Thesis of doctoral (Ph.D.) dissertation

Synthesis of aminoflavones and flavone-amino acid hybrids by Buchwald-Hartwig reaction

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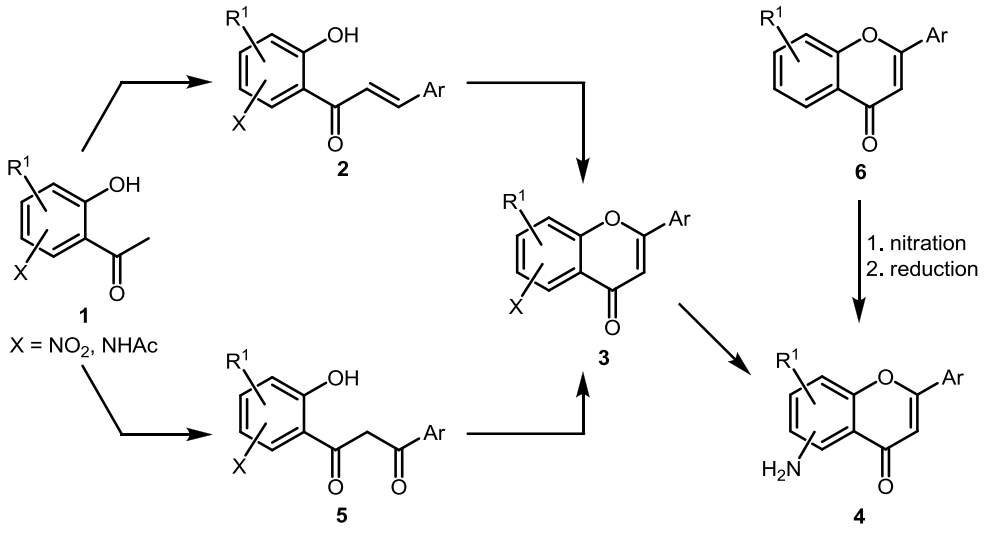
1. Introductions and objectives

Flavones are widespread in nature mostly as plant metabolites. Beside their frequent occurrence, flavones also show versatile biological activities, e. g. antioxidant, anti-inflammatory, and antimicrobial properties. It is worth noting that many aminoflavone derivatives have considerable enzyme inhibitory effects as α-glucosidase, tyrosin-kinase, and cyclin-dependent kinase inhibitors; in addition, antiproliferative, antitumor, cytotoxic effects and central nervous system protective properties were also observed.

Most of the syntheses of flavones are based on the corresponding chromone (4*H*-1-benzopyran-4-one) core structure by conventional methods. However, the synthesis of aminoflavones is suffering from limitations, especially in the case of derivatives which have their amine substituents linked to Ring A. The most common synthetic methods are based on either the Baker-Venkataraman rearrangement or the Claisen-Schmidt condensation (Scheme 1). In the first case an aroylation is followed by the Baker-Venkataraman rearrangement under strongly basic conditions. The same intermediates **3** are available by a base-induced aldol-type condensation giving the corresponding chalcone **2**, which, in turn, can be cyclized into a nitro- or acetamidoflavone **3**.

The targeted aminoflavones **4** can be prepared by hydrolysis or reduction, in this latter transformation harsh conditions are usually required (Scheme 1).

The main problem of these methods is the synthesis of the corresponding acetophenone **1** which is limited by the regioselectivity of the nitration of the acetophenone and the sensitivity of the R^1 substituents under harsh conditions. An alternative way is the nitration and reduction of a previously formed flavone moiety but the possibilities are also limited by the regioselectivity and reactivity patterns of the nitration step.



Scheme 1: Synthesis of aminoflavones by conventional methods

Synthesising flavone derivatives with alkyl/arylamino function represents a much greater challenge than the formation of the amino group. Due to the lack of selectivity, direct alkylation leads to low yields.

In the last few decades Buchwald-Hartwig amination of halo substituted aryl/heteroaryl systems became a useful method for synthesising alkyl- and arylamino substituted arenes. Nevertheless, in the field of flavone derivatives only sporadic examples have been published. Caddick *et al.* used the Buchwald-Hartwig reaction for amination of bromo- or triflyloxy substituted flavones under microwave activation but only *n-hexylamine* was used as nitrogen source.

Considering these information we decided to widely investigate the Buchwald-Hartwig reaction of differently substituted bromo- and chloroflavones trying to systematically examine the limitations of the coupling.

In the case of positive results we planned to react amino acid and peptide derivatives during the coupling. Our aim was to achieve selective Buchwald-Hartwig reactions of dibromoflavone derivatives with amines and amino acid esters. We also intended to measure the biological activity of the synthesized new compounds against cancerous cell lines.

2. Methods

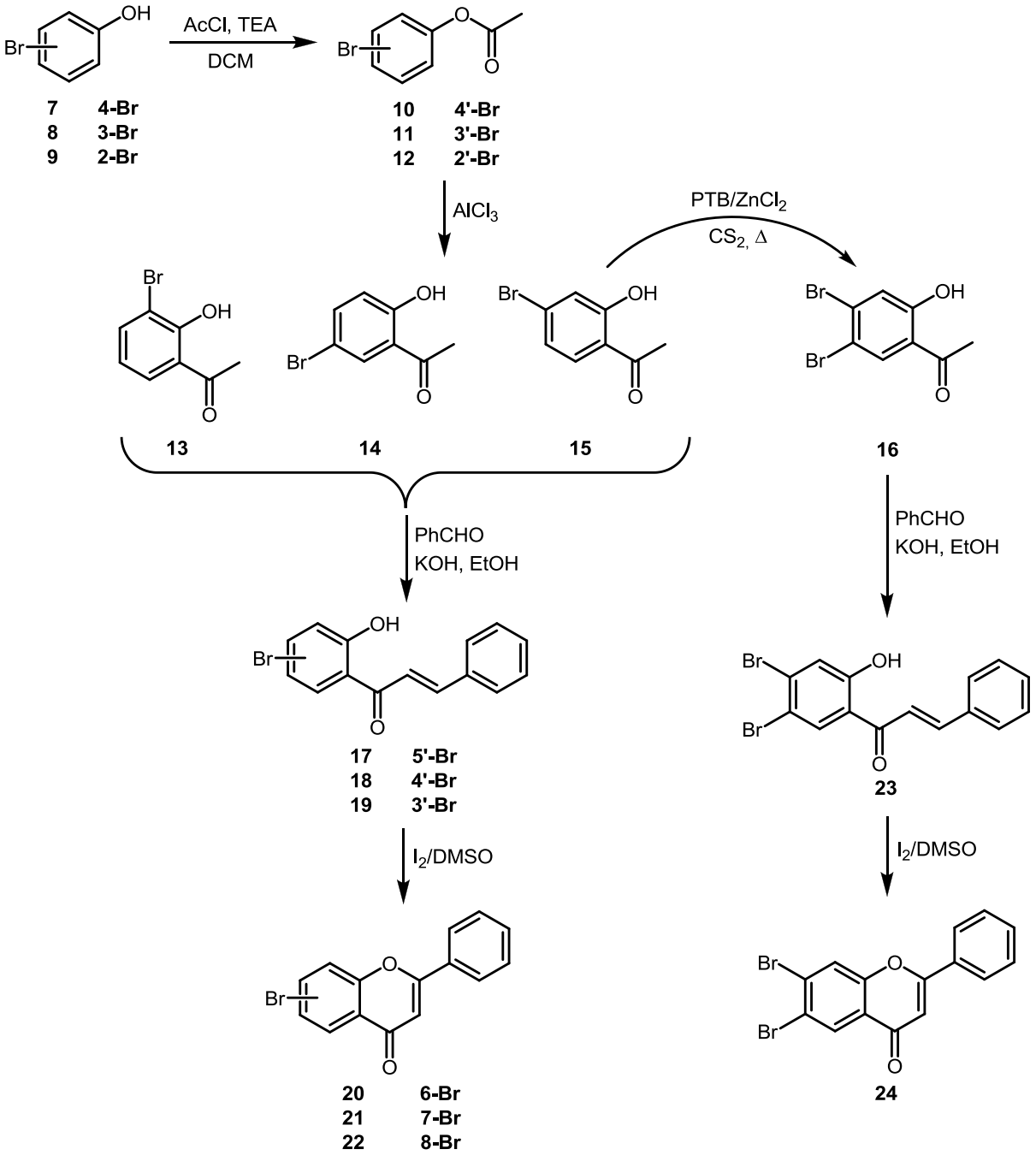
Methods of the modern preparative organic chemistry were applied in the course of the synthetic work. Reactions were monitored by thin-layer chromatography. Substrates and products of the reactions were purified by classic or flash column chromatography and/or crystallization. The products were characterised by classical analytical methods (melting point, elemental analysis), one and two dimensional NMR, IR spectroscopy, and mass spectrometry. The enantiomeric excess of the products coupled with amino acids was measured by chiral HPLC technique.

3. Results

3.1. Synthesis of the starting materials

During the synthetic work one dibromo- and three different monobromoflavones were used as starting materials. These substrates are not available commercially, so we had to synthesise them; starting from the corresponding bromophenol followed by the Claisen-Schmidt method (Scheme 2). While the Fries-rearrangement of 3'- and 4'-bromophenyl-acetate (**7** and **8**), under neat conditions provided the required acetophenones **10**, **11**, in the

case of 2'-bromophenyl-acetate (**9**), the neat conditions resulted the *para* rearranged product. In case of this latter molecule using 1,2-dichlorobenzene as solvent the *ortho* rearrangement became dominant. The 4',5'-dibromo-2'-hydroxyacetophenone (**16**) was synthesised by the bromination of the 4'-bromo-2'-hydroxyacetophenone, then the Claisen-Schmidt condensation followed by a ring closure provided the 6,7-dibromoflavone (**24**) in good yield (Scheme 2).



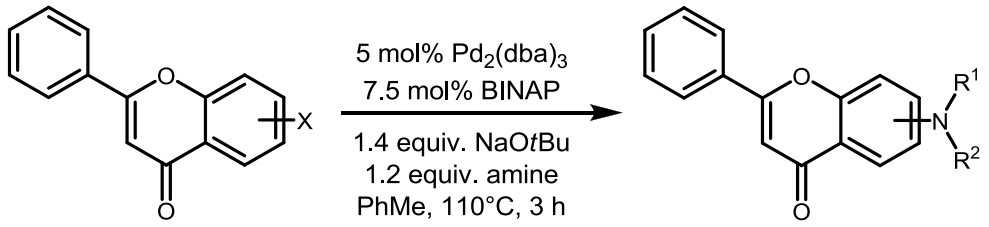
Scheme 2: Synthesis of the substrates through Claisen-Schmidt condensation

3.2 Buchwald-Hartwig reaction of monobromo- and monochloroflavones with amines

Using the conditions published by Caddick *et. al.*, we reacted the 6-, 7-, and 8-bromoflavone (**20-22**) with systematically chosen primary and secondary amines, just as with different aniline derivatives. We proved position 6 to be the least reactive; almost the same or better yields were achieved during the coupling into position 8, while the position 7 turned out to be the most reactive.

Aliphatic amines having an α-branching showed steric hindrance in the cross-coupling reactions, which was proved by using various butyl amine isomers.

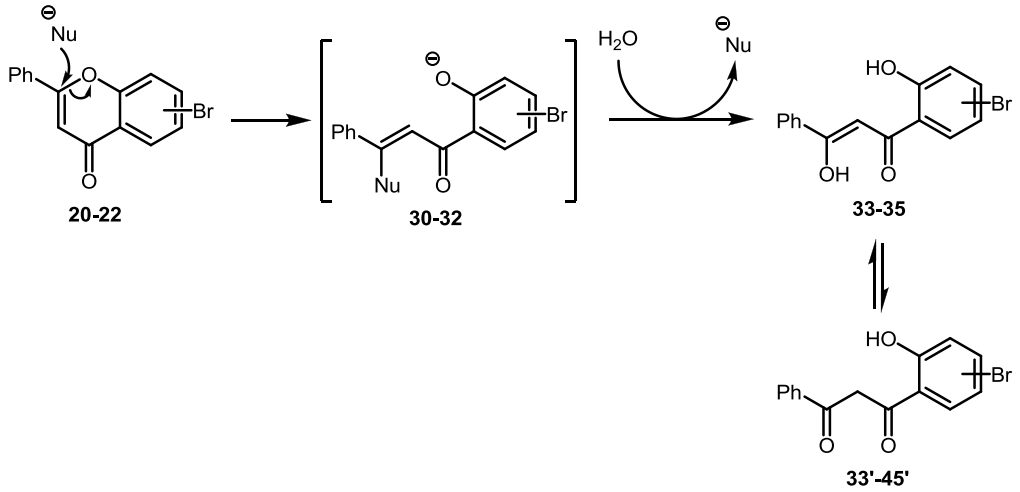
We also compared the reactivity of 6- (**25**) and 7-chloroflavone (**26**) with the corresponding bromo derivatives and determined that the reactivity of bromoflavones (**20**, **21**) significantly exceeds the reactivity of chloro derivatives (**25**, **26**); in addition, the reactivity difference between the position 6 and 7 between chloroflavones became even more obvious. (Table 1).



			27		**28**		**29**
Amine	R^1	R^2	6-Br (**20**)	6-Cl (**25**)	7-Br (**21**)	7-Cl (**26**)	8-Br (**22**)
a	Bu	H	46%	7.7%	81%	32%	51%
b	*i*Bu	H	46%	---	79%	---	---
c	*s*Bu	H	38%	---	70%	---	---
d	*t*Bu	H	0%	---	26%	---	---
e	Bn	H	59%	8.8%	89%	41%	66%
f	2-Me-C_6H_4	H	53%	---	---	---	---
g	4-Cl-C_6H_4	H	48%	6.5%	55%	36%	59%
h	4-MeO-C_6H_4	H	75%	---	74%	---	65%
i	Bu	Bu	18%	---	16%	---	---
j	-$(CH_2)_4$-		40%	7.3%	79%	38%	---
k	-$(CH_2)_5$-		58%	---	55%	---	---
l	-$(CH_2)_2O(CH_2)_2$-		51%	---	74%	---	47%
m	Ph	Me	31%	---	41%	---	---

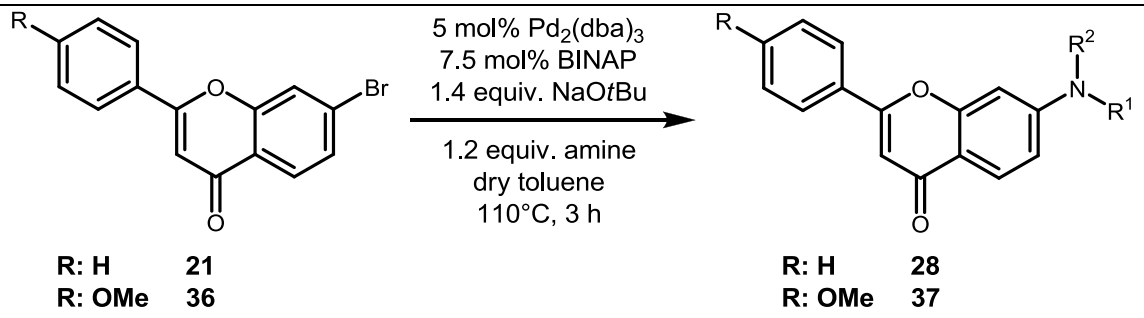
Table 1: Isolated yields of the Buchwald-Hartwig reaction of monobromo- and monochloroflavones with various amines

Under these reaction conditions a base induced side reaction was observed; the flavone ring was opened at carbon 2 by a nucleophile anion (Scheme 3). During the workup, the suppositional intermediate (**30**-**32**) was transformed into hydroxyl derivative (**33-35**), which was isolated. The rate of the ring-opening reaction showed the following tendency: 6-Br < 7-Br << 8-Br



Scheme 3: Ring opening side reaction of bromoflavones

The Buchwald-Hartwig reaction of 7-bromo-4'methoxyflavone was performed using the same reaction conditions. Almost the same or better yields were achieved; this could be explained by the minor ring-opening side-reaction, as well as the better crystallization properties of the products (Table 2).



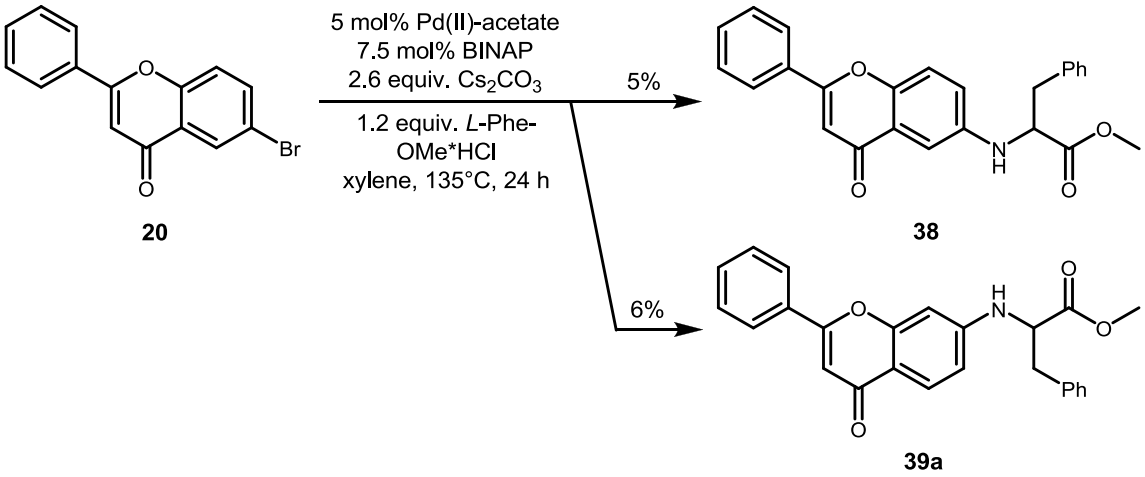
			28	**37**
Amine	R^1	R^2	7-Br (**21**)	7-Br-4'-OMe (**36**)
a	Bu	H	81%	84%
e	Bn	H	89%	71%
g	4-Cl-C_6H_4	H	55%	80%
h	4-MeO-C_6H_4	H	74%	70%
i	Bu	Bu	16%	13%
k	-$(CH_2)_5$-		55%	61%
m	Ph	Me	41%	60%

Table 2: Isolated yields in the case of 7-bromo- and 7-bromo-4'-methoxyflavone

3.3 Buchwald-Hartwig reaction of monobromoflavones with amino acid esters

3.3.1. Buchwald-Hartwig coupling of 6-bromoflavone with *L*-phenylalanine methyl ester

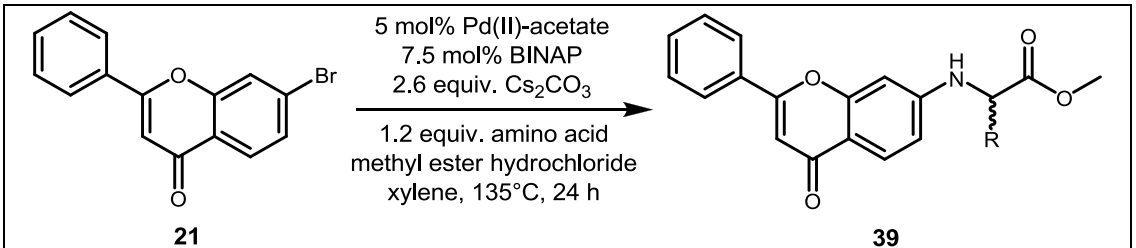
First we tested the coupling of 6-bromoflavone (**20**) with *L*-phenylalanine methyl ester, but the conditions previously used with the amines turned out to be inappropriate in this case. After a long optimisation procedure we found a condition where the corresponding 6-{[2-phenyl-1-(methoxycarbonyl)ethyl]amino}flavone (**38**) was isolated with a yield of 5%. In addition, the regioisomer 7-{[2-phenyl-1-(methoxycarbonyl)ethyl]amino}flavone (**39a**) was also isolated with 6% of yield (Scheme 4). This observation was explained by the halogen dance effect.



Scheme 4: Reaction of 6-bromoflavone with *L*-phenylalanine methyl ester

3.3.2. Buchwald-Hartwig coupling of 7-bromoflavone with amino acid esters

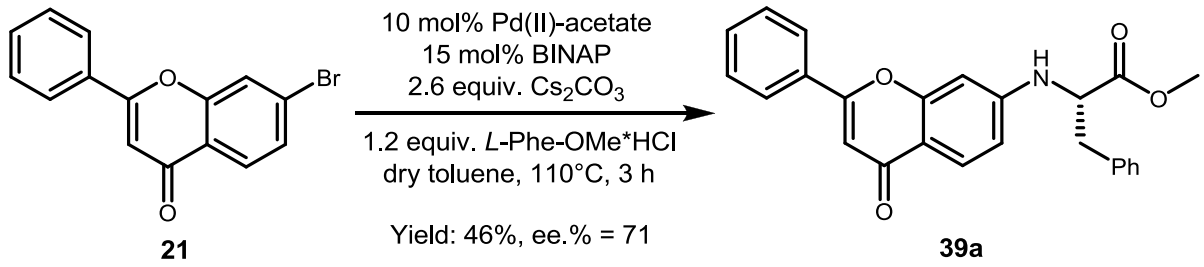
Under the modified conditions we also carried out the coupling reactions using 7-bromoflavone (**21**). The products were isolated with moderate to good yields. Under the used conditions the racemisation of the products **39** occurred. (Table 3)



39	**a**	**b**	**c**	**d**	**e**
R	Bn	Me	*i*Bu	$MeSCH_2CH_2$	CH_3OOCCH_2
Yield (%)	64	30	56	40	11

Table 3: Coupling of 7-bromoflavone with amino acid methyl ester under the yield optimised condition

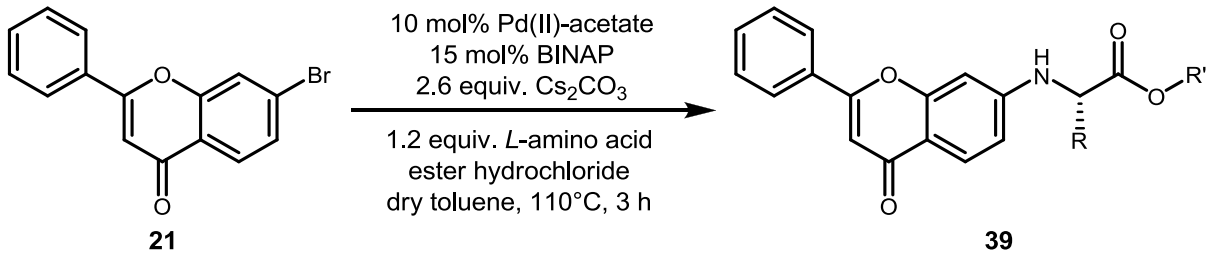
During a wide range optimisation in the case of *L*-phanylalanine methyl ester we found a condition where the coupled product was isolated in 46% yield with 71% enantiomeric excess. Although the high temperature and long reaction time increased the yield, the rate of the racemisation also increased. The BINAP appeared to be the most effective phosphane; not only good yields were achieved, but the isolated products also showed considerably higher enantiomeric excess (Scheme 5).



Scheme 5: Coupling of 7-bromoflavone with *L*-phenylalanine methyl ester

The 7-bromoflavone (**21**) was successfully coupled with many other amino acids as well under the optimised conditions; mostly moderate to high yields, and high to excellent enantiomeric excesses were achieved. The increase of the carbanion stabilizing effect of the sidechains resulted in the decrease of the enantiomeric excess of the products. (Table 4)

We also observed the coagulation of the catalyst during the reaction, caused by the overheating of the heterogenic system at the wall of the flask. Changing the flask to a pressure tube, we managed to prevent the coagulation and increase the isolated yields while keeping the good enantiomeric excesses.

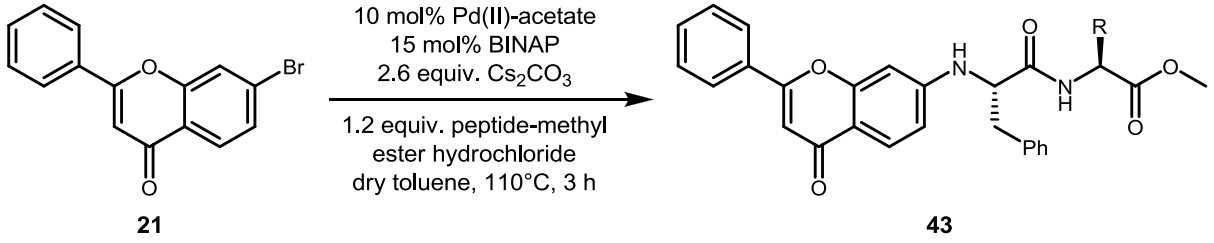


		a	**b**	**c**	**d**	**e**	**f**	**g**	**h**
	R	Bn	Me	*i*Bu	$MeSCH_2CH_2$	CH_3OOCCH_2	*i*Pr	$-CH_2CH_2CH_2-(N$	Ph
R': OMe	Yield (%)	74	65	66	75	30	37	26	59
	ee (%)	75	86	93	87	56	98	96	0
R': O*t*Bu	Yield (%)	78	73						
	ee (%)	11	36						

Table 4: Coupling of 7-bromoflavone with amino acid esters in the enantiomeric excess optimised condition

3.3.3. Coupling of 7-Bromoflavone with dipeptide esters

The coupling was extended to dipeptide methyl ester type compounds (**42a-c**) as well, made by peptide synthesis. The coupling with **42a,b** dipeptide methyl ester was successfully made (Table 5). Using the peptide containing a serine unit (**42c**), no successful reaction was achieved, which can be explained by the protic function group in the side-chain of the serine unit.



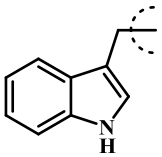
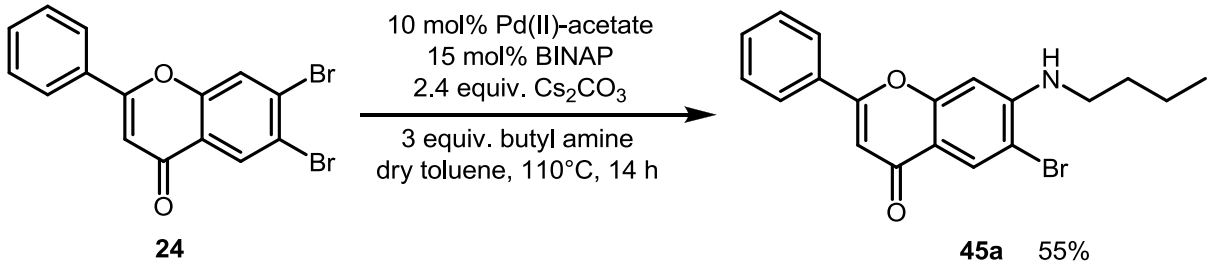
42	R	**43**	Yield (%)
42a	*i*Bu	**43a**	75
42b		**43b**	53
42c	CH_2OH	**43c**	0

Table 5: Coupling of 7-bromoflavone with peptide methyl ester

3.4. Buchwald-Hartwig reaction of 6,7-dibromoflavone with amines and amino acid esters

3.4.1. Coupling of 6,7-dibromoflavone with butyl amine – optimization reactions

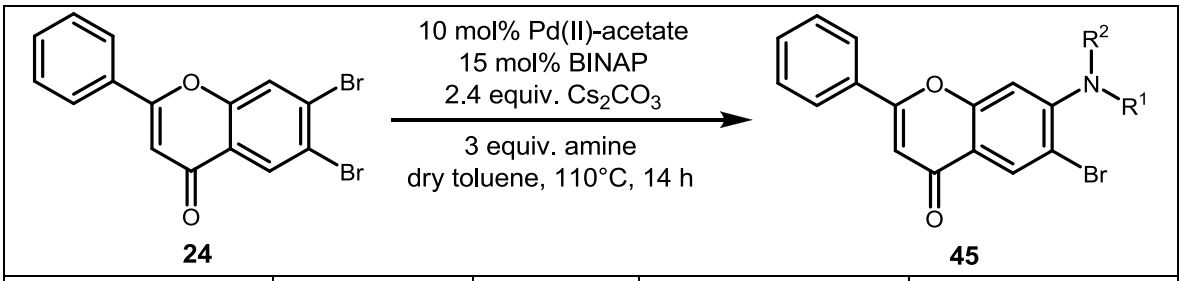
We optimized the Buchwald-Hartwig reaction of 6,7-dibromoflavone (**24**) with *n*-butyl amine. The reaction proved to be selective and only one regioisomer (**45a**) (coupled at position 7) was isolated (Scheme 6). This fact is another proof of the higher reactivity of the position 7. The structure of the product **45a** was confirmed by two-dimensional NMR and X-ray crystal structure analysis as well. The disubstituted product was not detected at all, however, the dehalogenated by-product 7-butylaminoflavone (**28a**) appeared.



Scheme 6: Coupling of 6,7-dibromoflavone with butyl amine – optimal condition

3.4.2. Coupling of 6,7-dibromoflavone with amines

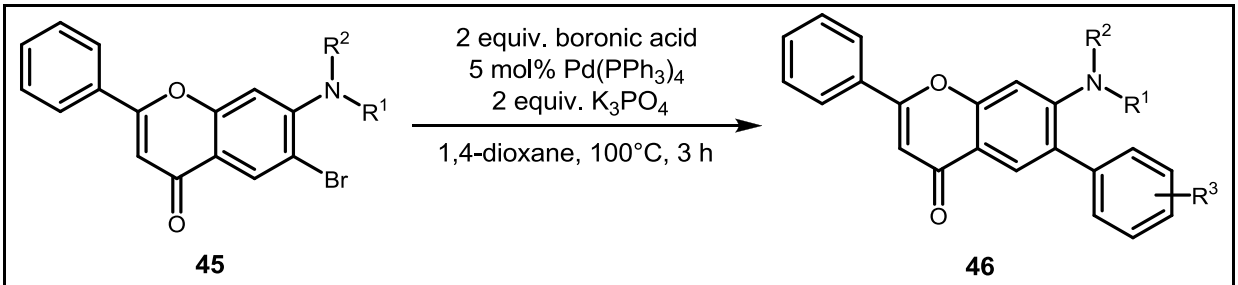
Under the optimised conditions different primary, secondary, open-chained and cycled amines were successfully used for coupling. The products were isolated with low and moderate yields (Table 6). The regioselectivity of the coupling was confirmed by NOESY NMR spectroscopy in all cases.



Amine	R^1	R^2	**45**	Yield (%)
a	Bu	H	**45a**	55%
e	Bn	H	**45b**	35%
g	4-Cl-C_6H_4	H	**45c**	25%
j	-$(CH_2)_4$-		**45d**	39%

Table 6: Coupling of 6,7-dibromoflavone with amines

Disubstituted product was not observed, not even in the presence of high excess of amine. Therefore, we performed the Suzuki reaction of the isolated monocoupled products, in order to examine the capability of transformation of the molecules in a different cross-coupling reaction which is less sensitive to the electronic effects. Both electron-poor and electron-rich arylboronic acids were successfully employed (Table 7).

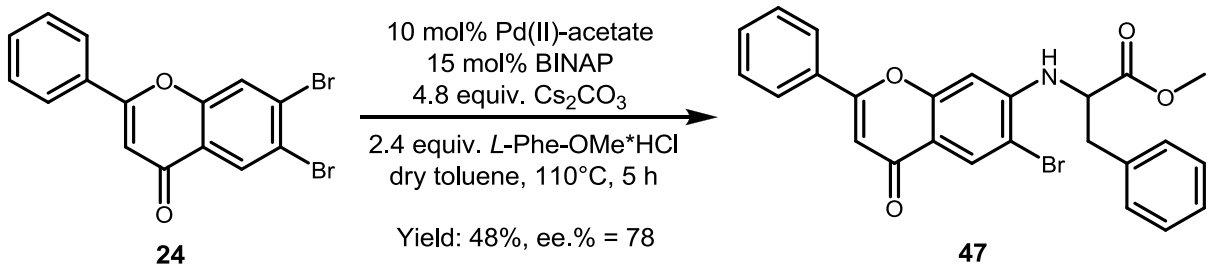


Amine	R^1	R^2	**45**	R^3	**46**	Yield (%)
a	Bu	H	**45a**	3,4-$(OMe)_2$	**46a**	86
				4-Cl	**46b**	80
e	Bn	H	**45b**	3,4-$(OMe)_2$	**46c**	83
				4-Cl	**46d**	79
g	4-Cl-C_6H_4	H	**45c**	3,4-$(OMe)_2$	**46e**	78
				4-Cl	**46f**	72
j	-$(CH_2)_4$-		**45d**	3,4-$(OMe)_2$	**46g**	93
				4-Cl	**46h**	88

Table 7: Suzuki reaction of the aminated monocoupled products

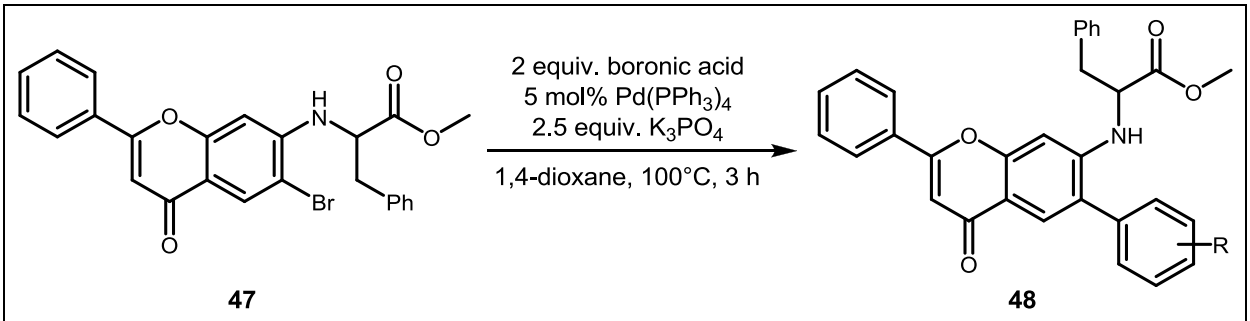
3.4.3. The Buchwald-Hartwig reaction of 6,7-dibromoflavone with *L*-phenylalanine methyl ester

The best conditions from the optimization experiments of the reaction of 6,7-bromoflavone (**24**) with *L*-phenylalanine methyl ester is represented in Scheme 7. The amino acid reacted at the position 7 selectively; the structure of product **47** was clearly proved by two-dimensional NMR spectroscopy. The enantiomeric excess decreased during the reaction.



Scheme 7: Coupling of 6,7-dibromoflavone with *L*-phenylalanine methyl ester

The formation of the disubstituted compounds was not detected in this case either. The isolated monocoupled product (**47**) reacted with both electron-poor and electron-rich arylboronic acids in Suzuki reaction, providing the corresponding products in high yields (Table 8).

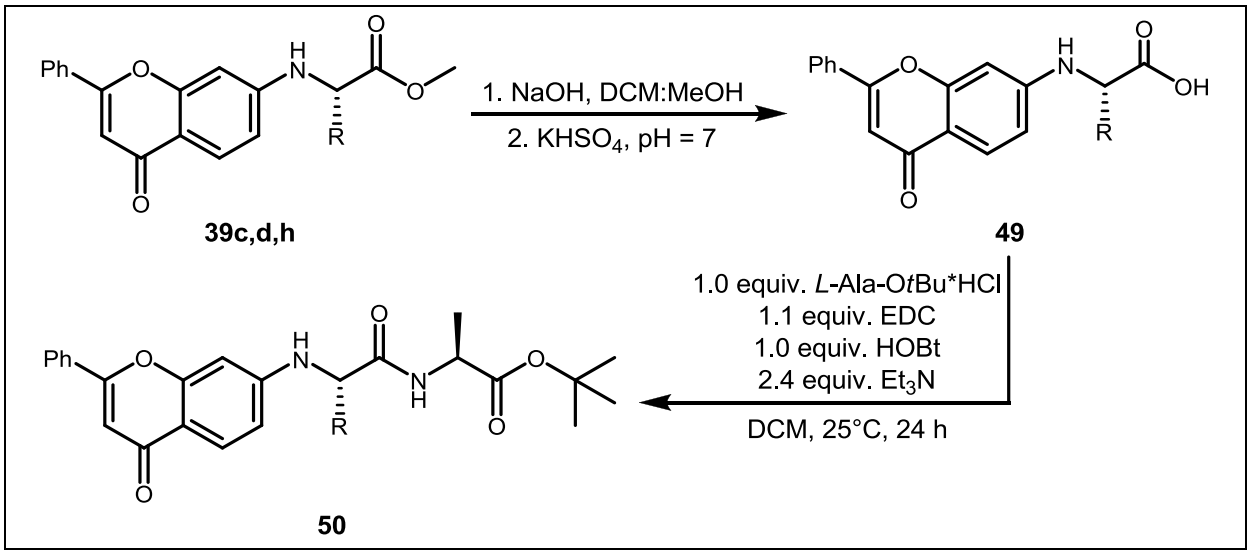


48	R	Yield (%)
48a	4-CH_3	98
48b	4-OMe	90
48c	4-Cl	86
48d	H	98

Table 8: Suzuki reaction of the flavone-amino acid hybrid (47)

3.5. Synthesis of flavone-peptide hybrids from flavone-amino acid hybride molecules

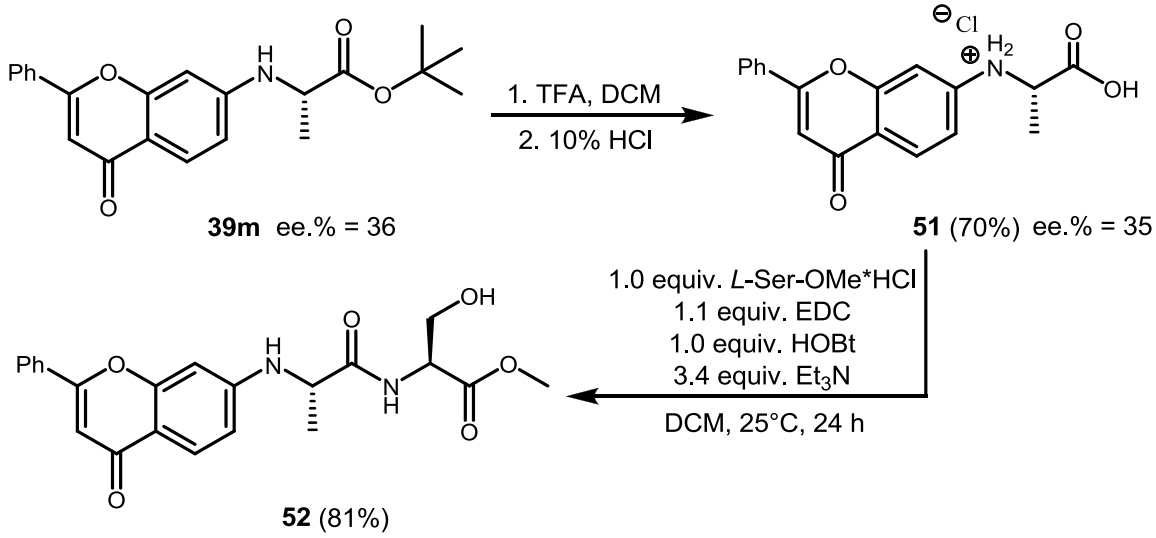
We managed to synthesise flavone-peptide hybrids in an alternative way hydrolysing the flavone-amino acid ester molecules following by a peptide synthesis of the isolated **49** amino acid transforming into flavone-dipeptide derivatives **50**. Due to the basic condition the enantiomeric excess decreased during the hydrolysis (Table 9).



R	**39**	**39** ee. %	**49**	**49** Yield (%)	**49** ee. %	**50**	**53** Yield (%)
*i*Bu	**39c**	93	**49a**	83	82	**50a**	72
C_2H_4-SCH_3	**39d**	74	**49b**	79	46	**50b**	75
*i*Pr	**39h**	97	**49c**	73	81	**50c**	55

Table 9: Synthesis of flavone-dipeptide molecules through peptide synthesis

To avoid the disadvantages of the basic hydrolysis, we performed the acidic hydrolysis of the *tert*-butyl ester compound, which is represented here by one example (Scheme 8).



Scheme 8: Peptide synthesis after acidic deprotection

Publikációs jegyzék

Az értekezés témájához tartozó közlemények/Publications related to the dissertation:

1. Kónya, K.; ***Pajtás, D.***; Kiss-Szikszai, A.; Patonay, T.: Buchwald-Hartwig reactions of monohaloflavones; *Eur. J. Org. Chem.* **2015**, 828–839.
   [Impakt faktor: 3.065 (2014/2015)]

2. ***Pajtás, D.***; Patonay, T.; Kónya, K.: Synthesis of 8-bromoflavone and its Buchwald-Hartwig Reaction; *Synthesis* (elfogadva)
   [Impakt faktor: 2.689 (2014/2015)]

3. ***Pajtás, D.***; Patonay, T.; Kónya, K.: Optimization of flavone-amino acid and flavone-dipeptide hybrids' synthesis in Buchwald-Hartwig reaction; *Eur. J. Org. Chem.* (közlésre előkészítve)

Egyéb közlemények/Other publications:

1. ***Pajtás, D.***; Dihen, K.; Patonay, T.; Kónya, K.; Villinger, A.; Langer, P.: Site-selective Suzuki-Miyaura reaction of 6,8-dibromoflavone; *Synlett* (elfogadva)
   [Impakt faktor: 2.419 (2014/2015)]

Az értekezés témakörében tartott előadások/Lectures related to the dissertation:

1. Kónya Krisztina, Fekete Szabolcs, ***Pajtás Dávid***, Kiss-Szikszai Attila, Patonay Tamás: Különbözően szubsztituált aminoflavonok hatékony előállítása Buchwald-Hartwig aminálási reakcióval és aminoflavonok alkilezésével; MTA Flavonoidkémia Munkabizottság Előadóülése, Debrecen, 2008. 10. 20.

2. Kónya Krisztina, ***Pajtás Dávid***, Patonay Tamás: Palládium-katalizált aminálási reakciók oxigéntartalmú heterociklusok körében; Heterociklusos Kémiai Munkabizottság ülése, Balatonszemes, 2009. 05. 20-22.

3. ***Pajtás Dávid***: Szubsztituált halogénflavonok Buchwald-Hartwig reakciója; Hatvani István Szakkollégium hallgatói konferenciája, Debrecen, 2009. 11. 26.

4. ***Pajtás Dávid***: Flavonszármazékok aminálási reakciói; Debreceni Egyetem házi Tudományos Diákköri Konferencia, Debrecen,2009. 12. 07.

5. ***Pajtás Dávid***, Kónya Krisztina, Kiss-Szikszai Attila, Patonay Tamás: Aminoflavonok és aminosavval kapcsolt flavonok előállítása Buchwald-Hartwig reakcióval; Heterociklusos Kémiai Munkabizottság ülése, Balatonszemes, 2010. 05. 21.

6. ***Pajtás Dávid***: Aminoflavonok szintézise Buchwald-Hartwig reakcióval, Hatvani István Szakkollégium hallgatói konferenciája; Debrecen, 2010. 11. 18.

7. ***Pajtás Dávid***: Aminoflavonok és aminosavval kapcsolt flavonok szintézise Buchwald-Hartwig reakcióval; Debreceni Egyetem házi Tudományos Diákköri Konferencia, Debrecen 2010. 11. 25.

8. ***Pajtás Dávid***: Aminoflavonok és aminosavval kapcsolt flavonok szintézise Buchwald-Hartwig reakcióval; XXX. jubileumi Országos Tudományos Diákköri Konferencia, Pécs, 2011. 04. 27-29.

9. ***Pajtás Dávid***, Kónya Krisztina, Kiss-Szikszai Attila, Patonay Tamás: Flavon-aminosav hibridek szintézise Buchwald-Hartwig reakcióval; Heterociklusos Kémiai Munkabizottság ülése, Balatonszemes, 2011. 09. 26-28.

10. ***Pajtás Dávid***: Flavon-aminosav hibridek szintézise Buchwald-Hartwig reakcióval; Hatvani István Szakkollégium hallgatói konferenciája, Debrecen, 2011. 12. 2.

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12. ***Pajtás Dávid***, Kónya Krisztina, Kiss-Szikszai Attila, Patonay Tamás: 8-Brómflavon előállítása és Buchwald-Hartwig reakcióinak tanulmányozása; Heterociklusos és Elemorganikus Kémiai Munkabizottság ülése, Balatonszemes, 2013. 06. 5-7.

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Az értekezés témájában bemutatott poszterek/Posters related to the dissertation:

1. Krisztina Kónya, ***Dávid Pajtás***, Szabolcs Fekete, Tamás Patonay: Syntheses of substituted N-alkyl/arylflavones by palladium catalyzed Buchwald-Hartwig amination and by classic alkylation methods; 16th European Symposium on Organic Chemistry (ESOC), Prague, 2009. 07. 12-16.

2. Krisztina Kónya, ***Dávid Pajtás***, Attila Kiss-Szikszai, Tamás Patonay: C-N bond formation: a new route to flavones with potential pharmacological activity; Natural and artificial ecosystems in Somes-Cris-Mures and Tisa river basins, Arad, 2010. 05. 7-8.

3. ***Dávid Pajtás***, Krisztina Kónya, Attila Kiss-Szikszai, Tamás Patonay: The formation of flavone-amino acid hybrids by Buchwald-Hartwig reaction; 4th German-Hungarian Workshop, Debrecen, 2011. 06. 14-16.

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5. ***Dávid Pajtás***, Attila Kiss-Szikszai, Peter Langer, Tamás Patonay: Selective Suzuki and Buchwald-Hartwig reaction of 6,7- and 6,8-dibromoflavones; 20th International Conference of Organic Synthesis, Budapest, 2014. 06. 29.-07. 02.

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List of publications related to the dissertation

Foreign language scientific article(s) in international journal(s) (2)

1. **Pajtás, D.**, Patonay, T., Kónya, K.: Synthesis of 8-Bromoflavone and its Buchwald-Hartwig Reaction.
   Synthesis. "Accepted by Publisher" (2015) ISSN: 0039-7881.
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   Eur. J. Org. Chem. 2015 (4), 828-839, 2015. ISSN: 1434-193X.
   DOI: http://dx.doi.org/10.1002/ejoc.201403108
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