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Syntheses of natural products and molecules for RNA imaging

Ivan M. Geraskin
Iowa State University

Syntheses of natural products and molecules for RNA imaging

by

Ivan M. Geraskin

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George A. Kraus, Major Professor
L. Keith Woo
Arthur Winter
Levi Stanley
Gregory J. Phillips

The student author, whose presentation of the scholarship herein was approved by the program of study committee, is solely responsible for the content of this dissertation. The Graduate College will ensure this dissertation is globally accessible and will not permit alterations after a degree is conferred.

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TABLE OF CONTENTS

ABSTRACT

The synthesis of natural products and biologically active molecules is one of the main applications of organic chemistry. Many natural products with valuable biological properties have low abundance in natural sources. Therefore, they need to be synthesized in sufficient quantities that their properties and activities can be studied. Chapter 1 describes synthesis of a library of biologically active compounds which are currently used in RNA imaging studies. Chapter 2 describes the synthesis of the procyanidin A skeleton. Procyanidins are important natural products with various biological activities. Chapter 3 describes a method for the preparation of anthocyanidins, natural products which possess valuable biological properties.

GENERAL INTRODUCTION

The synthesis of natural products and biologically active molecules is one of the main applications of organic chemistry. Many natural products with valuable biological properties have low abundance in natural sources. Therefore, they need to be synthesized in sufficient quantities that their properties and activities can be studied. Bioactive small molecules can be utilized in studying different biological processes such as transcription of RNA. Understanding these processes will allow the development of new therapies and drugs. The synthesis should be efficient, simple, with the use of environmentally friendly reagents and follow the principles of green chemistry. Therefore, products should be synthesized in good yields and be easy to purify.

In this thesis, we report new synthetic methods that were used towards the syntheses of natural products and molecules for RNA imaging. Chapter 1 describes synthesis of a library of biologically active compounds which are currently used in RNA imaging studies by our collaborators in the Department of Biochemistry, Biophysics and Molecular Biology at Iowa State University. Also, we have developed a method for labelling of aminoglycosides with fluorinated esters. This method is used by our collaborators at the University of California San Francisco who use radiolabeled esters to track bacteria in soil. Some of this work has been published in *Methods* in 2016 (Ilgu, M.; Ray, J.; Bendickson, L.; Wang, T.; Geraskin, I. M.; Kraus, G. A.; Nilsen-Hamilton, M. *Methods*, **2016,** *98*, 26-33.). Chapter 2 describes the synthesis of the procyanidin A-skeleton. Procyanidins are important natural products with various biological activities. This method is operationally simple, efficient, and can be performed on a large scale and used for the total synthesis of A-type procyanidins. This work has been published in *Tetrahedron Letters* in 2017 (Kraus, G. A.; Geraskin, I. M. *Tetrahedron Letters*, **2017**, *58*, 4609–4611.). In chapter 3 we report the method for the preparation of anthocyanidins, natural products which possess valuable

biological properties, from benzopyrans. This method is efficient one-pot transformation which can be used for the synthesis of anthocyanidins on a large scale. This work has been published in *Natural Product Communications* in 2016 (Kraus, G. A.; Geraskin, I. M. *Natural Product Communications* **2016**, *11*, 1649-1650.).

CHAPTER 1. SYNTHESIS OF SMALL MOLECULES FOR RNA IMAGING

Some of this work has been published in *Methods* in 2016 (Ilgu, M.; Ray, J.; Bendickson, L.; Wang, T.; Geraskin, I. M.; Kraus, G. A.; Nilsen-Hamilton, M. *Methods*, **2016,** *98*, 26-33.).

Introduction

RNA molecules participate in and regulate many fundamental cellular processes. To gain better understanding of the variety of RNA functions, the entire cellular life of RNAs, from their synthesis to their decay, needs to be examined. Thus, the ability to image RNA transcription and trafficking in real time and in living cells will allow researchers to investigate many cellular processes involving RNAs. The RNA molecule is not fluorescent by itself. There are several strategies for fluorescent imaging of RNA. One of them requires the expression of an exogenous transcript of interest fused to an aptamer sequence, known as chimeric transcript. The aptamer itself is not fluorescent, but when it binds a specific protein partner fused to an autofluorescent protein (chimeric protein), the entire system becomes fluorescent after excitation at appropriate wavelength. The main drawbacks of aptamer-protein systems are the strong background signal produced by the constant fluorescence of the chimeric protein not bound to the aptamer and proteins bound to RNA can participate in interactions with other cell elements. One of the examples of proteins which was extensively used for RNA imaging is green fluorescent protein (GFP).[1]

Another strategy utilizes fluorescent molecules, ligands, which have affinity to certain regions of RNA, so-called light-up aptamers.[2] These ligands become more fluorescent upon binding to aptamers and the ligand-aptamer complex can be visualized by fluorescence

microscopy. There are many reports on the discovery of aptamers that bind fluorophores which enables RNA imaging. Some of them include aptamers that bind triphenylmethanes, bisbenzimides, benzothiazolidene asymmetric cyanine dye, and thiazole orange.[3-5] Recently, Jaffrey and coworkers reported RNA aptamers, denoted as Spinach and Spinach 2, that bind (Z)-5-(4-HydroxyBenzylidene)-2,3-dimethyl-3,5-dihydro-4H-Imidazol-4-one (HBI), compound **1**, and (Z)-4-(3,5DiFluoro-4-HydroxyBenzylidene)-1,2-dimethyl-1H-Imidazol-5(4H)-one DFHBI, compound **2**, fluorophores mimicking the fluorophore present in GFP (Figure 1).[6] HBI corresponds to the fragment of GFP responsible for its fluorescence.[7]

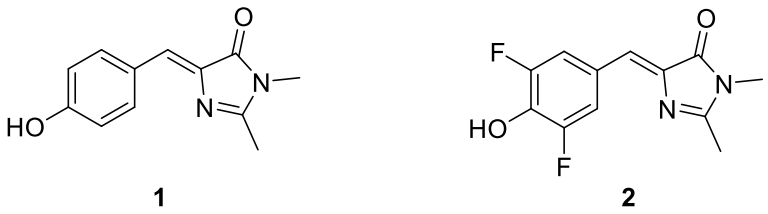


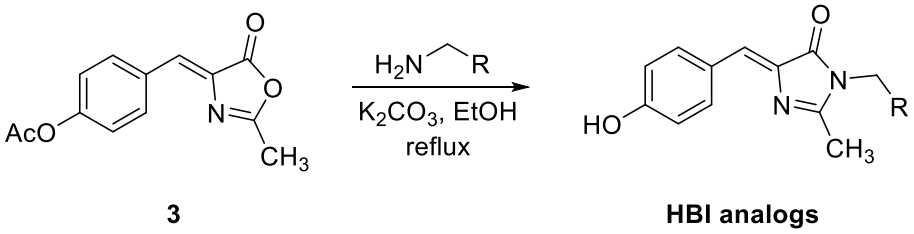
Figure 1. Strucures of HBI (**1**) and DFHBI (**2**)

The advantage of this strategy is that ligands are small molecules which typically do not participate in non-RNA binding interactions and can be modified to produce better fluorescence and affinity.

Another technique to utilize small molecules for RNA imaging is FRET. FRET, Förster resonance energy transfer, is a mechanism describing energy transfer between two light-sensitive molecules.[8] The mechanism of transfer occurs when the donor, in its excited state, transfers energy non-radiatively to a neighboring molecule, the acceptor, in its ground state via dipole-dipole interactions.[9] The advantage of FRET is that there is no background fluorescence coming from ligands not bound to RNA because fluorescent visualization occurs only when two molecules, donor and acceptor, are in close proximity to each other.[10]

Results and discussion

We have been exploring DFHBI platform in order to create better fluorescent probes for RNA imaging in live cells. First, we have synthesized HBI analogs with R group being long chain and branched alkyl, alkynyl and aryl in order to test binding affinities of synthesized ligands to Spinach, Spinach 2 aptamers, Scheme 1. A library of HBI analogs was synthesized by the reaction of azalactone **3**, which is readily available from 4-hydroxy-benzaldehyde and N-acetylglycine, with the corresponding primary amines in the presence of base, as shown in Scheme 1.



3 **HBI analogs**

Scheme 1. Synthesis of HBI analogs

The synthesized analogs and yields are listed in Table 1.

Table 1. Synthesized HBI analogs.

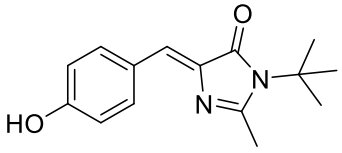
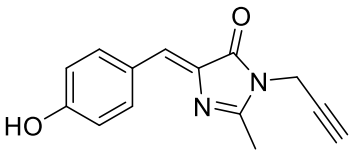
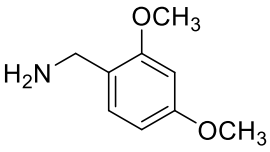
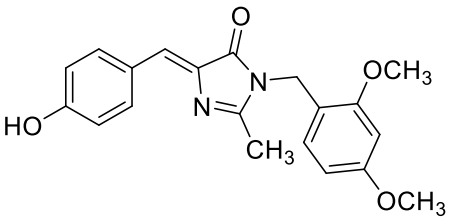
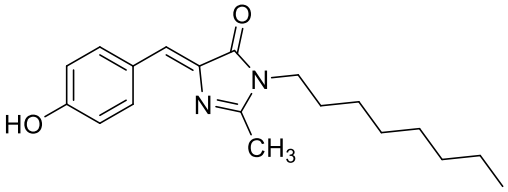
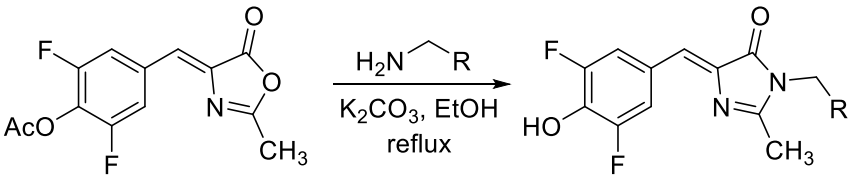
Entry	Amine	Product	Compound	Yield, %
1	*t*-BuNH$_2$		**4**	52
2	Propargyl amine		**5**	57

Table 1. (continued)

3			**6**	73
4	$C_8H_{17}NH_2$		**7**	85

It was found that compound **6**, HBI analog with R = 2,4-dimethoxybenzene, had the highest binding affinity towards Spinach and Spinach 2 aptamers (Table 1, entry 3). Considering this result, various DFHBI analogs with different aryl groups, replacing methyl group on amide nitrogen of DFHBI azalactone ring, were synthesized in a similar fashion in order to find one which will give the highest fluorescence, as shown in Table 2.

Table 2. Synthesized DFHBI analogs.

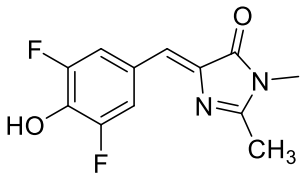
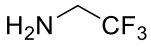
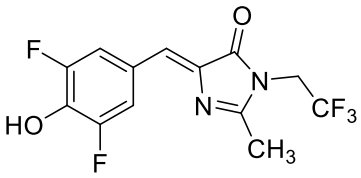
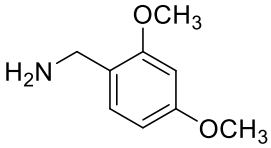
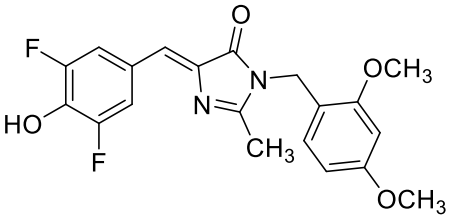
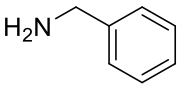
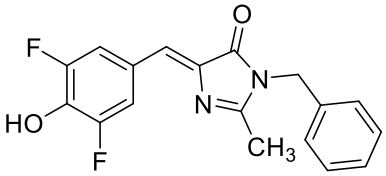
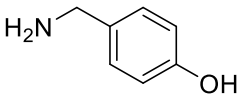
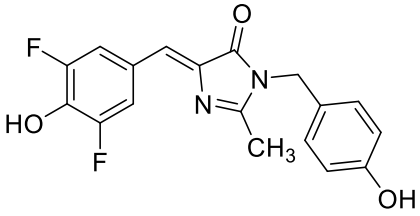
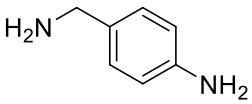
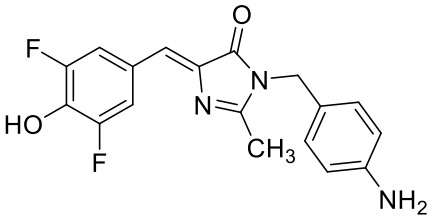


8
azalactone

DFHBI analogs

Entry	Amine	Product	Compound	Yield, %

Table 2. (continued)

1	CH_3NH_2		**9**	61
2			**10**	43
3			**11**	71
4			**12**	75
5			**13**	52
6			**14**	72

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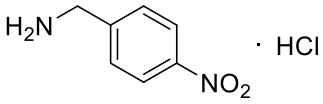
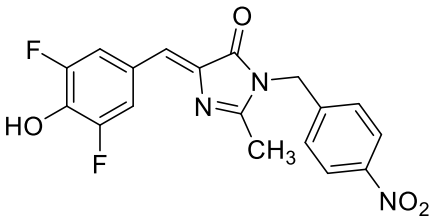
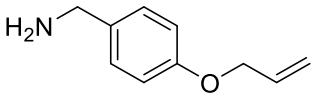
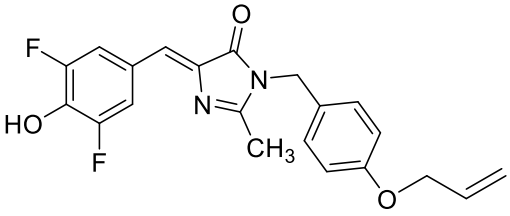
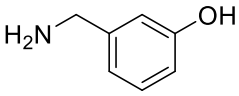
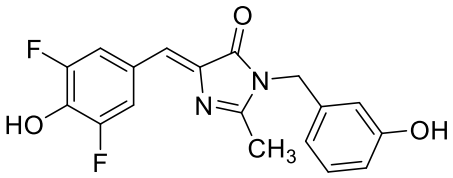
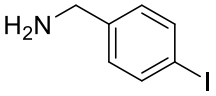
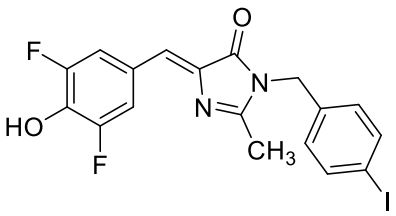
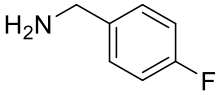
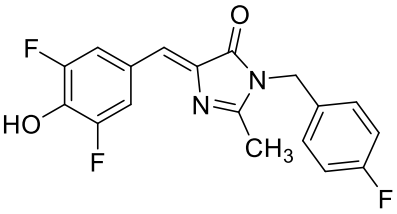
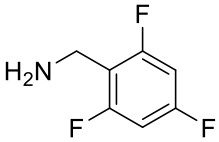
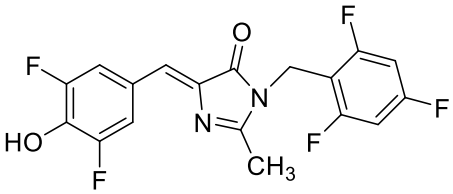
7			**15**	78
8			**16**	77
9			**17**	53
10			**18**	73
11			**19**	73
12			**20**	75

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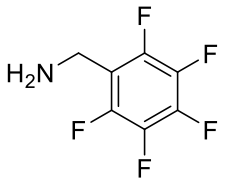
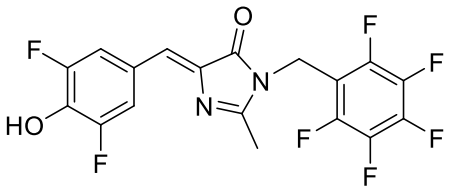
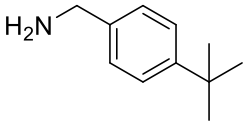
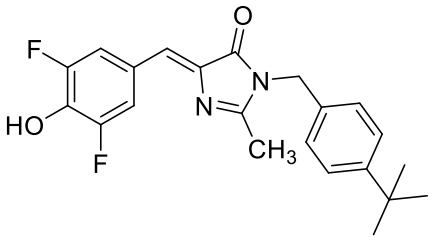
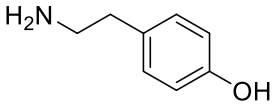
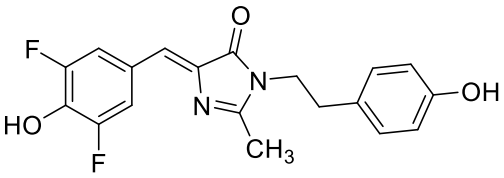
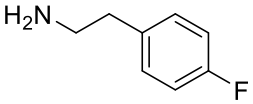
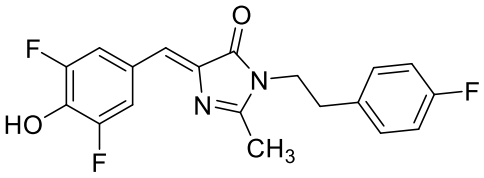
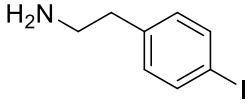
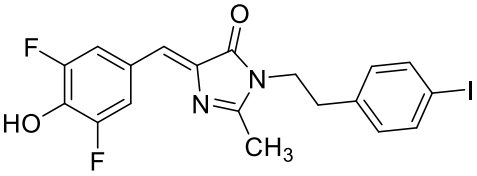
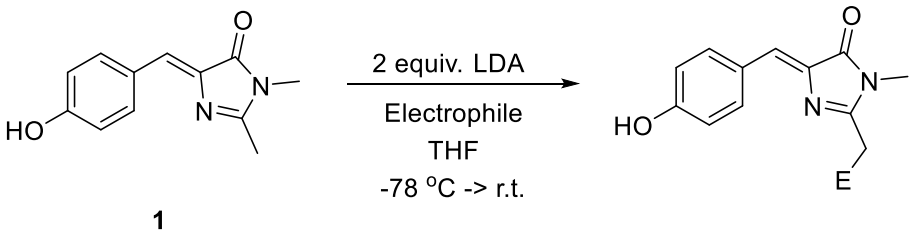
13			**21**	71
14			**22**	63
15			**23**	73
16			**24**	69
17			**25**	70

The best DFHBI analog, with the highest affinity towards Spinach and Spinach 2 RNA aptamers, and fluorescent yield, was found to be PFP-DFHBI, compound **21**.[11, 12] This new ligand has superior qualities compared to commonly used DFHBI and can be used for fluorescent imaging of RNA. It has three-fold higher fluorescence compared to DFHBI which makes it to be more useful ligand.

In order to further explore DFHBI platform we were able to functionalize the allylic position in HBI. Treatment of HBI with two equivalents of LDA formed the dianion which selectively reacted with various aldehydes at the allylic position forming aldol condensation products (Scheme 3, Table 3, Entries 1-3). The high selectivity of the reaction can probably be best explained by electrostatic repulsion of negative charges in dianion making second negative charge to be formed selectively at the allylic position, as far away as possible from the phenolate.



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Scheme 3. Functionalization of allylic position in HBI

The scope of the reaction can be extended further by using various electrophiles. Allylation of HBI using allyl bromide afforded compound **28** in moderate yield (Table 3, entry 4). Similarly, treatment of HBI with two equivalents of LDA followed by addition of diphenyl disulfide or diphenyl diselenide gave compounds **29**, **30**, **31** respectively (Table 3, entries 5, 6). Interestingly, when diphenyl disulfide was used as an electrophile small portion of bis-organo-sulfidation product was isolated due to sulfur lowering the pKa of adjacent methylene hydrogens (Table 3, entry 6).

Table 3. Synthesized DFHBI analogs.

Entry	Electrophile	Product	Compound	Yield (%)

Table 3. (continued)

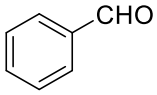
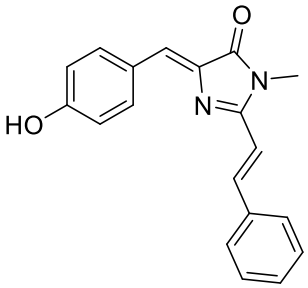
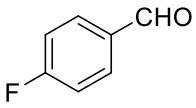
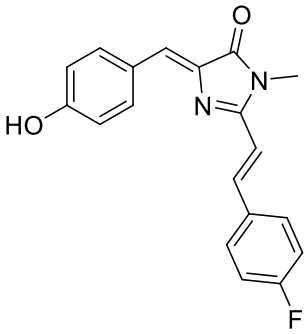
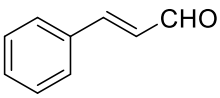
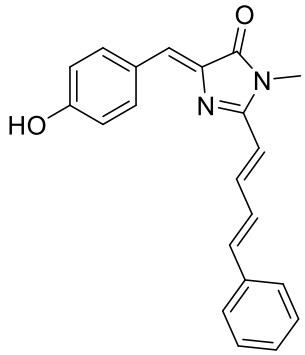
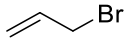
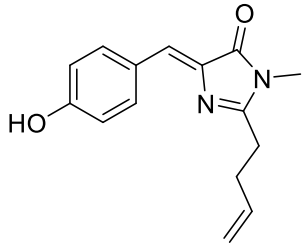
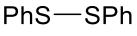
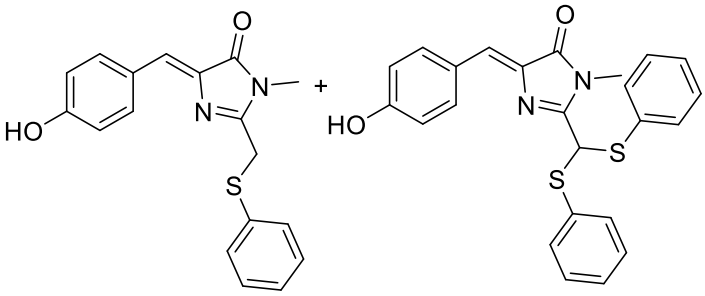
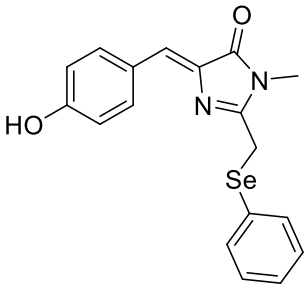
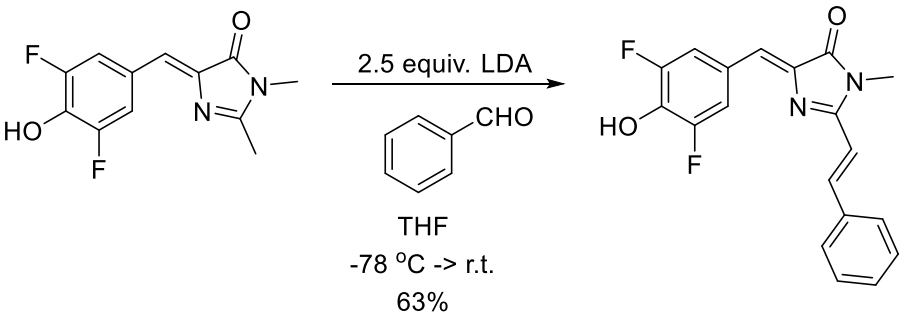
1			**26**	91
2			**27**	48
3			**28**	63
4			**29**	44
5		 (5 : 1)	**30 31**	88

Table 3. (continued)

6	PhSe−SePh		**32**	55

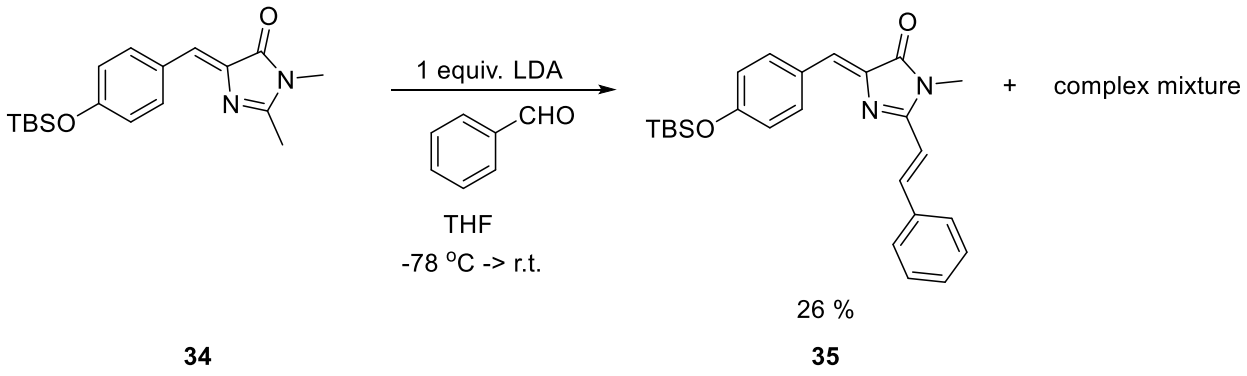
The same reaction conditions can be applied for the functionalization of the allylic position in DFHBI (Scheme 4).



2 **33**

Scheme 4. Functionalization of allylic position in DFHBI

When TBS-protected HBI, compound **34**, was treated with one equivalent of LDA, followed by the addition of benzaldehyde, very low selectivity was observed, Scheme 5. Compound **35** was isolated in 26% yield and complex mixture of products was obtained.



Scheme 5. Functionalization of allylic position in TBS-protected HBI

These compounds, DFHBI analogs modified at the allylic position, were found to have less binding affinity towards Spinach, Spinach 2 RNA aptamers than PFP-DFHBI, compound **21**. Fortunately, compounds **26**, **27**, **28**, **33** have been found to have interesting photophysical properties due to extended conjugation. Namely, they have different excitation and emission wavelengths compared to DFHBI and were found to have longer fluorescence lifetime. The studies are currently conducted to explore the full potential of these compounds.

After finding DFHBI ligands with the highest affinity towards Spinach 1 and Spinach 2 aptamers, the ones having substituted benzyl groups in place of methyl group on amide nitrogen of DFHBI azalactone ring, we have attached cyanine dyes to generate the desired FRET pair. Cy3, Cy5 fluorescent dyes were employed to form the FRET pair, where Cy3 acts as the donor and Cy5 as the acceptor, Figure 2. Both non-sulfonated and sulfonated versions of the dyes are commercially available in their NHS ester forms and were employed for the labelling of the DFHBI derivatives. The best FRET signal was observed in vitro when sulfonated Cy3, Cy5 dyes were used, most likely because of their better water-solubility.

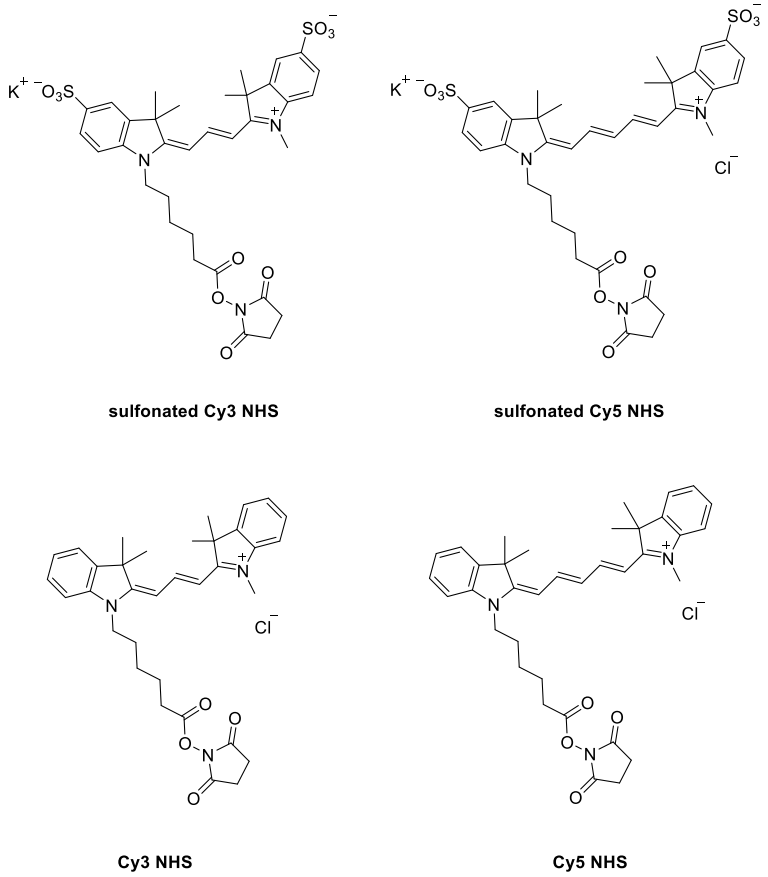
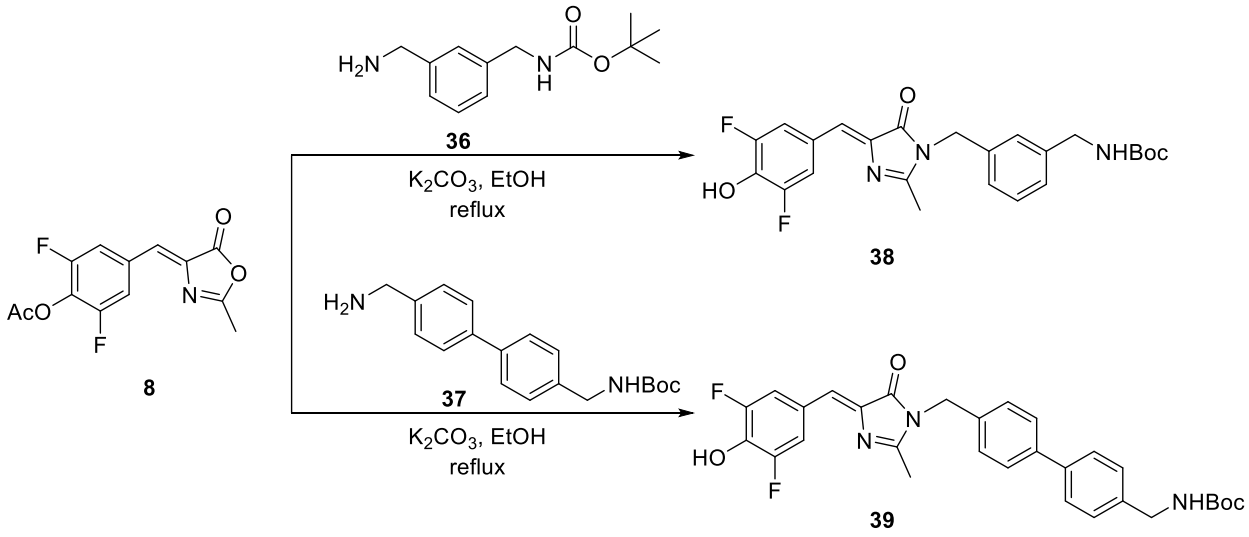


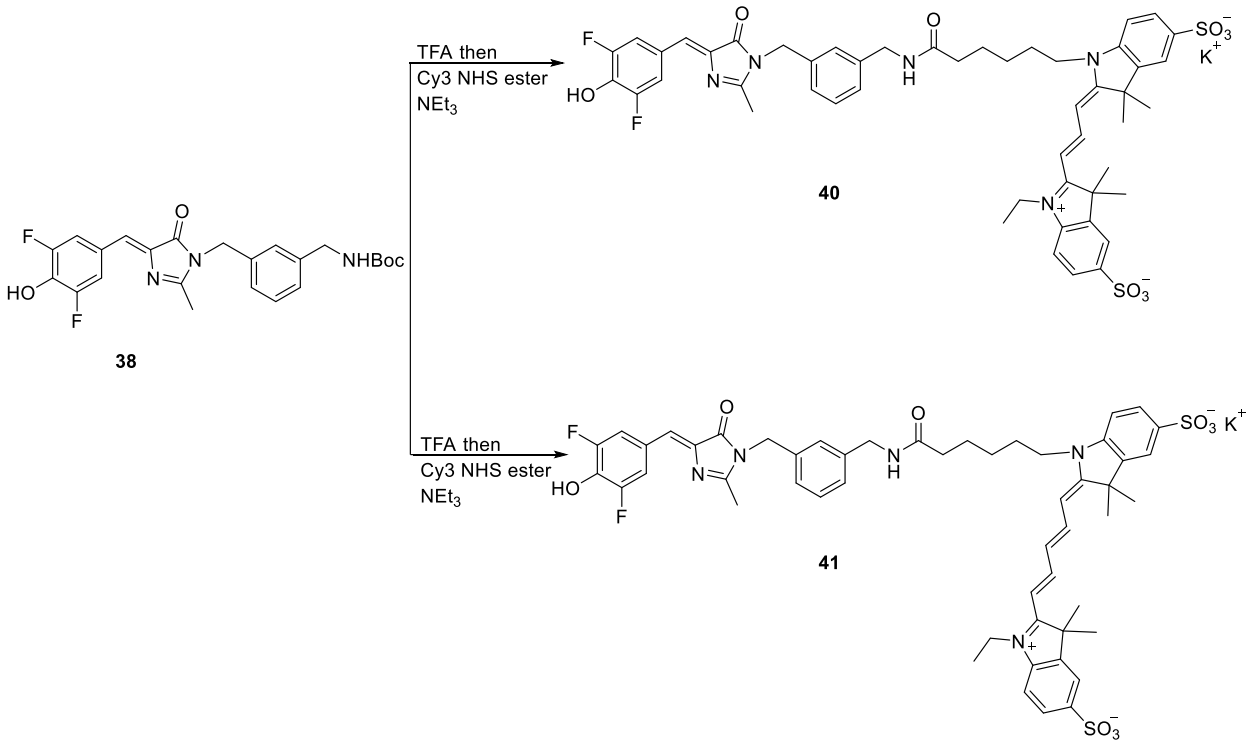
Figure 2. Commercially available cyanine dyes

NHS esters are known for their selective reactivity towards primary amino groups in the presence of other nucleophiles. DFHBI ligands with protected primary amino group on benzyl ring were synthesized, Scheme 6. Monoprotected amines **36**, **37** were prepared in good yields by the reaction of the corresponding diamines with one equivalent of di-tert-butyl decarbonate in dioxane. Azalactone **8** was treated with monoprotected amines **36**, **37** in the presence of potassium carbonate at reflux in ethanol to afford 3-bocaminobenzylamine DFHBI **38** and 4-bocaminophenylbenzylamine DFHBI **39**.

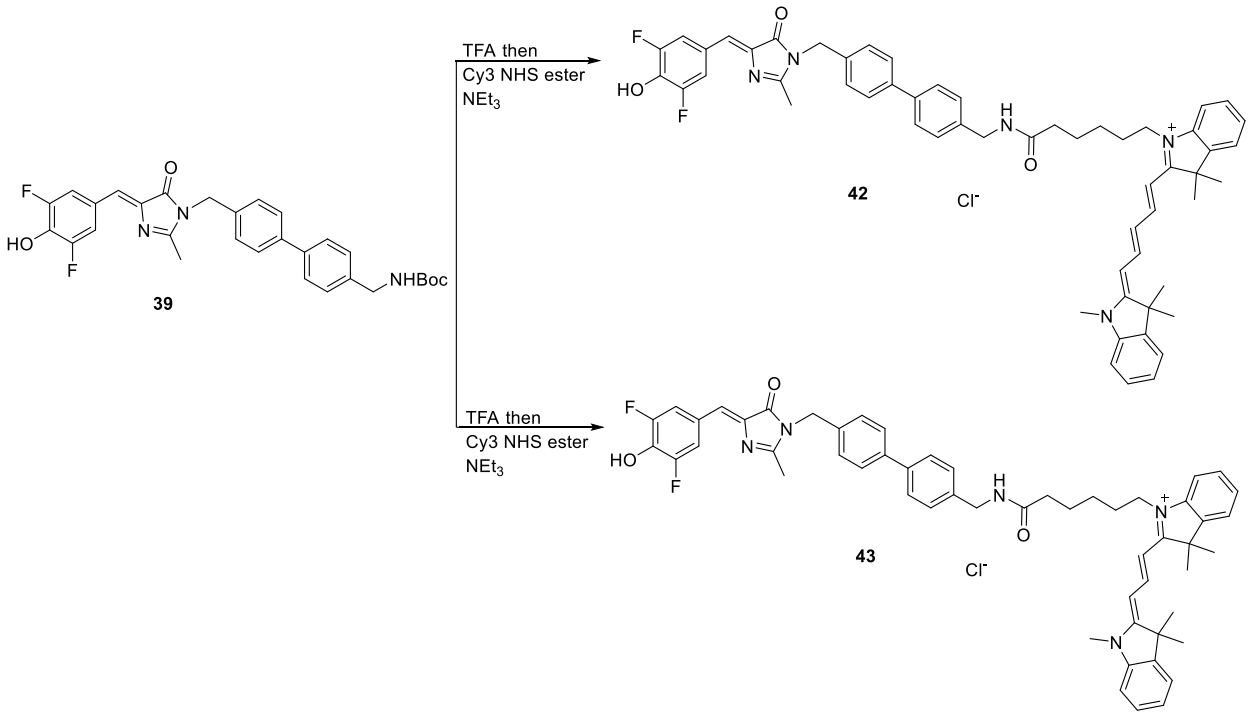


Scheme 6. Synthesis of Boc-protected 3-aminobenzylamine DFHBI **38** and 4-aminophenylbenzylamine DFHBI **39**

Deprotection of Boc group in 3-aminobenzylamine DFHBI **38** with trifluoroacetic acid liberated primary amino group which selectively reacted with Cy3 NHS ester or Cy5 NHS ester to give 3-bocaminobenzylamine DFHBI-Cy3 **40** and 3-aminobenzylamine DFHBI-Cy5 **41**, Scheme 7. Similarly, another FRET pair, 4-aminophenylbenzylamine DFHBI-Cy3 **42** and 4-aminophenylbenzylamine DFHBI-Cy5 **43**, was generated by deprotection of primary amino group in 4-bocaminophenylbenzylamine DFHBI **39**, followed by the reaction with Cy3 or Cy5 NHS esters, Scheme 8.

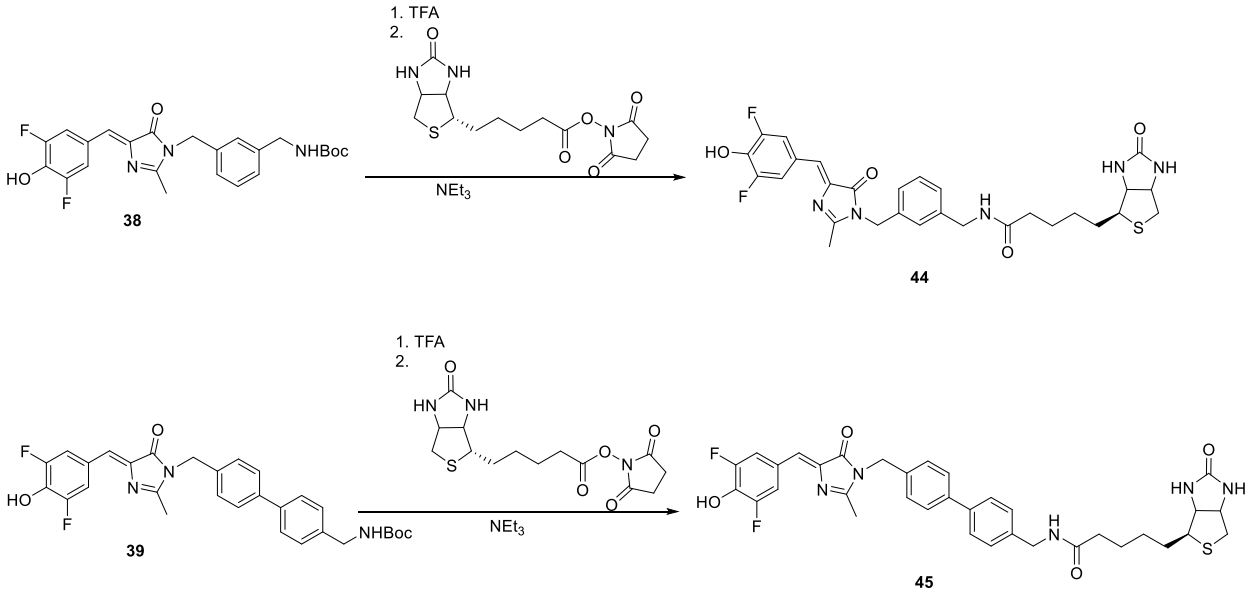


Scheme 7. Synthesis of FRET pair 3-aminobenzylamine DFHBI-Cy3 **40** and 3-aminobenzylamine DFHBI-Cy5 **41**



Scheme 8. Synthesis of FRET pair 4-aminophenylbenzylamine DFHBI-Cy3 **42** and 4-aminophenylbenzylamine DFHBI-Cy5 **43**

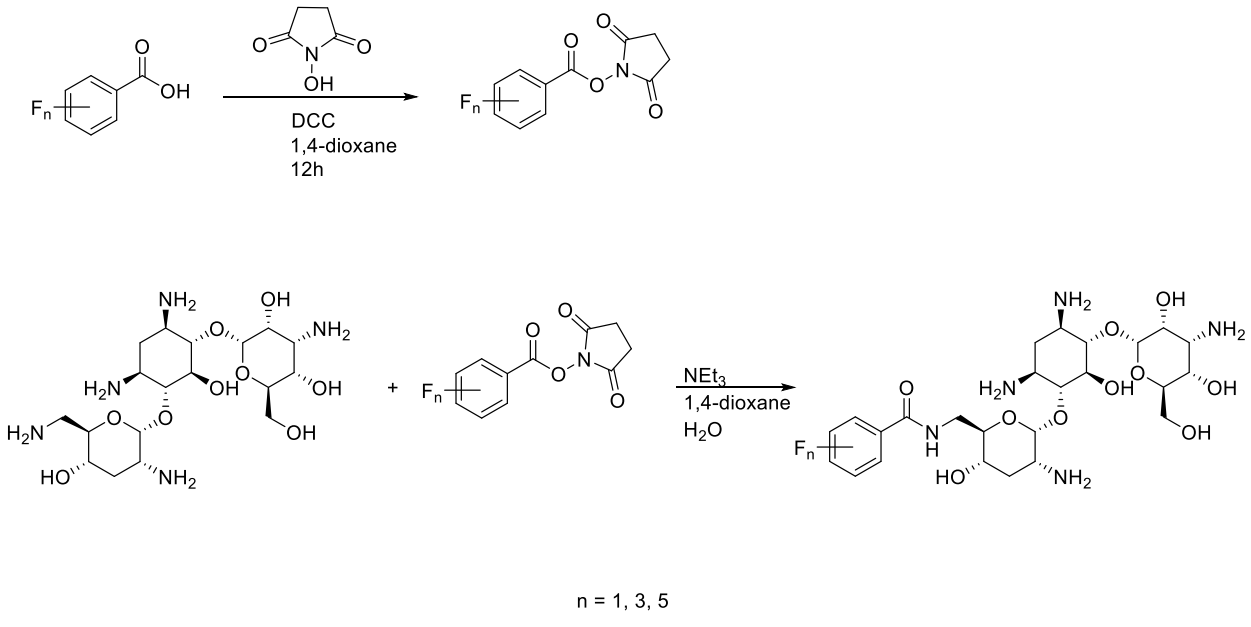
In order to select novel RNA aptamers from pool of RNAs, which bind DFHBI ligands and can expand their applications, biotin needs to be attached to these ligands. The same synthetic strategy as one for attaching cyanine dyes to DFHBI analogs, utilizing selective affinity of NHS esters to primary amino groups, was employed. Treatment of 3-bocaminobenzylamine DFHBI **38** with TFA followed by the reaction with biotin NHS ester in the presence of triethyl amine afforded 3-aminobenzylamine DFHBI-biotin **44** in quantitative yield, Scheme 9. Similarly, 4-aminophenylbenzylamine-biotin **45** was synthesized by treatment of 4-bocaminophenylbenzylamine DFHBI **39** followed by reaction with biotin NHS ester in the presence of triethyl amine.



Scheme 9. Synthesis of 3-aminobenzylamine DFHBI-biotin **44** and 4-aminophenylbenzylamine-biotin **45**

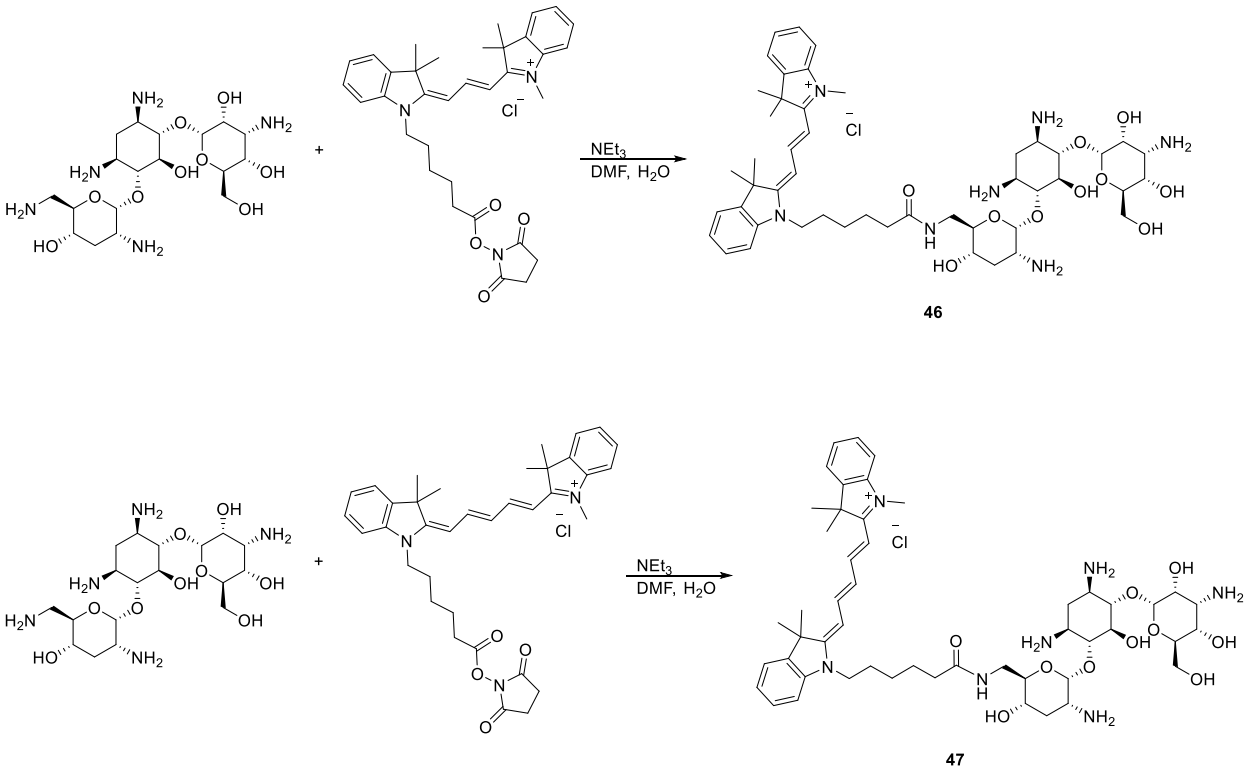
The studies are currently conducted by our collaborators at Iowa State University, Department of Biochemistry, Biophysics and Molecular Biology to select new RNA aptamers for newly synthesized DFHBI ligands using compounds **44** and **45**.

Our collaborators from the University of California in San Francisco study various bacteria in soil. In order to track these bacteria different techniques can be used such as fluorescence, radiolabeling. We have developed procedures for labelling of aminoglycosides which can be applied for tracking bacteria in soil. NHS ester protocol employed for coupling of DFHBI to cyanine dyes and biotin was utilized to attach fluorobenzoyl moiety to tobramycin, as shown in Scheme 10. NHS esters of fluorobenzoic acids are readily available by treatment of the corresponding fluorobenzoic acids with N-hydroxysuccinimide in the presence of DCC. NHS esters of fluorobenzoic acids reacted with primary amino group of tobramycin with complete regioselectivity. Other reaction conditions (benzoyl chloride/triethyl amine) employed for functionalization of primary amino group did not provide satisfactory selectivity and resulted in mixture of products. Tobramycin can be radiolabeled with fluorobenzoyl moiety bearing radioactive isotope ^{18}F using this method and can be used to study bacteria in soil.



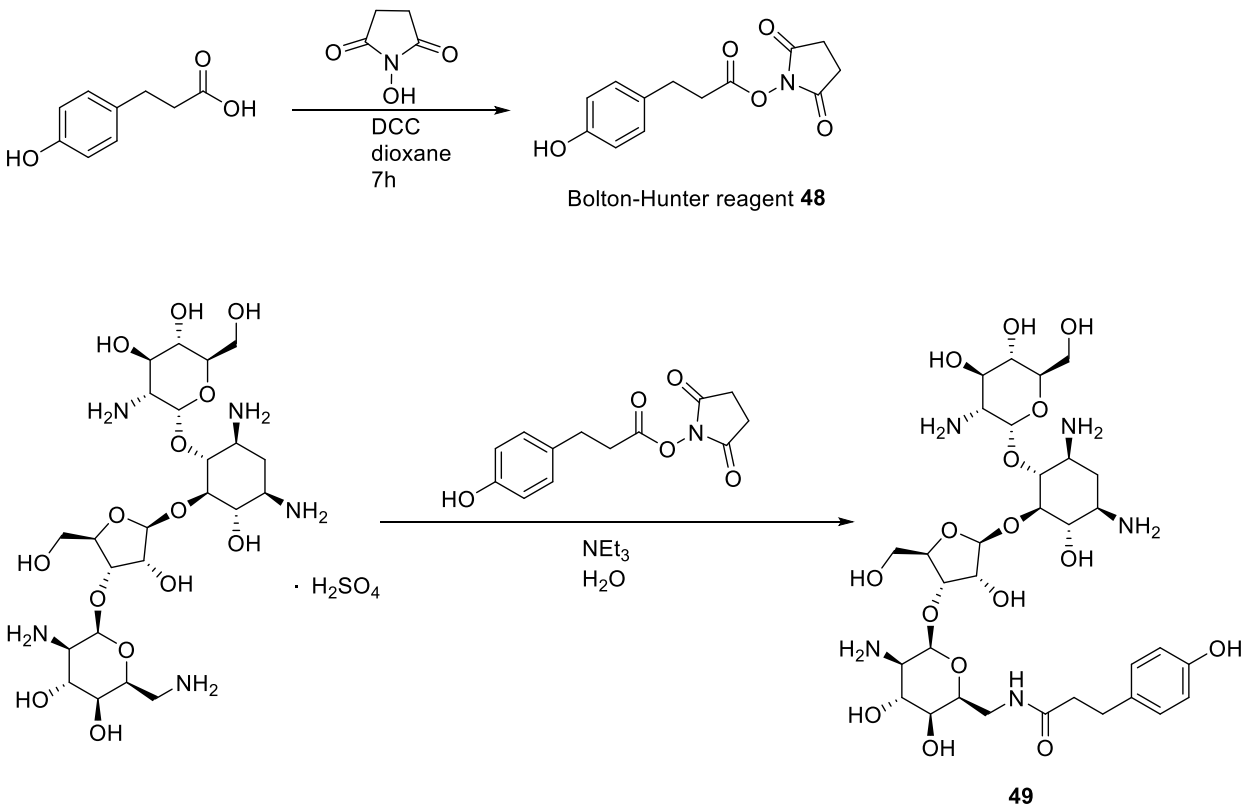
Scheme 10. Regioselective labelling of tobramycin with fluorobenzoyl moiety

Tobramycin was coupled with Cy3, Cy5 cyanine dyes to generate FRET pair, Scheme 11. Tobramycin reacted with Cy3 NHS ester or Cy5 NHS ester regioselectively at primary amino group to yield tobramycin-Cy3 **46** or tobramycin-Cy5 **47**.



Scheme 11. Synthesis of FRET pair tobramycin-Cy3 **46**, tobramycin-Cy5 **47**

Primary amino group in paromomycin, another aminoglycoside, was functionalized with 4-hydroxyphenylpropionate using Bolton-Hunter reagent, Scheme 12. Compound **48** is readily available by treatment of 4-hydroxyphenylpropionic acid with N-hydroxysuccinimide in the presence of DCC.



Scheme 12. Regioselective labelling of paromomycin with Bolton-Hunter reagent

Studies of interactions of these functionalized aminoglycosides and bacteria in soil are currently conducted by our collaborators at the University of California in San Francisco.

Conclusion

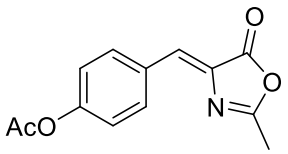
In summary, we have synthesized a number of DFHBI analogs. Some of the synthesized analogs, compounds **11-22**, have superior qualities compared to DFHBI. The best DFHBI analog, PFP-DFHBI, compound **21**, has three-fold higher fluorescence compared to DFHBI which makes it to be more useful for fluorescent visualization of gene expression. The synthesized analogs can be used as either light-up aptamers or via FRET for the visualization of gene expression. We have successfully coupled Cy3 and Cy5 dyes to newly synthesized ligands, compounds **38**, **39**, to generate a FRET pair for imaging. Biotin has been attached to these ligands to select novel RNA aptamers from pool of RNAs.

A new chemistry has been developed allowing functionalization of DFHBI at the allylic position. Some of the synthesized compounds have been found to have different excitation and emission wavelengths compared to DFHBI and longer fluorescence lifetime. These properties can be useful for imaging of biological molecules. The studies are currently conducted to explore the full potential of these compounds.

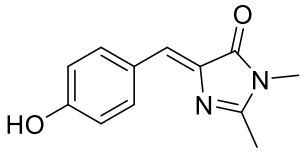
We have developed procedures for labelling aminoglycosides which can be applied for tracking bacteria in soil. We have developed procedure to radiolabel tobramycin with fluorobenzoyl moiety bearing ^{18}F fluorine isotopes. Also, tobramycin was coupled with Cy3, Cy5 cyanine dyes to generate FRET pair. Paromomycin was labelled with Bolton-Hunter reagent. These labelled aminoglycosides are being studied how they interact with bacteria in soil.

Experimental Section

All starting materials were purchased from Sigma-Aldrich; solvents were purchased from Fisher Scientific and used without further purification. All reactions were carried out in flame-dried glassware under argon with dry solvents under anhydrous conditions. All yields refer to chromatographically isolated products. Reactions were monitored by thin-layer chromatography (TLC) carried out on 0.20 mm silica gel plates using UV light as a visualizing agent. Silica gel 60A, particle size 0.032 – 0.063 mm, was used for flash column chromatography. ^{1}H and ^{13}C NMR spectra were acquired in $CDCl_3$ on a Varian MR-400 spectrometer. ^{1}H and ^{13}C chemical shifts (δ) are given in ppm relative to the residual protonated solvent peak ($CDCl_3$: $\delta H = 7.26$ ppm, $\delta C = 77.0$ ppm; CD_3OD: $\delta H = 3.31$ ppm, $\delta C = 49.0$ ppm) as an internal reference. High-resolution mass spectra (HRMS) were recorded on an Agilent 6540 QTOF (quadrupole time of flight) mass spectrometer using ESI (electrospray ionization) or APCI (atmospheric-pressure chemical ionization), or EI (electron ionization) on an Agilent 6890 GC/MS. IR were recorded on Nicolet Fisher Scientific iS5. Melting points were measured on Mel-Temp II melting point apparatus.

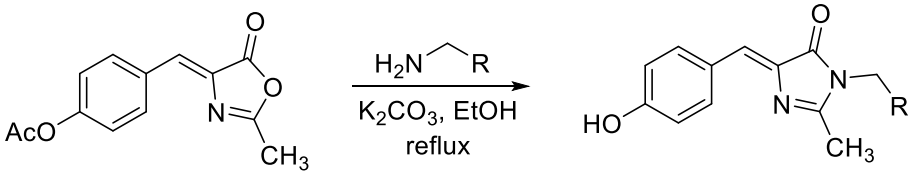


(Z)-4-((2-methyl-5-oxooxazol-4(5H)-ylidene)methyl)phenyl acetate 3. Yellow solid. 86% yield. This compound was prepared according to the literature procedure and showed spectroscopic data identical to previously reported.[13]



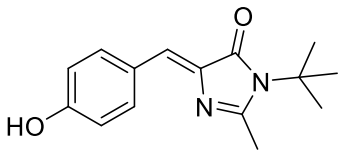
(Z)-5-(4-hydroxybenzylidene)-2,3-dimethyl-3,5-dihydro-4H-imidazol-4-one 1. Yellow solid. 56% yield. This compound was prepared according to the literature procedure and showed spectroscopic data identical to previously reported.[13, 14]

Procedure 1. General procedure for the synthesis of HBI analogs.

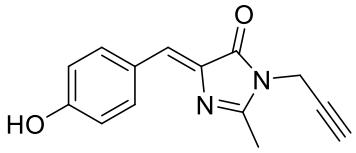


3 **HBI analogs**

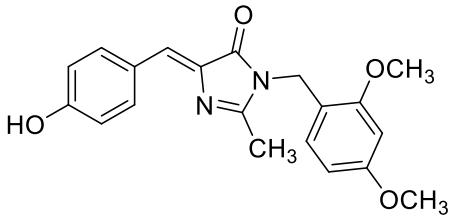
To azalactone **3** (0.5 g, 2.04 mmol) in ethanol (10 mL) the corresponding amine (2.45 mmol) was added followed by potassium carbonate (0.4227 g, 3.06 mmol). The reaction was refluxed overnight. The solvent was evaporated. Water was added (30 mL). pH was adjusted to 3 with 1M HCl. The product precipitated out of the solution. The crude product is filtered, dried. The product was purified by preparative TLC.



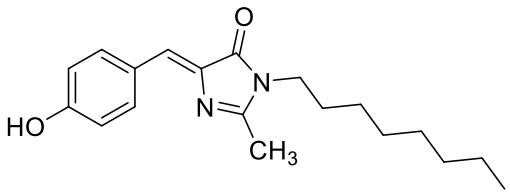
(Z)-3-(tert-butyl)-5-(4-hydroxybenzylidene)-2-methyl-3,5-dihydro-4H-imidazol-4-one 4. Yellow solid. 52 % yield. ^{1}H NMR (400 MHz, CD_3OD): δ 7.49 (d, *J* = 8.5 Hz, 2H, ArH), 7.39 (s, 1H), 6.81 (d, *J* = 8.5 Hz, 2H, ArH), 2.16 (s, 3H), 1.29 (s, 9H).



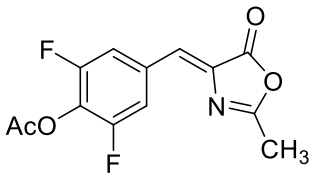
(Z)-5-(4-hydroxybenzylidene)-2-methyl-3-(prop-2-yn-1-yl)-3,5-dihydro-4H-imidazol-4-one 5. Yellow solid. 57% yield. ^{1}H NMR (400 MHz, CD_3OD): δ 8.01 (d, *J* = 8.8 Hz, 2H, ArH), 7.04 (s, 1H), 6.89 (d, *J* = 8.8 Hz, 2H, ArH), 4.47 (s, 2H, CH_2), 2.80 (s, 1H), 2.48 (s, 3H).



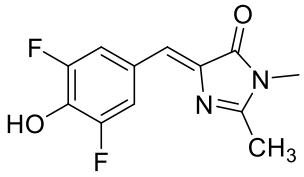
(Z)-3-(2,4-dimethoxybenzyl)-5-(4-hydroxybenzylidene)-2-methyl-3,5-dihydro-4H-imidazol-4-one 6. Yellow solid. 73% yield. ^{1}H NMR (400 MHz, CD_3OD): δ 8.01 (d, *J* = 8.8 Hz, 2H, ArH), 7.04 (s, 1H), 7.01 (s, 1H, ArH), 6.85 (d, *J* = 8.8 Hz, 2H, ArH), 6.56 (s, 1H, ArH), 6.47-6.50 (m, 1H) 4.77 (s, 2H, CH_2), 3.84 (s, 3H, OCH_3), 3.78 (s, 3H, OCH_3), 2.28 (s, 3H).



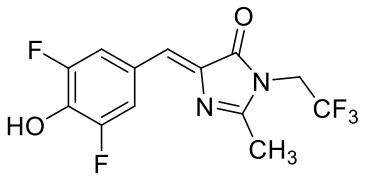
(Z)-5-(4-hydroxybenzylidene)-2-methyl-3-octyl-3,5-dihydro-4H-imidazol-4-one 7. Yellow solid. 85% yield. ^{1}H NMR (400 MHz, CD_3OD): δ 8.00 (d, *J* = 8.6 Hz, 2H, ArH), 7.01 (s, 1H), 6.85 (d, *J* = 8.7 Hz, 2H, ArH), 3.59 – 3.66 (m, 2H), 3.14 (t, *J* = 7.1 Hz, 1H, ArH), 2.39 (s, 3H), 1.63 (p, *J* = 7.3 Hz, 2H), 1.48 (p, *J* = 7.2 Hz, 1H), 1.27 – 1.35 (m, 11H), 0.87 – 0.92 (m, 2H).



(Z)-2,6-difluoro-4-((2-methyl-5-oxooxazol-4(5H)-ylidene)methyl)phenyl acetate 8. Yellow solid. 57% yield. This compound was prepared according to the literature procedure and showed spectroscopic data identical to previously reported.[14]

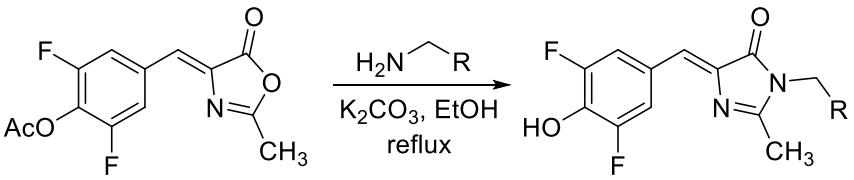


(Z)-5-(3,5-difluoro-4-hydroxybenzylidene)-2,3-dimethyl-3,5-dihydro-4H-imidazol-4-one 9. Yellow solid. 61% yield. This compound was prepared according to the literature procedure and showed spectroscopic data identical to previously reported.[14]



(Z)-5-(3,5-difluoro-4-hydroxybenzylidene)-2-methyl-3-(2,2,2-trifluoroethyl)-3,5-dihydro-4H-imidazol-4-one 10. 43% yield. This compound was prepared according to the literature procedure with a few modifications. Modifications: DMF was used instead of ethanol as a solvent. Reaction was run in a sealable tube at 40 ºC. The compound showed spectroscopic data identical to previously reported.[15]

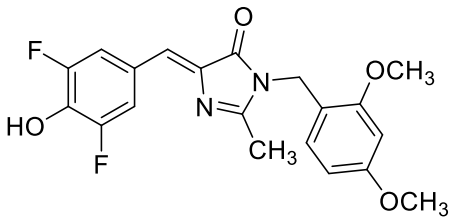
Procedure 2. General procedure for the synthesis of DFHBI analogs.



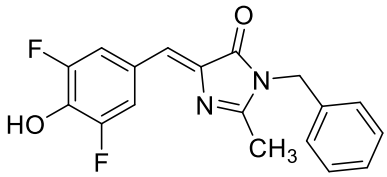
8
azalactone

DFHBI analogs

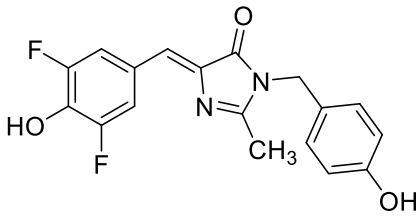
To azalactone **8** (0.200 g, 0.711 mmol) in ethanol (10 mL) was added solution of amine (0.853 mmol) in ethanol (5 mL) followed by potassium carbonate (0.1474 g, 1.07 mmol). The reaction mixture was refluxed for 12 hours. After cooling down to room temperature solvent was evaporated. Water (15 mL) was added, pH was adjusted to 3 using 1M HCl. The solution was set overnight at 4 °C. The product precipitated out of the solution. The crude product is filtered, dried. The product was purified by preparative TLC.



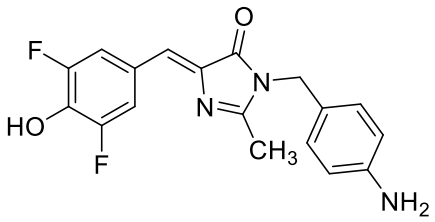
(Z)-3-(2,4-dimethoxybenzyl)-5-(4-hydroxybenzylidene)-2-methyl-3,5-dihydro-4H-imidazol-4-one 11. Orange solid. 71% yield. ^{1}H NMR (400 MHz, CD_3OD): δ 8.01 (d, *J* = 8.8 Hz, 2H, ArH), 7.04 (s, 1H), 7.01 (s, 1H, ArH), 6.85 (d, *J* = 8.8 Hz, 2H, ArH), 6.56 (s, 1H, ArH), 6.47-6.50 (m, 1H) 4.77 (s, 2H, CH_2), 3.84 (s, 3H, OCH_3), 3.78 (s, 3H, OCH_3), 2.28 (s, 3H).



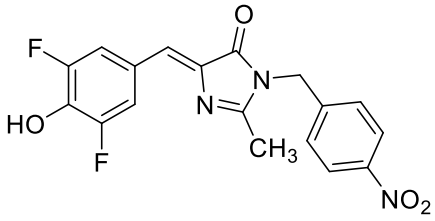
(Z)-3-benzyl-5-(3,5-difluoro-4-hydroxybenzylidene)-2-methyl-3,5-dihydro-4H-imidazol-4-one 12. Orange solid. 75% yield. ^{1}H NMR (400 MHz, CD_3OD): δ 7.81 (d, *J* = 9.8 Hz, 2H, ArH), 7.37 (t, *J* = 7.2 Hz, 2H, ArH), 7.31 (d, *J* = 7.3 Hz, 1H, ArH), 7.26 (t, *J* = 7.3 Hz, 2H), 6.99 (s, 1H), 2.28 (s, 3H). HRMS ESI (m/z): calcd. for $C_{18}H_{14}F_2N_2O_2$ $[M + H]^+$, 329.1096; found 329.1104.



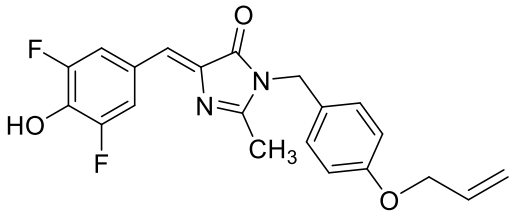
(Z)-3-(4-aminobenzyl)-5-(3,5-difluoro-4-hydroxybenzylidene)-2-methyl-3,5-dihydro-4H-imidazol-4-one 13. Yellow solid. 52% yield. ^{1}H NMR (400 MHz, CD_3OD): δ 7.81 (d, *J* = 9.2 Hz, 2H, ArH), 7.10 (d, *J* = 8.4 Hz, 2H, ArH), 6.96 (s, 1H), 6.77 (d, *J* = 8.4 Hz, 2H, ArH), 4.75 (s, 2H), 2.29 (s, 3H). HRMS ESI (m/z): calcd. for $C_{18}H_{15}F_2N_2O_3$ $[M+H]^+$, 345.1045; found 345.1045.



(Z)-3-(4-aminobenzyl)-5-(3,5-difluoro-4-hydroxybenzylidene)-2-methyl-3,5-dihydro-4H-imidazol-4-one 14. Orange solid. 72% yield. ^{1}H NMR (400 MHz, CD_3OD): δ 7.79 (d, *J* = 9.6 Hz, 2H, ArH), 7.01 (d, *J* = 8.4 Hz, 2H, ArH), 6.95 (s, 1H), 6.70 (d, *J* = 8.4 Hz, 2H, ArH), 4.71 (s, 2H), 2.29 (s, 3H). HRMS ESI (m/z): calcd. for $C_{18}H_{16}F_2N_3O_2$ $[M+H]^+$, 344.1205; found 344.1214.

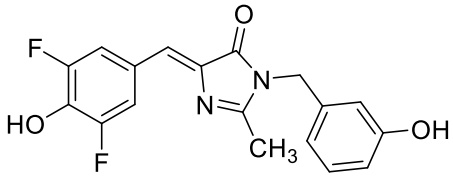


(Z)-5-(3,5-difluoro-4-hydroxybenzylidene)-2-methyl-3-(4-nitrobenzyl)-3,5-dihydro-4H-imidazol-4-one 15. Orange solid. 78% yield. ^{1}H NMR (400 MHz, CD_3OD): δ 8.26 (d, *J* = 8.4 Hz, 2H, ArH), 7.82 (d, *J* = 9.6 Hz, 2H, ArH), 7.52 (d, *J* = 8.4 Hz, 2H, ArH), 7.01 (s, 1H), 5.01 (s, 2H), 2.31 (s, 3H). HRMS ESI (m/z): calcd. for $C_{18}H_{14}F_2N_3O_4$ $[M+H]^+$, 374.0947; found 374.0950.

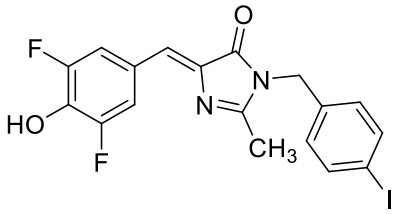


(Z)-3-(4-(allyloxy)benzyl)-5-(3,5-difluoro-4-hydroxybenzylidene)-2-methyl-3,5-dihydro-4H-imidazol-4-one 16. Yellow solid. 77% yield. ^{1}H NMR (400 MHz, CD_3OD): δ 7.75 (d, *J* = 8.0 Hz, 2H, ArH), 7.16 (d, *J* = 8.4 Hz, 2H, ArH), 6.96 (s, 1H), 6.88 (d, *J* = 8.4 Hz, 2H, ArH), 5.99-6.09

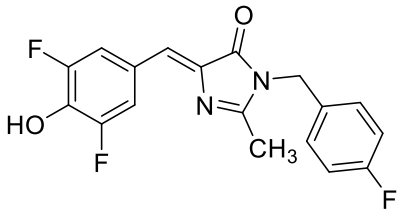
(m, 1H), 5.41 (d, *J* = 17.2 Hz, 1H), 5.29 (d, *J* = 10.4 Hz, 1H), 4.76 (s, 2H), 4.51 (s, 2H), 2.29 (s, 3H). HRMS ESI (m/z): calcd. for $C_{21}H_{19}F_2N_2O_3$ $[M+H]^+$, 385.1379; found 385.1377.



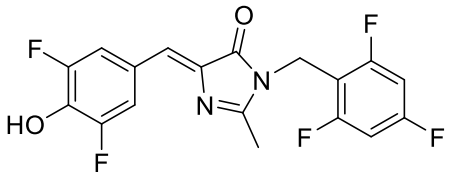
(Z)-5-(3,5-difluoro-4-hydroxybenzylidene)-3-(3-hydroxybenzyl)-2-methyl-3,5-dihydro-4H-imidazol-4-one 17. Yellow solid. 53% yield. ^{1}H NMR (400 MHz, CD_3OD): δ 7.82 (d, *J* = 10.0 Hz, 2H, ArH), 7.17 (t, *J* = 8.0 Hz, 1H, ArH), 6.98 (s, 1H), 6.72 (d, *J* = 8.0 Hz, 2H, ArH), 6.64 (s, 1H, ArH), 4.79 (s, 2H), 2.28 (s, 3H). HRMS ESI (m/z): calcd. for $C_{18}H_{15}F_2N_2O_3$ $[M+H]^+$, 345.1045; found 345.1050.



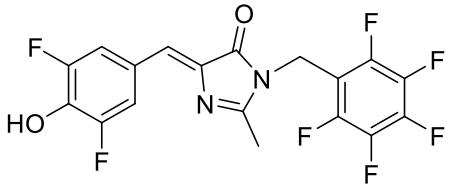
(Z)-5-(3,5-difluoro-4-hydroxybenzylidene)-3-(4-iodobenzyl)-2-methyl-3,5-dihydro-4H-imidazol-4-one 18. Yellow solid. 73% yield. ^{1}H NMR (400 MHz, CD_3OD): δ 7.82 (d, *J* = 10.0 Hz, 2H, ArH), 7.73 (d, *J* = 8.4 Hz, 2H, ArH), 7.06 (d, *J* = 8.4 Hz, 2H, ArH), 6.98 (s, 1H), 4.83 (s, 2H), 2.29 (s, 3H). HRMS ESI (m/z): calcd. for $C_{18}H_{14}F_2IN_2O_2$ $[M+H]^+$, 455.0063; found 455.0074.



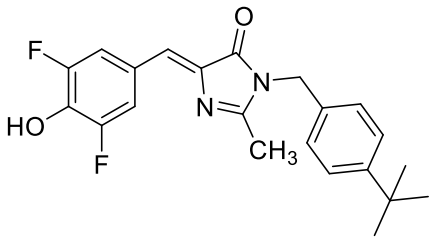
(Z)-5-(3,5-difluoro-4-hydroxybenzylidene)-3-(4-fluorobenzyl)-2-methyl-3,5-dihydro-4H-imidazol-4-one 19. Yellow solid. 73% yield. ^{1}H NMR (400 MHz, CD_3OD): δ 7.81 (d, *J* = 9.6 Hz, 2H, ArH), 7.28-7.32 (m, 2H, ArH), 7.10 (t, *J* = 8.8 Hz, 2H, ArH), 6.98 (s, 1H), 4.85 (s, 2H), 2.30 (s, 3H). HRMS ESI (m/z): calcd. for $C_{18}H_{14}F_3N_2O_2$ $[M+H]^+$, 347.1002; found 347.1008.



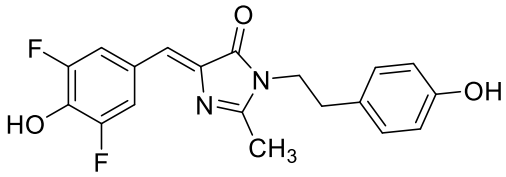
(Z)-5-(3,5-difluoro-4-hydroxybenzylidene)-2-methyl-3-(2,4,6-trifluorobenzyl)-3,5-dihydro-4H-imidazol-4-one 20. Yellow solid. 75% yield. ^{1}H NMR (400 MHz, CD_3OD): δ 7.79 (d, *J* = 9.6 Hz, 2H, ArH), 6.90-6.95 (m, 2H, ArH), 6.89 (s, 1H), 4.91 (s, 2H), 2.38 (s, 3H). HRMS ESI (m/z): calcd. for $C_{18}H_{12}F_5N_2O_2$ $[M+H]^+$, 383.0813; found 383.0820.



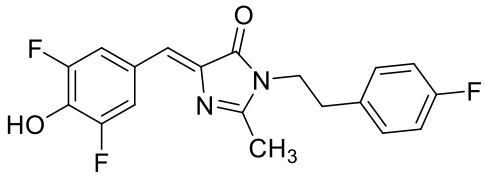
(Z)-5-(3,5-difluoro-4-hydroxybenzylidene)-2-methyl-3-((perfluorophenyl)methyl)-3,5-dihydro-4H-imidazol-4-one 21. Yellow solid. 71% yield. ^{1}H NMR (400 MHz, CD_3OD): δ 7.82 (d, *J* = 9.6 Hz, 2H, ArH), 6.89 (s, 1H), 4.98 (s, 2H), 2.42 (s, 3H). HRMS ESI (m/z): calcd. for $C_{18}H_{10}F_7N_2O_2$ $[M+H]^+$, 419.0625; found 419.0633.



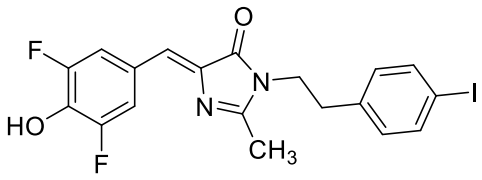
(Z)-3-(4-(tert-butyl)benzyl)-5-(3,5-difluoro-4-hydroxybenzylidene)-2-methyl-3,5-dihydro-4H-imidazol-4-one 22. Yellow solid. 71% yield. ^{1}H NMR (400 MHz, CD_3OD): δ 7.80 (d, *J* = 9.6 Hz, 2H, ArH), 7.40 (d, *J* = 8.4 Hz, 2H, ArH), 7.17 (d, *J* = 8.4 Hz, 2H), 6.97 (s, 1H), 4.83 (s, 2H), 2.28 (s, 3H), 1.30 (s, 9H). HRMS ESI (m/z): calcd. for $C_{22}H_{23}F_2N_2O_2$ $[M+H]^+$, 385.1722; found 385.1727.



(Z)-5-(3,5-difluoro-4-hydroxybenzylidene)-3-(4-hydroxyphenethyl)-2-methyl-3,5-dihydro-4H-imidazol-4-one 23. Yellow solid. 73% yield. ^{1}H NMR (400 MHz, CD_3OD): δ 7.77 (d, *J* = 9.6 Hz, 2H, ArH), 6.97 (d, *J* = 8.4 Hz, 2H, ArH), 6.90 (s, 1H), 6.72 (d, *J* = 8.4 Hz, 2H, ArH), 3.81 (t, *J* = 6.8 Hz, 2H), 2.84 (t, *J* = 6.8 Hz, 2H), 1.96 (s, 3H). HRMS ESI (m/z): calcd. for $C_{19}H_{17}F_2N_2O_3$ $[M+H]^+$, 359.1202; found 359.1210.

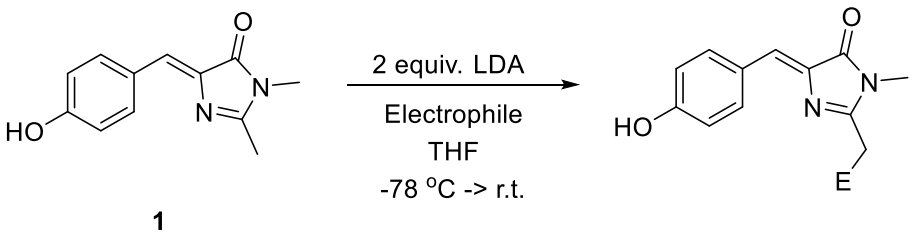


(Z)-5-(3,5-difluoro-4-hydroxybenzylidene)-3-(4-fluorophenethyl)-2-methyl-3,5-dihydro-4H-imidazol-4-one 24. Yellow solid. 69% yield. ^{1}H NMR (400 MHz, CD_3OD): δ 7.77 (d, *J* = 10.0 Hz, 2H, ArH), 7.17-7.21 (m, 2H, ArH), 7.02 (t, *J* = 8.8 Hz, 2H, ArH), 6.89 (s, 1H), 3.85 (t, *J* = 6.8 Hz, 2H), 2.93 (t, *J* = 6.8 Hz, 2H), 2.06 (s, 3H).

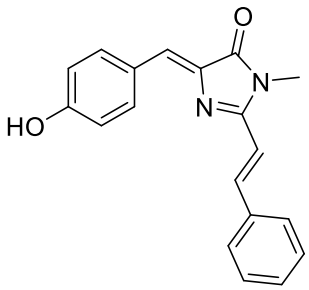


(Z)-5-(3,5-difluoro-4-hydroxybenzylidene)-3-(4-iodophenethyl)-2-methyl-3,5-dihydro-4H-imidazol-4-one 25. Yellow solid. 70% yield. ^{1}H NMR (400 MHz, CD_3OD) δ 7.77 (d, *J* = 9.6 Hz, 2H, ArH), 7.65 (d, *J* = 8.4 Hz, 2H, ArH), 6.99 (d, *J* = 8.4 Hz, 2H, ArH), 6.89 (s, 1H), 3.85 (t, *J* = 6.8 Hz, 2H), 2.93 (t, *J* = 6.8 Hz, 2H), 2.08 (s, 3H). HRMS ESI (m/z): calcd. for $C_{19}H_{16}F_2IN_2O_2$ $[M+H]^+$, 469.0219; found 469.0227.

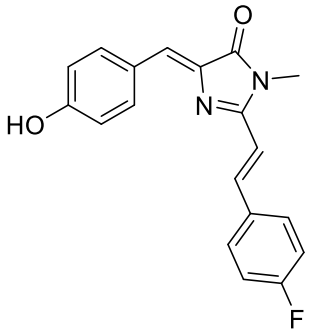
Procedure 3. General procedure for the functionalization of HBI at the allylic position.



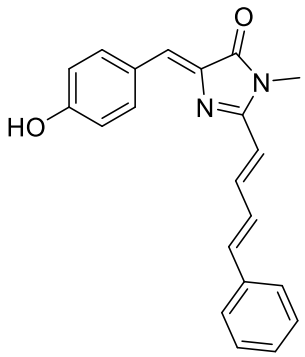
To solution of diisopropylamine (0.1 mL, 0.694 mmol) in THF (5 mL) was added 0.23 mL (0.578 mmol) of 2.5 M n-BuLi in hexane at -78 ºC under argon. In 10 min solution of HBI (0.050 g, 0.231 mmol) in THF (20 mL) was added slowly. The reaction was stirred for 30 min. The appropriate electrophile (0.231 mmol) in THF (3 mL) was added slowly. The reaction was allowed to warm up to room temperature overnight. The reaction was quenched with 1M HCl. 50 mL of water was added. The product was extracted with ethyl acetate (3×50 mL). The organic layer was dried with $MgSO_4$, filtered. The solvent was evaporated. The crude product was purified by preparative TLC.



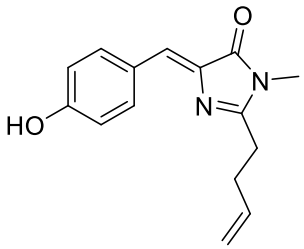
5-((Z)-4-hydroxybenzylidene)-3-methyl-2-((E)-styryl)-3,5-dihydro-4H-imidazol-4-one 26. Orange solid. 91% yield. ^{1}H NMR (400 MHz, CD_3OD): δ 8.18 (d, *J* = 8.9 Hz, 2H, ArH), 8.09 (d, *J* = 15.7 Hz, 1H), 7.65 (dd, J = 7.5, 1.9 Hz, 2H, ArH), 7.43 (d, *J* = 7.8 Hz, 3H, ArH), 7.14 (s, 1H), 6.91 (d, *J* = 8.8 Hz, 2H, ArH), 6.84 (d, *J* = 15.8 Hz, 1H), 3.34 (s, 3H). HRMS ESI (m/z): calcd. for $C_{19}H_{17}N_2O_2$ $[M+H]^+$, 305.1285; found 305.1285.



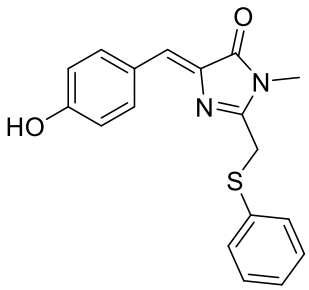
2-((E)-4-fluorostyryl)-5-((Z)-4-hydroxybenzylidene)-3-methyl-3,5-dihydro-4H-imidazol-4-one 27. Orange solid. 48% yield. ^{1}H NMR (400 MHz, CD_3OD): δ 8.19 (d, *J* = 8.8 Hz, 2H, ArH), 8.07 (d, *J* = 15.9 Hz, 1H), 7.76 (d, *J* = 7.6 Hz, 2H, ArH), 7.41 – 7.46 (m, 4H), 7.12 (d, *J* = 15.9 Hz, 1H), 7.08 (s, 1H), 3.34 (s, 3H). HRMS ESI (m/z): calcd. for $C_{19}H_{16}FN_2O_2$ $[M+H]^+$, 323.1190; found 305.1199.



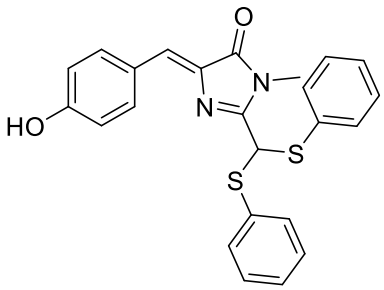
5-((Z)-4-hydroxybenzylidene)-3-methyl-2-((1E,3E)-4-phenylbuta-1,3-dien-1-yl)-3,5-dihydro-4H-imidazol-4-one 28. Orange solid. 63% yield. ^{1}H NMR (400 MHz, CD_3OD): δ 8.12 (d, *J* = 8.6 Hz, 2H); 7.83 (dd, *J* = 15.0, 10.9 Hz, 1H); 7.57 (d, *J* = 7.3 Hz, 2H); 7.35 – 7.42 (m, 3H), 7.14 – 7.23 (m, 1H), 7.09 (s, 1H), 7.03 (s, 1H), 6.87 (d, *J* = 8.7 Hz, 2H), 6.62 (d, *J* = 15.0 Hz, 1H), 3.26 (s, 3H). HRMS ESI (m/z): calcd. for $C_{21}H_{19}N_2O_2$ $[M+H]^+$, 331.1441; found 331.1448.



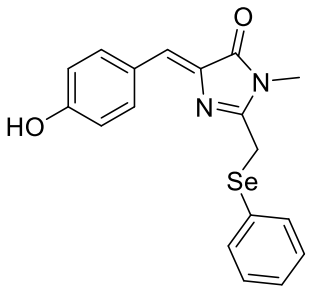
(Z)-2-(but-3-en-1-yl)-5-(4-hydroxybenzylidene)-3-methyl-3,5-dihydro-4H-imidazol-4-one 29. Yellow solid. 44% yield. ^{1}H NMR (400 MHz, CD_3OD): δ 8.05 (d, *J* = 8.7 Hz, 2H, ArH), 7.01 (s, 1H), 6.84 (d, *J* = 8.8 Hz, 2H, ArH), 6.00 (td, *J* = 16.9, 6.6 Hz, 1H), 5.17 (dd, *J* = 17.2, 1.7 Hz, 1H), 5.06 (dd, *J* = 10.2, 1.5 Hz, 1H), 3.18 (s, 3H), 2.72 – 2.81 (m, 2H), 2.55 – 2.63 (m, 2H). ^{13}C NMR (100 MHz, $CDCl_3$): 26.8, 28.9, 30.7, 116.3, 116.7, 119.9, 127.1, 129.4, 135.8, 138.2, 161.5, 165.3, 172.8. HRMS ESI (m/z): calcd. for $C_{15}H_{17}N_2O_2$ $[M+H]^+$, 257.1285; found 257.1289.



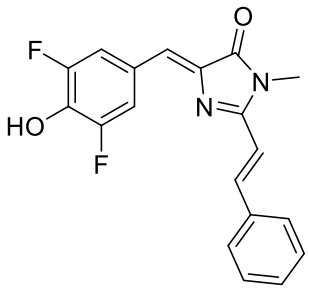
(Z)-5-(4-hydroxybenzylidene)-3-methyl-2-((phenylthio)methyl)-3,5-dihydro-4H-imidazol-4-one 30. Yellow solid. 73% yield. ^{1}H NMR (400 MHz, CD_3OD): δ 7.87 (d, *J* = 8.7 Hz, 2H, ArH), 7.50 (d, *J* = 6.9 Hz, 2H, ArH), 7.30 (t, *J* = 7.2 Hz, 2H, ArH), 7.26 (d, *J* = 7.1 Hz, 1H, ArH), 7.01 (s, 1H), 6.79 (d, *J* = 8.7 Hz, 2H, ArH), 4.12 (s, 2H), 3.25 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): 27.2, 33.06, 116.7, 126.8, 128.9, 130.1, 130.8, 133.2, 134.7, 136.0, 136.9, 161.1, 161.8, 172.4. HRMS ESI (m/z): calcd. for $C_{18}H_{17}N_2O_2S$ $[M+H]^+$, 325.1005; found 325.1005.



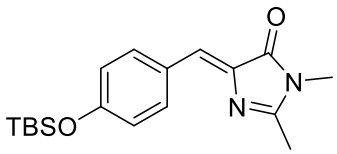
(Z)-2-(bis(phenylthio)methyl)-5-(4-hydroxybenzylidene)-3-methyl-3,5-dihydro-4H-imidazol-4-one 31. Yellow solid. 15% yield. ^{1}H NMR (400 MHz, CD_3OD): δ 7.86 (d, *J* = 8.8 Hz, 2H, ArH), 7.53 (dd, *J* = 6.4, 3.2 Hz, 4H, ArH), 7.32 (dd, *J* = 4.9, 1.8 Hz, 6H, ArH), 7.01 (s, 1H), 6.77 (d, *J* = 8.7 Hz, 2H, ArH), 5.66 (s, 1H) 3.36 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): 27.8, 55.5, 116.7, 126.8, 128.8, 130.0, 130.2, 131.7, 133.2, 135.1, 136.3, 160.0, 162.0, 172.4. HRMS ESI (m/z): calcd. for $C_{24}H_{20}N_2O_2S_2$ $[M+H]^+$, 433.1039; found 433.1039.



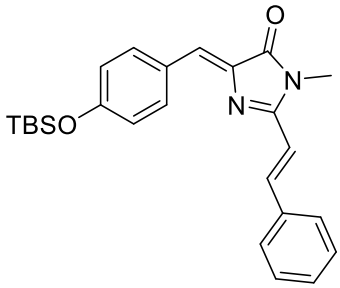
(Z)-5-(4-hydroxybenzylidene)-3-methyl-2-((phenylselanyl)methyl)-3,5-dihydro-4H-imidazol-4-one 32. Yellow solid. 55% yield. ^{1}H NMR (400 MHz, CD_3OD): δ 7.80 (d, *J* = 8.7 Hz, 2H, ArH), 7.56 – 7.63 (m, 2H, ArH), 7.27 (dd, *J* = 5.1, 1.8 Hz, 3H, ArH), 6.97 (s, 1H), 6.77 (d, *J* = 8.8 Hz, 2H, ArH), 4.05 (s, 2H), 3.20 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): 23.6, 27.1, 116.7, 126.9, 129.0, 129.6, 130.2, 132.6, 135.9, 136.2, 137.0, 161.7, 162.2, 172.3. HRMS ESI (m/z): calcd. for $C_{18}H_{17}N_2O_2Se$ $[M+H]^+$, 367.0509; found 367.0513.



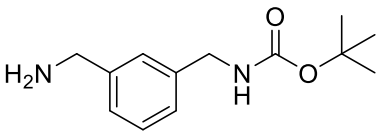
5-((Z)-3,5-difluoro-4-hydroxybenzylidene)-3-methyl-2-((E)-styryl)-3,5-dihydro-4H-imidazol-4-one 33. This compound was synthesized using procedure 3 with the exception that DFHBI was used in place of HBI. Yellow solid. 63% yield. ^{1}H NMR (400 MHz, CD_3OD): δ 8.17 (d, *J* = 15.9 Hz, 1H), 8.06 (d, *J* = 9.9 Hz, 2H, ArH), 7.84 (dd, *J* = 7.6, 1.6 Hz, 2H, ArH), 7.47 (d, *J* = 7.6 Hz, 3H, ArH), 7.26 (d, *J* = 15.8 Hz, 1H), 6.91 (s, 1H). HRMS ESI (m/z): calcd. for $C_{19}H_{15}F_2N_2O_2$ $[M+H]^+$, 341.1096; found 341.1102.



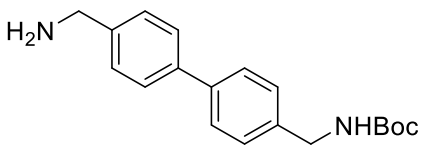
5-((Z)-4-((tert-butyldimethylsilyl)oxy)benzylidene)-3-methyl-2-((E)-styryl)-3,5-dihydro-4H-imidazol-4-one 34. Yellow solid. 55% yield. This compound was prepared according to the literature procedure and showed spectroscopic data identical to previously reported.[16]



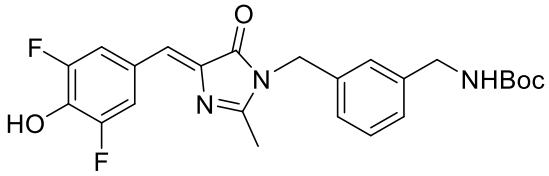
5-((Z)-4-((tert-butyldimethylsilyl)oxy)benzylidene)-3-methyl-2-((E)-styryl)-3,5-dihydro-4H-imidazol-4-one 35. Yellow solid. 26% yield. ^{1}H NMR (400 MHz, CD_3OD): δ 8.16 (d, *J* = 8.7 Hz, 2H, ArH), 8.09 (d, *J* = 15.8 Hz, 1H), 7.64 (d, *J* = 6.4 Hz, 2H, ArH), 7.40-7.44 (m, 3H, ArH), 7.15 (s, 1H), 6.92 (d, *J* = 8.7 Hz, 2H), 6.84 (d, *J* = 15.8 Hz, 1H), 3.34 (s, 3H), 0.99 (s, 9H), 0.24 (s, 6H). HRMS ESI (m/z): calcd. for $C_{25}H_{31}N_2O_2Si$ $[M+H]^+$, 419.2149; found 419.2161.



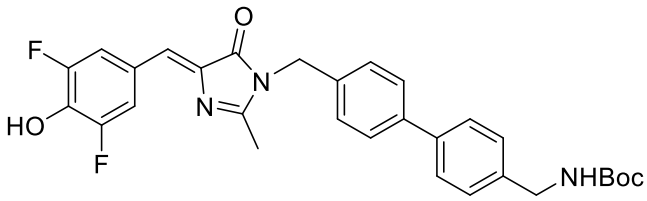
tert-butyl (3-(aminomethyl)benzyl)carbamate 36. This compound was prepared according to the literature procedure and showed spectroscopic data identical to previously reported.[17]



tert-butyl ((4'-(aminomethyl)-[1,1'-biphenyl]-4-yl)methyl)carbamate 37. This compound was prepared according to the literature procedure.[17]



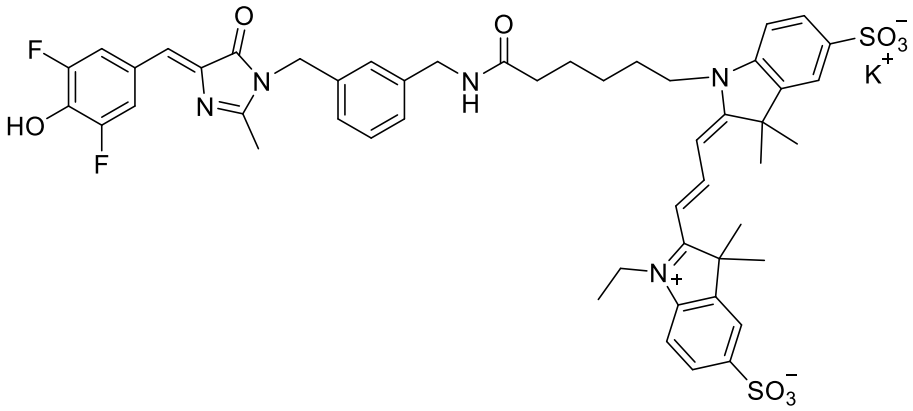
tert-butyl (Z)-(3-((4-(3,5-difluoro-4-hydroxybenzylidene)-2-methyl-5-oxo-4,5-dihydro-1H-imidazol-1-yl)methyl)benzyl)carbamate 38. This compound was prepared according to procedure 1. Yellow solid. 71% yield. ^{1}H NMR (400 MHz, CD_3OD): δ 7.81 (d, *J* = 9.8 Hz, 2H), 7.32 (t, *J* = 7.9 Hz, 1H), 7.14-7.22 (m, 3H), 6.98 (s, 1H), 4.58 (bs, 2H, NH_2), 4.17-4.23 (m, 3H), 2.27 (s, 3H), 1.41 (s, 9H).



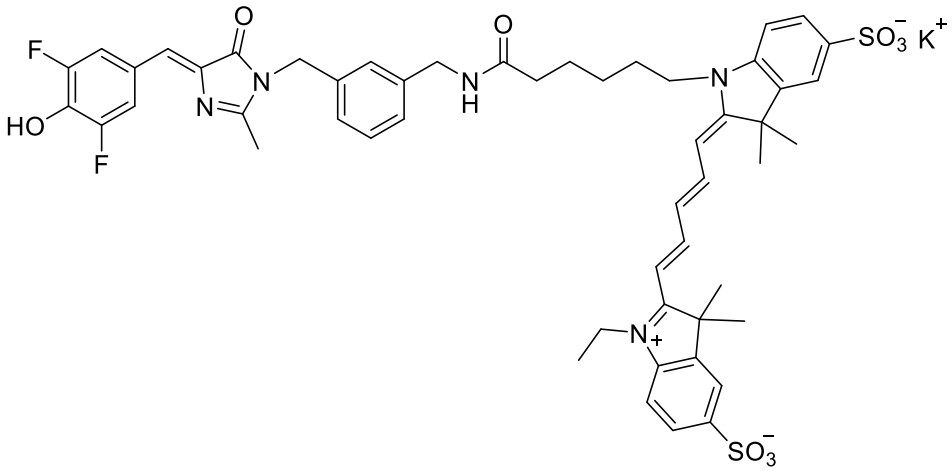
tert-butyl (Z)-((4'-((4-(3,5-difluoro-4-hydroxybenzylidene)-2-methyl-5-oxo-4,5-dihydro-1H-imidazol-1-yl)methyl)-[1,1'-biphenyl]-4-yl)methyl)carbamate 39. This compound was prepared according to procedure 1 and was used for coupling with cyanine dyes and biotin without further purification. Yellow solid. 71% yield. ^{1}H NMR (400 MHz, CD_3OD): δ 7.62 (d, *J* = 7.5 Hz, 2H), 7.53 – 7.60 (m, 4H), 7.31 – 7.38 (m, 4H), 7.02 (s, 1H), 4.39 (s, 2H), 4.26 (s, 2H), 2.00 (s, 3H), 1.41 (s, 9H).

Procedure 3. General procedure for coupling of DFHBI derivatives with cyanine Cy3, Cy5 dyes, biotin.

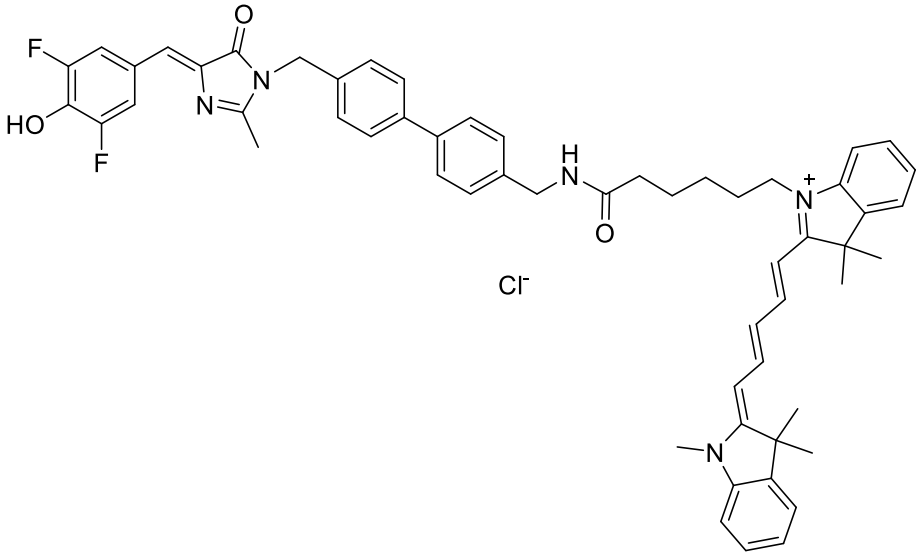
To DFHBI derivative (0.1 mmol) was added trifluoroacetic acid (2-3 mL). The reaction was stirred for 5 min. Trifluoroacetic acid was evaporated. The product was dried in vacuum. The crude was dissolved in DMF (2 mL). Triethylamine was added (0.1 mL) followed by the corresponding NHS ester (0.1 mmol in 2 mL of DMF). The reaction was stirred for 36 hours. The solvent was evaporated. The crude was purified by preparative TLC yielding the products in quantitative yields.



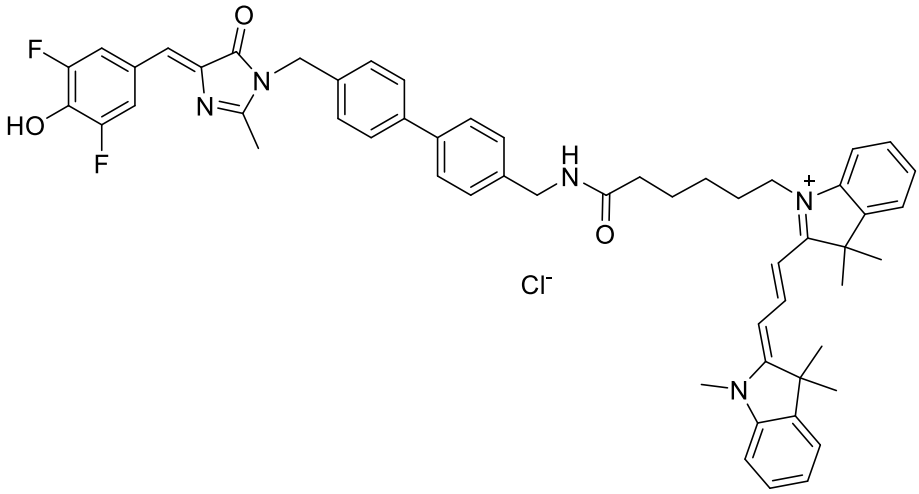
DFHBI-3-aminobenzylamine-Cy3 40. HRMS ESI (m/z): calcd. for $C_{50}H_{52}F_2N_5O_9S_2$ $[M\text{-}K^+]^+$, 968.3180; found 968.3196.



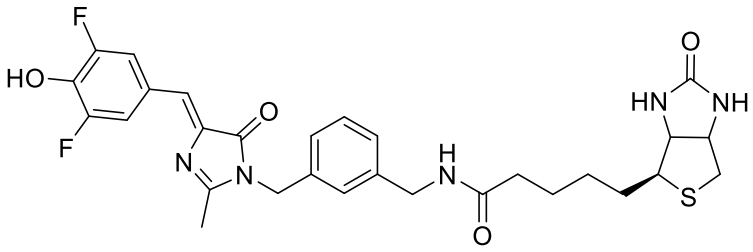
DFHBI-3-aminobenzylamine-Cy5 41. HRMS ESI (m/z): calcd. for $C_{52}H_{54}F_2KN_5O_9S_2$ $[M\text{-}K^+]^+$, 994.3336; found 994.3304.



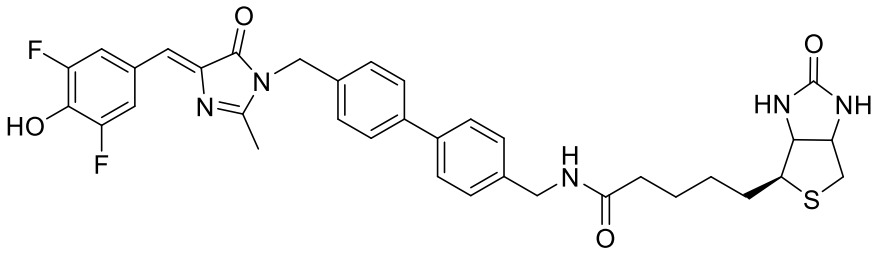
DFHBI-diphenyldiamine-Cy5 42. HRMS ESI (m/z): calcd. for $C_{57}H_{58}F_2N_5O_3$ $[M]^+$, 898.4502; found 898.4498.



DFHBI-diphenyldiamine-Cy3 43. HRMS ESI (m/z): calcd. for $C_{55}H_{56}F_2N_5O_3$ $[M]^+$, 872.4346; found 872.4348.

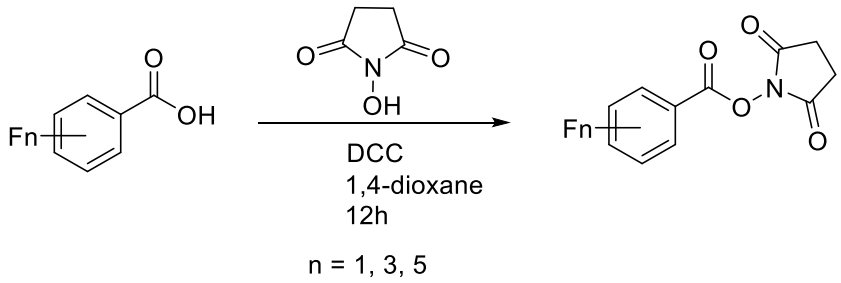


DFHBI-3-aminobenzylamine-biotin 44. HRMS ESI (m/z): calcd. for $C_{29}H_{32}F_2N_5O_4S$ $[M+H]^+$, 583.2143; found 584.2147.



DFHBI-diphenyldiamine-biotin 45. HRMS ESI (m/z): calcd. for $C_{29}H_{32}F_2N_5O_4S$ $[M+H]^+$, 660.2456; found 660.2435.

Procedure 4. General procedure for synthesis of NHS esters of fluorobenzoic acids.

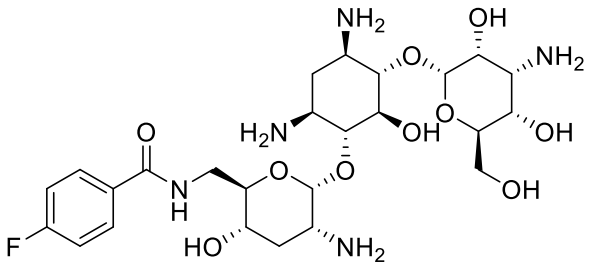


To a solution of fluorobenzoic acid (3.41 mmol) in dioxane (7 mL) was successively added *N*-hydroxysuccinimide (0.4121 g, 3.58 mmol) and *N,N'*-dicylohexylcarbodiimide (0.7388 g, 3.58 mmol). The reaction was stirred overnight. The formed white precipitate of *N,N'*- dicyclohexylurea

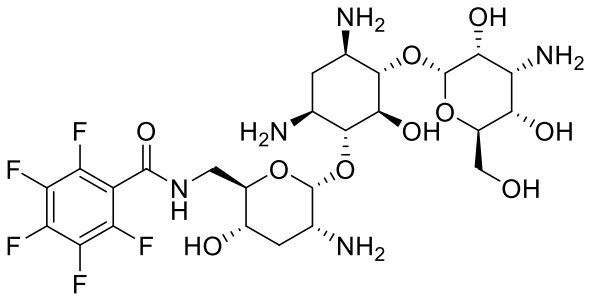
was filtered off. The solution of NHS ester in 1,4-dioxane was used in the next step. The yield of the synthesized NHS ester is quantitative according to TLC and 1H NMR analysis.

Procedure 5. General procedure for labelling of tobramycin with fluorobenzoyl moiety.

To a solution of tobramycin (0.1210 g, 0.259 mmol), triethylamine (0.18 mL) in water (3 mL) was added fluorobenzoic acid NHS ester (0.30 mL of 0.775 M solution in 1,4-dioxane) dropwise. The reaction mixture was stirred overnight under argon. The volatiles were evaporated in vacuum. The crude material was purified by preparative TLC (water - n-butanol – MeOH - NH_4OH, (4:5:2:1) to afford the desired products in 85-93 % yields.



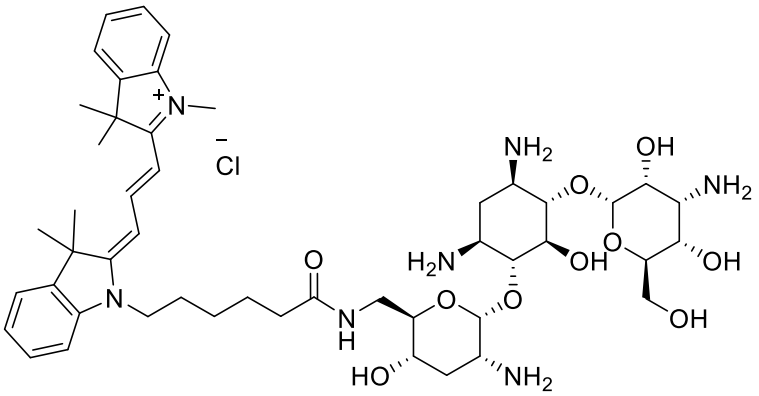
Tobramycin 4-fluorobenzoic acid conjugate. HRMS ESI (m/z): calcd. for $C_{25}H_{41}FN_5O_{10}$ $[M+H]^+$, 590.2837; found 590.2825.



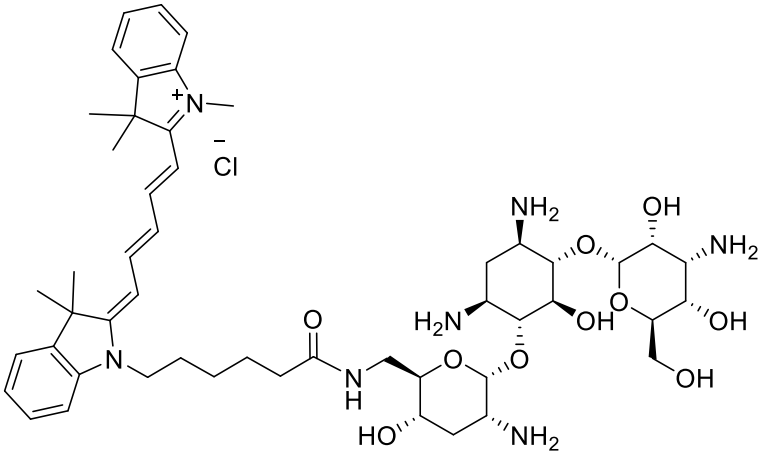
Tobramycin pentafluorobenzoic acid conjugate. HRMS ESI (m/z): calcd. for $C_{25}H_{37}F_5N_5O_{10}$ $[M+H]^+$, 662.2461; found 662.2455.

Procedure 5. General procedure for labelling of tobramycin with cyanine Cy3, Cy5 dyes.

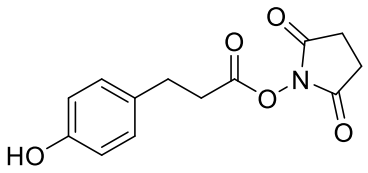
To tobramycin (3.8 mg, 0.811 mmol) in water (0.5 mL) was added triethylamine (0.2 mL) followed by Cy3 or Cy5 NHS ester (8.11 mmol) in DMF (0.5 mL). The reaction was stirred for 24 hrs. The volatiles were evaporated in vacuum. The crude material was purified by preparative TLC to yield cyanine-labelled tobramycin conjugates in quantitative yields.



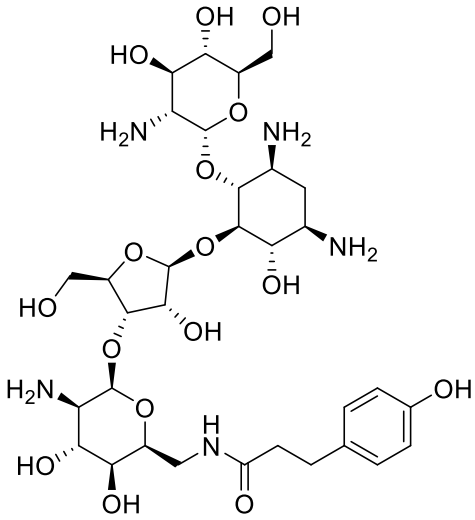
Tobramycin Cy3 conjugate 46. HRMS ESI (m/z): calcd. for $C_{47}H_{69}N_7O_9$ $[M^+-CH_2OH]^+$, 875.5151; found 875.5918.



Tobramycin Cy3 conjugate 47. HRMS ESI (m/z): calcd. for $C_{50}H_{74}N_7O_{10}$ $[M+H^+ +MeOH]^+$, 965.5832; found 965.5807.



NHS ester of 4-hydroxyphenylpropionic acid 48. This compound was prepared using procedure 4.



Paromomycin 4-hydroxypropionic acid conjugate 49. White solid. Yield 76%. HRMS ESI (m/z): calcd. for $C_{25}H_{37}F_5N_5O_{10}$ $[M+H]^+$, 764.3566; found 764.3552.

References

(1) (a) Bertrand, E.; Chartrand, P.; Schaefer, M.; Shenoy, S. M.; Singer, R. H.; Long, R. M. *Mol Cell* **1998**, *2*, 437-445. (b) Larson, D. R.; Zenklusen, D.; Wu, B.; Chao, J. A.; Singer, R. H. *Science* **2011**, *332*, 475-478.

(2) Armitage, B. A. *Current Opinion in Chemical Biology* **2011**, *15*, 806-812.

(3) Babendure, J. R.; Adams, S. R.; Tsien, R. Y. *J. Am. Chem. Soc.* **2003**, *125*, 14716–14717.

(4) Sando, S.; Narita, A.; Hayami, M.; Aoyama, Y. *Chem. Commun.* **2008**, 3858–3860.

(5) Dolgosheina, E. V.; Jeng, S. C.; Panchapakesan, S. S.; Cojocaru, R.; Chen, P. S.; Wilson, P. D. *ACS Chem. Biol.* **2014**, *9*, 2412–2420.

(6) Paige, J. S.; Wu, K. Y.; Jaffrey, S. R. *Science*, **2011**, *333*, 642-646.

(7) Chudakov, D. M.; Matz, M. V.; Lukyanov, S.; Lukyanov, K. A. *Physiol. Rev.* **2010**, *90*, 1103-1163.

(8) Helms, Volkhard Fluorescence Resonance Energy Transfer Principles of Computational Cell Biology Weinheim: Wiley-VCH, **2008**, 202.

(9) Clegg, R. M. The History of FRET Reviews in Fluorescence Springer, **2006**, 1.

(10) Jares-Erijman, E. A.; Jovin, T. M. *Nat. Biotechnol*. 2003, 21, 1387-1395.

(11) Ilgu, M.; Ray, J.; Bendickson, L.; Wang, T.; Geraskin, I. M.; Kraus, G. A.; Nilsen-Hamilton, M. *Methods*, **2016,** *98*, 26-33.

(12) Anisuzzaman S.; Geraskin I. M.; Ilgu M.; Bendickson L.; Kraus G. A.; Nilsen-Hamilton M. "Identification and properties of a super-ligand for the spinach and broccoli light-up aptamers" Manuscript in preparation.

(13) Dong, J.; Abulwerdi, F.; Baldridge, A.; Kowalik, J.; Solntsev, K. M.; Tolbert, T. M. *J. AM. CHEM. SOC.*, **2008**, *130*, 14096–14098.

(14) Paige, J. S.; Wu, K. Y.; Jaffrey, S. R. *Science*, **2011**, *333*, 642-646.

(15) Song, W.; Strack, R. L.; Svensen, N.; Jaffrey, S. R. *J. Am. Chem. Soc.* **2014**, *136*, 1198−1201.

(16) Ivashkin, P. E.; Lukyanov, K. A.; Yampolsky, I. V. *Russian Journal of Bioorganic Chemistry* **2011**, *37*, 411-420.

(17) Barazza, A.; Gotz, M.; Cadamuro, S. A.; Goettig, P.; Willem, M.; Steuber, H.; Kohler, T.; Jestel, A.; Reinemer, P.; Renner, C.; Bode, W.; Moroder, L. *ChemBioChem* **2007**, *8*, 2078–2091.

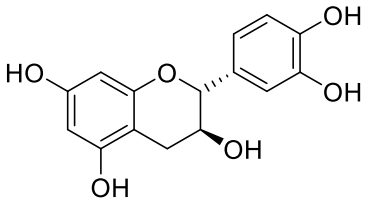
CHAPTER 2. RAPID ASSEMBLY OF THE PROCYANIDIN A-SKELETON

This work has been published in *Tetrahedron Letters* in 2017 (Kraus, G. A.; Geraskin, I. M. *Tetrahedron Letters*, **2017**, *58*, 4609–4611.).

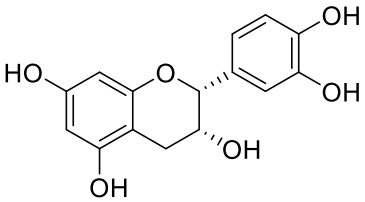
Introduction

Procyanidins are polyphenols that are present in various fruits, vegetables, plants, nuts, grains, grapes, red wine, green tea. They are very potent bioactive compounds. Procyanidins have been reported to exhibit antioxidative and free-radical scavenging,[1] anti-inflammatory,[2,3] anti-infectious,[4] antiviral,[5] cardiovascular protective,[6] anticancer properties.[7,8] Procyanidins started to be used as natural and alternative medicines and products containing procyanidins appeared in the natural product market as dietary supplements in the 1980s.[9] They are used as natural preservatives, stabilizers in foods and as natural antioxidants to prevent degradation of fatty acids.[10,11] Procyanidins have been reported to be effective in the prevention of various degenerative diseases.[12,13]

Procyanidins are a class of proanthocyanidins, which contain only catechin or epicatechin subunits structures of which are shown in Figure 1.



catechin (**1**)



epicatechin (**2**)

Figure 1. Structures of catechin (**1**) and epicatechin (**2**)

Procyanidins are classified into two types, A-type and B-type, depending on type of bonding between monomers. Subunits are linked by single bond between carbon-4 of one subunit and either carbon-8 or carbon-6 of another subunit in B-type procyanidins, as shown in Figure 2.

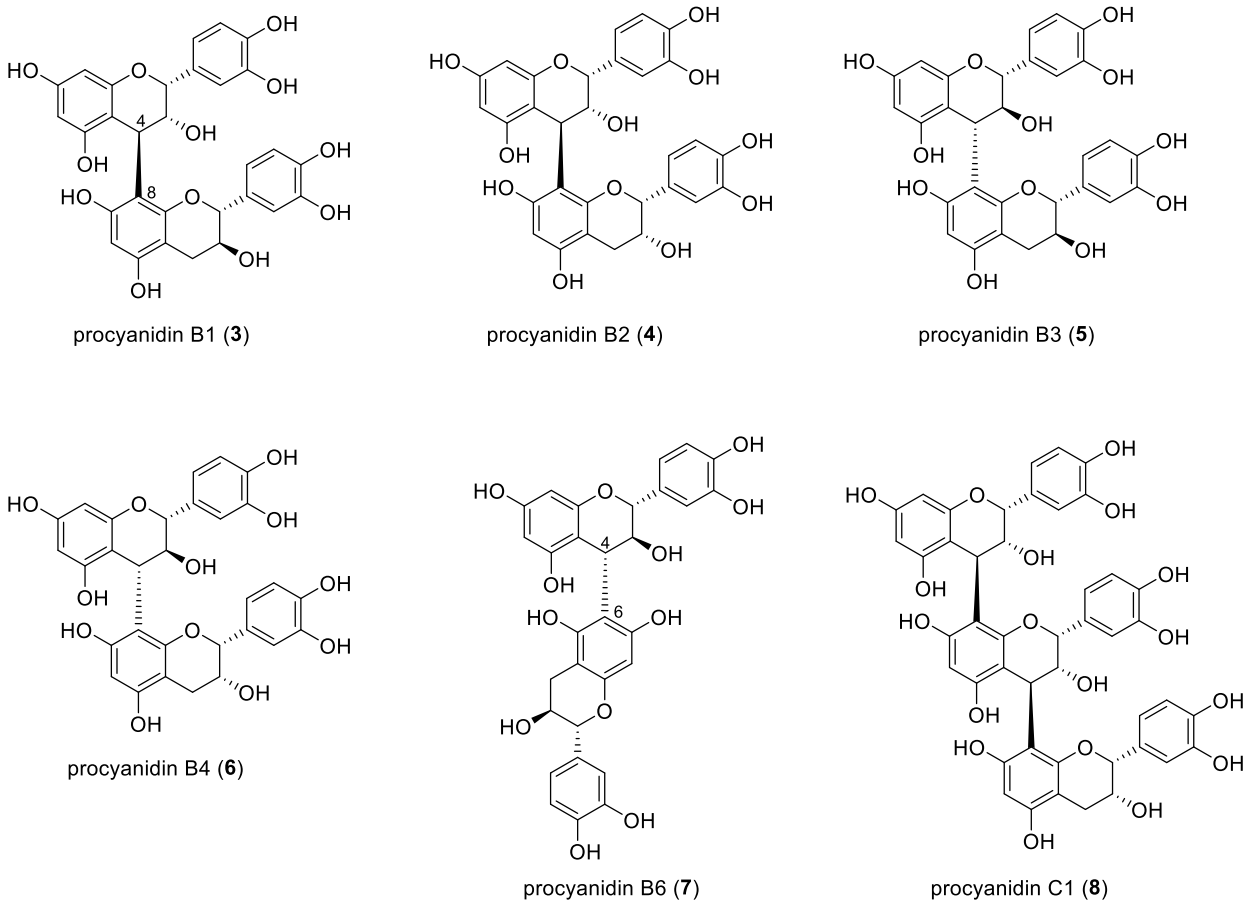
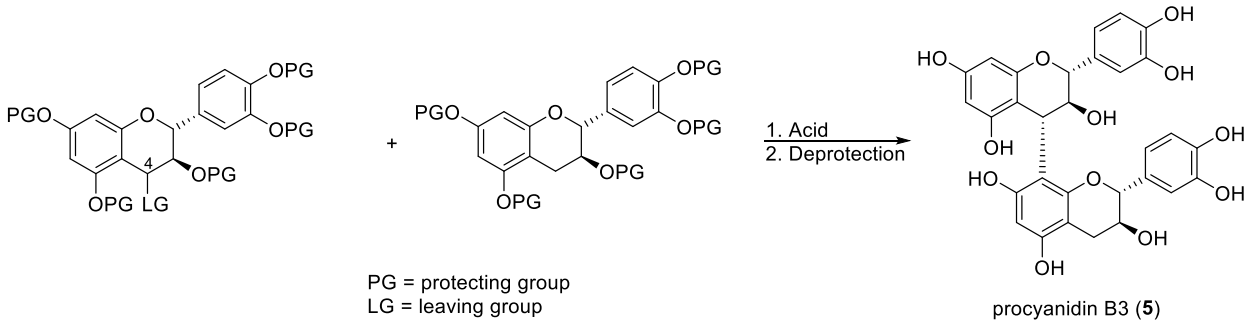


Figure 2. Structures of B-type procyanidins

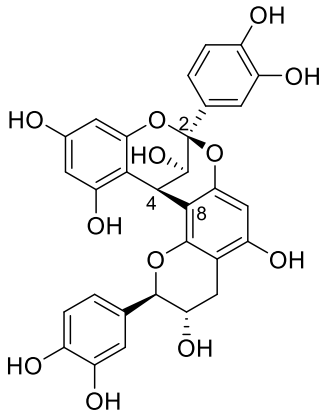
B-type procyanidins are the most abundant in nature and procyanidins B1, B2, B3 and B4 encounter most frequently. Being the largest type of procyanidins B-type procyanidins received considerably more attention compared to A-type procyanidins. There are many reports on the synthesis of B-type procyanidins. Most of the reported syntheses use the strategy outlined in Scheme 1. Two protected catechin or epicatechin molecules, with one bearing leaving group at

carbon-4, condense under acidic conditions forming the dimer. Upon deprotection the desired product, procyanidin is formed.

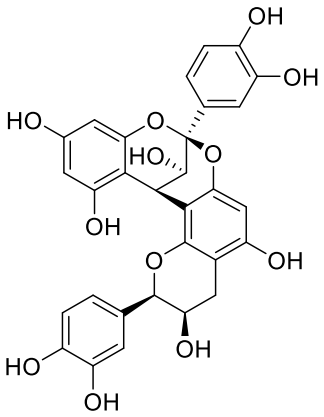


Scheme 1. General stategy for the synthesis of B-type procyanidins

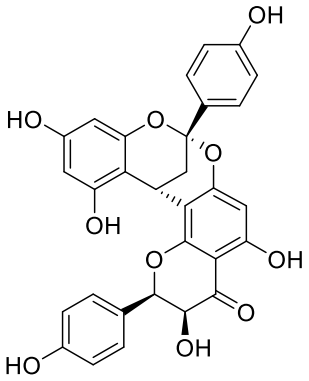
A-type procyanidins are less abundant in nature and have received less attention from scientific community. Besides having bond between carbon-4 of one subunit and either carbon-8 or carbon-6 of another subunit A-type procyanidins have second ether linkage between hydroxyl group of one subunit and carbon-2 of another subunit. The structures of some A-type procyanidins are depicted in Figure 3. Procyanidin A_1, procyanidin A_2 are the most common A-type procyanidins. Mahuannin D (**11**) showed hypotensive activity.[14] Mahuannin E (**12**) exhibited cytotoxicity against SGC-7901, HepG2, and HeLa tumor cell lines.[15] Geranin A (**13**) exhibits antiprotozoal activity in the mg/mL level.[16] Ephedrannin B (**14**) suppressed the transcription of tumor necrosis factor-α and interleukin-1β.[17]



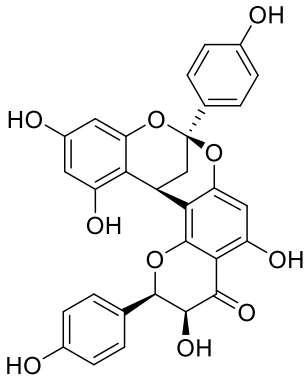
procyanidin A_1 (**9**)



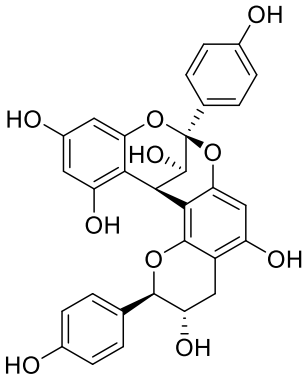
procyanidin A_2 (**10**)



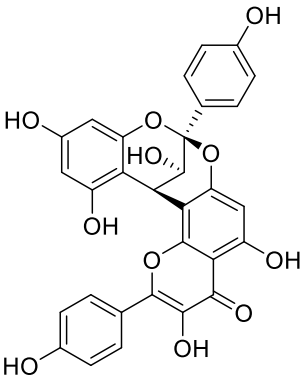
Mahuannin D (**11**)



Mahuannin E (**12**)



Geranin A (**13**)



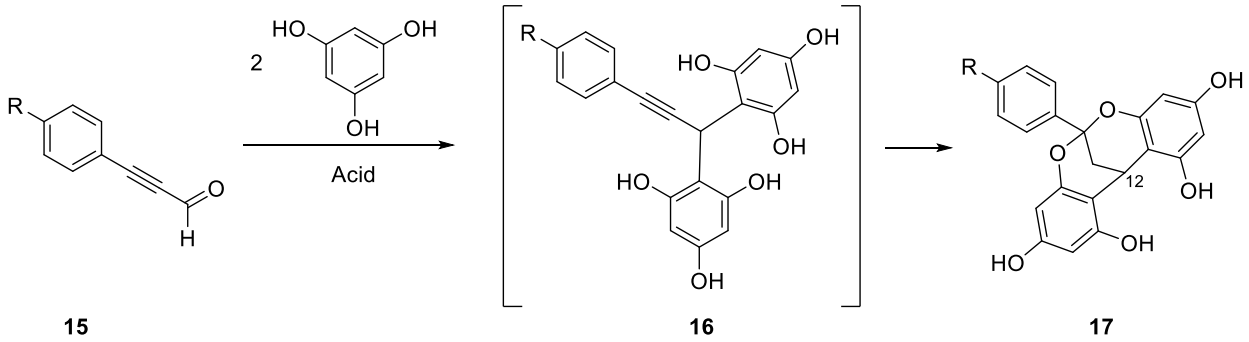
Ephedrannin B (**14**)

Figure 3. Structures of A-type procyanidins

Because of valuable biological properties and low availability of A-type procyanidins from natural sources several synthetic approaches have been reported. Stereoselective synthesis of procyanidin A_2 using Lewis acid-mediated annulation approach was reported by Ito, Ohmori and Suzuki in 2014.[18] Sharma and coworkers reported synthesis of proanthocyanidins A_1 and A_2 in 2015.[19] Convenient synthesis of A-type procyanidins from benzopyrilium salts was reported by Kraus, Yuan and Kempema in 2009.[20]

Results and discussion

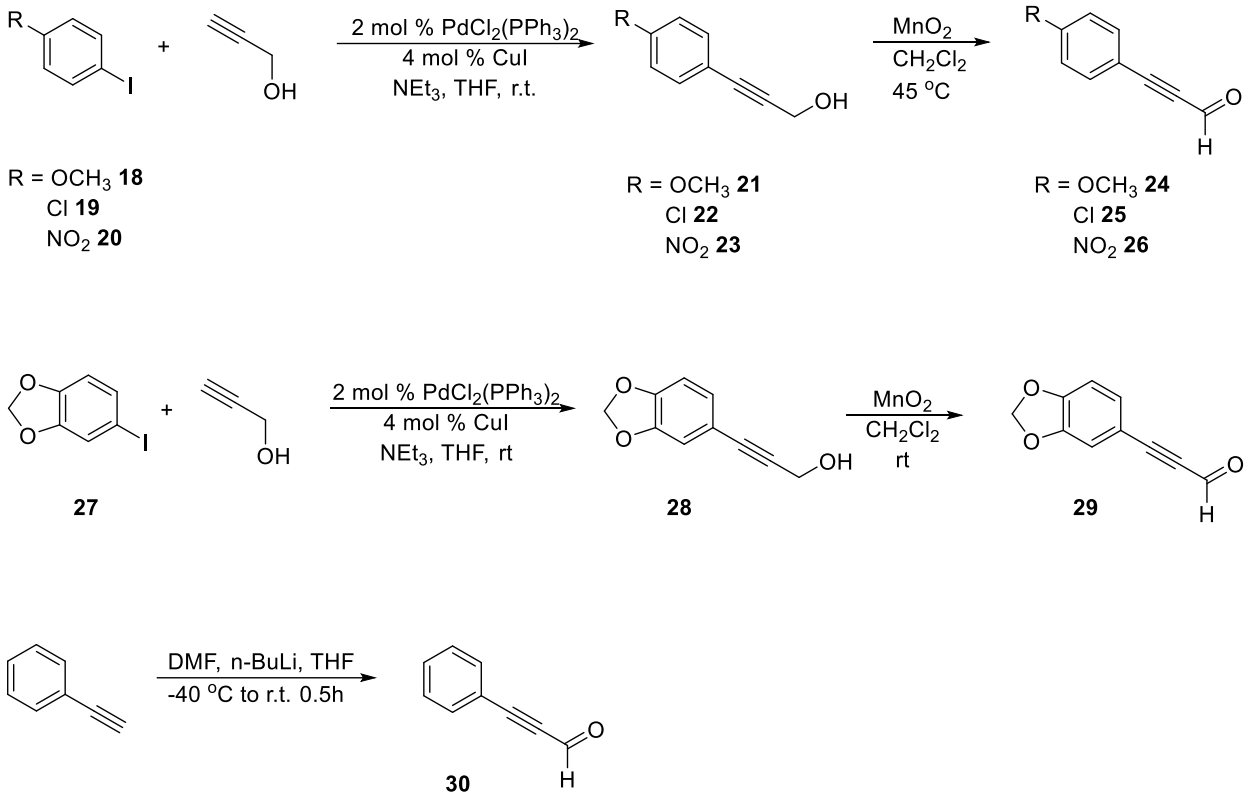
Valuable biological properties combined with low abundance in nature of type-A procyanidins encouraged us to develop convenient synthetic strategy towards this important class of compounds. We envisioned that retrosynthetically procyanidin A skeleton **17** can be derived from two molecules of phloroglucinol and acetylenic aldehyde **15**. Our synthetic strategy is outlined in Scheme 2.



15 **16** **17**

Scheme 2. General Approach to the Procyanidin A Skeleton

The starting aldehydes **24**, **25**, **26**, **29** are readily available via a Sonogashira reaction of the corresponding aryl halide with propargyl alcohol followed by oxidation with MnO_2, as illustrated in Scheme 3. Aldehyde **30** was obtained in good yield by treatment of phenylacetylene with n-butyl lithium followed by the addition of *N,N*-Dimethylformamide.

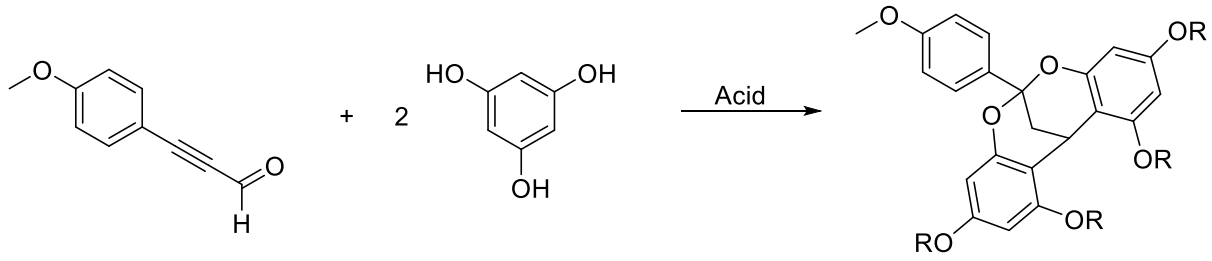


Scheme 3. Synthesis of acetylenic aldehydes

We studied **17**, the product from the reaction of **15** (R = OMe) with phloroglucinol and PTSA, using ^{1}H NMR, ^{13}C NMR, and 2D NMR. The resonance at 99.9 ppm in the ^{13}C NMR spectrum confirmed the presence of an acetal or ketal. The correlation between the bridgehead carbon atom C_{12} at 21.2 ppm and hydrogen atom on C_{12} at 4.31 ppm in the HSQC spectrum confirmed that structure **17** was formed. The cross peaks at 4.31; 2.19 and 2.17; 4.33 in COSY indicate correlation between hydrogen atom on C_{12} and methylene hydrogens on adjacent carbon atom. In addition, the predicted NMR spectrum for ketal **17** resembled the spectrum we obtained from experiment. We think that the reaction occurs via two acid catalyzed additions of phloroglucinol to the carbonyl carbon of the acetylenic aldehyde followed by an intramolecular ketalization.

To find the best reaction conditions we evaluated a number of Lewis acid catalysts in the reaction of acetylenic aldehyde **15** with phloroglucinol, as depicted in Table 1.

Table 1. Optimization of the reaction conditions



15

R = H **17**
Ac **31**

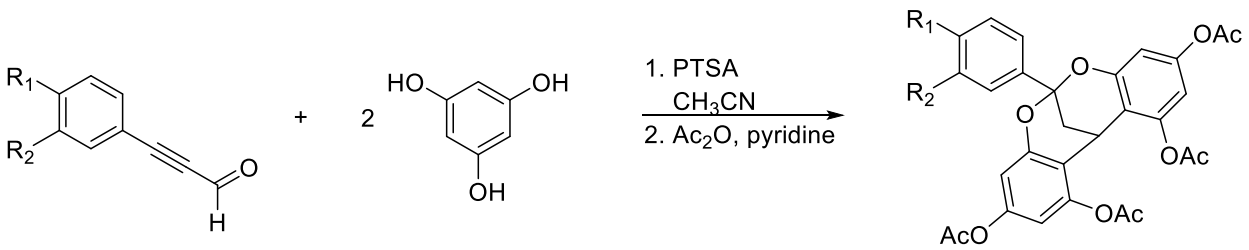
Entry	Acid	Solvent	Percent yield of **17**	Percent yield of **31**[a] (acetylated product)
1	PTSA·H_2O	CH_3CN	26	82
2	PTSA·H_2O	THF	0	
3	PTSA·H_2O	DMF	0	
4	BF_3·Et_2O	CH_3CN	25	77
5	TFA	CH_3CN	10	
6	HCl	CH_3CN		78
7	H_2SO_4	CH_3CN	12	

[a] Acetylation with acetic anhydride and pyridine.

The product was obtained in the highest yield when PTSA was used as acid catalyst in acetonitrile, entry 1, Table 1. As seen in entries 1, 4, and 6, acetylation prior to purification significantly improved the isolated yields, presumably due to improved stability and solubility of the acetylated products. All reactions were carried out in acetonitrile at room temperature. There was no reaction in DMF or THF. Compound **31** can be prepared on a gram scale using this method.

To extend the scope of the reaction we evaluated different substitution patterns on the aromatic ring of the aldehyde using the best reaction conditions, PTSA/CH_3CN. All reactions afforded good yields of products, as shown in Table 2. Compounds **32** and **33** were unstable on silica gel and should not be exposed to silica gel for prolonged period of time.

Table 2. Reaction scope



substrate 1 **substrate 2**

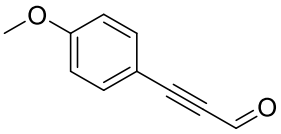
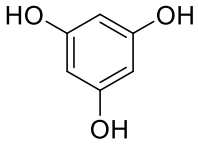
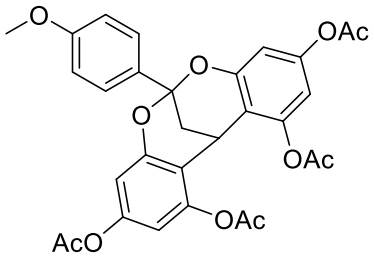
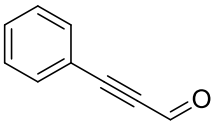
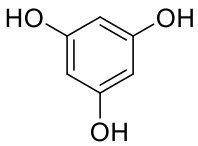
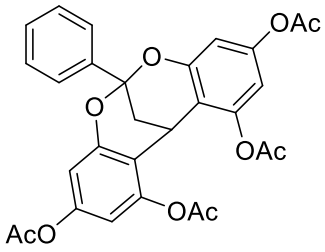
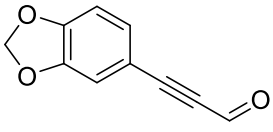
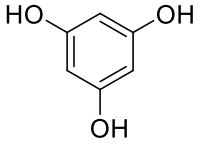
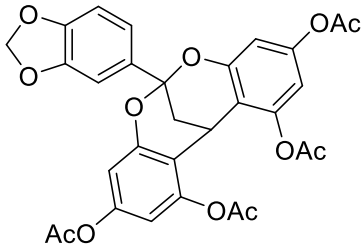
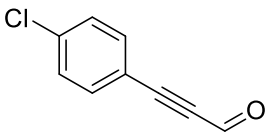
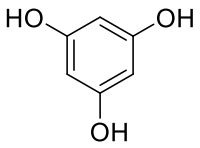
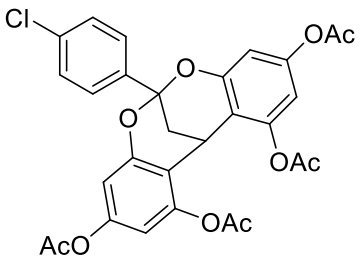
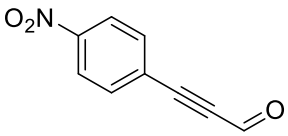
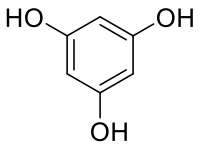
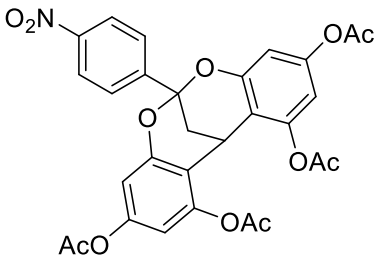
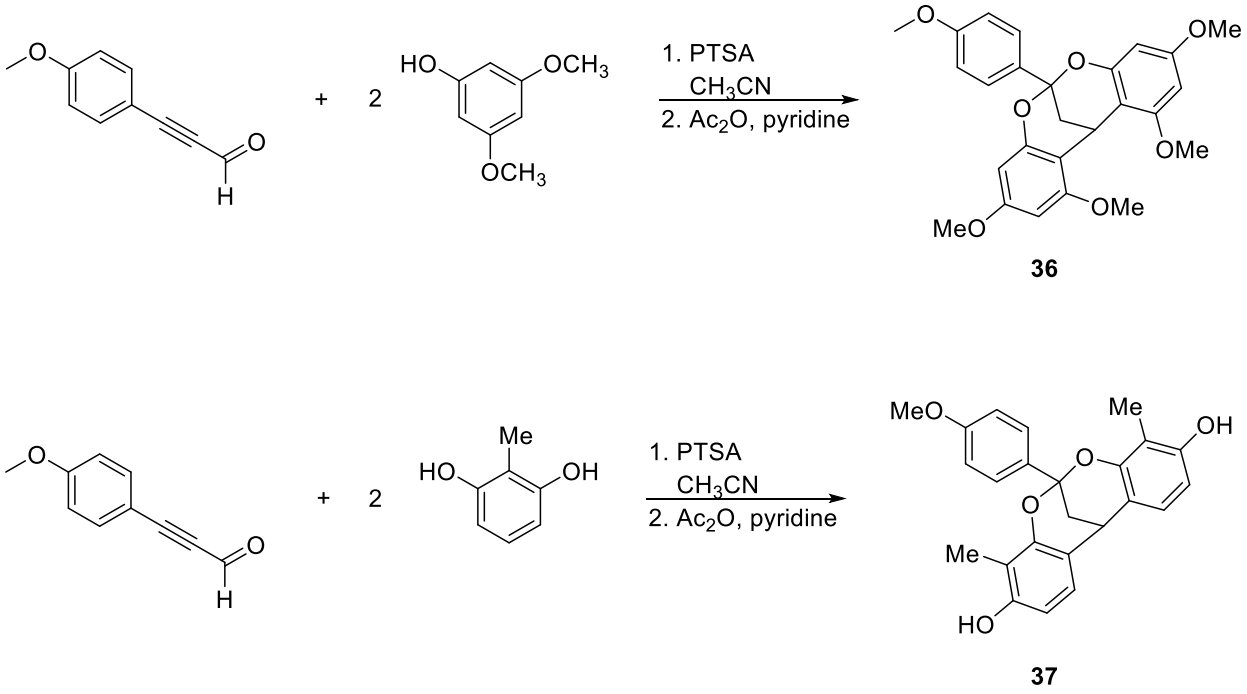
Entry	Substrate 1	Substrate 2	Product	Yield, %	
1				82	**31**
2				54	**32**
3				56	**33**

Table 2. (continued)

4				61	**34**
5				67	**35**

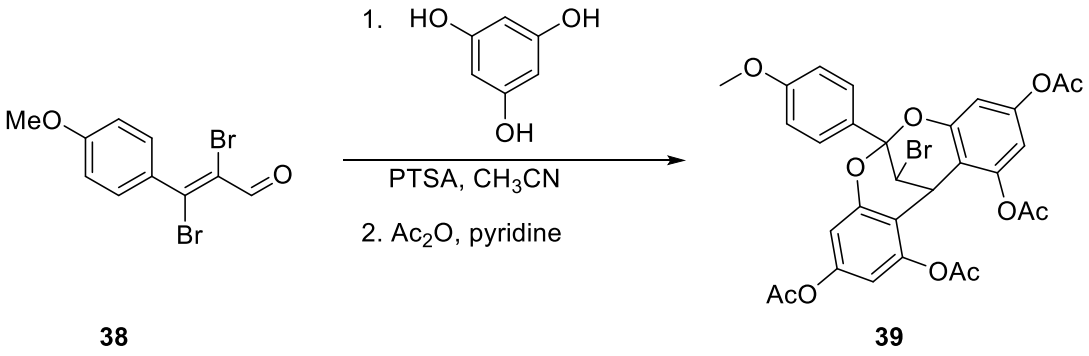
To further extend reaction scope phloroglucinol dimethyl ether and 2-methyl resorcinol were used as substrates under best reaction conditions, as shown in Scheme 4.



Scheme 4. Condensation reaction using phloroglucinol dimethyl ether and 2-methyl resorcinol

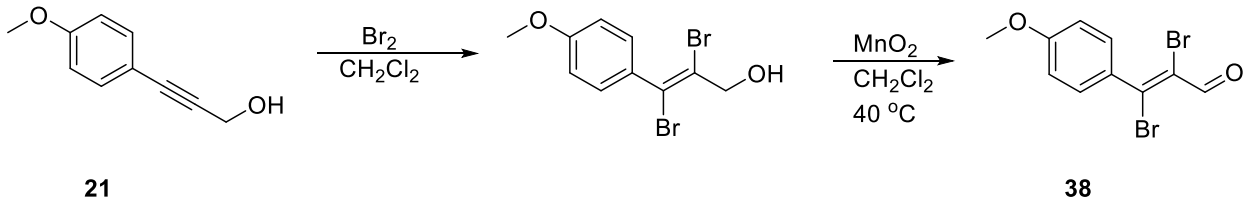
Unfortunately, products **36**, **37** were unstable to column chromatography but reaction afforded good yields of products based on ^{1}H NMR analysis of the crude reaction mixture.

Many procyanidins such as **9** and **10** possess hydroxyl group on the one-carbon bridge. Efforts to oxidize the tetraacetate **31** by C-H activation using Cp_2VCl_2 and TBHP failed.[21] Fortunately, we found that dibromo aldehyde **38** reacts with two equivalents of phloroglucinol under best reaction conditions, PTSA/CH_3CN, followed by acetylation to give tetraacetate **39** in 57% yield, as shown in Scheme 5.



Scheme 5. Synthesis of bromotetraacetate

The dibromo aldehyde **38** can be synthesized in good yield by bromination of alkyne **21** followed by oxidation with MnO_2, as depicted in Scheme 6.



Scheme 6. Synthesis of dibromoaldehyde

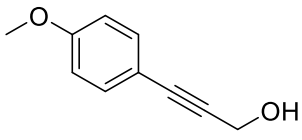
Conclusion

In summary, procyanidins are important class of natural products with valuable biological properties. A-type procyanidins are not abundant in nature and have to be synthesized in order for scientists to explore the full potential of these natural products. While there are many reports on the synthesis of B-type procyanidins A-type procyanidins are overlooked by scientific community and there are only a few reports on the synthesis of A-type procyanidins.

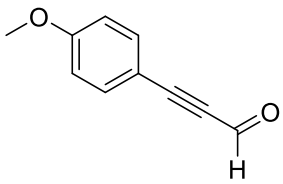
We have developed convenient one-step procedure for the synthesis of procyanidin A-skeleton. Different alkynylaldehydes or dibromocinnamaldehydes and phloroglucinol derivatives can be used to make procyanidin A skeleton with various substitution patterns on aromatic rings using this procedure. The method is efficient and simple and can be useful for the total synthesis of A-type procyanidins. Some compounds were prepared on a gram scale using this method.

Experimental Section

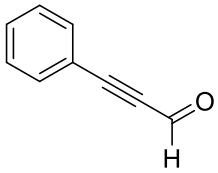
All starting materials were purchased from Sigma-Aldrich; solvents were purchased from Fisher Scientific and used without further purification. All reactions were carried out in flame-dried glassware under argon with dry solvents under anhydrous conditions. All yields refer to chromatographically isolated products. Reactions were monitored by thin-layer chromatography (TLC) carried out on 0.20 mm silica gel plates using UV light as a visualizing agent. Silica gel 60A, particle size 0.032 – 0.063 mm, was used for flash column chromatography. ^{1}H and ^{13}C NMR spectra were acquired in $CDCl_3$ on a Varian MR-400 spectrometer. ^{1}H and ^{13}C chemical shifts (δ) are given in ppm relative to the residual protonated solvent peak ($CDCl_3$: $\delta H = 7.26$ ppm, $\delta C = 77.0$ ppm; CD_3OD: $\delta H = 3.31$ ppm, $\delta C = 49.0$ ppm) as an internal reference. High-resolution mass spectra (HRMS) were recorded on an Agilent 6540 QTOF (quadrupole time of flight) mass spectrometer using ESI (electrospray ionization) or APCI (atmospheric-pressure chemical ionization), or EI (electron ionization) on an Agilent 6890 GC/MS. IR were recorded on Nicolet Fisher Scientific iS5. Melting points were measured on Mel-Temp II melting point apparatus.



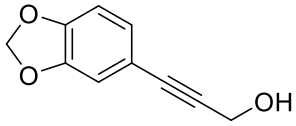
3-(4-methoxyphenyl)prop-2-yn-1-ol (21). Solid (5.64 g, 87% yield). This compound was prepared according to the literature procedure and showed spectroscopic data identical to previously reported.[22]



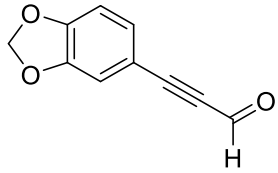
3-(4-methoxyphenyl)propiolaldehyde (24). Yellow solid (0.8202 g, 83% yield). This compound was prepared according to the literature procedure and showed spectroscopic data identical to previously reported.[22]



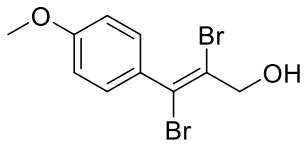
3-phenylpropiolaldehyde (30). Yellowish oil (1.2491 g, 78% yield). This compound was prepared according to the literature procedure and showed spectroscopic data identical to previously reported.[23]



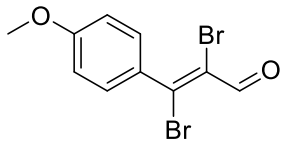
3-(benzo[d][1,3]dioxol-5-yl)prop-2-yn-1-ol (28). Grey solid (0.3435 g, 65% yield). This compound was prepared according to the literature procedure and showed spectroscopic data identical to previously reported.[24]



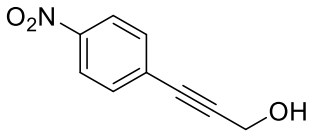
3-(benzo[d][1,3]dioxol-5-yl)propiolaldehyde (29). Yellow solid (0.1742 g, 75% yield). This compound was prepared according to the literature procedure and showed spectroscopic data identical to previously reported.[25]



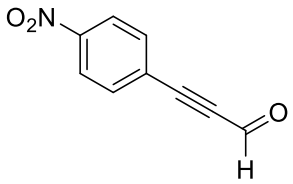
(E)-2,3-dibromo-3-(4-methoxyphenyl)prop-2-en-1-ol. Grey solid (1.0308 g, 91% yield). This compound was prepared according to the literature procedure and showed spectroscopic data identical to previously reported.[26]



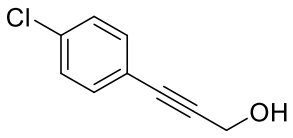
(E)-2,3-dibromo-3-(4-methoxyphenyl)acrylaldehyde (38). Grey solid (0.4923 g, 79% yield). This compound was prepared according to the literature procedure and showed spectroscopic data identical to previously reported.[26]



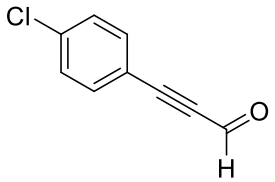
3-(4-nitrophenyl)prop-2-yn-1-ol (23). Light-brown solid (0.7146 g, 81% yield). This compound was prepared according to the literature procedure and showed spectroscopic data identical to previously reported.[27]



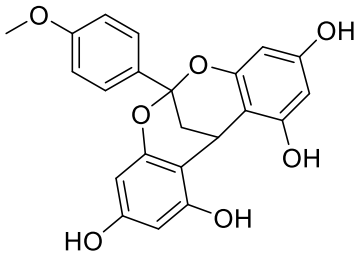
3-(4-nitrophenyl)propiolaldehyde (26). Yellow solid (0.7146 g, 81% yield). This compound was prepared according to the literature procedure and showed spectroscopic data identical to previously reported.[27]



3-(4-chlorophenyl)prop-2-yn-1-ol (22). Yellow solid (6.32 g, 91% yield). This compound was prepared according to the literature procedure and showed spectroscopic data identical to previously reported.[28]

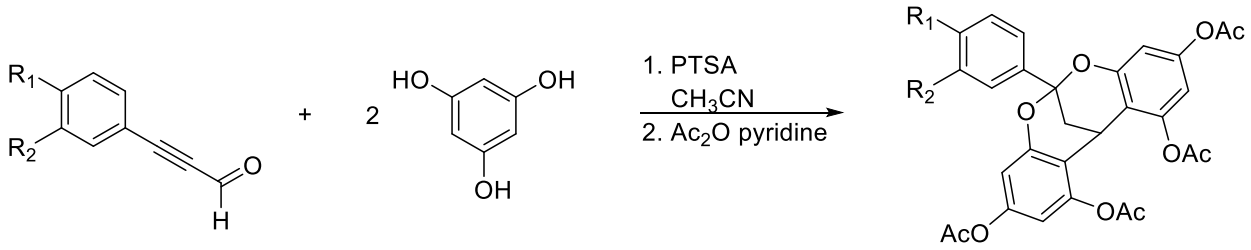


3-(4-chlorophenyl)propiolaldehyde (25). Orange crystalline solid (1.4 g, 89% yield). This compound was prepared according to the literature procedure and showed spectroscopic data identical to previously reported.[28]



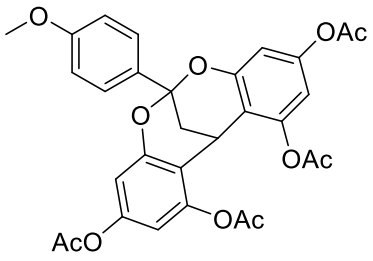
6-(4-methoxyphenyl)-12H-6,12-methanodibenzo[d,g][1,3]dioxocine-1,3,9,11-tetraol (17): To a solution of 3-(4-methoxyphenyl)propiolaldehyde (0.085 g, 0.53 mmol) and phloroglucinol (0.268 g, 2.12 mmol) in acetonitrile (10 mL) was added PTSA · H_2O (0.0101 g, 0.053 mmol). The solution immediately changed color to dark red. The reaction was stirred at room temperature under argon for 12 hours. The solvent was evaporated. The crude was purified by preparative TLC (CH_2Cl_2 – MeOH, 92:8) to yield **5** (0.0544 g, 26% yield) as red solid, m.p. 176-178 °C. IR: 3311, 2942, 2830, 1021. ^{1}H NMR (400 MHz, CD_3OD): δ 2.19 (2H, d, J = 3.2 Hz, CH_2), 3.83 (3H, s, OCH_3), 4.31 (1H, t, J = 3.2 Hz, CH), 5.98 (2H, d, J = 2.4 Hz, ArH), 6.02 (2H, d, J = 2.4 Hz, ArH), 6.98 (2H, d, J = 8.8 Hz, ArH), 7.60 (2H, d, J = 8.8 Hz, ArH). ^{13}C NMR (100 MHz, CD_3OD): 21.7, 34.7, 55.7, 96.7, 97.4, 99.8, 107.8, 114.4, 128.1, 135.5, 154.5, 154.6, 158.2, 161.3. HRMS ESI (m/z): calcd. for $C_{22}H_{18}O_7$ $[M + H]^+$, 395.1125; found 395.1109.

General procedure for the condensation reaction



31-35

The preparation of compound **7** is representative.

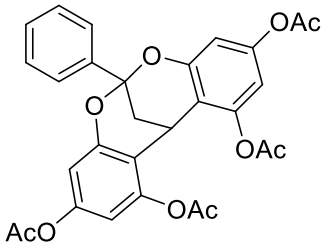


6-(4-methoxyphenyl)-12H-6,12-methanodibenzo[d,g][1,3]dioxocine-1,3,9,11-tetrayl tetraacetate (31): To a solution of 3-(4-methoxyphenyl)propiolaldehyde (0.085 g, 0.53 mmol) and phloroglucinol (0.268 g, 2.12 mmol) in acetonitrile (10 mL) was added PTSA · H_2O (0.0101 g, 0.053 mmol). The reaction was stirred at room temperature under argon for 12 hours. The solvent was evaporated. The crude was dissolved in a solution of pyridine (1.9 mL) and acetic anhydride (2 mL). The resulting solution was stirred overnight at room temperature under argon. The reaction solution was poured into ice-cold water (20 mL) and extracted with ethyl acetate (3 × 25 mL). The combined organic fractions were washed with water (2 × 20 mL), brine (20 mL), dried over $MgSO_4$, filtered, solvent was evaporated. The crude product was purified by preparative TLC (EtOAc – Hex, 70:30) to yield tetraacetate **7** (0.245 g, 82% yield) as grey solid, m.p. 156-158 °C. IR: 3056, 2933, 1768, 1264, 1194. ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.24 (6H, s, $2CH_3$), 2.27-2.28 (2H, m, CH_2), 2.41 (6H, s, $2CH_3$), 3.83 (3H, s, OCH_3), 4.25 (1H, t, J = 2.8 Hz, CH), 6.53 (2H, d,

J = 2.4 Hz, ArH), 6.73-6.74 (2H, m, ArH), 6.93-6.96 (2H, m, ArH), 7.55-7.59 (2H, m, ArH). ^{13}C NMR (100 MHz, $CDCl_3$): 21.2, 21.6, 23.1, 33.0, 55.5, 99.1, 108.4, 109.4, 113.8, 115.8, 127.1, 132.3, 147.7, 150.0, 154.0, 160.2, 169.0, 169.2. HRMS ESI (m/z): calcd. for $C_{30}H_{27}O_{11}$ $[M + H]^+$, 563.1553; found 563.1548.

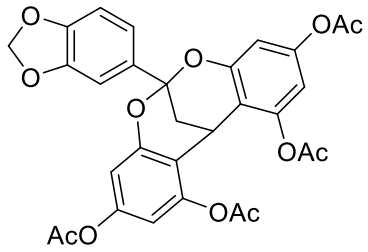
Table 3. Screening of different acids for the condensation reaction.

Entry	Acid	Isolated yield, %	Isolated yield after acetylation, %
1	PTSA ·H_2O	26	82
2	BF_3·Et_2O	25	77
3	TFA	10	
4	HCl		78
5	H_2SO_4	12	

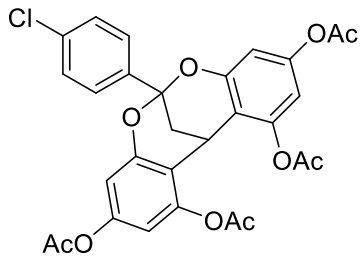


6-phenyl-12H-6,12-methanodibenzo[d,g][1,3]dioxocine-1,3,9,11-tetrayl tetraacetate (**32**): Grey solid (0.0977 g, 54% yield), m.p. 89-91 °C. IR: 3057, 1766, 1185. ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.25 (6H, s, $2CH_3$), 2.29-2.30 (2H, m, CH_2), 2.42 (6H, s, $2CH_3$), 4.25 (1H, t, J = 3.0 Hz, CH), 6.53 (2H, d, J = 2.4 Hz, ArH), 6.75 (2H, d, J = 2.4 Hz, ArH), 7.40-7.44 (3H, m, ArH), 7.66 (2H, dd, J = 8.0 Hz, ArH). ^{13}C NMR (100 MHz, $CDCl_3$): 21.2, 21.6, 23.0, 33.0, 53.6, 99.1, 108.4,

109.5, 115.8, 125.7, 128.6, 129.3, 140.1, 147.7, 150.1, 153.9, 168.9, 169.1. HRMS ESI (m/z): calcd. for $C_{29}H_{24}O_{10}$ $[M + H]^+$, 533.1442; found 533.1441.

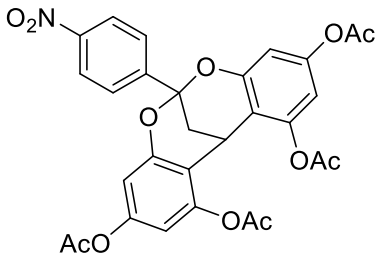


6-(benzo[d][1,3]dioxol-5-yl)-12H-6,12-methanodibenzo[d,g][1,3]dioxocine-1,3,9,11-tetrayl tetraacetate (33): Light brown solid (0.0561 g, 56% yield), m.p. 108-110 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.89 (3H, s, CH_3), 2.04 (3H, s CH_3), 2.27 (2H, br s, CH_2), 2.38 (3H, s, CH_3), 4.68-4.70 (1H, m, CH), 5.92-5.93 (2H, m, CH_2), 6.31-6.32 (1H, m, ArH), 6.69 (2H, d, J = 6.8 Hz, ArH), 6.72-6.73 (1H, m, ArH), 6.94-6.96 (2H, m, ArH), 7.01-7.02 (1H, m, ArH). ^{13}C NMR (100 MHz, $CDCl_3$): 19.7, 20.8, 20.9, 21.3, 29.9, 46.2, 101.3, 102.2, 105.4, 114.0, 114.4, 114.7, 115.2, 119.9, 121.1, 134.6, 137.1, 140.1, 146.6, 147.0, 149.3, 149.5, 149.7, 150.3, 150.4, 150.7, 168.1, 168.4, 169.1, 169.3. HRMS ESI (m/z): calcd. for $C_{30}H_{25}O_{12}$ $[M + H]^+$, 577.1341; found 577.1335.

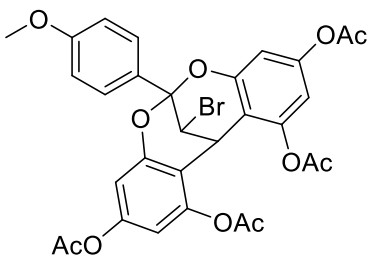


6-(4-chlorophenyl)-12H-6,12-methanodibenzo[d,g][1,3]dioxocine-1,3,9,11-tetrayl tetraacetate (**34**). Yellow solid (0.1066 g, 61% yield), m.p. 238-239 °C. ^{1}H NMR (400 MHz,

$CDCl_3$): δ 2.04 (6H, s, $2CH_3$), 2.04-2.25 (2H, m, CH_2), 2.42 (6H, s, $2CH_3$), 4.24-4.26 (1H, m, CH), 6.53-6.54 (1H, m, ArH), 6.74-6.75 (1H, m, ArH), 7.42 (2H, d, J = 8.7 Hz, ArH), 7.58 (2H, d, J = 8.7 Hz, ArH). ^{13}C NMR (100 MHz, $CDCl_3$): 21.2, 21.5, 22.9, 32.9, 98.7, 108.4, 109.6, 115.7, 127.3, 128.7, 135.3, 138.7, 147.8, 150.1, 153.6, 168.9, 169.1. HRMS ESI (m/z): calcd. for $C_{28}H_{27}O_7$ $[M + H]^+$, 567.1053; found 567.1061.



6-(4-nitrophenyl)-12H-6,12-methanodibenzo[d,g][1,3]dioxocine-1,3,9,11-tetrayl tetraacetate (**35**): Light-grey crystalline solid (0.024 g, 67% yield), m.p. 173-175 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.86 (6H, s, $2CH_3$), 2.25 (6H, s, $2CH_3$), 2.28-2.29 (2H, m, CH_2), 4.11-4.17 (1H, m, CH), 6.82 (2H, s, ArH), 6.89-6.90 (2H, m, ArH), 7.16-7.18 (2H, m, ArH), 7.35 (2H, d, J = 8.0 Hz, ArH), 8.09 (2H, d, J = 8.0 Hz, ArH). ^{13}C NMR (100 MHz, $CDCl_3$): 169.0, 168.6, 150.8, 150.1, 149.6, 146.9, 134.8, 129.7, 127.7, 123.9, 123.0, 120.6, 114.7, 36.0, 29.8, 21.2, 20.9. HRMS ESI (m/z): calcd. for $C_{29}H_{22}NO_{12}$ $[M - H^+]^-$, 576.1147; found 576.1158.



13-bromo-6-(4-methoxyphenyl)-12H-6,12-methanodibenzo[d,g][1,3]dioxocine-1,3,9,11-tetrayl tetraacetate (**39**): Grey solid (0.1448 g, 57% yield), m.p. 53-55 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.25 (3H, s, CH_3), 2.27 (3H, s, CH_3), 2.27 (3H, s, CH_3), 2.40 (3H, s, CH_3), 2.45 (3H, s, CH_3), 3.86 (3H, s, OCH_3), 4.52 (2H, s, CHBr, CH), 6.56-6.57 (1H, m, ArH), 6.66-6.68 (1H, m, ArH), 6.87-6.89 (1H, m, ArH), 6.95 (2H, d, J = 8.0 Hz, ArH), 7.52-7.55 (1H, m, ArH), 7.60 (2H, d, J = 8.0 Hz, ArH). ^{13}C NMR (100 MHz, $CDCl_3$): 21.3, 21.6, 32.6, 46.2, 55.5, 98.8, 108.1, 112.0, 113.6, 116.0, 128.2, 129.7, 146.9, 149.4, 150.5, 150.7, 152.2, 153.5, 160.6, 168.6, 169.1. HRMS ESI (m/z): calcd. for $C_{30}H_{26}BrO_{11}$ $[M + H]^+$, 643.0638; found 643.0673.

References

(1) Seeram, N.; Aviram, M.; Zhang, Y.; Hennings, S. M.; Feng, L.; Dreher, M.; Heber, D. J. *Agric. Food Chem.* **2008**, *56*, 1415.

(2) Gentile, C.; Allegra M.; Angileri, F. *Eur. J. Nutr.* **2012**, *51*, 353-363.

(3) Tatsuno, T.; Jinno, M.; Arima, Y. *Biol. Pharm. Bull*. **2012**, *35*, 909-916.

(4) Chaves F. C.; Gianfagna T. J. *Physiol. Mol. Plant Pathol.* **2007**, *70*, 174-179.

(5) Cheng, H. Y.; Lin, T. C.; Yang, C. M.; Shieh, D. E.; Lin, C. C. *J. Sci. Food Agric.* **2005**, *85*, 10.

(6) de Pascual-Teresa, S.; Moreno, D. A.; Garcia-Viguera, C. *Int. J. Mol. Sci.* **2010**, *11*, 1679-1703.

(7) Gosse, F.; Guyot, S.; Roussi, S. *Carcinogenesis* **2005**, 1291-1295.

(8) Martin, M. A.; Goya, L.; Ramos, S. Food Chem. Toxicol. **2013**, *56*, 336–351.

(9) Fine, A. M. *Oligomeric proanthocyanidin* **2003**, *8*, 442–450.

(10) Li, H. J.; Deinzer M. L.; Proanthocyanidins in hops. Beer in health and disease prevention. **2009**, Elsevier, Amsterdam, 333–348.

(11) Malien-Aubert, C.; Dangles, O.; Amiot M. J. *J Agric. Food Chem.* **2002**, *50*, 3299-3305.

(12) Shi, J.; Yu, J.; Pohorly, J. E.; Kakuda, Y. *J. Med. Food* **2003**, *6*, 291– 299.

(13) Faria, A.; Calhau, C.; de Freitas V.; Mateus, N. *J. Agric. Food Chem.* **2006**, *54*, 2392–2397.

(14) Kasahara, Y.; Hikino, H. *HETEROCYCLES* **1983**, *20*, 1953-1956.

(15) Tao, H.; Wang, L.; Cui, Z.; Zhao, D.; Liu, Y. *Planta Medica* **2008**, *74*, 1823.

(16) Calzada, F.; Cerda-Garcia-Rojas, C.; Meckes, M.; Cedillo-Rivera, R.; Bye, R.; Mata, R. *J. Natural Prod.* **1999**, *62*, 705.

(17) Kim, I.; Park, Y.; Yoon, S.; Lee, H. *Internat. Immunopharmacology* **2010**, *10*, 1616.

(18) Ito, Y.; Ohmori, K.; Suzuki, K. *Angew. Chem. Int. Ed.* **2014**, *53*, 10129-10133.

(19) Sharma, P. K.; Romanczyk, L. J., Jr.; Kondaveti, L.; Reddy, B.; Arumugasamy, J.; Lombardy, R.; Gou, Y.; Schroeter, H. *Org. Lett.* **2015**, *17*, 2306.

(20) Kraus, G. A.; Yuan, Y.; Kempema, A. *Molecules* **2009**, *14*, 807-815.

(21) Newhouse, T.; Baran, P. S. *Angew. Chem. Int. Ed*. **2011**, *50*, 3362.

(22) Albuquerque, H. M.; Santos, C. M.; Cavaleiro, J. A.; and Silva, A. M. *Eur. J. Org. Chem.* **2015**, 4732.

(23) Paioti, P. H.; Abboud, K. A.; Aponick, A. *J. Am. Chem. Soc.* **2016**, 138, 2150.

(24) Gudla, V.; Balamurugan, R. *J. Org. Chem.* **2011**, 76, 9919.

(25) Lebel, H. Davi, M. *Adv. Synth. Catal.* **2008**, *350*, 2352.

(26) Ziegler, C. B.; Harris, S. M. *J. Org. Chem.* **1987**, *52*, 443.

(27) Yong Guan, Y.; Lopez-Alberca, M. P.; Lu, Z.; Zhang, Y.; Desai, A. A.; Patwardhan, A. P.; Dai, Y.; Vetticatt, M. J.; Wulff, W. D. *Chem. Eur. J.* **2014**, *20*, 13894.

(28) Hermann, D.; Arlcan, D.; Relnhard, B. *Synthesis* **2017**, *49*, 326.

CHAPTER 3. SYNTHETIC ANTHOCYANIDINS FROM NATURAL BENZOPYRANS

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Introduction

Anthocyanins are ionic polycyclic natural products. They are present in a variety of fruits and vegetables including blueberries, currants, cherries, grapes, raspberries, gooseberries and certain varieties of sweet potatoes. This class of compounds has been reported to exhibit a broad array of biological activities. There are number of excellent reviews describing antioxidant properties of anthocyanins.[1-3] Anthocyanins from red cabbage were reported to show a strong protective effect against oxidative damage of platelet biomolecules induced by biological oxidants.[4] Blueberry anthocyanins showed inhibition of acrylamide-induced toxicity in mice by preventing oxidative stress and cytochrome P450 2E1 activation.[5] Cherry anthocyanins were reported to inhibit polyphenol oxidase enzyme activity.[6] Anthocyanins were suggested as potential candidates for the treatment of diabetic retinopathy.[7] During World War II British pilots were fed blueberries to improve their night vision. The French paradox, observation that there is relatively low occurrence of coronary heart disease among French people who consume red wine on regular basis, was attributed to the presence of anthocyanins present in red wine which act as radical scavengers. Because of their valuable biological properties several methods of isolation and purification of anthocyanins from plant extracts have been developed. Researchers employed various techniques such as mechanical shaking, countercurrent chromatography, and enrichment on macroporous resins to purify anthocyanins.[8-10] All these purification methods were not effective

in separating procyanidins from other polyphenols. Furthermore, because of low abundance of procyanidins in natural sources large amounts of natural materials are required to obtain necessary amounts of procyanidins for research studies.

Anthocyanidins are aglycones of anthocyanins, sugar-free counterparts of anthocyanins. Some representative compounds are shown in Figure 1.

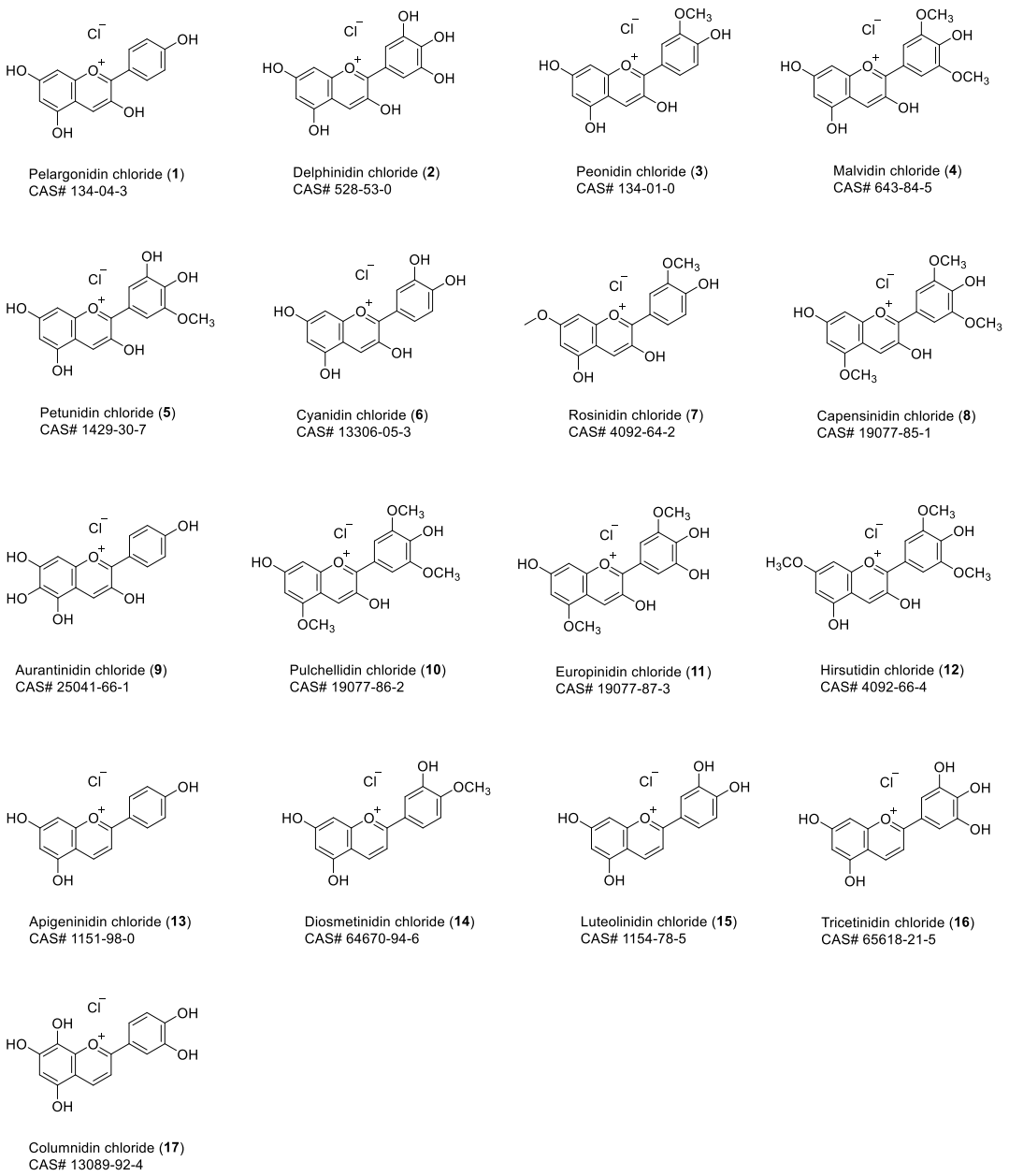
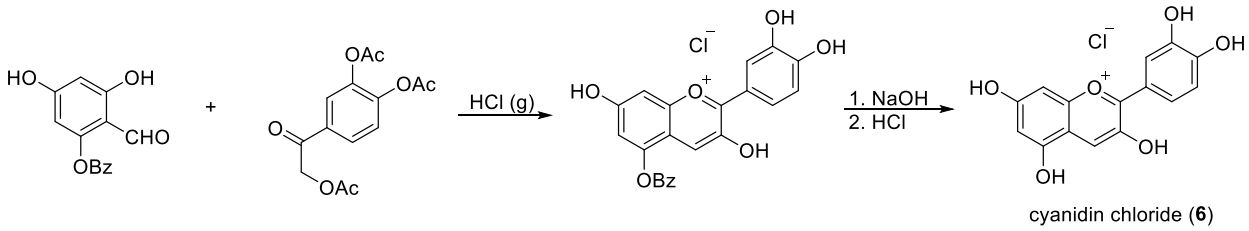


Figure 1. Natural anthocyanidins

About 30% of anthocyanidins found in nature contain cyanidin, 22% contain delphinidin, and 18% contain pelargonidin. Around 20% of the anthocyanins are based on peonidin, malvidin, and petunidin.[11] Most of the anthocyanidins contain hydroxyl group at carbon-3 through which they are connected to sugar moiety. Some anthocyanins, mostly those present in bryophytes, contain 3-desoxyanthocyanidins, apigenidin, diosmetnidin, luteolinidin, tricetinidin, columidin, compounds **13-17**.

Because of the valuable biological properties and low abundance of anthocyanidins from natural sources to explore the full potential of these compounds several syntheses were reported. Most of them used synthetic approach developed by Robinson which involves the reaction of a substituted acetophenone with an aromatic aldehyde in the presence of a concentrated solution of gaseous hydrochloric acid.[12] The representative example is shown in Scheme 1.



Scheme 1. Robinson's anthocyanidin synthesis

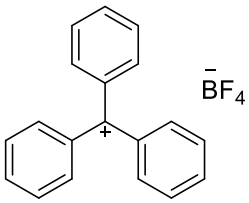
While this method worked on a small scale and a number of anthocyanidins were prepared by this method, there are potential hazards associated with the use of gaseous hydrochloric acid. We sought for a more convenient procedure, not requiring the use of hazardous reagents and preparation of aromatic precursors.

Results and discussion

There are many benzopyran-containing natural products which are more readily available than anthocyanidins and have very similar structural motives to anthocyanidins. These products can be possibly converted to anthocyanidins under the right reaction conditions. There a few transformations of this type which have been reported. Rutin, a flavonol glycoside, was reduced by Bruillard and coworkers with zinc amalgam in 3% absolute methanolic hydrochloric acid to give the corresponding cyanidin glycoside.[13] The synthesis of cyanidin 3-*O*-β-D-glucoside from (+)-catechin was reported by Kondo and coworkers.[14] The key intermediate, dihydroanthocyanin, was oxidized by air in hydrogen chloride-methanol to generate cyanidine glycoside. Although air was employed to oxidize dihydroanthocyanidins, there are no reports of its application to oxidize more reduced precursors. Dihydroanthocyanidin was oxidized to anthocyanidin with chloranil in moderate yield by Sweeny and Iacobucci.[15]

The optimal oxidant would convert benzopyrans cleanly into anthocyanidins without traces of the oxidant contaminating the product. The product anthocyanidin should be easily separated and purified from the crude reaction mixture because this type of compounds is very labile to many purification methods such as silica gel column chromatography. To be sustainable, the oxidants should be used in catalytic amounts and must be capable to be regenerated by environmentally friendly oxidants.

We explored triphenylcarbenium tetrafluoroborate, also called trityl tetrafluoroborate, a well-known hydride abstractor, shown in Figure 2. Trityl tetrafluoroborate is a yellow solid which is soluble in most organic solvents such as tetrahydrofuran, dichloromethane and reacts with nucleophilic solvents.

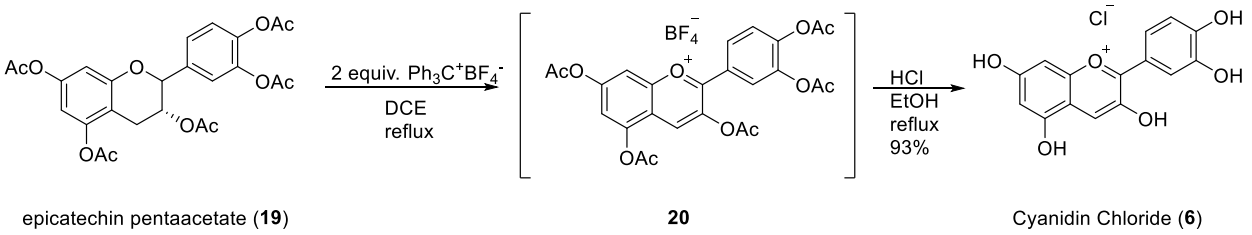


18

Figure 2. Triphenylcarbenium tetrafluoroborate (**18**)

Trityl tetrafluoroborate has been used in organic syntheses in dehydrogenation reactions, deprotection of ketone acetals, oxidation of silyl enol ethers.[16-19] It had not been used in the synthesis of anthocyanidins. The byproduct of the oxidation is triphenylmethane, a hydrocarbon, which can be simply separated from the highly polar products by trituration. Also, triphenylmethane can be possibly re-oxidized to make the process catalytic.

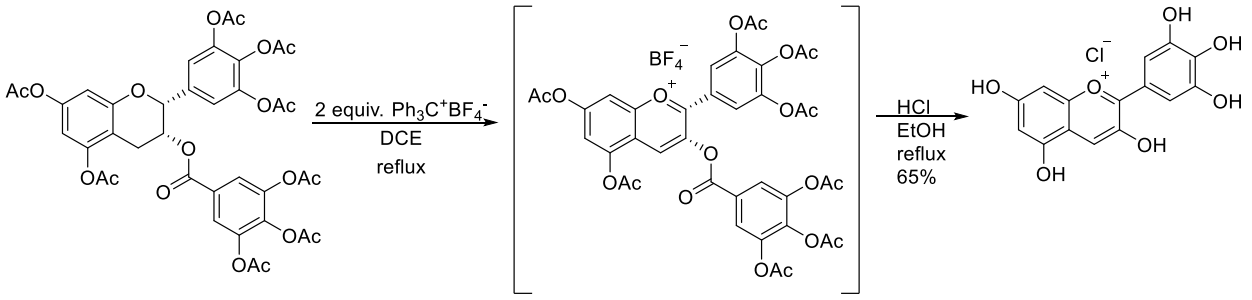
Reaction of epicatechin directly with trityl tetrafluoroborate afforded mostly trityl aryl ether formation plus small amounts of product. Acetylation of hydroxyl groups prior to the hydride abstraction significantly increased the yield of the flavylium salt. Also, changing the solvent from dichloromethane to 1,2-dichloroethane improved the yield of the desired product. Treatment of epicatechin pentaacetate **19** with trityl tetrafluoroborate in 1,2-dichloroethane afforded flavylium tetrafluoroborate salt **20** that is upon treatment with hydrochloric acid in ethanol produced cyanidin chloride **6** in 93% yield, as depicted in Scheme 2.



epicatechin pentaacetate (**19**) **20** Cyanidin Chloride (**6**)

Scheme 2. Synthesis of cyanidin chloride (**6**)

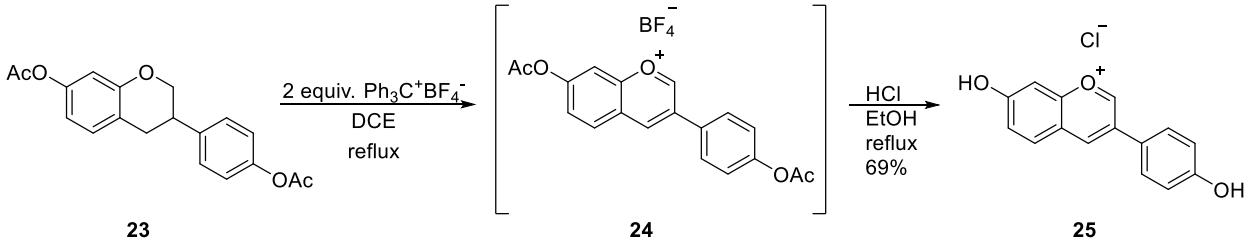
Similarly, delphinidin chloride **2** was synthesized in 65% yield by treatment of epigallocatechin gallate peracetate **21** with trityl tetrafluoroborate followed by treatment of the resulting tetrafluoroborate salt **22** with hydrochloric acid in ethanol as illustrated in Scheme 3.



Epigallocatechin Gallate Peracetate (**21**) **22** Delphinidin chloride (**2**)

Scheme 3. Synthesis of Delphinidin chloride (**2**)

This process can be extended to the preparation of isoflavylium salts. The diacetate of commercially available equol **23** was refluxed with trityl tetrafluoroborate in dichloroethane followed by treatment with hydrochloric acid in ethanol to afford isoflavylium salt **25** in 69 % yield, as shown in Scheme 4.



Scheme 4. Synthesis of **25**

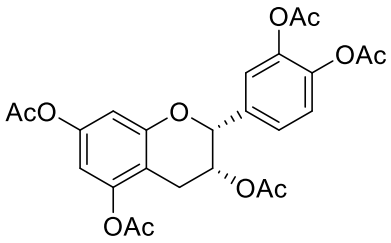
Conclusion

In summary, anthocyanins are important class of potent biological molecules. They were reported to have tremendous number of biological activities. There are hundreds of known anthocyanins which have been isolated from natural sources. Scientists isolate about 20-30 new anthocyanins yearly. Anthocyanins structurally consist of anthocyanidin part, which is believed to be responsible for all valuable biological properties anthocyanins possess, and sugar moiety. Because of valuable biological properties and low abundance from natural sources efficient syntheses of anthocyanins need to be developed to study this important class of natural products.

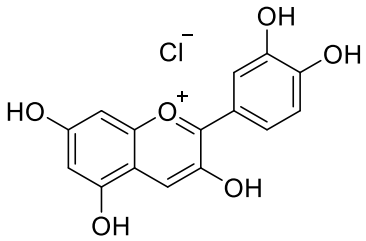
There are many readily available benzopyran-containing natural products which have chemical structures very close to structures of anthocyanidins. We have explored the reactivity of different benzopyran derivatives which are structurally close to known anthocyanidins. We have discovered that trityl tetrafluoroborate can be used to synthesize anthocyanidins in good yields. The process is operationally convenient and can be used to prepare anthocyanidins in gram quantities for biological evaluation.

Experimental Section

All starting materials were purchased from Sigma-Aldrich; solvents were purchased from Fisher Scientific and used without further purification. All reactions were carried out in flame-dried glassware under argon with dry solvents under anhydrous conditions. All yields refer to chromatographically isolated products. Reactions were monitored by thin-layer chromatography (TLC) carried out on 0.20 mm silica gel plates using UV light as a visualizing agent. Silica gel 60A, particle size 0.032 – 0.063 mm, was used for flash column chromatography. ^{1}H and ^{13}C NMR spectra were acquired in $CDCl_3$ on a Varian MR-400 spectrometer. ^{1}H and ^{13}C chemical shifts (δ) are given in ppm relative to the residual protonated solvent peak ($CDCl_3$: $\delta H = 7.26$ ppm, $\delta C = 77.0$ ppm; CD_3OD: $\delta H = 3.31$ ppm, $\delta C = 49.0$ ppm) as an internal reference. High-resolution mass spectra (HRMS) were recorded on an Agilent 6540 QTOF (quadrupole time of flight) mass spectrometer using ESI (electrospray ionization) or APCI (atmospheric-pressure chemical ionization), or EI (electron ionization) on an Agilent 6890 GC/MS. IR were recorded on Nicolet Fisher Scientific iS5. Melting points were measured on Mel-Temp II melting point apparatus.

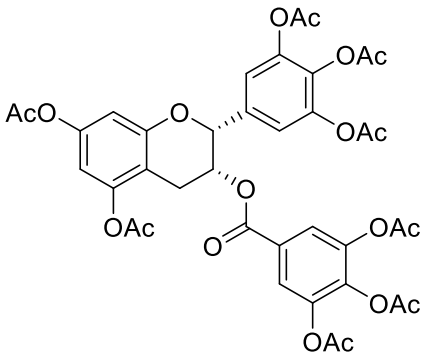


Pentaacetyl (-)-epicatechin (19). To 0.5000 g of (-)epichatechin (1.72 mmol) in 6 mL of pyridine was added 2 mL of acetic anhydride at 0 °C. The reaction was stirred overnight at room temperature under argon. The solution was then partitioned between 50 mL of ethyl acetate:50 mL of 1N HCl. The organic layer was washed with 1N HCl (3 × 50 mL), water (50 mL), brine (50 mL). The washed organic layer was dried over $MgSO_4$, filtered, solvent was evaporated. The crude product was triturated with hexane to provide 0.7286 g of (-)-epicatechin pentaacetate at 85% yield. ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.92 (3H, s, $COCH_3$), 2.28 (3H, s, $COCH_3$), 2.30 (9H, s, $COCH_3$), 2.87 (1H, dd, *J* = 18.0 Hz, H-4a), 2.99 (1H, dd, *J* = 18.0 Hz, H-4b), 5.11 (1H, s, H-2), 5.38-5.40 (1H, m, H-3), 6.57 (1H, d, *J* = 2.2 Hz, H-6 or H-8), 6.67 (1H, d, *J* = 2.2 Hz, H-6 or H-8), 7.20 (1H, d, *J* = 8.4 Hz, H-9), 7.27 (1H, dd, *J* = 8.4 Hz, H-10), 7.36 (1H, d, *J* = 2.0 Hz, H-11).

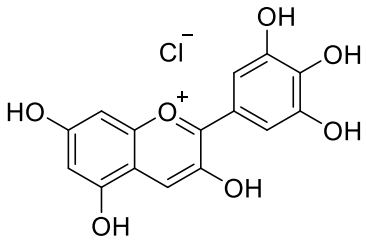


Cyanidin chloride (6). To a solution of (-)-epicatechin pentaacetate (0.1000 g, 0.2 mmol) in DCE (2 mL) was added solution of triphenylcarbenium tetrafluoroborate (0.1319 g, 0.4 mmol) in DCE (5 mL). The reaction was refluxed overnight. DCE was evaporated. The residue was dissolved in ethanol (7 mL) and concentrated HCl (2 mL) was added. The reaction was refluxed overnight.

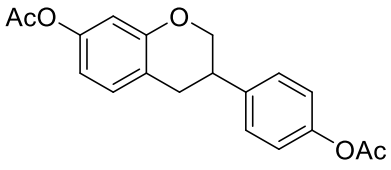
Volatiles were evaporated. Crude was triturated with diethyl ether to yield 0.0603 g of cyanidin chloride in 93% yield. ^{1}H NMR (400 MHz, CD_3OD): δ 6.61 (1H, s, ArH), 6.85 (1H, s, ArH), 7.01 (1H, d, *J* = 8.8 Hz, ArH), 8.10 (1H, s, ArH), 8.22 (1H, dd, *J* = 8.8 Hz, ArH), 8.55 (1H, s, ArH). HRMS ESI (m/z): calcd. for $C_{15}H_{11}O_6^+$ $[M]^+$, 287.0550; found 287.0550.



Epigallocatechin gallate (EGCG) peracetate (21). EGCG (0.0407 g, 0.089 mmol) was dissolved in a solution of pyridine (0.6 mL) and acetic anhydride (0.7 mL). The reaction was stirred at 45 °C in an oil bath under argon for 20 h. The reaction was then poured into ice-cold water (10 mL) and was left to stand for 1 h to form a white precipitate. The resulting precipitate was filtered, dried. The crude product was purified by preparative TLC (EtOAc – Hex, 70:30) to yield 0.0466g of EGCG peracetate in 67 % yield. ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.23 (6H, s, $COCH_3$), 2.25 (3H, s, $COCH_3$), 2.27 (3H, s, $COCH_3$), 2.28 (9H, s, $COCH_3$), 2.29 (3H, s, $COCH_3$), 2.98 (1H, dd, *J* = 17.8 Hz, H-4a), 3.08 (1H, dd, *J* = 17.8 Hz, H-4b), 5.19 (1H, s, H-2), 5.62-5.64 (1H, m, H-3), 6.61 (1H, d, *J* = 2.2 Hz, H-6 or H-8), 6.73 (1H, d, *J* = 2.2 Hz, H-6 or H-8), 7,24 (2H, s, H-7), 7.62 (2H, s, H-9).

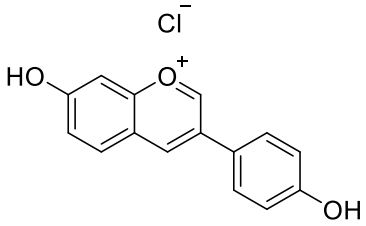


Delphinidin chloride (2). To a solution of EGCG peracetate (0.0466 g, 0.059 mmol) in DCE (1 mL) was added solution of triphenylcarbenium tetrafluoroborate (0.0388 g, 0.118 mmol) in DCE (3 mL). The reaction was refluxed overnight. DCE was evaporated. The residue was dissolved in ethanol (5 mL) and concentrated HCl (1 mL) was added. The reaction was refluxed overnight. Volatiles were evaporated. Crude was triturated with diethyl ether to yield 0.0603 g of delphinidin chloride in 65% yield. ^{1}H NMR (400 MHz, CD_3OD): δ 5.80 (1H, s, ArH), 5.99 (1H, s, ArH), 6.60 (1H, s, ArH), 6.83 (2H, s, ArH). HRMS ESI (m/z): calcd. for $C_{15}H_{11}O_7^+$ $[M]^+$, 303.0499; found 303.0500.



4',7-diacetoxyisoflavan (23). (±)-Equol (0.0430 g, 0.177 mmol) was dissolved in a solution of pyridine (0.7 mL) and acetic anhydride (0.7 mL). The reaction was stirred at room temperature overnight. The reaction solution was poured into ice-cold water (10 mL) and extracted with dichloromethane (3 × 10 mL). The combined organic layers were washed with water (2 × 20 mL), brine (20 mL), dried over $MgSO_4$, filtered, solvent was evaporated to yield 0.0424g (73%) of 4',7-diacetoxyisoflavan. ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.29 (3H, s, $COCH_3$), 2.31 (3H, s, $COCH_3$), 3.01 (2H, d, J = 8.4 Hz, $ArCH_2$), 3.21-3.29 (1H, m, ArCH), 3.98-4.03 (1H, m, OCH), 4.35 (1H,

dd, *J* = 10.4 Hz, OCH), 6.61-6.64 (2H, m, ArH), 7.06-7.09 (3H, m, ArH), 7.25 (2H, d, *J* = 7.2 Hz, ArH).



7-hydroxy-3-(4-hydroxyphenyl)chromenylium chloride (25). To a solution of (±)-equol diacetate (0.0416 g, 0.127 mmol) in DCE (2 mL) was added solution of triphenylcarbenium tetrafluoroborate (0.0842 g, 0.255 mmol) in DCE (5 mL). The reaction was refluxed overnight. DCE was evaporated. The residue was dissolved in ethanol (7 mL) and concentrated HCl (1.5 mL) was added. The reaction was refluxed overnight. Volatiles were evaporated. Crude was triturated with diethyl ether to yield 0.0603 g of 7-hydroxy-3-(4-hydroxyphenyl)chromenylium chloride in 69% yield. ^{1}H NMR (400 MHz, CD_3OD): δ 7.08-7.2 (3H, m, ArH), 7.16-7.21 (3H, m, ArH), 7.24-7.29 (3H, m, ArH). HRMS ESI (m/z): calcd. for $C_{15}H_{11}O_6^+$ $[M^+ - H^+ + Na^+ + C_2H_5OH]^+$, 307.0934; found 307.1332.

References

1. Del Bo, C.; Martini, D.; Porrini, M.; Klimis-Zacas D.; Riso P. *Food Function* **2015**, *6*, 2890-2917.
2. Wu, X. *Anthocyanins in Health and Disease* **2014**, 141-164.
3. Clifford, M. N. *J. Sci. Food Agric*. **2000**, *80*, 1063-1072.
4. Saluk, J.; Bijak, M.; Posmyk, M. M.; Zbikowska H. M. *International Journal of Biological Macromolecules* **2015**, *80*, 702-709.
5. Zhao, M.; Wang, P.; Zhu, Y.; Liu, X.; Hu, X.; Chen, F. *Journal of Functional Foods* **2015**, *14*, 95-101.
6. Demir, T.; Sonmez, F.; Bilen, C.; Gencer, N. *Journal of Food, Agriculture & Environment*, **2013**, *11*, 572-575.
7. Nabavi, S. F.; Habtemariam, S.; Daglia, M.; Shafighi, N.; Barber, A. J.; Nabavi S. M. *Current Medicinal Chemistry* **2015**, *22*, 52-58.
8. Jiang, H.; Shi, D.; Wang, X.; Gou, X.; Yin, L.; Yang, G. (2014) *Shipin Kexue* **2014**, *35*, 67-72.
9. Kostadinovic, V. S.; Mirhosseini, H.; Bogeva, E. *Journal of Nutrition & Food Sciences* **2013**, *3*, 1000243/1-1000243/7.
10. Ma, T.; Hu, N.; Ding, C.; Zhang, Q.; Li, W.; Suo, Y.; Wang, H.; Bai, B.; Ding, C. *Food Chemistry* **2016**, *194*, 296-303.
11. Andersen, O. M.; Markham, K. R. **2006** CRC, Taylor & Francis. **2006**, 471-553.
12. Siegel A. *Ambix* **2008**, *55*, 62-82.
13. Elhabiri, M.; Figueiredo, P.; Fougerousse A.; Brouillard, R. *Tetrahedron Letters* **1995**, *36*, 4611-4614.

14. Kondo, T.; Oyama, K.; Nakamura, S.; Yamakawa, D.; Tokuno, K.; Yoshida, K. *Organic Letters* **2006**, *8*, 3609-3613.

15. Sweeny, J. G.; Iacobucci, G. A. *Tetrahedron* **1977**, *33*, 2890-2917.

16. Fu, P. P.; Harvey, R. G. *Chem. Rev.* **1978**, *78*, 317.

17. Ichikawa, J.; Yokota, M.; Kudo, T.; Umezaki, S. *Angew. Chem. Int. Ed.* **2008**, *47*, 4870.

18. Varin, M.; Barre, E.; Iorga, B.; Guillou, C. *Chem. Eur. J.* **2008**, *14*, 6606.

19. Orellana, A.; Rovis, T. *Chem. Commun.* **2008**, 730.

GENERAL CONCLUSION

New methods for the syntheses of natural products and molecules for RNA imaging have been described in this dissertation. In Chapter 1 we have shown the synthesis of library of small molecules which can be used for RNA imaging. PFP-DFHBI, the best synthesized compound, has been found to have superior qualities compared to commonly used DFHBI ligand. The synthesized compounds can be used as either light-up aptamers or in FRET for the visualization of gene expression. We have successfully coupled some of the most promising compounds to cyanine dyes Cy3 and Cy5 to generate FRET pairs and to biotin to select new RNA aptamers from a pool of RNAs. We have developed the procedure to functionalize allylic position in DFHBI with different electrophiles. This method was used for the synthesis of DFHBI analogs with extended conjugation which have been found to have different excitation and emission wavelengths and longer fluorescence lifetime compared to DFHBI. The studies are currently ongoing to explore the full potential of these compounds. We have developed an efficient method for labelling of aminoglycosides. This procedure is used by our collaborators at the University of California San Francisco to track bacteria in soil.

In chapter 2 we describe one-step procedure for the synthesis of procyanidin A-skeleton. Different alkynylaldehydes or dibromocinnamaldehydes were coupled with phloroglucinol derivatives to afford procyanidin A skeleton with various substitution patterns on aromatic rings using this method. The method is simple, efficient, was performed on a large scale and will be used for the total synthesis of A-type procyanidins.

I chapter 3 we explored the reactivity of natural benzopyrans and have found that they can be converted in one pot to anthocyanidins in good yields with the use of trityl tetrafluoroborate. The synthesized anthocynidins are easily purified from non-polar byproduct triphenylmethane.

The method is efficient and simple and can be used to prepare anthocyanidins in gram quantities for biological evaluation.

This work resulted in three articles published in peer-reviewed journals. Two or three additional publications are in preparation.

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