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Diploma thesis

Development of peptidomimetics on alpha(v)beta(3) integrin receptor for tumor imaging.

Heidelberg 2008 **Jiří Fuxa**

I declare that this graduation thesis is my own author work and all the sources are quoted in references.

Jihlava, 15.5.2009 Signature

I would like to thank Dr. Holger Kubas and his team at the Department of Radiopharmaceutical Chemistry in DKFZ in Heidelberg for valuable advice and help during the work on this publication.

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CONTENT

1 Abbreviations

ADDP	1,1'-(azodicarbonyl)dipiperidine
DCAD	Di-*p*-chlorobenzyl azodicarboxylate
DCM	Dichloromethane
DEAD	Diethyl azodicarboxylate
DIPEA	Diisopropylethylamine
DMAP	Dimethylaminopyridin
DMF	Dimethylformamid
DMSO	Dimethyl Sulfoxide
EDC	1-ethyl-3-(3-dimethylaminopropyl)carbodiimide
EtOAc	Ethylacetate
HOBT	Hydroxybenzotriazole
HPLC	High-performance liquid chromatography
MeOH	Methanol
NMM	N-methyl morpholine
NMR	Nuclear magnetic resonance
PBu_3	Tributylphosphine
PET	Positron emission tomography
PPh_3	Triphenylphosphine
TFA	Trifluoroacetic acid
THF	Tetrahydrofurane
TMAD	N,N,N',N'-tetramethylazodicarboxamide

2 Introduction

2.1 Integrins

The integrins are a family of α,β heterodimeric receptors and are expressed by all multicellular animals, but their diversity varies widely among species; for example, in mammals, 19 α and 8 β subunit genes encode polypeptides that combine to form 25 different receptors, whereas the *Drosophila* and *Caenorhabditis g*enomes encode only five and two integrin *a* subunits respectively.[1]

Molecular mass is usually between 90 kDa and 160 kDa and both subunits penetrate the plasma membrane. The α and β subunits have distinct domain structures, with extracellular domains from each subunit contributing to the ligand-binding site of the heterodimer. The cytoplasmic tails of human integrin subunits are less than 75 amino acids long.[2] The technology progress allowed us to specify exactly amino acids sequence and steric arrangement of some important integrin, for example αvβ3.[3] According to binding specifity of extracelular subunit we can divide integrins in main groups, laminin-binding integrins, collagen-binding integrins, leukocyte integrins and RGD-recognizing integrins.[2]

Integrin receptors provide communication between the cell and other cells or extracelular matrix. They participate on information exchange about outside environment and at the same time regulate response to changes inside the cells. Among other functions there are regulation of cellular shape, cell adhesion and a very important role in cell migrations.

2.2 Integrins in relation to cancer

On the beginning carcinoma genesis is malfunction of control mechanism cells. Consequently, an uncontrolled cell proliferation starts and expresses many different proteins and receptors specific for this process. Integrins play a key role in cell survival, proliferation, migration, expression of genes, and activation of growth factor receptors. Their functions and expressions are deregulated in several types of cancer including prostate cancer[4,5,] breast cancer[6], gastric cancer[7], colorectal cancer[8] and melanoma.[9]

Next research was focused on involvment of integrins in cell movement and adhesion by tumor methastasis. In this process it is necessary for the cell to survive, to save the adhesive interaction between tumor cells and surrounding, which are host cells or extracelular matrix. As a result there was disclosed the presence of integrins with affinity to vitronectin. The extracellular matrix protein vitronectin is recognized as an adhesive substrate for four known integrins: alpha(v)beta(1), alpha(v)beta(3), alpha(v)beta(5) or alpha(IIb)beta(3).[10] By studying the mechanism of interaction between vitronectin and receptors was find out that the ability of fibronectin to bind cells can be accounted for by the tetrapeptide L-arginyl-glycyl-L-aspartyl-L-serine sequence which is part of the cell attachment domain of fibronectin[11]. The tripeptide Arg-Gly-Asp (RGD) appears to be irreplaceable for maintenance of the activity of this peptide, whereas the serine residue can be replaced with some, but apparently not all, possible residues.[12]

Occupation of these receptors, from which the most investigated is alpha(v)beta(3), with RGD derivates significantly inhibited the experimental lung and liver cancer metastasis.The regulation of adhesive interaction of tumor cells with extracelular matrix or host cells may provide a promising approach for the prevention of tumor metastasis.[13] It is also possible to use this RGD sequence incorporated in suitable radioactive marked molecules for imaging early stadium of cancer metastases by positron emission tomography.

2.3 RGD sequence in tumor imaging

From the first structure models of the extracellular segment of the alpha(v)beta(3) integrin receptor with a RGD ligand bound to the active place, structural models for the interactions of known ligands with the alpha(v)beta(3) integrin receptor were generated by automated computing docking. The obtained complexes were classified for their consistency with structure-activity relationships and site-directed mutagenesis data.[14]

Following these models, there were synthetised different peptide with RGD triplet in ground to advance binding capacity and affinity. On both ends the attachment with another peptides different size and sequence was attempted. For affinity is better the cyclization of pentapeptides and hexapeptides than keeping the linear structure.[15]

To potentionally use these peptides for tumor imaging on PET scanners it is necessary to incorporate or bind radioactivelly labelled element. For keeping good affinity is always used suitable linker which holds down steric obstruction. As a linker it is possible to put in reasonably large molecule for example glycoside[16] or another organic molecule. On the other side of the linker is radioactive labelled element ^{125}I, ^{18}F, ^{111}In, ^{99m}Tc, ^{64}Cu.[17]

2.4 Peptidomimetics

Potential improvement of RGD peptide and other analogues in clinical practice reduce many inconvenient attributes typical for proteins. Resulted compounds are relatively large and lowly absorbable into circulation. They are very sensitive to pH changes in surroundings and quickly decomposed with proteolytic enzymes.

Secondary structure peptidomimetic approach enables to prepare non-peptidic antagonists which to a certain extent remove these negative properties. Non-peptidic molecules are usually smaller and better penetrate into circulation. They are not frequently decomposed with proteolytic enzymes and also better pH stability improves the possibilities of their use.

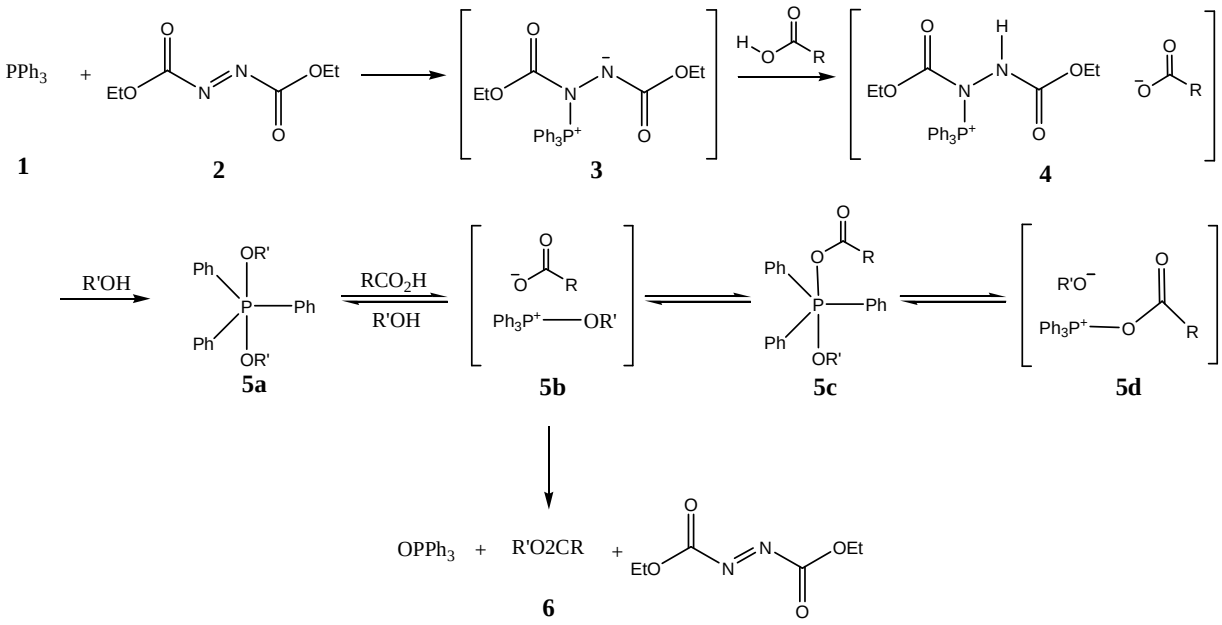
According to computing accounted models are synthetized potentional high affinity compounds library. These substances´ library are screened in vitro to identify the best ones and to detect exact integrin receptor subtype.

For diagnostic use on PET scanners it is necessary to get radioactive form of these chosen molecules. Unlike to RGD peptide there is not used a linker for binding radioactive element, but the straight incorporation into main structure. In the first step of this reaction, radioactive element is substituted and receptor affinity is checked again, because this new element in molecule can change its three-dimensional orientation and be the cause of affinity decrease. Just because of affinity decrease after substitution it is not possible to use some otherwise very good molecules for tumor imaging. If the high affinity is saved, this compound is radioactively labelled and serves as the contrasting substance by investigation.

3 Methodics

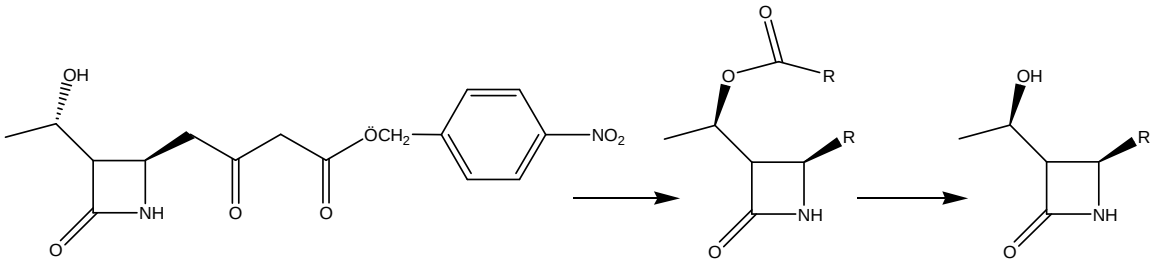
The Mitsunobu reaction was discovered in 1967[18] and gained countenance for possibility of wide using in organic synthesis. It is a reaction of alcohol with different nucleophilic groups in presence of phosphine and azadicarboxyl compound. Mechanism was always studied by esterification with PPh_3 and DEAD, which is often used through stereochemistry conversion (Scheme 1).[19,20]

At first the triphenyl phosphine (**1**) and DEAD (**2**) create an intermediate (**3**), which attacks the carboxyl acid and deprotonates it by forming ion pair (**4**). The generated carboxylate anion takes away proton from alcohol and establish alcoxide. In the next step thanks this alcoxide originate four different intermediates (**5a-d**) from which just oxyphosphonium ion (**5b**) provides the final product, ester (**6**) in opposite stereomerical configuration. The amount of forming oxyphosphonium ion is highly depending on solvation and basicity of nucleophyle.[21]



Scheme 1

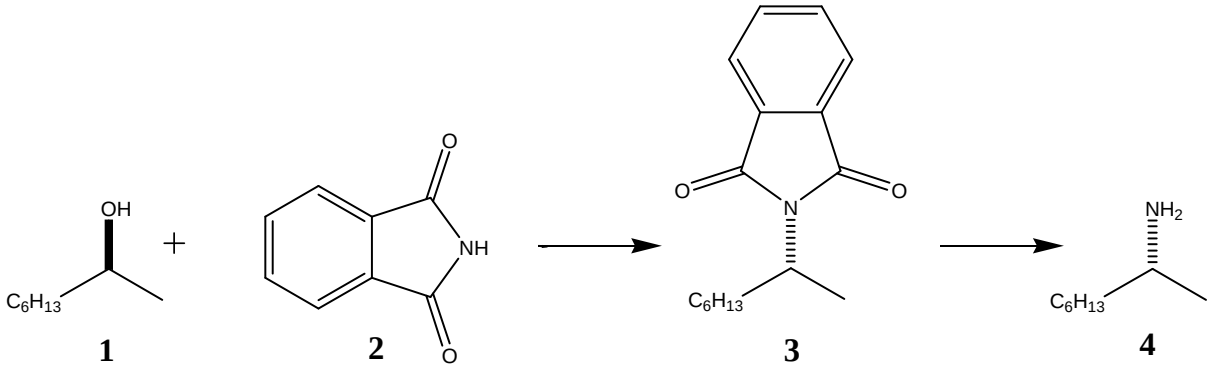
The Mitsunobu reaction is also used in another specific synthesis with different nucleofilic groups. Change of stereochemic configuration hydroxyl group is the key in the synthesis of the new antibiotic thienamycin (**1**) (Scheme 2). Reaction kinetics study showed that only under strictly controlled conditions could a good yield of inverted product (**2)** be obtained.[21]



1 **2**

Scheme 2

Reaction of alcohol (**1**) with pthalamide (**2**) as a nucleofil obtaines useful intermediate (**3**) which can be converted with hydrazine to amine(**4**) (Scheme 3).[22]



1 **2** **3** **4**

Scheme 3

Very well applicable is the Mitsunobu reaction for preparation alky-aryl ethers. But by syntheses of pyridine ethers yields significantly lowered and another by-product was observed. Through the research in literature the pK_a was recogonized as reason of this trouble. As a resolution was used the replacement of DEAD by ADDP[23] which provided higher basic anion.[24] Next options for replacement of DEAD are another compounds di-*p*-chlorobenzyl azodicarboxylate (DCAD)[25] and N,N,N',N'-tetramethylazodicarboxamide (TMAD).[26] These were developed for Mitsunobu reaction to optimize its yields.

4 Aim of the work

The main objective of my work was implementation of iodine in nonpeptidic high affinity compounds to alpha(v)beta(3) integrin receptors. After preparation this compounds I will check by the cell test the effect of this substitution on affinity to the receptors. In case of retention of good affinity it is possible to radioactively label these molecules and to use them for tumor imaging on PET scanners.

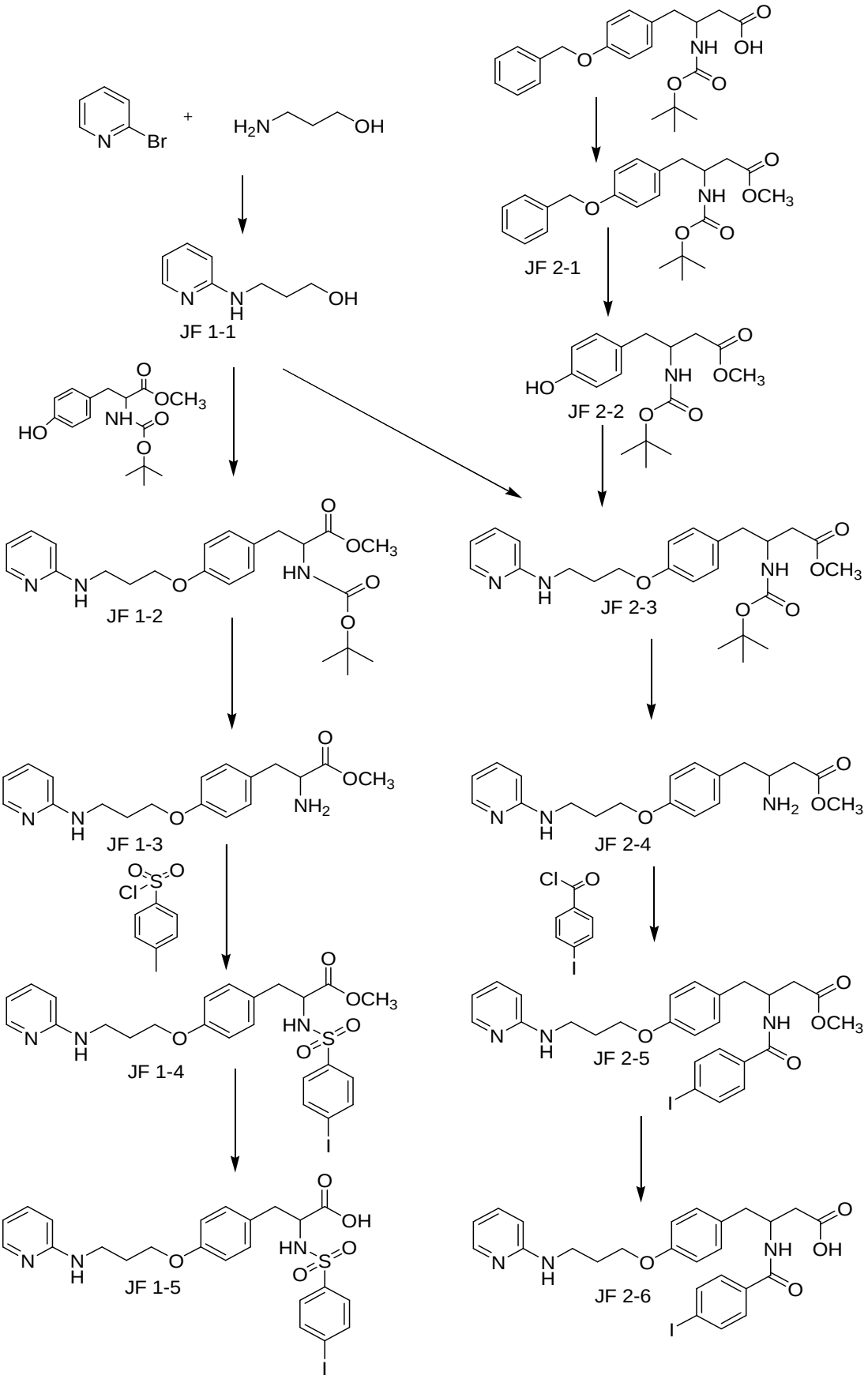
5 Experimental section

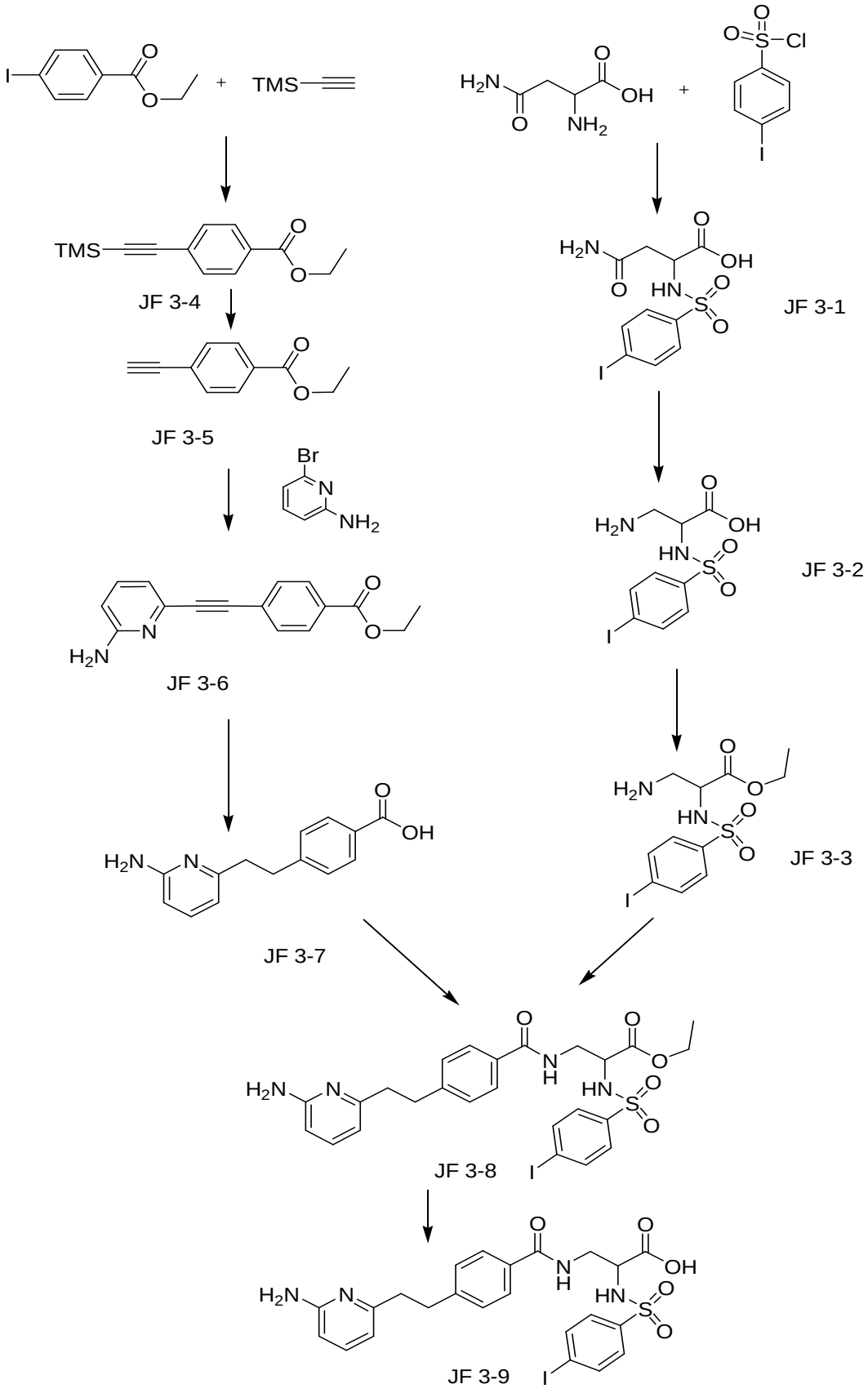
All chemicals used in this work were purchased from Fluka, Aldrich, Afa Aesar, Merck, ABCR and AppliChem. Solvents weren't destilled before use.

Thin layer chromatography was performed on Polygram SilG/UV254 (Macherey-Nagel, Düren, Germany) with UV detection.

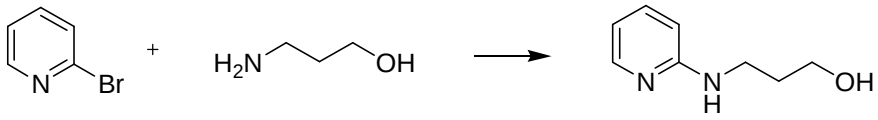
HPLC was performed on an Agilent 1100 Series system using water with 0.1% TFA as solvent A and acetonitrile with 0.1% TFA as solvent B. The elution profile was monitored by UV absorbance at 214 and 254 nm. For analysis was used Chromolith® Performance RP-18e 100 x 4.6 column (Merck, Darmstadt, Germany); gradient elution protocol: 100% solvent A to 100% solvent B in 5 min; flow rate: 4.0 mL/min. For purification was used Chromolith® SemiPrep RP-18e (100 x 10) column (Merck, Darmstadt, Germany); flow rate: 8.0 mL/min.

Mass spectra were recorded on a Bruker Daltonics microflex® benchtop MALDI-TOF MS (Bruker Daltonics, Bremen, Germany).


JF 1-1
JF 2-1
JF 2-2
JF 1-2
JF 2-3
JF 1-3
JF 2-4
JF 1-4
JF 2-5
JF 1-5
JF 2-6


JF 3-4
JF 3-5
JF 3-6
JF 3-7
JF 3-1
JF 3-2
JF 3-3
JF 3-8
JF 3-9

5.1 Preparation of 3-(pyridin-2-ylamino)propan-1-ol (JF 1-1)



Reagents

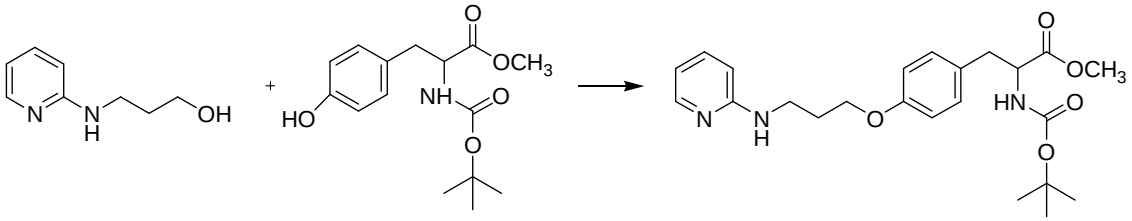
	2-bromopyridine	3-aminopropan-1-ol	JF1-1
Chemical formula	C_5H_8BrN	C_3H_9NO	$C_8H_{12}N_2O$
Mw	158,00	75,11	152,19
m[g]	2,00	1,90	1,15
n[mmol]	12,7	25,4	
d[g/ml]		0,9880	
V[ml]		1,92	

Procedure

2-Brompyridine (2,0g; 12,7mmol) was heated with 3-aminopropanol (1,92ml) at 150 °C for 7h. The resulting mixture was purified by column chromatography on silica gel (DCM/methanol, 90:10) to give 1,15g (59,6 %) of ligt yellow oil.[27]

MS (ESI): m/z= 153,1 $[M+H]^+$

5.2 Preparation of 2-(tert-butoxycarbonylamino)-3-(4-(3-(pyridin-2-ylamino)propoxy)phenyl)propanoate (JF 1-2)



Reagents

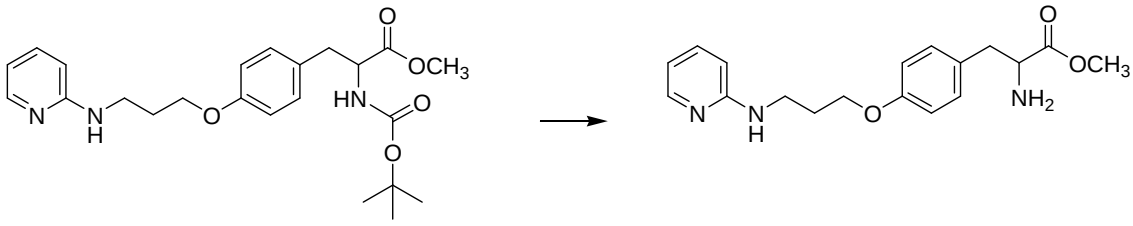
	JF1-1	N-Boc-tyrosine methyl ester	THF	PBu_3	ADDP	JF 1-2
Chemical formula	$C_8H_{12}N_2O$	$C_{15}H_{21}NO_5$				$C_{23}H_{31}N_3O_5$
Mw	152,19	295,34		202,32	252,32	429,53
m[g]	1,15	2,00		1,79	2,23	1,58
n[mmol]	7,5	6,8		8,84	8,84	3,7
d[g/ml]				0,820		
V[ml]			120	2,20		

Procedure

N-Boc-tyrosine methyl ester(2,0g; 6,8mmol), 3-(pyridin-2-ylamino)propan-1-ol (1,15g; 7,5mmol) and tributylphosphine (1,79g; 8,84mmol) were dissolved in dry THF (120ml) in flask and stirred at 0°C under argon atmosphere. Azodicarboxydipiperidid (2,23g; 8,84mmol) was dissolved in dry THF (35ml) and added dropwise to the reaction mixture in 4 h time. The resulting yellow suspension was allowed to warm to room temperature and stirred for 20 h. After addition of silica gel and evaporation of THF the mixture was purified by column chromatography on silica gel (DCM/ethylacetate, 2:1) to give 1,58g (54,5 %) of colorless oil.[27]

MS (ESI): m/z= 430,3 $[M+H]^+$

5.3 Preparation of methyl 2-amino-3-(4-(3-(pyridin-2-ylamino)propoxy)phenyl)propanoate (JF 1-3)



Reagents

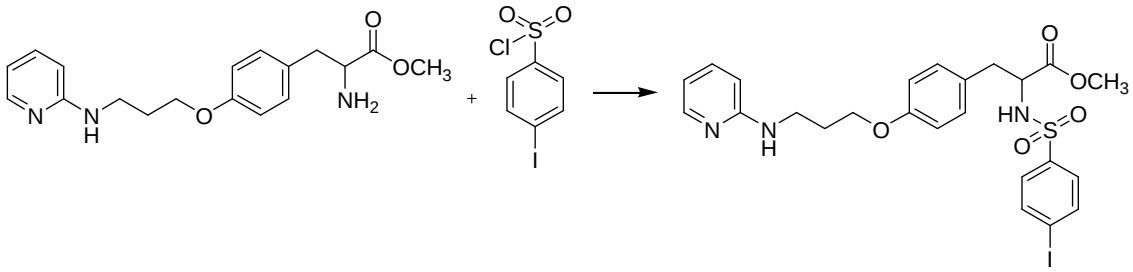
	JF 1-2	Dioxane	HCl	JF 1-3
Chemical formula	$C_{23}H_{31}N_3O_5$			$C_{18}H_{23}N_3O_3$
Mw	429,53			329,53
m[mg]	800			680
n[mmol]	1,86			
d[g/ml]				
V[ml]		16	5	

Procedure

The starting material (800mg; 1,86 mmol) was dissolved in 16 ml of dioxane. Then 5ml of HCl was added and the mixture was stirred at room temperature and monitored by thin-layer chromatography. After 3 h the solvent was evaporated and the resulting amine hydrochloride (680 mg; 94%) was used for next reaction without purification.[27]

MS (ESI): m/z= 219,1; 330,2 $[M+H]^+$; 437,4

5.4 Preparation of methyl 2-(4-iodophenylsulfonamido)-3-(4-(3-(pyridin-2-ylamino)propoxy)phenyl)propanoate (JF 1-4)



Reagents

	JF 1-3	DMF	4-I-benzen-sulfonyl chloride	DIPEA	JF 1-4
Chemical formula	$C_{18}H_{23}N_3O_3$				$C_{24}H_{26}N_3O_5SI$
Mw	329,53		302,3	129,25	595,43
m[mg]	200		550	390	127
n[mmol]	0,6		1,8	3	
d[g/ml]				0,755	
V[ml]		8		0,52	

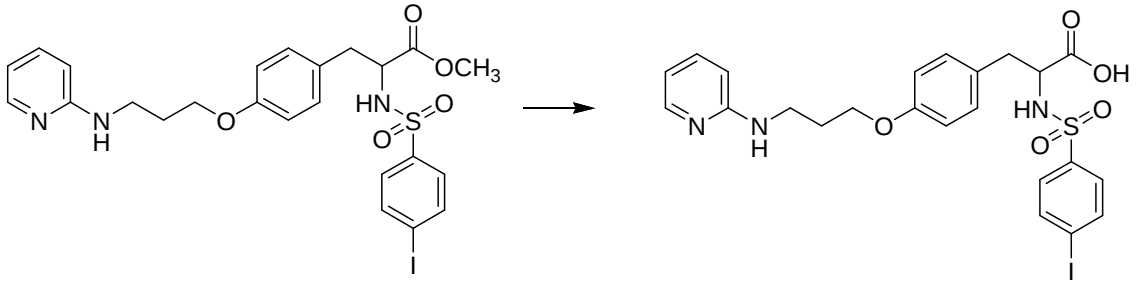
Procedure

The amine hydrochloride (200 mg; 0,6 mmol; 1 eq) was dissolved in 8 ml of THF, 4-I-benzen-sulfonyl chloride (550 mg;1,8 mmol; 3eq) and DIPEA (0,52 ml; 3 mmol; 5 eq) were added and the resulting mixture was stirred for 26 h. Then the solvent was evaporated and a part (27 mg) was separeted and purified for analyses. Second part (100 mg) was used for next step without purification.[27]

MS (ESI): m/z= 462,1 $[M_1+H]^+$; 596,2 $[M_2+H]^+$

HPLC (0-100%; 4min): t_R=2,997

5.5 Preparation of 2-(4-iodophenylsulfonamido)-3-(4-(3-(pyridin-2-ylamino)propoxy)phenyl)propanoic acid (JF 1-5)



Reagents

	JF 1-4	LiOH	MeOH/H_2O 3/1	JF 1-5
Chemical formula	$C_{24}H_{26}N_3O_5SI$			$C_{23}H_{24}N_3O_5SI$
Mw	595,43	23,95		581,43
m[mg]	100	20+10		15
n[mmol]	0,17	0,85		
d[g/ml]				
V[ml]			16	

Procedure

The methyl ester was dissolved in 16 ml of methanol/water 3:1 and LiOH was added (20mg; 0,85 mmol; 5eq). After 48 h next LiOH (10 mg) was added and the reaction was always monitored with HPLC. After 73 h the reaction was terminated and the solvent was evaporated. The crude product was purified by preparative HPLC to give a final product as TFA salt (15 mg; 15 %).[27]

MS (ESI): m/z= 582,2 $[M+H]^+$

HPLC (0-100%; 4min): t_R=2,787 min

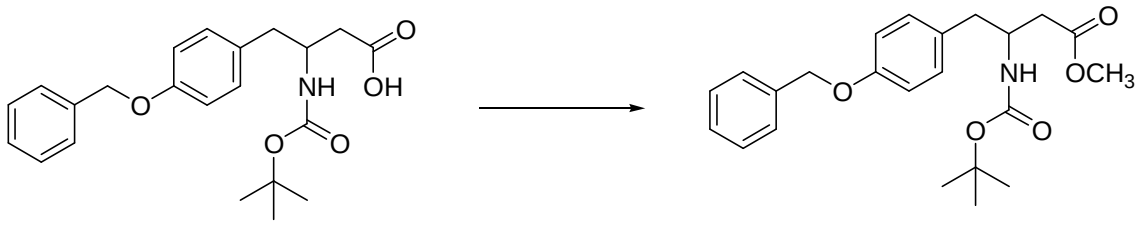
^{1}H-NMR (300 MHz, DMSO): δ= 8,32 (bs, 1H), 7,90 (d, 1H), 7,74 (t, 1H), 7,28 (d, 2H), 7,13 (d, 2H), 7,01 (d,2H), 6,93 (d, 2H) 6,71 (d, 2H), 4,01 (t, 2H), 3,77 (dt, 1H), 3,44 (m, 2H), 2,88(d, 1H), 2,60 (d, 1H), 2,03(m, 2H)

Elementary analysis:

Found: C 43,20; H 3,92; N 6,05

Counted: C 43,18; H 3,62; N 6,04; S 4,61; I 18,25; O 16,10; F 8,20

5.6 Preparation of methyl 4-(4-(benzyloxy)phenyl)-3-(tert-butoxycarbonylamino)butanoate (JF 2-1)



Reagents

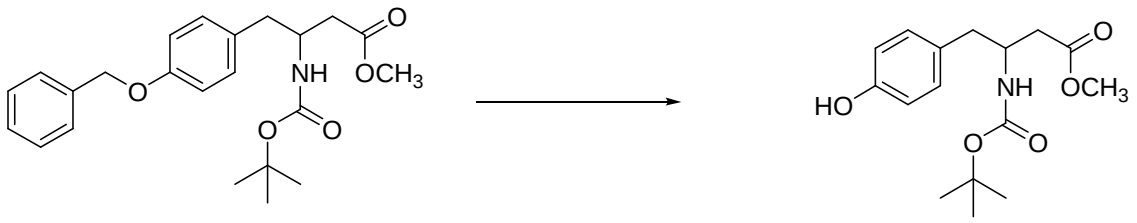
	Boc-O-benzyl homotyrosine	MeOH	DMAP	EDC	DCM	JF 2-1
Chemical formula	$C_{22}H_{27}NO_5$					$C_{23}H_{29}NO_5$
Mw	385,45	32,04	122,17	191,7		399,46
m[mg]	500	83,5	160	498,5		440
n[mmol]	1,3	2,6	0,13	2,6		
d[g/ml]		0,792				
V[ml]		0,11			10	

Procedure

Boc-O-benzyl homotyrosine (500 mg; 1,3 mmol; 10eq), methanol (0,11 ml; 2,6 mmol; 20 eq) and DMAP (160 mg; 0,13 mmol; 1 eq) were dissolved in 10 ml of dichloromethane. To this solution was added EDC (498,5 mg; 2,6 mmol; 20eq) and the resulting mixture was stirred at room temperature. After 4,25 h was 1N HCl added and the product was extracted three times with 10ml dichloromethane. Extract was dried over anhydrous $MgSO_4$ and then was the solvent evaporated. Crude product was purified by column chromatography on silica gel (DCM/MeOH, 97:3) to give 440 mg (84,6 %) of white solid.[28]

MS (ESI): m/z= 400,2 $[M+H]^+$

5.7 Preparation of methyl 3-(tert-butoxycarbonylamino)-4-(4-hydroxyphenyl)butanoate (JF 2-2)



Reagents

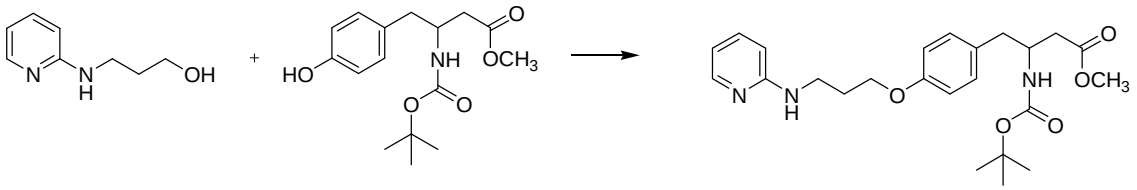
	JF 2-1	10% Pd-C	MeOH	Ammonium formate	JF 2-2
Chemical formula	$C_{23}H_{29}NO_5$				$C_{16}H_{23}NO_5$
Mw	399,46			63,03	309,46
m[mg]	440	440		350	330
n[mmol]	1,1			5,5	
d[g/ml]					
V[ml]			12		

Procedure

Boc-O-benzyl homotyrosine methyl ester (440 mg; 1,1 mmol; 1 eq) was suspended with an equal weight of 10% Pd-C in dry methanol (12 ml) under argon atmosphere. Anhydrous ammonium formate (350 mg; 5,5 mmol; 5 eq) was added in a single portion and the mixture was stirred at reflux temperature (70-80°C) for 0,5 h. After it was the catalyst filtered through a celite pad, washed with methanol and the solvent evaporated to give final product (330 mg, 97%).[29]

MS (ESI): m/z= 332,2 $[M+Na]^+$, 641,4 $[2M+Na]^+$

5.8 Preparation of methyl 3-(tert-butoxycarbonylamino)-4-(4-(3-(pyridin-2-ylamino)propoxy)phenyl)butanoate (JF 2-3)



Reagents

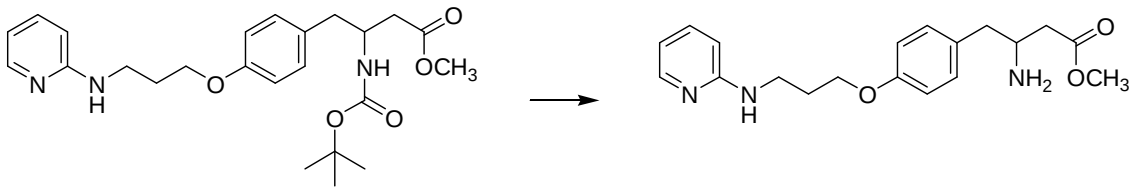
	JF1-1	N-Boc-homotyrosine methyl ester	THF	PBu_3	ADDP	JF 2-3
Chemical formula	$C_8H_{12}N_2O$	$C_{16}H_{23}NO_5$				$C_{24}H_{33}N_3O_5$
Mw	152,19	309,46		202,32	252,32	443,65
m[mg]	293	540			574	120
n[mmol]	1,92	1,75		2,275	2,275	
d[g/ml]				0,820		
V[ml]			40	0,56		

Procedure

N-Boc-homotyrosine methyl ester(540 mg; 1,75 mmol), 3-(pyridin-2-ylamino)propan-1-ol (293 mg; 1,92 mmol) and tributylphosphine (0,56 ml; 2,275 mmol) were dissolved in dry THF (4O ml) in flask and stirred at 0°C under argon atmosphere. Azodicarboxydipiperidid (574 mg; 2,275 mmol) was dissolved in dry THF (20 ml) and added dropwise to the reaction mixture in 3 h time. The resulting yellow suspension was allowed to warm to room temperature and stirred for 22 h. After addition of silica gel and evaporation of THF was the mixture purified by column chromatography on silica gel (DCM/ethylacetate, 2:1) to give 120 mg (15 %) of colorless oil.[27]

MS (ESI): m/z= 444,3 $[M+H]^+$,

5.9 Preparation of methyl 3-amino-4-(4-(3-(pyridin-2-ylamino)propoxy)phenyl)butanoate (JF 2-4)



Reagents

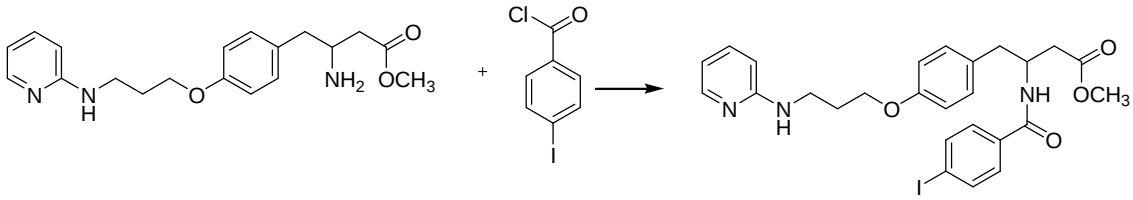
	JF 2-3	Dioxane	HCl	JF 2-4
Chemical formula	$C_{24}H_{33}N_3O_5$			$C_{19}H_{25}N_3O_3$
Mw	443,65			343,65
m[mg]	100			80
n[mmol]	0,225			
d[g/ml]				
V[ml]		5	2	

Procedure

The starting material (100mg; 0,225 mmol) was dissolved in 5 ml of dioxane. Then was added 2ml of HCl and the mixture was stirred at room temperature and monitored by thin-layer chromatography. After 1:45 h the solvent was evaporated and the resulting amine hydrochloride (80 mg; 93,46%) was used for the next reaction without purification.[27]

MS (ESI): m/z= 344,3 $[M_1+H]^+$; 330,2 $[M_2+H]^+$

5.10 Preparation of methyl 3-(4-iodobenzamido)-4-(4-(3-(pyridin-2-ylamino)propoxy)phenyl)butanoate (JF 2-5)



Reagents

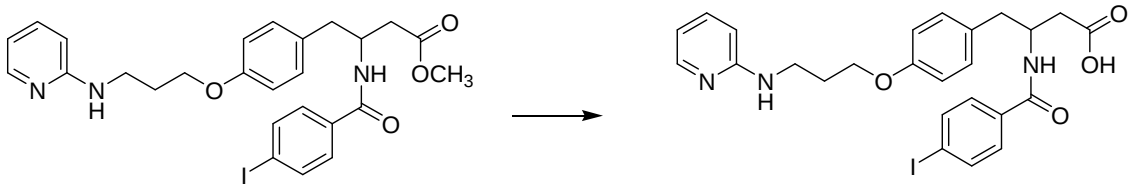
	JF 2-4	4-iodobenzoyl chloride	Dioxane/Water 1:1	$NaHCO_3$	JF 2-5
Chemical formula	$C_{19}H_{25}N_3O_3$				$C_{26}H_{28}N_3O_4I$
Mw	343,65	266,46		84,01	573,55
m[mg]	80	60		50	40
n[mmol]	0,2	0,23		0,6	
d[g/ml]					
V[ml]			6		

Procedure

The amine hydrochloride (80 mg; 0,2 mmol; 1eq) was dissolved in 6 ml of dioxane/water (1:1) then 4-iodobenzoyl chloride (60 mg; 0,23 mmol; 1,1eq) and $NaHCO_3$ (50 mg; 0,6 mmol; 3 eq) were added and the resulting mixture was stirred at room temperature. After 4 h the solvent was evaporated and the crude product purified by column chromatography on silica gel (DCM/MeOH (NH_3) 95:5) to give 40mg (33 %) of white solid.[27]

MS (ESI): m/z= 574,2 $[M+H]^+$

5.11 Preparation of 3-(4-iodobenzamido)-4-(4-(3-(pyridin-2-ylamino)propoxy)phenyl)butanoic acid (JF 2-6)



Reagents

	JF 2-5	LiOH	MeOH/H_2O 3/1	JF 2-6
Chemical formula	$C_{26}H_{28}N_3O_4I$			$C_{25}H_{26}N_3O_4I$
Mw	573,55	23,95		559,55
m[mg]	30	6		6
n[mmol]	0,05	0,25		
d[g/ml]				
V[ml]			16	

Procedure

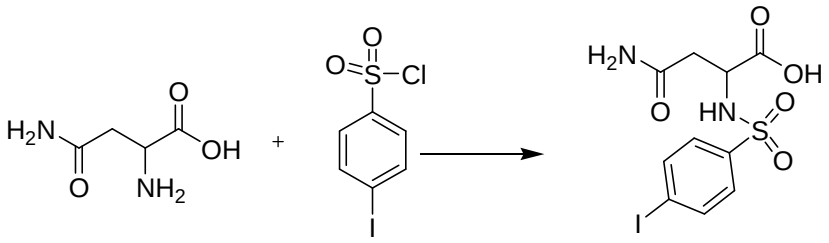
The methyl ester was dissolved in 16 ml of methanol/water 3:1 and LiOH (6mg; 0,25 mmol; 5eq) was added. After 25,5 h the reaction was terminated and the solvent was evaporated. The crude product was purified by preparative HPLC to give a final product as TFA salt (6 mg; 17 %).[27]

MS (ESI): m/z= 560,2 $[M+H]^+$

HPLC (0-100%; 4min): t_R=2,824 min

^{1}H-NMR (300 MHz, DMSO): δ= 8,37 (bs, 1H), 7,87 (d, 1H), 7,84 (t, 1H), 7,79 (d, 2H), 7,57 (t, 2H), 7,11 (t, 2H), 7,08 (d, 1H), 6,82 (t, 2H), 6,78 (d, 1H), 4,37 (m, 1H), 3,99 (t, 2H), 3,42 (m, 2H), 2,74 (m, 2H), 1,99 (m, 2H)

5.12 Preparation of 4-amino-2-(4-iodophenylsulfonamido)-4-oxobutanoic acid JF (3-1)



Reagets

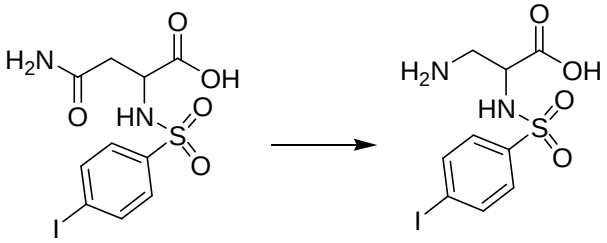
	L-asparagine	NaOH	Dioxane	4-iodophenylsulfonyl chloride	NaOH	JF 3-1
Chemical formula	$C_4H_8N_2O_3$					$C_{10}H_{11}N_2O_5SI$
Mw	132,12			302,52		398,08
m[g]	1,25	0,425		3,17	0,425	1,78
n[mmol]	9,4			10,4		
d[g/ml]						
V[ml]		6,25 (H_2O)	6,25		6,25 (H_2O)	

Procedure

To a stirred solution of L-asparagine (1,25 g; 9,4 mmol), NaOH (0,425 g;), H_2O (6,25 ml) and dioxane (6,25 ml) at 0 °C was added 4-iodophenylsulfonyl chloride (3,17 g; 10,4 nmol). After 1 min. NaOH (0,425 g; 6,25ml) was added and the reaction mixture stirred for 1,5 h. Dioxane was evaporated and the mixture washed with EtOAc. The aqueous phase was then cooled to 0°C and acidified to pH 5,0 with concentrated HCl to effect product precipitation. The resulting solid was collected by filtration and dried at 50°C to give 1,78 g (47,3 %) of white solid.[30]

MS (ESI): m/z= 399,1 $[M+H]^+$; 421,0 $[M+Na]^+$; 819,0 $[2M+Na]^+$

5.13 Preparation of 3-amino-2-(4-iodophenylsulfonamido) propanoic acid (JF 3-2)



Reagents

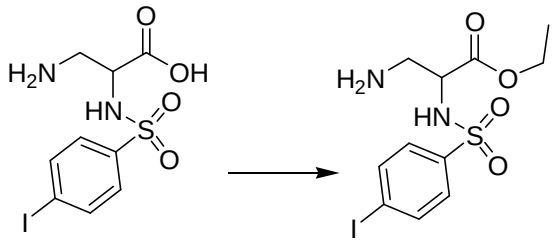
	JF 3-1	NaOH	Bromine	NaOH	JF 3-2
Chemical formula	$C_{10}H_{11}N_2O_5SI$		Br_2		$C_9H_{11}N_2O_4SI$
Mw	398,08		159,81		370,08
m[g]	1,78	0,4		1,4	1,12
n[mmol]	5				
d[g/ml]			3,1		
V[ml]		5 (H_2O)	0,26	6,5 (H_2O)	

Procedure

To a stirred solution of NaOH (1,4 g;) in H_2O (6,5 ml) cooled to 0°C was added bromine (0,26ml) dropwise. After 5 min a cold solution of JF 3-1 (1,78 g; 5 mmol) and NaOH (0,4 g) in H_2O (5 ml) was added in one portion. The solution was stirred for 20 min at 0°C and then 30 min at 90°C. The mixture was recooled to 0°C and acidified to pH 7,0 with concentrated HCl. The white precipitate was collected by filtration and dried to give 1,12g (67,3%) of white solid.[30]

MS (ESI): m/z= 370,9 $[M+H]^+$

5.14 Preparation of ethyl 3-amino-2-(4-iodophenylsulfonamido) propanoate (JF 3-3)



Reagents

	JF 3-2	EtOH	JF 3-3
Chemical formula	$C_9H_{11}N_2O_4SI$		$C_{11}H_{15}N_2O_4SI$
Mw	370,08		398,08
m[g]	1,12		0,77
n[mmol]			
V[ml]		30	

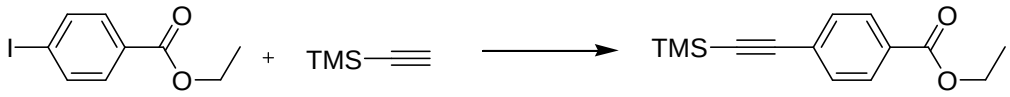
Procedure

NaCl in 30% HCl with H_2SO_4 prepared HCl gas which was rapidly bubbled through a suspension of JF 3-2 (1,12 g; 3mmol) in 30 ml EtOH at 5°C. After 15 min the cooling bath was removed and the reaction mixture was heated to 55-60°C for 8,5 h. Then the reaction was concentrated to give 0,77 g (65,3%) of ester as white solid.[30]

MS (ESI): m/z= 398,9$[M+H]^+$

HPLC (0-100%; 4min): t_R=2,346 min

5.15 Preparation of ethyl 4-((trimethylsilyl)ethynyl)benzoate (JF 3-4)



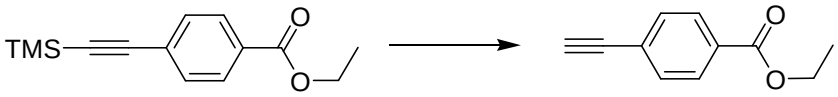
Reagents

	Ethyl 4-iodobenzoate	TMS-actylene	Et_3N	CH_3CN	$(Ph_3P)_2PdCl_2$	CuI	JF 3-4
Chemical formula	$C_8H_9O_2I$						$C_{14}H_{18}SiO_2$
Mw	276,08	98,22	101,19		701,89	190,4	246,08
m[g]	1,9	0,69			0,039	0,019	1,55
n[mmol]	7	7	28		0,055	0,1	
d[g/ml]	1,63	0,69	0,726				
V[ml]	1,18	1	3,9	10			

Procedure

TMS-acetylene (1 ml; 7 mmol), ethyl-4iodobenzoate (1,18 ml; 7 mmol) and Et_3N (3,9 ml; 28 mmol) were combined in 10 ml CH_3CN in a glass pressure tube. $(Ph_3P)_2PdCl_2$ (0,039 g; 0,055 mmol) and CuI (0,019 g; 0,1mmol) were added and the reaction was sealed and heated at 100°C for 19 h. After dilution with EtOAc the mixture was washed three times with 100 ml water then three times with 150 ml brine and concentrated to provide 1,55 g (92,3%) of brown oil.[30]

5.16 Preparation of ethyl 4-ethynylbenzoate (JF 3-5)



Reagents

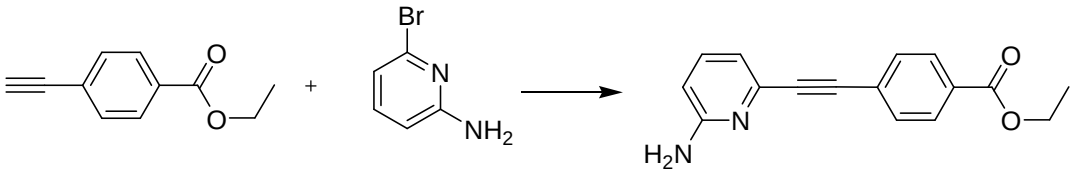
	JF 3-4	EtOH	K_2CO_3	JF 3-5
Chemical formula	$C_{14}H_{18}SiO_2$			$C_{11}H_{10}O_2$
Mw	246,08		138,21	174
m[g]	4,62		0,15	3,7
n[mmol]	18,7		1,1	
d[g/ml]				
V[ml]		60		

Procedure

JF 3-4 (4,62 g; 18,7 mmol) was dissolved in 60 ml EtOH. K_2CO_3 (0,15 g; 1,1mmol) was added and the mixture was stirred at room temperature. After 23,5 h the solvent was evaporated and the crude product purified by column chromatography (10 % EtOAc/n-hexane) to give 3,7 g (76%) of JF 3-5.[30]

MS (ESI): m/z= 175,0$[M+H]^+$; 300,2; 328,2

5.17 Preparation of ethyl 4-((6-aminopyridin-2-yl)ethynyl) benzoate (JF 3-6)



Reagents

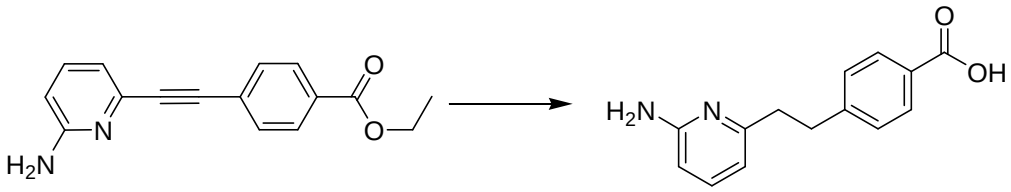
	JF 3-5	2-Amino-6-bromopyridine	Et_3N	CH_3CN	$(Ph_3P)_2PdCl_2$	CuI	JF 3-6
Chemical formula	$C_{11}H_{10}O_2$						$C_{16}H_{14}N_2O_2$
Mw	174,0	173,01	101,19		701,89	190,4	266,01
m[g]	1,392	1,66			0,308	0,106	1,13
n[mmol]	8	2,6			0,44	0,55	
d[g/ml]			0,726				
V[ml]			4	15			

Procedure

JF 3-5 (1,392 g; 8 mmol), 2-Amino-6-bromopyridine (1,66 g; 2,6 mmol), Et_3N (15 ml), $(Ph_3P)_2PdCl_2$ (0,308 g; 0,44 mmol) and CuI (0,106 g; 0,55 mmol) were combined in 15 ml CH_3CN sealed in a glass pressure tube and heated to 100°C for 3,5 h. Then the mixture was diluted with EtOAc, washed three times with water and three times with brine, dried with $MgSO_4$ and concentrated under reduced pressure. The crude product was purified by column chromatography (20 % Acetone/n-Hexane) to give 1,13 g (53%) of JF 3-6[30]

MS (ESI): m/z= 267,1$[M+H]^+$

5.18 Preparation of 4-(2-(6-aminopyridin-2-yl)ethyl)benzoic acid (JF 3-7)



Reagents

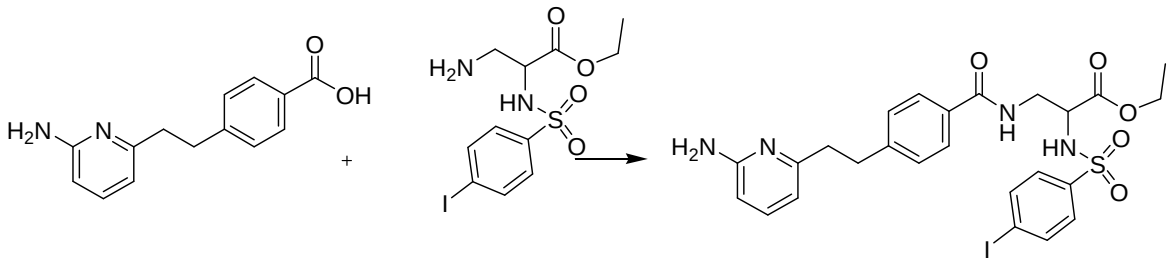
	JF 3-6	Pd-C	EtOH	HCl 10N	JF 3-7
Chemical formula	$C_{16}H_{14}N_2O_2$				$C_{14}H_{14}N_2O_2$
Mw	266,01				242,01
m[g]	1,17	0,585			0,99
n[mmol]	4,4				
d[g/ml]					
V[ml]			50	20	

Procedure

Mixture of JF 3-6 (1,17 g; 4,4 mmol), 10% Pd-C (0,585 g) in 50 ml EtOH was stirred under 1 atm H_2. After 23 h the mixture was filtered through a celite pad and concentrated under reduced pressure. A suspension of crude ester in 10N HCl (20 ml) was heated to 65°C for 2 h. After evaporation of solvent was obtained 0,99 g (93,4%) of JF 3-7 as a tan solid.[30]

MS (ESI): m/z= 243,1$[M+H]^+$

5.19 Preparation of ethyl 3-(4-(2-(6-aminopyridin-2-yl) ethyl)benzamido)-2-(4-iodophenylsulfonamido) propanoate (JF 3-8)



Reagents

	JF 3-7	JF 3-3	EDC	HOBT	NMM	DMF	JF 3-8
Chemical formula	$C_{14}H_{14}N_2O_2$	$C_{11}H_{15}N_2O_4SI$					$C_{25}H_{27}N_4O_5SI$
Mw	242,01	398,08		153,1			622,09
m[mg]	200	340	180	125			221
n[mmol]	0,8	0,85		0,82			
d[g/ml]							
V[ml]					0,315	6	

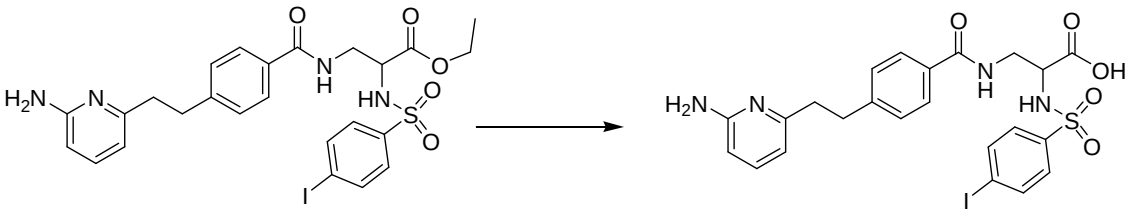
Procedure

The solution of JF 3-7 (200 mg; 0,8 mmol), JF 3-3 (340 mg; 0,85 mmol), EDC (180 mg), HOBT (125 mg; 0,82 mmol), NMM (0,315 ml) in DMF (6 ml) was stirred for 30 h. Then the reaction was diluted with EtOAc and washed twice with 80 ml saturated $NaHO_3$, twice with 80 ml brine, dried with $MgSO_4$ and concentrated under reduced pressure. The crude product was purified with column chromatography (10% 2-propanol/EtOAc) to give 221 mg (43,1 %) of JF 3-8.[30]

MS (ESI): m/z= 623,2 $[M+H]^+$

HPLC (0-100%; 4min): t_R=2,902 min

5.20 Preparation of 3-(4-(2-(6-aminopyridin-2-yl)ethyl) benzamido)-2-(4-iodophenylsulfonamido)propanoic acid (JF 3-9)



Reagents

	JF 3-8	30% HCl	JF 3-9
Chemical formula	$C_{25}H_{27}N_4O_5SI$		$C_{23}H_{23}N_4O_5SI$
Mw	622,09		594,09
m[mg]	221		
n[mmol]	0,36		
V[ml]		30	

Procedure

The solution of JF 3-8 (221 mg; 0,36 mmol) and 30% HCl (30 ml) was heated to 65°C . After 20,5 h was the mixture concentrated under reduced pressure and purified on HPLC to give JF 3-9 as TFA salt.[30]

MS (ESI): m/z= 595,1 $[M+H]^+$

HPLC (0-100%; 4min): t_R=2,616 min

^{1}H-NMR (300 MHz, DMSO): δ= 8,34 (t, 1H), 8,27 (s, 1H), 7,79 (d, 2H), 7,74 (d, 2H), 7,64 (d, 2H), 7,45(d, 2H), 7,31 (d, 2H), 6,75 (d, 1H), 4,15 (s, 2H), 3,48 (m, 2H), 3,32 (m, 2H)

Elementary analysis:

Found (with 1,1 TFA): C 42,05; H 3,37; N 7,78

Counted: C 42,38; H 3,41; N 7,91; S 4,53; I 17,91; O 15,81; F 8,05

6 Discussion

At first the aminopyridinyl alcohol JF1-1 was synthesized from 2-bromopyridine and aninopropanol at 150°C in around 60% yield, which was little bit lower than expected on based literature. While N-Boc-tyrosin methyl ester was good comercionally accessible N-Boc-homotyrosin methyl ester (JF 2-2) had to be prepared with two-step reaction from O-benzoyl-N-boc-homotyrosin. First the hydroxyl group was protected with methylation by using methanol, DMAP and EDC in DCM and then the benzoyl group was removed using ammonium formate and Pd/C as catalyst in methanol. Both reaction passed with good yields around 85-95%. Both these compounds were coupled with aminopyridinyl alcohol through Mitsunobu reaction by addition PBu_3 and ADDP in THF to give heterogeneous yields. We obtained almost 55% yield of JF 1-2 but just 15% yield of JF 2-3. By repetition of this coupling for obtaininig a bigger amount of product the reaction didn't pass in agreement with this scheme and we didn't get any required product. In regard to uncertainity of this reaction as option for this coupling is possible to use reaction of 1,3-dibrompropan with aromatic hydroxyl from N-Boc-homotyrosin methyl ester and subsequently have let react the formed product with aminopyridin. Based on published literature promises this method better yields.[31] Before next reactions both precursors were Boc-deprotected with HCl in dioxane by indoor temperature to get high yields above 90%. JF 1-3 compound based on tyrosin structure was acylated with 4-iodine-sulfonyl chlorid with DIPEA in DMF. In contrast to literature we tried purification of resulting product on column chromatography, but the same mobile phase used by thin layer chromatography didn't separated obtained product enough. In case of use with bigger amount of starting substance we recommend to pay attention to specification of mobile phase for facilitation of the last step of synthesis. JF 2-4 compound based on homotyrosin structure was acetylated with 4-iodobenzoyl chloride with $NaHCO_3$ in mixture of dioxane/water. Resulting crude product was successfully purified by column chromatography. Both compounds, JF1-4 and JF 2-5, were in the last step demethylated with LiOH in methanol. While the reaction with purified JF 2-5 passed relatively quickly to the reaction with JF 1-4 was necessary to add next LiOH and also the reaction time was three-times longer. At the same time purification facilitated a lot preparative HPLC with final product.

Preparation of final structure JF 3-9 was divided in two parts. At first was synthetized 3-amino-2-arylsulfonylaminopropanoic acid derivat which was coupled with aminopyridilethylbenzoic acid.

At first L-asparagine was acetylated on amino group with 4-iodophenylsulfonyl chloride in aquesous NaOH and dioxane to get JF 3-1 with yield 47%. Resulting product was shorted on carbon skelet through reaction with bromine to give JF 3-2 in yield 67%. At the end was the hydroxyl group protected to obtain ethyl ester JF 3-3. From all these three reactions were obtained products clearly enough which weren't necessary to purify before next use. Except of preparation of JF 3-2, it failed to get so high yields as was presented in literature.[30]

Second part of resulting structure was prepared with coupling TMS-acetylene and Ethyl 4-iodobenzoate with $(Ph_3P)_2PdCl_2$ and CuI in CH_3CN in a glass pressure tube to give JF 3-4 with yield 92%. From resulting product was TMS group broken away with K_2CO_3 in ethanol. The resulted free acetylen was coupled with 2-Amino-6-bromopyridine with the help of $(Ph_3P)_2PdCl_2$ and CuI in CH_3CN to give JF 3-6. The mobile phase for column chromatography for better product separation was found out to improve fraction of aceton in hexane to 20% from 10% as featured in instruction. Reduction of double bond with Pd/C under hydrogen atmosphere by normal atmospheric pressure and consequentive elimination methylester group via acid hydrolysis passed with high yield of 93% JF 3-7 compound. To released hydroxyl group was coupled the free amino group from JF 3-3 in presence of EDC, HOBT and NMM in DMF. After purification by column chromatography was resulting compound JF 3-8 diluted in concentrated HCl and by elimination ethylester group the final product JF 3-9 was obtained. All steps in this synthesis except for some lower yields corresponded with literature.

7 Conclusions

Within my work I prepared two new iodized compounds

2-(4-iodophenylsulfonamido)-3-(4-(3-(pyridin-2-ylamino)propoxy)phenyl) propanoic acid (JF 1-5)

3-(4-iodobenzamido)-4-(4-(3-(pyridin-2-ylamino)propoxy)phenyl) butanoic acid (JF 2-6)

and one earlier known compound which was used in tests as reference

3-(4-(2-(6-aminopyridin-2-yl)ethyl) benzamido)-2-(4-iodophenylsulfonamido) propanoic acid (JF 3-9)

All these substances were described with Mass spectrometry, ^{1}H-NMR spectroscopy and HPLC.

By in vitro cell-binding assay we established IC_{50} values.[32] For JF 1-5 IC_{50}=195,6 (before substitution 3,4), for JF 2-6 IC_{50}=9924,8 (before substitution 1,2) and for JF 3-9. IC_{50}=2,4. Because the affinity of new substituted compounds was clearly lower radioactive labelling wasn't made.

8 Abstract

The integrins are family of α,β heterodimeric receptors with high importance in many cell processes. They are expressed by all multicellular animals. Mainly alpha(v)beta(3) subset plays important role in cells adhesion with surroundings. Adhesion decrease by occupation of these receptors is used as restriction for tumor metastasis or for early tumor imaging. Description of structure and three-dimensional orientation of binding place of this subset were set RGD (Arg-Gly-Asp) peptide as high affinite ligands. According to computing accounted models of interaction between RGD peptide and binding place were also nonpeptide ligands prepared.

In this publication we tried to prepare potentionally usable molecules for tumor imaging on PET scanners. For the base we chose already known molecules with high affinity to alpha(v)beta(3) integrins and with iodine substitution we got possibility for radioactive labelling. Unfortunately, this incorporation of iodine into the molecule decreased IC_{50} to value preclusive practical using.

9 Abstrakt

Integríny jsou α,β heterodimerní skupinou receptorů s velkým významem v mnoha buněčných procesech. Vyskytují se ve všech mnohobuněčných organismech. Především podskupina alpha(v)beta(3) hraje významnou roli v adhezi buněk s okolím. Snížení adheze obsazením těchto receptorů se využívá k omezení metastazování rakovinných buněk nebo k jejich včasné diagnostice. Popsáním struktury a prostorového uspořádání vazebného místa této podskupiny byly jako vysoce afinitní ligandy určeny peptidy obsahující RGD (Arg-Gly-Asp) triplet. Podle počítačových modelů interakce RGD peptidů a vazebného místa receptoru byly postupně připravovány i nepeptidové ligandy.

V této práci jsme se pokusili připravit molekuly potencionálně využitelé k diagnostice rakoviny pomocí pozitronové emisní tomografie. Jako předlohu jsme zvolili již dříve připravené molekuly s vysokou afinitou k alpha(v)beta(3) integrínovým receptorům a přípravou jejich jodovaných analogů jsme získali možnost jejich radioaktivního značení. Bohužel zavedení jódu do molekuly snížilo IC_{50} na hodnoty znemožňující jejich reálné využití.

10 Shrnutí

V rámci mé práce jsem připravil dvě nové jodované molekuly

kyselinu 2-(4-iodophenylsulfonamido)-3-(4-(3-(pyridin-2-ylamino)propoxy)phenyl) propanovou (JF 1-5)
kyselinu 3-(4-iodobenzamido)-4-(4-(3-(pyridin-2-ylamino)propoxy)phenyl) butanovou (JF 2-6)

a jednu dříve popsanou molekulu, která sloužila jako srovnání při testech in vitro

kyselinu 3-(4-(2-(6-aminopyridin-2-yl)ethyl) benzamido)-2-(4-iodophenylsulfonamido) propanovou (JF 3-9)

Všechny tyto látky byly charakterizovány hmotnostními a ^{1}H-NMR spektry a retenčními časy HPLC.

In vitro testováním afinity jsme určili hodoty IC_{50}. Pro JF 1-5 IC_{50}=195,6 (před substitucí 3,4), pro JF 2-6 IC_{50}=9924,8 (před substitucí 1,2) a pro JF 3-9. IC_{50}=2,4. Protože afinita substituovaných moleku byla výrazně nižší než u původních, radioaktivní značení látek nebylo provedeno.

11 References

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