolation by flash chromatography over silica gel.



Scheme 3. Synthesis of the endomorphin-1 analogues **6** and **7** starting from the dipeptides (*S*,*S*)-**3i** and (*S*,*R*)-**3i**.

Activation of the tripeptide acids with HOBt/EDC and coupling with H-Phe-NH_2 gave Boc-**6** and Boc-**7**, isolated by flash chromatography. Final deprotection with TFA gave the endomorphin-1 analogues **6** and **7** in good yields, 95% pure after semi-preparative RP-HPLC.

3. Conclusions

In summary, we have proposed a practical route for the preparation of protected dipeptides containing substituted tryptophans in either (*S*) or (*R*) configuration. The F-C alkylation of dipeptides containing Dha allowed the direct asymmetric synthesis of the tryptophanes within the peptidic structure.

The reaction gave (*S*,*S*)- or (*S*,*R*)-dipeptides, albeit with moderate stereoselectivity. After isolation by normal-phase chromatography, the diastereomeric dipeptides can be readily utilized for preparing peptides of pharmacological interest. As a preliminary demonstration, we synthesized analogues of endomorphin-1 with a 2-methyltryptophan in position 3.

Work is in progress to expand the scope of the reaction, in particular to improve the stereoselectivity. Further, the procedure will be utilized to synthesize a mini-library of endomorphin analogues, aiming to obtain new opioid peptides with improved performances as in vivo analgesic,[21] as well as new clues about the role of Trp in receptor recognition and activation.

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Supplementary data

Supplementary data (general experimental methods, typical reaction conditions and spectroscopic characterization of representative compounds) associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2010.03.017.

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