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TESIS DOCTORAL

Chemical probes for the study of the endogenous cannabinoid system

Sondas químicas para el estudio del sistema endocannabinoide endógeno

MEMORIA PARA OPTAR AL GRADO DE DOCTOR

PRESENTADA POR

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CHEMICAL PROBES FOR THE STUDY OF THE ENDOGENOUS CANNABINOID SYSTEM

SONDAS QUÍMICAS PARA EL ESTUDIO DEL SISTEMA CANNABINOIDE ENDÓGENO

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"Your assumptions are your windows on the world. Scrub them off every once in a while, or the light won't come in"

Isaac Asimov

A mi padre

El presente trabajo ha sido realizado en el Laboratorio de Química Médica del Departamento de Química Orgánica I de la Facultad de Ciencias Químicas de la Universidad Complutense de Madrid, bajo la supervisión de la ***Catedrática Dra. María Luz López Rodríguez*** *y de las* ***Dras. Silvia Ortega Gutiérrez*** *y* ***María del Mar Martín-Fontecha Corrales*** *a quienes deseo expresar mi más sincero agradecimiento por su calurosa acogida en este grupo de investigación, por sus continuas enseñanzas a lo largo de estos años, y muy especialmente, por todo el ánimo, apoyo y confianza depositados en este proyecto y en mí.*

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ABBREVIATIONS AND ACRONYMS

Throughout this manuscript, abbreviations and acronyms recommended by the American Chemical Society in the Organic Chemistry and Medicinal Chemistry areas have been employed (revised in the *Journal of Organic Chemistry* and *Journal of Medicinal Chemistry* on May 2015; http://pubs.acs.org/page/joceah/submission/authors.html and http://pubs.acs.org/paragonplus/submission/jmcmar/jmcmar_abbreviations.pdf). In addition, those indicated below have also been used.

2-AG	2-Arachidonoylglycerol
ABHD6	α/β-Hydrolase domain 6
ADDP	1,1'-(Azodicarbonyl)dipiperidine
AEA	*N*-Arachidonoylethanolamine (anandamide)
app	Apparent
BCA	Bicinchoninic acid
BOP-Cl	Bis(2-oxo-3-oxazolidinyl)phosphinic chloride
CBR	Cannabinoid receptor
CNS	Central nervous system
DAGL	Diacylglycerol lipase
DAPI	4'-6-Diamidino-2-phenylindole dihydrochloride
DBDMH	1,3-Dibromo-5,5-dimethylhydantoin
DIPEA	*N*,*N*-Diisopropylethylamine
DMEM	Dulbecco's modified Eagle medium
eCB	Endocannabinoid
ECS	Endogenous cannabinoid system
EDC	*N*-(3-Dimethylaminopropyl)-*N*'-ethylcarbodiimide
EGTA	Ethyleneglycoltetraacetic acid
ER	Estrogen receptor
FAAH	Fatty acid amide hydrolase
FBS	Fetal bovine serum
FS	Forward scatter
GO	Gene ontology

GPCR	G protein-coupled receptor
hCB_1R	Human type 1 cannabinoid receptor
hCB_2R	Human type 2 cannabbinoid receptor
HER2	Human epidermal growth factor 2 receptor
HOBt	1-Hydroxybenzotriazole
MAGL	Monoacylglycerol lipase
mDCs	Myeloid dendritic cells
MS	Mass spectrometry
$mtCB_1R$	Mitochondrial type 1 cannabinoid receptor
MTT	3-[4,5-Dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide
MW	Microwave
NAAA	*N*-Acylethanolamine-hydrolysing acid amidase
NAPE-PLD	*N*-Arachidonoylphosphatidylethanolamine phospholipase D
NF-κB	Nuclear factor kappa B
PANTHER	Protein analysis through evolutionary relationships
PBMCs	Peripheral blood mononuclear cells
pDCs	Plasmacytoid dendritic cells
PPAR	Peroxisome proliferator-activated receptor
PR	Progesterone receptor
p-TSA	*p*-Toluenesulfonic acid
qt	Quintet
RP	Reverse phase
SEM	Standard error of the mean
sept	Septet
SILAC	Stable isotope labelling by amino acids in cell culture
S-PHOS	2-Dicyclohexylphosphino-2',6'-dimethoxybiphenyl
TBAI	Tetrabutylammonium iodide
TBTA	Tris[(1-benzyl-1*H*-1,2,3-triazol-4-yl)methyl]amine
TCEP	Tris(2-carboxyethyl)phosphine

TES	Triethylsilane
TFP	Tetrafluorophenyl
THC	(-)-Δ^9-Tetrahydrocannabinol
TMCs	Tonsil mononuclear cells
TNBC	Triple negative breast cancer
tPSA	Topological polar surface area
TRP	Transient receptor potential
UCM	Universidad Complutense de Madrid

RESUMEN

1. RESUMEN

1.1. Introducción y objetivos

La química biológica nació hace dos décadas con objeto de estudiar la interfase entre la química y la biología, utilizando para ello herramientas capaces de interrogar los distintos sistemas biológicos, facilitando así la comprensión de los mismos.[1-3] Sin embargo, existen sistemas biológicos de gran relevancia cuyo estudio no ha sido abordado hasta ahora. Uno de ellos es el sistema cannabinoide endógeno (*endogenous cannabinoid system*, ECS), que durante los últimos años ha sido relacionado con diversas funciones fisiológicas a nivel tanto central como periférico.[4-7] Sin embargo, no existe ningún fármaco en el mercado a día de hoy dirigido a la modulación de este sistema. En este contexto, una mayor comprensión de algunos de los aspectos de la biología del ECS, tales como la localización de los receptores de cannabinoides (*cannabinoid receptors*, CBRs) a nivel celular, su localización a nivel subcelular, la existencia de otras dianas distintas de los receptores conocidos y molecularmente caracterizados CB_1R y CB_2R, y el estudio de otros ligandos de los CBRs, podrían permitir una aplicación terapéutica real de este sistema. Así, los objetivos generales del presente trabajo de investigación son: i) el desarrollo de sondas para el estudio de la expresión de los CBRs en el sistema inmune, ii) el diseño y síntesis de herramientas para explorar las diferencias entre el CB_1R de la membrana plasmática y el mitocondrial, iii) el descubrimiento de las dianas adicionales de los agonistas sintéticos HU210 y HU308, y iv) la identificación de las dianas terapéuticas del producto de origen natural honokiol.

1.2. Resultados y discusión

1.2.1. Desarrollo de sondas para el estudio de la expresión de los CBRs en el sistema inmune

La amplia distribución de los CBRs en los diferentes órganos y tejidos, así como su implicación en la regulación de muchas funciones (fisio)patológicas, abre una ventana de oportunidades para el tratamiento de diversas enfermedades. En este sentido, el sistema inmune además de ofrecer un gran potencial terapéutico, representa una excelente oportunidad para la búsqueda de biomarcadores que reflejen la existencia de tales desórdenes y su gravedad. Así, es bien conocido que las células del sistema inmune expresan los CBRs, que dicha expresión cambia en función de diferentes condiciones, y que el ECS regula muchos aspectos relacionados con el correcto funcionamiento del sistema inmune.[8-10] Por lo tanto, el desarrollo de herramientas que nos permitan estudiar el papel de los CBRs en condiciones fisiológicas, así como poder establecer si estos pueden ser usados como biomarcadores de enfermedades o de su prognosis, sería muy interesante. Con este objetivo, se llevó a cabo el diseño de sondas moleculares formadas por: i) un grupo de unión basado en un esqueleto de alta afinidad por los CBRs, ii) una subunidad de marcaje (S.M.) que permita su detección y visualización, y iii) un espaciador apropiado con objeto de evitar un posible impedimento estérico por parte de la subunidad de marcaje, que podría traducirse en una pérdida de la afinidad (Figura 1). Con respecto al grupo de unión, se eligió el esqueleto del ligando sintético HU210, con alta afinidad por los CBRs. En lo que se refiere a la subunidad de marcaje, se seleccionaron la biotina y el fluoróforo Alexa Fluor 488 para la detección de los CBRs por microscopía confocal y citometría de flujo, respectivamente.

De este modo, se sintetizaron los derivados **1**, **2**, y **20** en los que se ha considerado la utilización de un grupo amida en los compuestos **1** y **2**, o un éter en el derivado **20**, como espaciador entre el grupo de unión y la subunidad de marcaje. Por otro lado, para la preparación del derivado fluorescente se seleccionó el espaciador tipo amida de la sonda **1** y se sustituyó la subunidad de biotina por el fluoróforo Alexa Fluor 488 en el compuesto **22** (Figura 1). Todos los compuestos sintetizados mostraron una elevada afinidad por los CBRs, con valores de la constante en el rango nanomolar. En concreto, el compuesto **1** presentó los mejores valores de afinidad [K_i (CB_1)=1.6±0.5 nM; K_i (CB_2)=0.36±0.02 nM], y el derivado **22** mostró una elevada selectividad por el CB_1R [K_i

(CB_1)=28±4 nM; K_i (CB_2)=0.7±0.2 μM], y por lo tanto fueron seleccionados para el estudio de los CBRs en sistemas celulares.

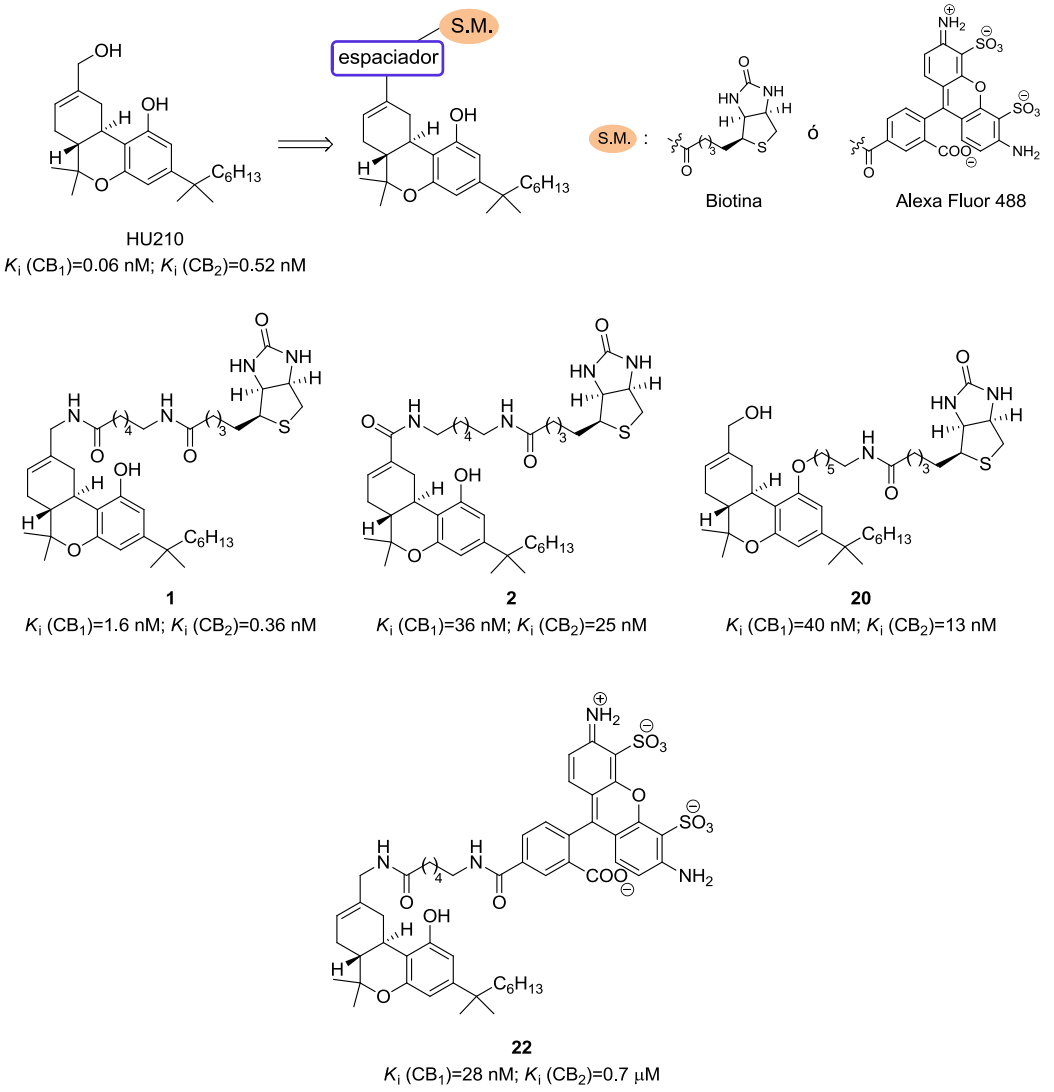

Figura 1. Diseño de las sondas derivadas de HU210 **1**, **2**, **20**, y **22**.

De entre las diferentes posibilidades con potencial terapéutico dentro del sistema inmune, inicialmente seleccionamos las enfermedades alérgicas, ya que se ha descrito que el ECS juega un papel protector en este tipo de desórdenes.[11,12] En este contexto, nos propusimos explorar la presencia de los CBRs en pacientes de distintas alergias frente a individuos sanos. Para ello se seleccionaron células mononucleares de sangre

periférica (*peripheral blood mononuclear cells*, PBMCs) y de órganos linfoides como las amígdalas (*tonsil mononuclear cells*, TMCs).

Así, la sonda **1** fue utilizada en diferentes células del sistema inmune para el estudio del CB_1R, demostrando la presencia de este receptor en pacientes alérgicos.[13] Por otro lado, la sonda fluorescente **22** permitió la visualización directa del CB_1R en PBMCs mediante citometría de flujo, destacando así como una valiosa herramienta para estudiar posibles modificaciones en la expression de este receptor en muestras con relevancia clínica, y también para estudiar su potencial como biomarcador.

1.2.2. Diseño y síntesis de herramientas para explorar las diferencias entre el CB_1R de la membrana plasmática y el mitocondrial

La complejidad de la señalización mediada por el CB_1R se ha visto incrementada como consecuencia de la identificación de un nuevo receptor localizado en la mitocondria, $mtCB_1R$,[14,15] existiendo hipótesis que sugieren que ambos receptores están involucrados en la regulación de distintos efectos.[16,17] Para poder diferenciar entre ambos receptores sería necesario el desarrollo de compuestos que, manteniendo una elevada afinidad por el CB_1R localizado en la membrana plasmática, fueran incapaces de atravesar dicha membrana, no pudiendo activar el $mtCB_1R$. Así, llevamos a cabo el diseño, síntesis, y evaluación biológica de los compuestos derivados de HU210 **26-28**, con subunidades de elevada polaridad como pueden ser una urea o una acilurea en los compuestos **26** y **27**, respectivamente, o un anillo de tetrazol en el derivado **28**, unidos al esqueleto a través de un grupo amida y el espaciador con la longitud óptima determinado con anterioridad (Figura 2). Los compuestos **26-28** mostraron buena afinidad por el CB_1R (K_i=4-12.1 nM), y por tanto destacan como prometedores candidatos para estudiar los efectos mediados por el CB_1R de la membrana plasmática, ya que estos derivados serán en principio incapaces de cruzar la membrana celular debido a su elevada polaridad, y por lo tanto, no podrán activar el $mtCB_1R$. Este trabajo se está llevando a cabo en colaboración con el profesor Giovanni Marsicano en la Universidad de Bordeaux (Francia).

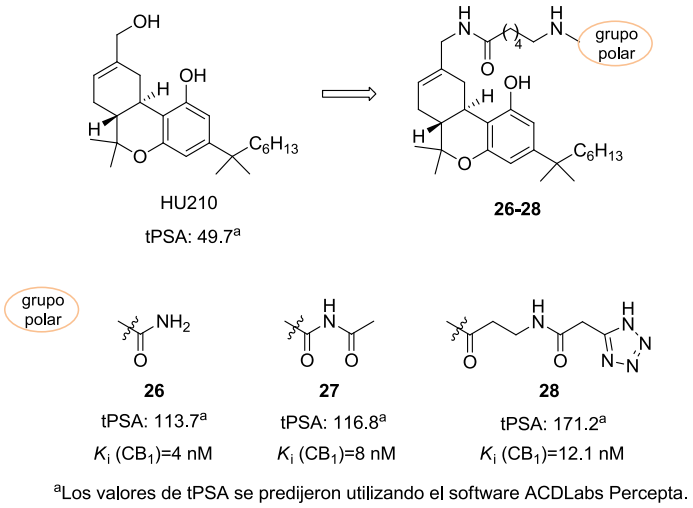

Figura 2. Derivados polares de HU210 **26-28**.

1.2.3. Descubrimiento de las dianas adicionales de los agonistas sintéticos HU210 y HU308

La identificación de dianas adicionales a los receptores CB_1 y CB_2 es posiblemente uno de los retos más importantes para el entendimiento del ECS. En este contexto, el cáncer y los desórdenes del sistema inmune son dos áreas que están recibiendo cada vez mayor atención debido al aumento en el número de efectos descritos que no están mediados por $CB_{1/2}$Rs.[18-24] Por tanto, el desarrollo de herramientas diseñadas específicamente para identificar las dianas de los cannabinoides reviste una gran importancia. Para lograr este objetivo se requieren sondas químicas que, además de presentar en su estructura el grupo de unión y la subunidad de marcaje, incorporen también un grupo de fotoentrecruzamiento como una subunidad de benzofenona, que tras irradiación con luz ultravioleta a la longitud de onda apropiada forme un enlace covalente con dichas proteínas, y éstas puedan ser posteriormente capturadas e identificadas.

Por tanto, se llevó a cabo el diseño y síntesis de las nuevas sondas **39-41** por incorporación directa de una subunidad de marcaje, que contenía tanto biotina como benzofenona, en los esqueletos de HU210 y HU308 (Figura 3). Puesto que la sonda **39** mostró los mejores valores de afinidad por ambos receptores [K_i (CB_1)=25.7±0.4 nM; K_i

(CB_2)=12.1±0.2 nM], se seleccionó para llevar a cabo experimentos de proteómica en membranas de células HEK-293-EBNA que sobreexpresan el CB_2R humano.

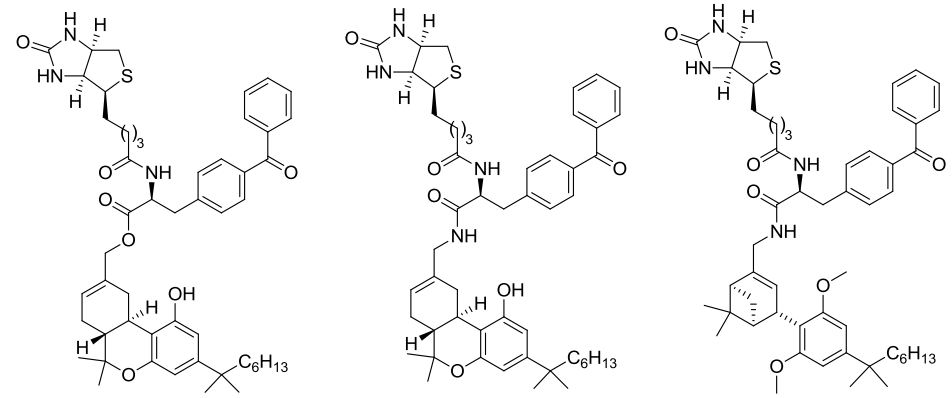

Figura 3. Estructura de las sondas **39-41**.

El empleo de **39** permitió la identificación de 15 proteínas de diferentes tipos, que participan en diversos procesos biológicos de interés. En concreto, la presencia del CB_2R entre ellas es especialmente importante, ya que pone de manifiesto la validez tanto de la plataforma como de la metodología.

1.2.4. Identificación de las dianas terapéuticas del producto de origen natural honokiol

El producto natural honokiol (Figura 4) muestra interesantes propiedades terapéuticas[25,26] entre las que destaca su capacidad antitumoral.[25,27] Sin embargo, se desconocen las dianas concretas en las que actúan tanto el honokiol como sus derivados, por lo que su identificación permitiría establecer nuevas estrategias farmacológicas para el tratamiento de esta enfermedad.

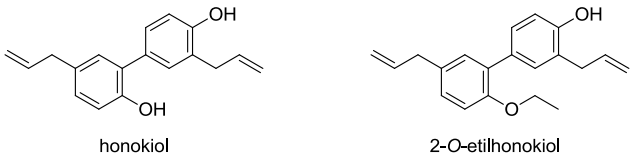

Figura 4. Estructura química del honokiol y 2-*O*-etilhonokiol.

Con el objetivo de identificar las dianas de estos compuestos, hemos llevado a cabo el diseño y síntesis de los derivados **56-59**. Estas sondas están basadas en el esqueleto de 2-*O*-etilhonokiol (Figura 4), un derivado sintético más activo que el honokiol,[28] e incorporan además las subunidades de benzofenona y biotina en su estructura. Adicionalmente, se han planteado también las sondas **64** y **65**, en las que se ha reemplazado la benzofenona por el grupo de menor tamaño diazirina, y la subunidad de biotina por un alquino terminal (Figura 5).

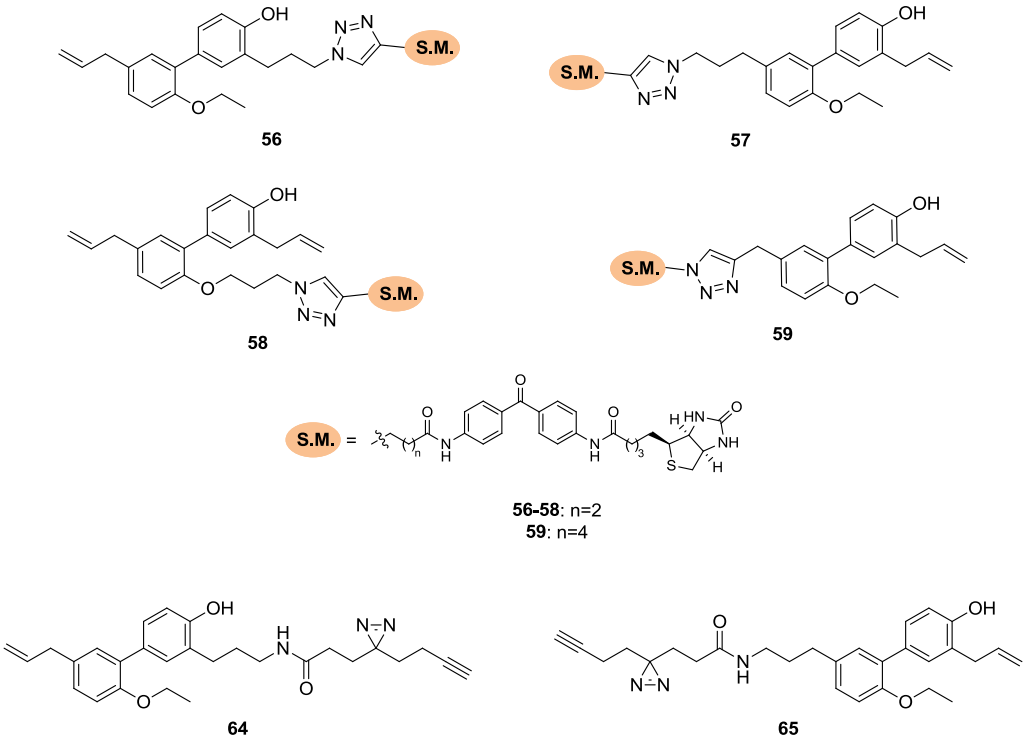

Figura 5. Estructura de las sondas **56-59**, **64** y **65**.

Entre los compuestos sintetizados, las sondas **64** y **65** mostraron la mayor capacidad para inhibir la proliferación celular en distintas líneas de cáncer de mama (MDA-MB-231) y ovario (SKOV3 y OVCAR3), por lo que se seleccionaron para experimentos posteriores. Así, se evaluó su potencial para marcar proteínas de forma específica. Puesto que la sonda **65** exhibió un mejor perfil de marcaje, se seleccionó ésta para identificar las proteínas capturadas con dicha sonda mediante espectrometría de masas cuantitativa SILAC (*stable isotope labelling by amino acids in cell culture*).[29,30] Los

resultados obtenidos han permitido identificar alrededor de 42 proteínas como dianas del honokiol, entre las que destaca la proteína LYRIC,[31] involucrada en mecanismos relacionados con la viabilidad de las células de cáncer tales como la apoptosis.

1.3. Conclusiones

En el presente trabajo de investigación se han desarrollado diferentes sondas para el estudio del ECS.

Así, se ha llevado a cabo la síntesis de la sonda biotinilada **1** y la sonda fluorescente **22** (Figura 1) que han permitido la detección del CB_1R en células humanas del sistema inmune.

Con respecto al estudio del $mtCB_1R$, se han sintetizado los ligandos de alta afinidad por el CB_1R **26-28** (Figura 2, K_i=4-12.1 nM). Dada la elevada polaridad de los mismos, estos compuestos constituyen un conjunto de herramientas prometedoras para el estudio de los diferentes efectos mediados por el CB_1R de membrana y el mitocondrial.

Por último, se han desarrollado una serie de sondas que están posibilitando descubrir las dianas de diferentes compuestos. Así, la sonda **39** (Figura 3) se está empleando para identificar las dianas adicionales del compuesto HU210, y la sonda **65** (Figura 5) está permitiendo revelar algunas de las dianas del honokiol en células de cáncer de mama MDA-MB-231, proporcionando por primera vez información directa sobre los mecanismos de acción de este producto natural.

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SUMMARY

2. SUMMARY

2.1. Introduction and objectives

Chemical biology emerged two decades ago aimed at the study of the interface between chemistry and biology, producing a great improvement in our understanding of complex biological systems by means of the development of chemical tools able to interrogate them.[1-3] In spite of these advances, there are relevant systems that hold great therapeutic promise but which have not been studied up to date. One of these cases is the endogenous cannabinoid system (ECS), which has been involved in almost every central and peripheral function.[4-7] However, there is no drug in the market targeting this system so far. In this context, it seems clear that there is a crucial need to understand some aspects of the biology of the ECS in a deeper manner. Among them, the cellular location of cannabinoid receptors (CBRs), as well as their localization within the cell, the existence of other cannabinoid targets different from the molecularly characterized CB_1R and CB_2R, and the study of interesting cannabinoid-related ligands, might help to exploit the therapeutic potential of the ECS. Thus, the overall objectives for the present work are: i) the development of probes to study the expression of CBRs in the immune system, ii) the design and synthesis of tools to explore the differences between plasma and mitochondrial CB_1Rs, iii) the discovery of the off-targets of the synthetic CBR agonists HU210 and HU308, and iv) the identification of the binding proteins of the natural product honokiol.

2.2. Results and discussion

2.2.1. Development of probes to study the expression of CBRs in the immune system

The widespread distribution of CBRs and their involvement in the regulation of different functions could offer a way of therapeutic intervention for many disorders. In this context, the immune system is of particular relevance because of its involvement in many diseases, and also for finding biomarkers that can reflect the presence or severity of different disorders. In this sense, it is known that immune cells express CBRs, that this expression changes under different conditions, and that the ECS regulates many aspects of the immune system.[8-10] Hence, there is an interest in studying the role of CBRs in the control of the immune function as well as in determining whether they can be biomarkers of diseases or prognosis. Therefore, we focused our attention on the development of small-molecule probes that could be used to detect CBRs in a direct manner. Such probes should have three features: i) a binding group based on a scaffold with high affinity for the CBR under study, ii) an appropriate tag that enables target detection and visualization, and iii) a suitable spacer in order to avoid potential steric interferences which could produce a loss of affinity (Figure 1). With regard to the binding group, we selected the scaffold of the synthetic high affinity ligand HU210 for the design of the new probes. As for the tag, we considered the biotin subunit and the fluorophore Alexa Fluor 488 for the detection of CBRs by confocal microscopy and flow cytometry, respectively.

Therefore, we synthesized compounds **1**, **2**, and **20** in which we have considered an amide in derivatives **1** and **2**, or an ether group in compound **20** as the linker between the binding group and the tag. With regard to the preparation of the fluorescent derivative, we selected the amide linker of probe **1** and replaced the biotin subunit by the fluorophore Alexa Fluor 488 in probe **22** (Figure 1). Interestingly, all compounds exhibited affinity values in the nanomolar range. In particular, compound **1** presented the best K_i values [K_i (CB_1)=1.6±0.5 nM; K_i (CB_2)=0.36±0.02 nM], and derivative **22** was selective for CB_1R [K_i (CB_1)=28±4 nM; K_i (CB_2)=0.7±0.2 μM]. Hence, these compounds were selected for the analysis of CBRs in cellular systems.

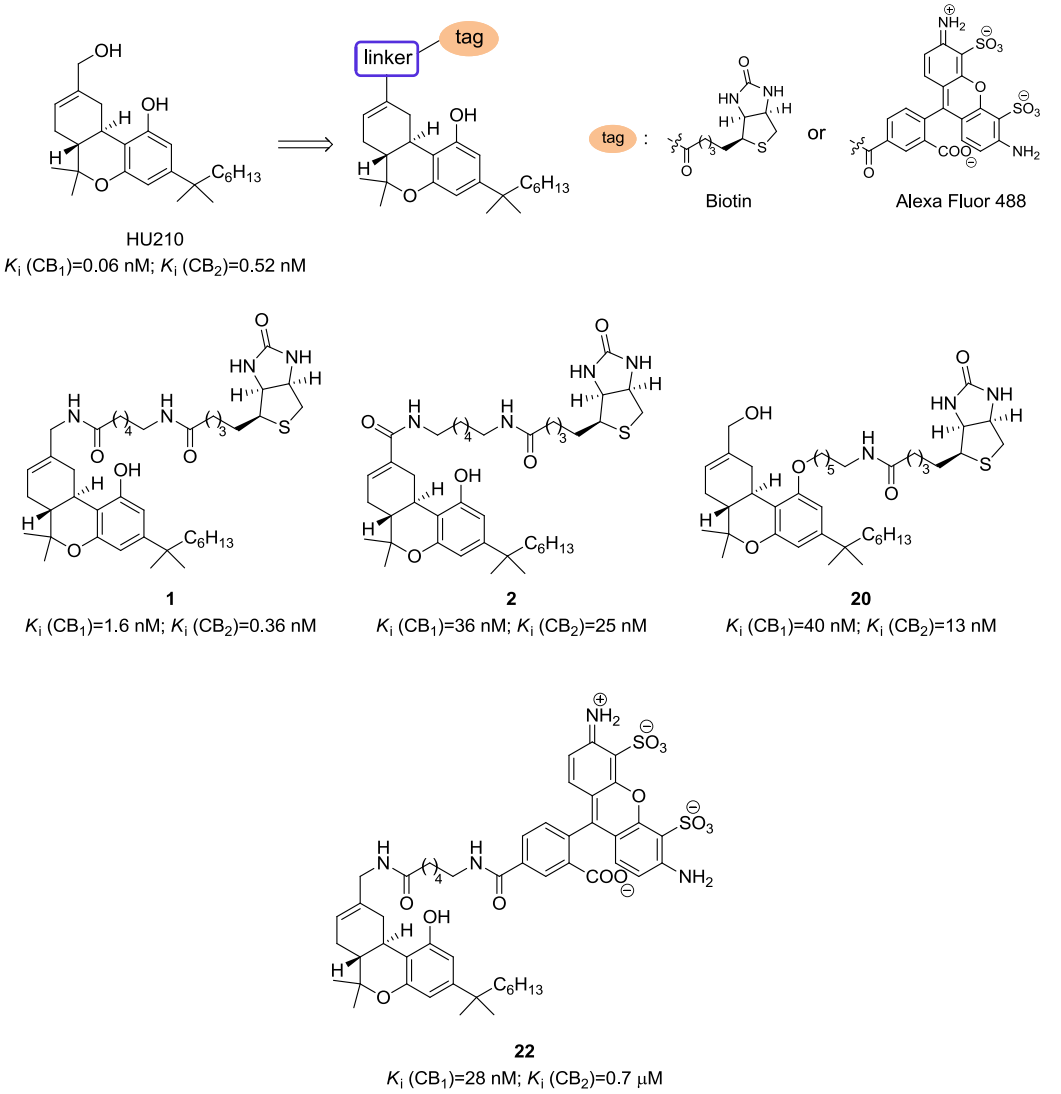


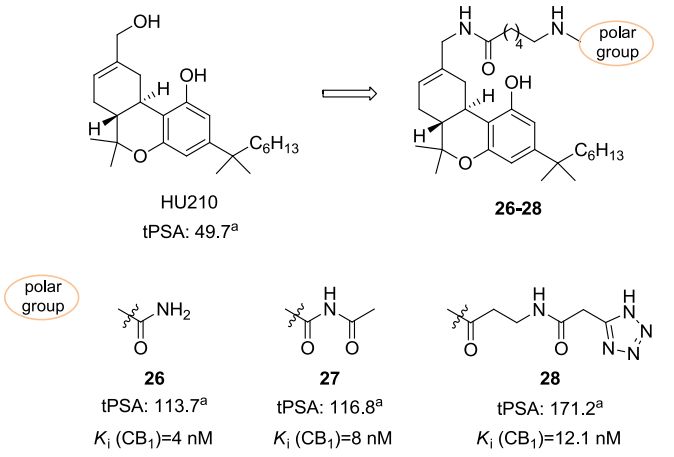
Figure 1. Design of new HU210-based probes **1**, **2**, **20**, and **22**.

Among the different possibilities with therapeutic relevance in the immune system, we initially focused on allergy, where the ECS has been suggested to play a protective role.[11,12] In this context, we aimed to explore the presence of CBRs in human allergic diseases of atopic patients and healthy subjects. To this end, mononuclear cells from peripheral blood (PBMCs) and from lymphoid organs such as tonsils (TMCs) were selected.

Thus, probe **1** was used in several cell subsets of immune cells to detect the CB_1R by confocal microscopy, proving for the first time that the CB_1R is highly expressed in tonsils of patients with different types of allergic diseases.[13] Alternatively, fluorescent probe **22** enabled the direct visualization of this receptor in human PBMCs by flow cytometry. Hence, this probe stands out as a valuable tool to study the modifications in the expression of this receptor in clinically relevant samples, and to establish its potential as a biomarker.

2.2.2. Design and synthesis of tools to explore the differences between plasma and mitochondrial CB_1Rs

The complexity of the CB_1R-mediated signalling has been recently increased by the identification of a new CB_1R located in the mitochondria, $mtCB_1$,[14,15] which has been hypothesized to be involved in the regulation of effects different from those mediated by the membrane CB_1R.[16,17] To differentiate between these two receptors, it would be necessary to develop compounds that keep the affinity towards the plasma membrane CB_1R but are unable to cross the cell membrane. Thus, we synthesized HU210-based compounds **26-28** with highly polar subunits such as an urea or an acylurea in compounds **26** and **27** respectively, and a tetrazole-containing chain in derivative **28**, attached through an amide linker and the optimal length of the spacer as determined before (Figure 2). Compounds **26-28** displayed good affinities towards CB_1R with K_i values in the nanomolar range (K_i=4-12.1 nM), and therefore they represent a promising tool to study the effects mediated by the plasma membrane CB_1R, as these derivatives are, in principle, unable to cross the cell membrane and activate the $mtCB_1R$. This work is currently ongoing in collaboration with Prof. Giovanni Marsicano at Université de Bordeaux (France).



[a]The tPSA values were predicted using the ACDLabs Percepta software.

Figure 2. Polar derivatives **26-28**.

2.2.3. Discovery of the off-targets of the synthetic CBR agonists HU210 and HU308

The identification of cannabinoid binding sites different from the known CBRs is possibly one of the foremost challenges to fully understand the ECS physiology. In this context, cancer and immune modulation are receiving increasing attention as non $CB_{1/2}R$-mediated effects have been described.[18-24] Hence, the development of analytical tools specifically tailored to enable the identification of cannabinoid targets would be of great value. To achieve this objective, we need to prepare chemical probes that besides bearing the binding group and the tag, present also a photocrosslinking group such as benzophenone, which after ultraviolet (UV) irradiation at the appropriate wavelength will form a covalent bond with the probe-target proteins, which in turn will be analyzed by multidimensional liquid chromatography altogether with mass spectrometry detection (LC-MS).

Therefore, probes **39-41** were designed and synthesized by direct incorporation of a tag bearing the biotin subunit and the benzophenone group into the HU210 and HU308 scaffolds, in order to study the differences between the two ligands (Figure 3). Since probe **39** presented the highest affinity towards both receptors [K_i (CB_1)=25.7±0.4 nM; K_i (CB_2)=12.1±0.2 nM], it was selected to carry out proteomic experiments. The use of this probe enabled the identification of 15 proteins of different functional classes which participate in diverse biological processes. Among them, the identification of the CB_2R

deserves special attention since it supports the solidness of the platform and therefore, validates the methodology.

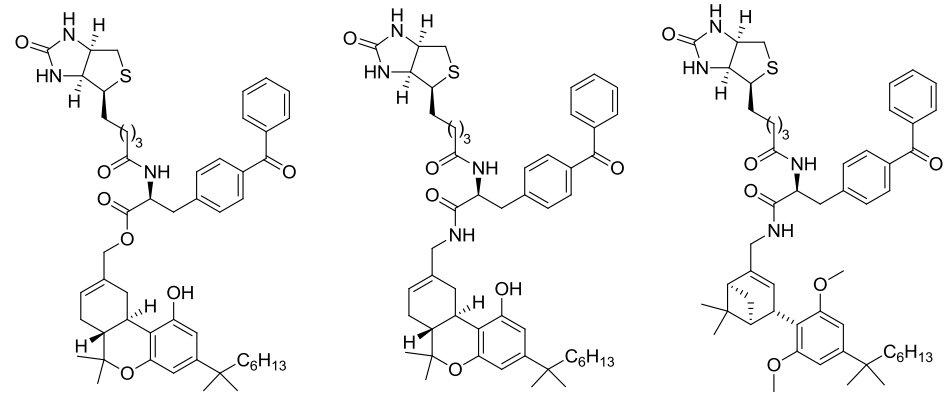


Figure 3. Structure of probes **39-41**.

2.2.4. Identification of the binding proteins of the natural product honokiol

Honokiol (Figure 4) is known due to its anxiolytic, analgesic, anti-depressant, anti-tumorigenic, and neuroprotective properties among others.[25,26] Among the different indications reported for honokiol and its derivatives, cancer has received increasing attention because of the significant activities shown by these compounds in several cancer models of different origins.[25,27] However, their actual targets are not known, and their identification could lead to the discovery of new therapeutic strategies. Hence, we have focused our efforts on the development of chemical probes based on the synthetic derivative 2-*O*-ethylhonokiol (Figure 4), which is more potent than honokiol in some assays.[28]

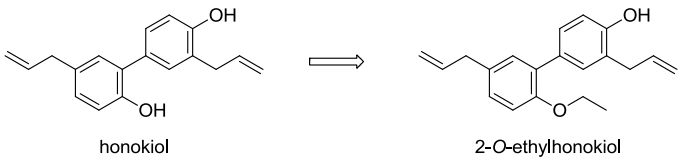


Figure 4. Chemical structure of honokiol and its synthetic derivative, 2-*O*-ethylhonokiol.

Thus, we designed and synthesized probes **56-59**, with a biotin and benzophenone containing tag, and probes **64** and **65**, where we have replaced the benzophenone by the less bulky diazirine group as the photocrosslinking moiety, and the biotin subunit by a terminal alkyne (Figure 5).

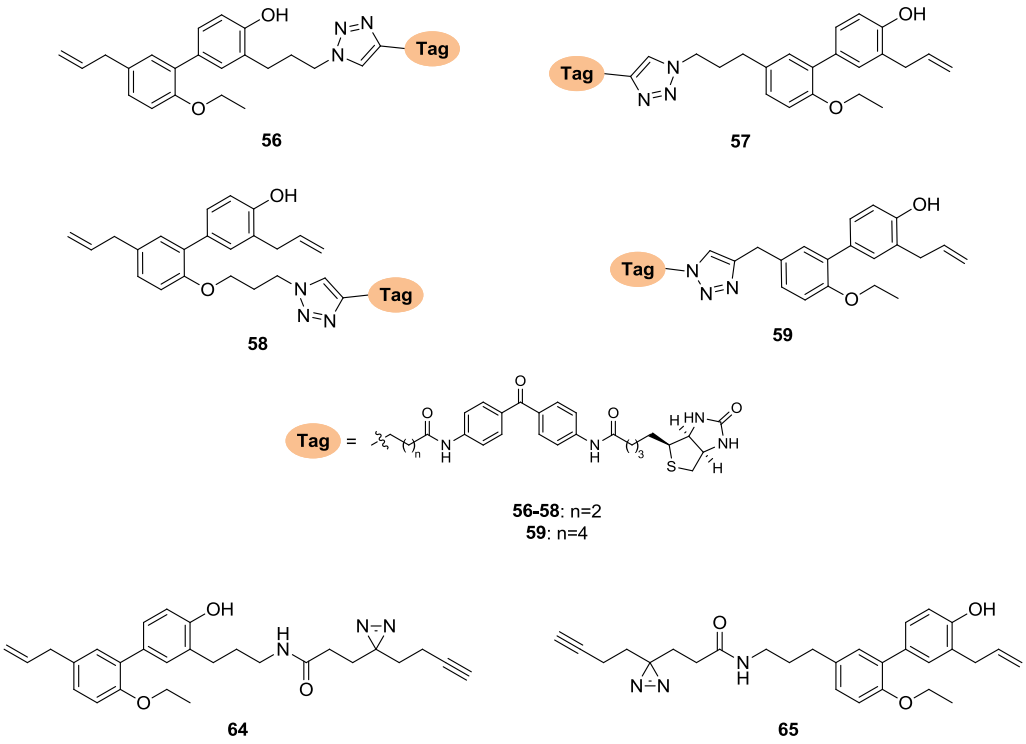


Figure 5. Structure of honokiol-based probes **56-59**, **64**, and **65**.

Among the synthesized compounds, probes **64** and **65** displayed the best cytotoxicity values in breast (MDA-MB-231) and ovarian (SKOV3 and OVCAR3) cancer cell lines, and hence were selected for further experiments. Then, we confirmed that these probes were able to label proteins in a specific way. Considering that probe **65** showed a slightly better labelling profile, it was selected to identify the targets of honokiol in MDA-MB-231 cells by SILAC quantitative MS.[29,30] This experiment enabled the identification of about 40 proteins that were significantly competed by honokiol. Among them, LYRIC protein[31] deserves particular attention since it is involved in mechanisms related to cell viability.

2.3. Conclusions

In the present work we have developed a set of chemical probes for the study of the ECS.

Biotinylated probe **1** (Figure 1), with high affinity toward both CBRs [K_i (CB_1)=1.6 nM; K_i (CB_2)=0.36 nM], enabled the visualization of the CB_1R in several subsets of human immune cells proving the expression of this receptor in patients with different types of allergic diseases. In addition, the fluorescent CB_1R-selective probe **22** has been successfully used to detect and semi-quantify the levels of expression of CB_1R in PBMCs. This result highlights the suitability of **22** to study possible modifications in such levels in clinically relevant systems, and to establish the potential of CB_1R as a biomarker.

Concerning the study of the $mtCB_1R$, highly polar compounds **26-28** (Figure 2), with high affinity values for the CB_1R (K_i=4-12.1 nM) represent promising tools to study the different effects mediated by the plasma membrane CB_1R and the $mtCB_1R$.

Finally, we have developed a series of chemical probes to enable the identification of the targets of different compounds. Thus, probe **39** (Figure 3) is being used to discover additional targets of the HU210 ligand, while probe **65** (Figure 5) is allowing the characterization of some of the direct targets of honokiol in MDA-MB-231 breast cancer cells, providing for the first time direct insights into the mechanisms of action of this natural product.

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INTRODUCTION AND OBJECTIVES

3. INTRODUCTION AND OBJECTIVES

During the last decades a vast amount of research has been devoted to the development of novel synthetic approaches, which altogether with undeniable advances in technology have made possible a constant increase in the number of new chemical entities designed for therapeutic applications.[1] However, despite the increasing investment in the drug discovery process, the rate of success continues to fall, fact only justifiable by the extraordinary complexity of the biological world implicated in the different pathological states.

In this regard, chemical biology emerged two decades ago aimed at the study of the interface between chemistry and biology, producing a great improvement in our understanding of complex biological systems by means of the development of chemical tools able to interrogate them.[2-4] In spite of these advances, there are relevant systems that hold great therapeutic promise but which have not been studied up to date. One of these cases is the endogenous cannabinoid system (ECS), which has been involved, during the last years, in almost every central and peripheral function.[5-8] However, no drug targeting this system remains in the market after the withdrawal of Rimonabant (Acomplia®), the only agent acting at the ECS ever approved. This compound, prescribed as an anti-obesity drug, was retired from the European market in 2008 due to safety concerns related with a high risk of serious psychiatric problems, including suicide.[9,10] This fact, rather than discourage researchers and pharmaceutical companies working on the cannabinoid field, inspired them to search for new alternatives in order to avoid the undesired side effects. As a result, different approaches based on the constituents of the

ECS have emerged.[6] The current view of the ECS includes, as the main elements, two cannabinoid receptors (CBRs), CB_1R[11,12] and CB_2R,[13] being the former mainly located in the central nervous system (CNS) and the later mostly expressed in tissues and cells of the immune system (Figure 1).[14,15] Nonetheless, other proteins have been related to the ECS such as the orphan G protein-coupled receptors (GPCRs) GPR18 and GPR55,[16,17] the transient receptor potential (TRP) of the type 1 vanilloid channel, or the nuclear peroxisome proliferator-activated receptors (PPARs).[18]

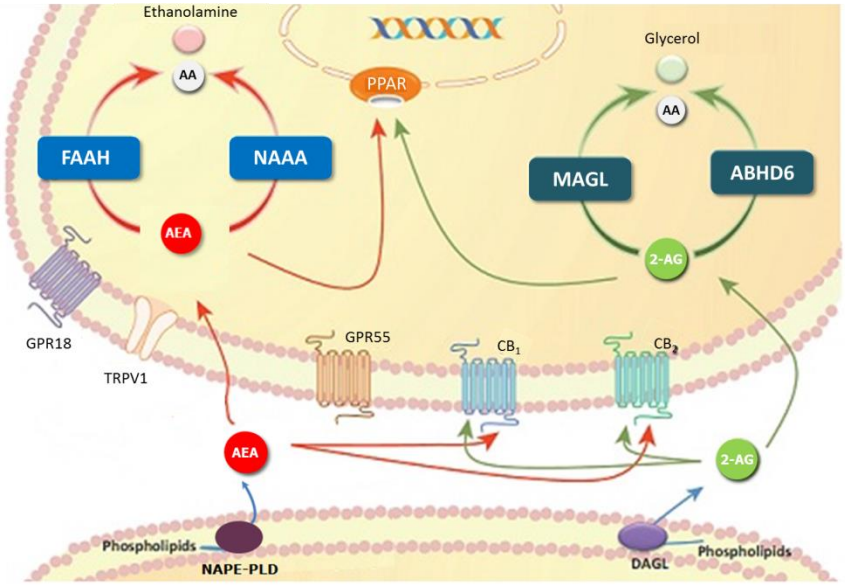


Figure 1. Schematic representation of the ECS (adapted from Alhouayek *et al.*[19]).

In addition, two molecules, *N*-arachidonoylethanolamine (AEA, anandamide)[20] and 2-arachidonoylglycerol (2-AG)[21,22] have been identified as the main endocannabinoids (eCBs), i.e., the endogenous ligands for these receptors (Figure 2). These derivatives of the arachidonic acid (AA) are biosynthesized and degraded by specific enzymes. The main responsible for their synthesis are *N*-arachidonoylphosphatidylethanolamine phospholipase D (NAPE-PLD) for AEA, and diacylglycerol lipase (DAGL) in the case of 2-AG.[18] Eventually, both eCBs are metabolized by different hydrolases, being fatty acid amide hydrolase (FAAH),[23,24] and monoacyl glycerol lipase (MAGL)[25] the ones accountable for the majority of AEA and 2-AG degradation, respectively. Nevertheless,

the enzymes *N*-acylethanolamine-hydrolysing acid amidase (NAAA)[18] and α/β-hydrolase domain 6 (ABHD6)[26] partially contribute to the degradation of these eCBs (Figure 1).

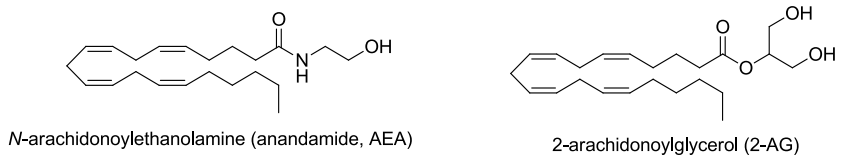


Figure 2. Structure of the main endocannabinoid ligands.

Since the discovery of the main psychoactive constituent of the plant *Cannabis sativa*, (-)-Δ^9-tetrahydrocannabinol (THC, Figure 3),[27] a great amount of research has been devoted to the synthesis and biological evaluation of cannabinoid ligands able to reproduce its effects. Among all the synthesized compounds, a few derivatives deserve special attention[28] due to their widespread use for the study of the functions of the ECS: i) the non-selective agonists HU210, CP55940, and the aminoalkylindol *R*-(+)-WIN552122, and ii) the selective agonists *R*-(+)-methanandamide and HU308. In particular, HU210 is a classical cannabinoid agonist with high affinity and potency for both CBRs widely used for the study of the ECS physiology,[29,30] while the tritiated derivative of CP55940,[28] which led to the discovery of the CB_1R, is the most widely used radiolabelled cannabinoid ligand. With respect to HU308,[31] it is one of the most selective ligands for the CB_2R,[28] whereas *R*-(+)-methanandamide,[32] the AEA-based ligand, represents the first generation of CB_1R selective compounds.

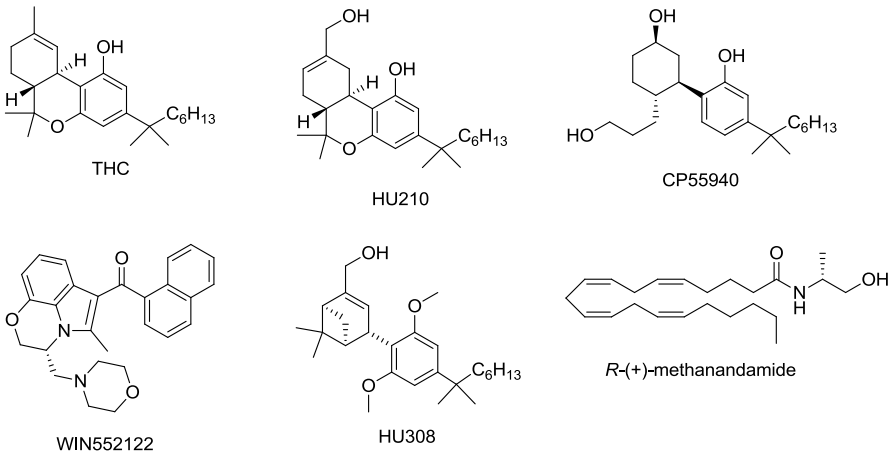


Figure 3. Representative CB_1R and/or CB_2R agonists.

Moreover, the study of the ECS would not have been possible without the development of selective antagonists/inverse agonists for each CBR, altogether with the use of CB_1R and/or CB_2R knock-out mice. In this sense, the most representative antagonists/inverse agonists of CBRs are the CB_1R-selective ligand SR141716A, and the CB_2R-selective one SR144528, both of them sharing a diarylpyrazole scaffold in their structure (Figure 4).[28]

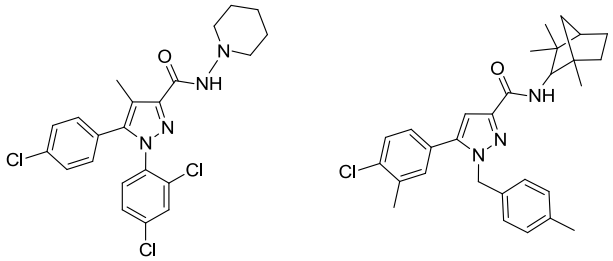


Figure 4. Representative CB_1R and CB_2R antagonists/inverse agonists.

According to the elements that form the ECS, potential approaches to exploit its therapeutic potential while keeping away the undesirable side effects have emerged. One of them has been the inhibition of the eCB metabolism in order to increase the eCB tone to achieve the benefits of cannabinoid agonists while avoiding psychotropic effects.[33-37] Also, a lot of research has pursued the identification of either peripherally restricted CB_1R inverse agonists/antagonists,[38] or CB_2R selective agonists,[39-41] since both would lack the side effects mainly mediated by the CB_1Rs present in the brain. Finally, a more recent approach has been the development of allosteric modulators for the CB_1R.[42-44] These are ligands that engage the CB_1R in a different binding site than that of the orthosteric ligand, modulating its binding and/or affinity. The major advantage of these ligands is that, as a consequence of a less conserved binding site in comparison with the orthosteric binding pocket, they present a better selectivity profile with respect to other proteins of the same family, with a consequent reduction of side effects.[45]

In spite of all the efforts made with regard to the development of new CBR ligands and new inhibitors for the metabolic enzymes of the eCBs, it is a fact that the therapeutic potential of the ECS can not be, still today, separated from the undesirable side effects. This is because there are some fundamental gaps in the understanding of the ECS that have not been resolved yet. Some of these aspects are:

- Cellular location

This has arisen as a critical aspect in CBR-induced effects. In this regard, CB_1Rs are mainly present in the presynaptic terminals of GABAergic interneurons, but also in other neuronal types such as glutamatergic, serotonergic, or cholinergic, among others. Besides, they are present in glial cells including microglia[46] and astrocytes.[47] Genetic mouse models are helping to determine which cell types are involved in which CB_1R-mediated effects. For example, CB_1R can both inhibit and stimulate food intake in fasting/refeeding conditions, depending on the specific neuronal population involved. CB_1Rs located in the ventral striatal neurons exerted a hypophagic action (that is, a reduction in food intake) through inhibition of GABAergic transmission. Conversely, brain CB_1Rs modulating excitatory transmission mediated the well-known effects of cannabinoids on appetite stimulation.[48,49] To further complicate the situation, recent studies suggest that the CB_1Rs localized in the peripheral nervous system are responsible not only for the appetite reduction induced by the CB_1R antagonist Rimonabant, but also for the behavioural negative effects associated to its administration.[50]

Different cellular localization of CB_1R has also been suggested as a potential way of dissociating therapeutic from undesirable side effects. In this regard, it has been described that peripheral CB_1R is involved in spasticity reduction whereas central CB_1R is responsible for the typical cannabinoid-induced hypothermia.[51] Similarly, CB_1Rs in GABAergic neurons are involved in the amnesic- and anxiogenic-like effects of THC,[52,53] whereas CB_1Rs in principal forebrain glutamatergic neurons are necessary for the anxiolytic properties of low doses of cannabinoids.[53]

Importantly, although CB_1R expression levels can vary between different populations, differential expression of CB_1Rs is not directly linked with equivalent functional relevance. The intracellular consequences of CB_1R signalling, and hence the resulting phenotypic effects, appear to emerge from its cellular localization, rather than being an intrinsic property of the protein. In fact, some of the signalling pathways appear to be specific of certain cell types.

In this context, it is evident that there is a clear need to establish where and when CBRs are expressed. As differences between in vitro and in vivo experiments have been detected,[47,54] it is important to develop sensitive methods that enable the study of CBR expression in native systems. In addition, most of the studies have been carried out in

the CNS, so it is essential to extend this knowledge to the periphery,[8] where the important role of the ECS is just starting to be established.

- Subcellular location

Another important aspect to consider in the search of new therapeutic opportunities for targeting CBRs is their localization within the cell. As G protein coupled receptors (GPCRs), CBRs are located in the plasma membrane of the cells, but their presence in other cell compartments has been proposed. In particular, the CB_1R has been found in the mitochondria ($mtCB_1R$),[55-57] adding a degree of complexity to the ECS physiology. The involvement of this new $mtCB_1R$ in the beneficial and/or psycotropic effects shown by cannabinoid ligands is currently under evaluation. Accordingly, the development of appropiate pharmacological tools that facilitate these studies could be of great interest.

- Other cannabinoid targets

There is evidence to suggest that binding to the molecularly characterized CB_1 and CB_2 receptors is not the only mechanism by which cannabinoid ligands exert some of their actions. In this regard, genetic deletion of CB_1R and CB_2R and/or administration of receptor antagonists do not eliminate some of the effects induced by eCBs, plant active principles (such as THC), or synthetic ligands such as HU210.[16,28,58-60] In this sense, the identification of these cannabinoid off-targets would undoubtedly contribute to a better understanding of the biology of the ECS, and hence, their uncovering is essential in order to be able to draw upon the therapeutic potential of this system.

- Other cannabinoid ligands

Besides the traditionally accepted cannabinoid ligands (summarized in Figures 3 and 4) other compounds have been suggested to induce cannabinoid-like effects.[39,61-63] Among them, the natural product honokiol (Figure 5) and its derivatives deserve special attention since they have been reported to have therapeutic relevance in important processes such as bone remodeling, and disorders such as neurodegenerative diseases or different types of cancer.[64-66] However, although honokiol and related compounds have a potential role in the treatment of several pathologies, a thorough undersanding of their mechanisms of action is imperative in order to use them for the treatment of human diseases.[66]

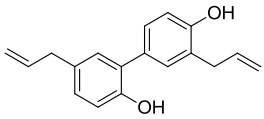


Figure 5. Natural product honokiol.

Considering that chemical probes could help to get deeper insights in all the above mentioned aspects, the overall objectives for the present work are:

1. Development of probes to study the expression of CBRs in the immune system
2. Design and synthesis of tools to explore the differences between membrane and mitochondrial CB_1Rs
3. Discovery of the off-targets of the synthetic CBR agonists HU210 and HU308
4. Identification of the target proteins of the natural product honokiol

RESULTS AND DISCUSSION

4. RESULTS AND DISCUSSION

4.1. Development of probes to study the expression of CBRs in the immune system

The widespread distribution of CBRs and their involvement in the regulation of different functions could offer a way of therapeutic intervention for many disorders. In this regard, many efforts have been devoted to establish the role of the ECS in the CNS,[5,67-69] although its therapeutic applications seem to be limited by the existence of undesirable side effects. However, the relevance of the peripheral ECS for human health and disease pathogenesis still remains to be completely elucidated.[8]

In this context, the immune system is of particular relevance because of its fundamental role in the body. The immune system's main function is to protect against disease by recognizing pathogens and distinguishing them from the organism's own healthy tissue. Accordingly, disorders of the immune system can result in immunodeficiency (when the immune system is less active than normal) or in autoimmune diseases, which result from a hyperactive immune system attacking normal tissues as if they were foreign organisms. In addition, malfunctioning of the immune system has also been linked to inflammatory diseases and cancer.[70,71] These facts convert the immune system not only in a system with therapeutic potential for many diseases but also for finding biomarkers that can reflect the presence or severity of different disorders. In this regard, the number of immune biomarkers is rapidly increasing specially in the fields of cancer, autoimmune disorders and neurodegenerative diseases.[72,73] However, the identification of immune biomarkers which can be analyzed in a straightforward manner from a simple blood test and in a multiplexed manner (i.e., more than one biomarker at the same time) by routine

techniques such as flow cytometry, remains a challenge.[72] In this context, it is known that immune cells express CBRs, that this expression changes under different conditions, and that the ECS regulates many aspects of the immune system.[74-76] Hence, there is an interest in studying the role of CBRs in controlling the immune function as well as in determining whether they can be biomarkers of diseases or prognosis. Towards this objective, it is necessary the development of tools that enable the visualization of CBRs in the immune system in a direct manner. Their availability would enable: (i) to detect up- or down-regulation of CBRs as biomarkers of diseases; (ii) to cluster patients and hence propose tailored therapeutic strategies; and (iii) to carry out multiplexed analysis with other clinically validated biomarkers.

However, the lack of appropriate tools has hampered these studies. In this regard, antibodies that recognize CBRs have been used, but they have important limitations due to their insufficient sensitivity and specificity.[77,78] In addition, the batch-to-batch variations inherent to the way in which antibodies are produced has been recognised as an important problem of reliability.[79,80]

Hence, our objective was to develop small molecule probes that could be used for detecting CBRs in blood cells. Towards this aim, we envisioned that the adequate probes should have three features: i) a binding group based on a scaffold with high affinity for the CBR under study, ii) an appropriate tag that enables target detection and visualization, and iii) a suitable spacer in order to avoid potential steric interferences which could produce a loss of affinity (Figure 6).

With regard to the tag, we have considered biotin and the green-fluorescent Alexa Fluor 488 dye (Figure 6). Biotin has been chosen because of its versatility, since it can be recognized by many different conjugates. In addition, it has been successfully used in other probes developed in our research group where it has enabled the visualization of different GPCRs by confocal microscopy in a variety of cells.[81-83] Alternatively, and in order to avoid the two-step labelling required by biotin, we have also conceived the bright dye Alexa Fluor 488 for flow cytometry experiments, as it can be directly visualized under the routinely used instruments, including microscopes and flow cytometers.

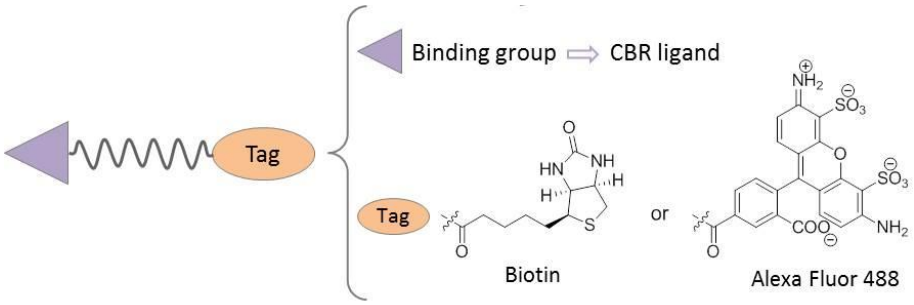


Figure 6. CBR probe design and selected tags.

With respect to the binding group, a variety of cannabinoid ligands could be initially considered. However, previous studies in our research group revealed that structural modification of the main eCBs involved decreases in affinity, although they could be used in transfected cell systems.[82] On the contrary, HU210 and HU308 scaffolds can be converted into probes that keep high affinity towards CBRs. In particular, probes UCM143 and UCM168 (Figure 7) have been successfully used for the visualization of CB_1 and CB_2 receptors in neurons and microglial cells, respectively.[83] However, preliminary studies aimed at the in vivo detection of CBRs with UCM143, evidenced the degradation of the probe. Interestingly, UCM143 produced typical HU210-induced behavioral effects such as catalepsy, antinociception or hypothermia, suggesting the hydrolysis of the ester group present in UCM143. This finding led us to consider other linkers with higher expected metabolic stability in the design of the new probes.

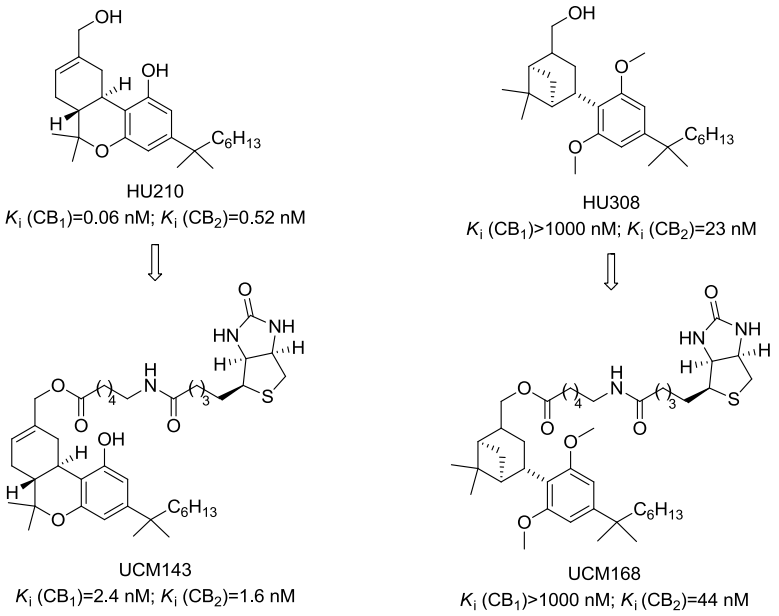


Figure 7. Structure of HU210 and HU308 and their previously designed probes.

Taking into account the above-mentioned results, the new series of probes were designed considering the scaffold of HU210 as binding moiety, amide and ether groups as linkers, and both the biotin subunit and the fluorophore Alexa Fluor 488 as tags (Figure 8).

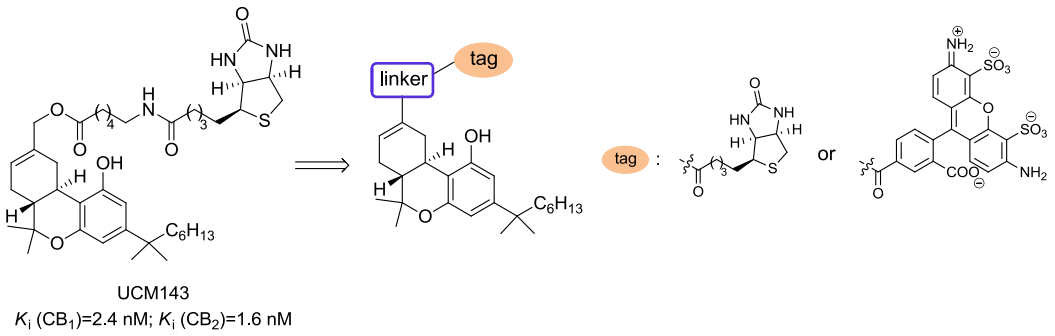


Figure 8. Design of new HU210-based probes.

Therefore, we designed compounds **1-3** in which we have replaced the ester linker of UCM143 by an amide in derivatives **1** and **2**, or by an ether group in compound **3** (Figure 9), keeping the same tag and length of the spacer as in UCM143.

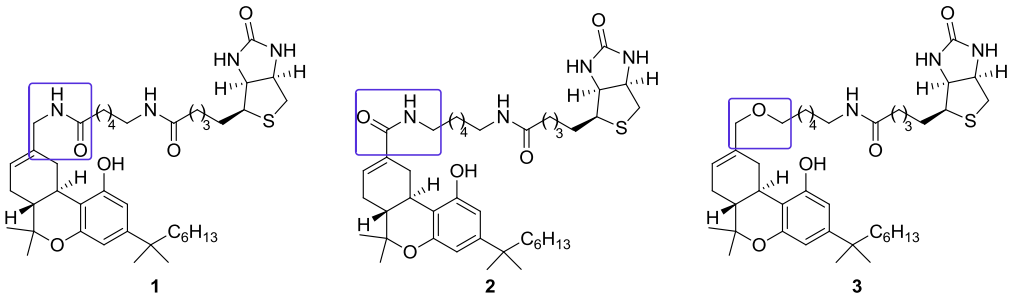
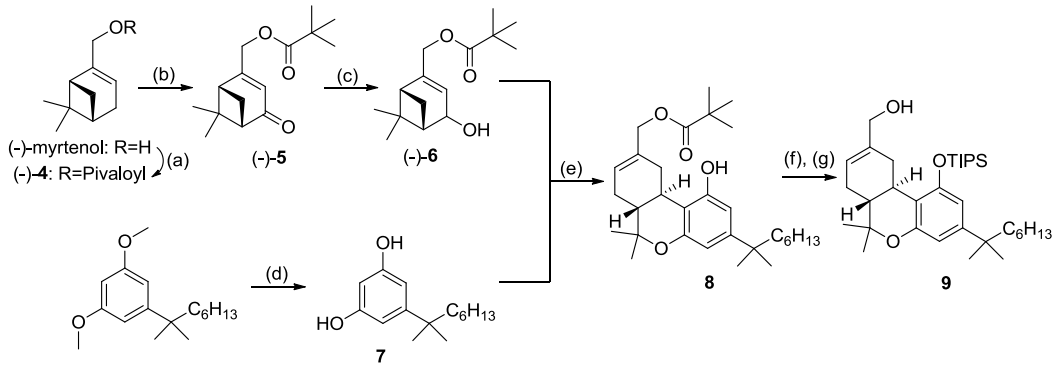


Figure 9. Design of HU210-based probes **1**-**3**.

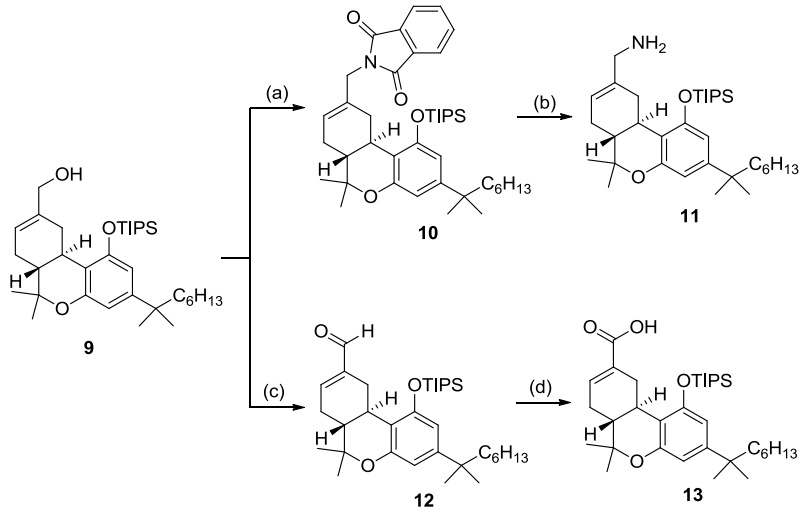
The preparation of the desired probes **1-3** involved the synthesis of the HU210 scaffold, carried out as previously reported by Mechoulam *et al.*[84] Esterification of commercially available (1*R*,5*S*)-6,6-dimethylbicyclo[3.1.1]hept-2-ene-2-methanol [(-)-myrtenol] with pivaloyl chloride provided ester (-)-**4**, which gave 4-oxo-myrtenyl pivalate [(-)-**5**] upon oxidation with chromium(VI) oxide (Scheme 1). Reduction of ketone (-)-**5** with lithium tri-*tert*-butoxyaluminium hydride led to alcohol (-)-**6**. With regard to resorcinol **7**, it was prepared by deprotection of the methoxy groups in the commercially available 1-(1,1-dimethylheptyl)-3,5-dimethoxybenzene by boron tribromide. Subsequent condensation of alcohol (-)-**6** with resorcinol **7** in the presence of boron trifluoride diethyl etherate afforded chromene **8**. Once the scaffold was prepared, phenol **8** was protected before carrying out any further of the allylic alcohol. Among the different protecting groups available for phenols, we selected the bulky triisopropylsilyl (TIPS) ether trying to avoid the high tendency of smaller analogs such as *tert*-butyldimethylsilyl (TBS) to be cleavaged under mild conditions. Thus, **8** was transformed into the corresponding silyl-ether **9** by treatment with TIPS chloride and imidazole under microwave (MW) irradiation, followed by removal of the pivaloyl group with lithium aluminium hydride.



Scheme 1. Reagents and conditions: (a) $(CH_3)_3CCOCl$, pyridine, DCM, 0 °C, 92%; (b) CrO_3, 3,5-dimethylpyrazole, DCM, -20 °C to 0 °C, 46%; (c) $LiAlH(t\text{-}BuO)_3$, THF, 0 °C to rt, 63%; (d) BBr_3, DCM, 0 °C to rt, 100%; (e) $BF_3{\cdot}Et_2O$, DCM, -20 °C to rt, 50%; (f) TIPS-Cl, imidazole, DMF, MW, 200 °C, 95%; (g) $LiAlH_4$, THF, 0 °C, 72%.

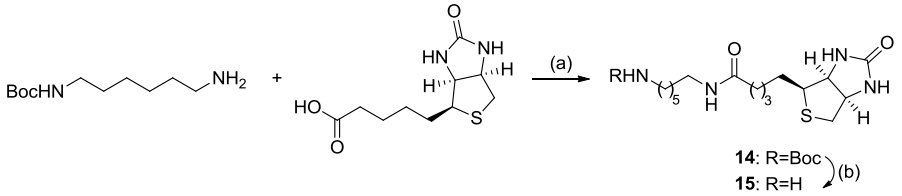
The preparation of amides **1**, **2** required the transformation of alcohol **9** into the corresponding amine or carboxylic acid, as well as the synthesis of the proper biotin derivatives functionalized as a carboxylic acid or an amine, respectively.

Hence, amine **11** was obtained through the Mitsunobu reaction of alcohol **9** with phthalimide in the presence of diethyl azodicarboxylate (DEAD) and triphenylphosphine and subsequent deprotection of the resulting phthalimide **10**, using hydrazine as the cleaving reagent and ethanol as solvent (Scheme 2). As for carboxylic acid **13**, it was prepared through the corresponding aldehyde **12** by consecutive oxidations of alcohol **9** with pyridinium chlorochromate (PCC) and sodium chlorite.



Scheme 2. Reagents and conditions: (a) phthalimide, PPh_3, DEAD, THF, rt, 93%; (b) i) $N_2H_4 \cdot H_2O$, EtOH, reflux; ii) HCl/H_2O 1:1 reflux to rt, 96%; (c) PCC, DCM, rt, 85%; (d) $NaClO_2$, 2-methyl-2-butene, KH_2PO_4, *t*-BuOH, rt, 74%.

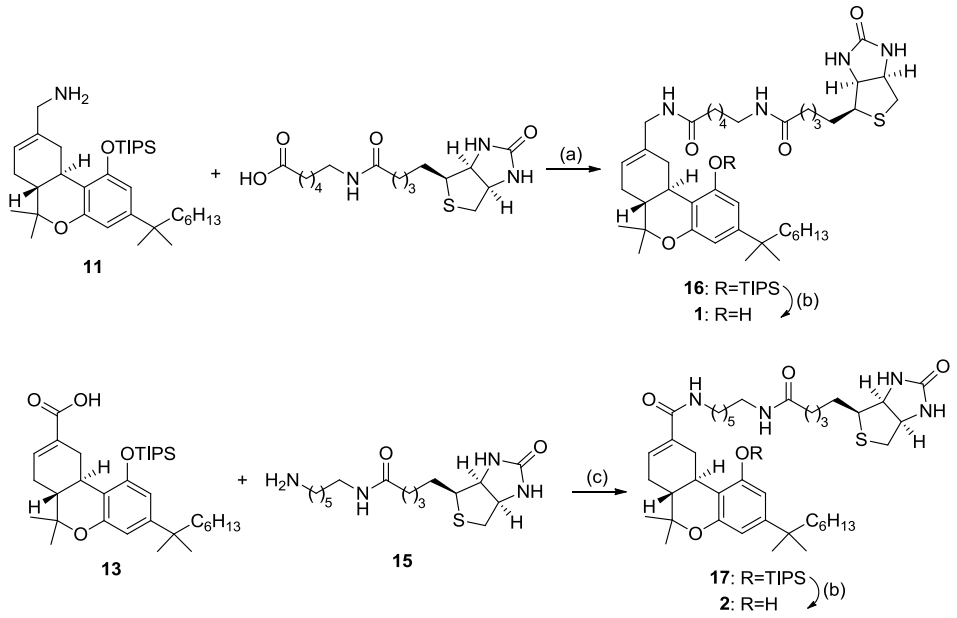
As for the corresponding biotinylated tags, amine **15** was prepared by condensation between *tert*-butyl (6-aminohexyl)carbamate with biotin in the presence of tributylamine and isobutyl chloroformate, followed by removal of the *tert*-butoxycarbonyl (Boc) protecting group of intermediate **14** with trifluoroacetic acid (TFA) (Scheme 3).



Scheme 3. Reagents and conditions: (a) Bu_3N, isobutyl chloroformate, DMF, 0 °C, 80%; (b) TFA, DCM, rt, 81%.

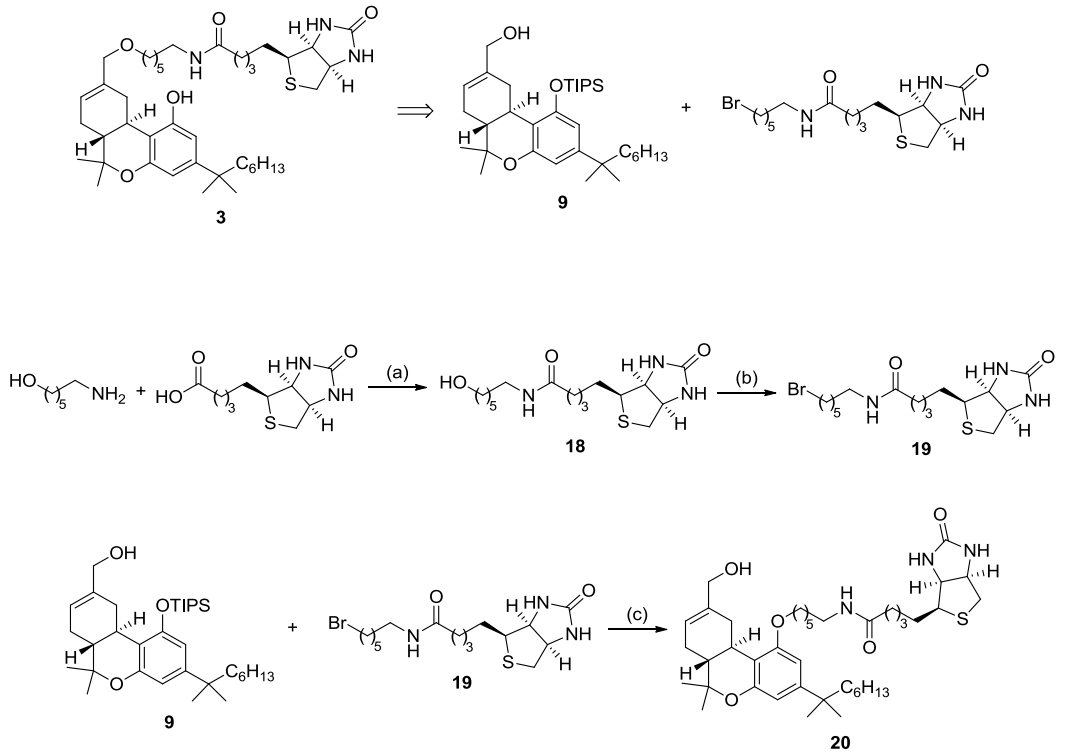
Once the fragments were synthesized, amide **16** was prepared through condensation between amine **11** and commercially available *N*-(+)-biotinyl-6-aminohexanoic acid using *N*-(3-dimethylaminopropyl)-*N*'-ethylcarbodiimide (EDC) and 1-hydroxybenzotriazole (HOBt) as coupling reagents in the presence of catalytic amounts of 4-dimethylaminopyridine (DMAP), and the same conditions were employed in the synthesis of amide **17** from carboxylic acid **13** and amine **15** (Scheme 4). Then, final

compounds **1** and **2** were obtained by removal of the TIPS protecting group with tetrabutylammonium fluoride (TBAF).



Scheme 4. Reagents and conditions: (a) EDC, HOBt, DMAP, DCM, DMF, 77 ºC to rt, 62%; (b) TBAF, THF, 0 ºC, 80-84%; (c) EDC, HOBt, DMAP, DCM, DMF, rt, 48%.

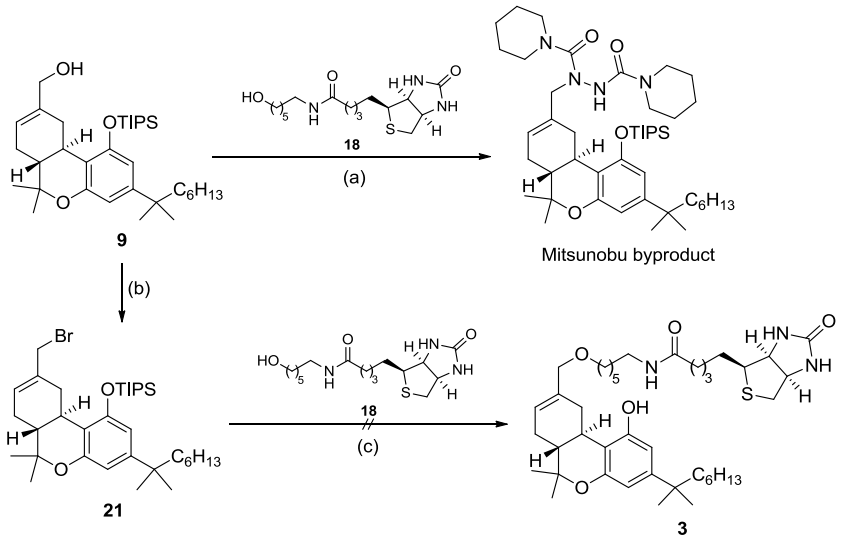
The synthesis of ether **3** was firstly envisioned as a Williamson alkylation of alcohol **9** with the corresponding biotinylated bromoderivative. This compound was synthesized following a previously described procedure,[85] through condensation between 6-aminohexanol and biotin followed by the Appel reaction of the obtained alcohol **18** (Scheme 5). Unfortunately, Williamson alkylation of alcohol **9** with bromoderivative **19** in the presence of sodium hydride and tetrabutylammonium iodide (TBAI) did not provide the desired allylic ether **3**, but instead phenolic ether **20** was isolated in all the assayed conditions. The obtention of **20** can be explained by in situ cleavage of the TIPS group followed by the alkylation of the resulting phenol (Scheme 5).



Scheme 5. Reagents and conditions: (a) Bu_3N, isobutyl chloroformate, DMF, 0 °C, 97%; (b) CBr_4, PPh_3, DMF, rt, 74%; (c) NaH, TBAI, THF, 0 °C to reflux, 75%.

Next, we approached the synthesis of **3** through the Mitsunobu reaction between alcohols **9** and **18** using tributylphosphine and 1,1'-(azodicarbonyl)dipiperidine (ADDP) as coupling reagents, conditions that have been commonly used with weakly acidic nucleophiles (Scheme 6). However, the formation of the byproduct of the addition of alcohol **9** to ADDP was always observed as previously reported for alcohols with pK_a>11,[86] which indicates the low acidity of allylic alcohol **9** for undergoing this reaction.

Finally, we tried to carry out the nucleophylic substitution of bromoderivative **21**, previously prepared through the Appel reaction of alcohol **9**, with alcohol **18** although we only observed a complex reaction mixture, which did not contain the desired product (Scheme 6).

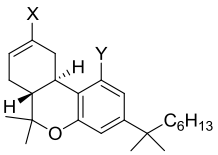


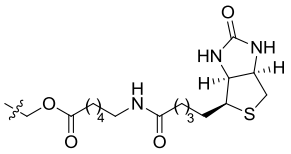
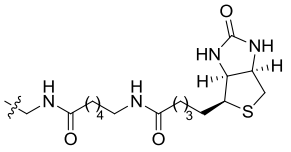
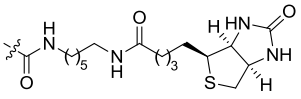
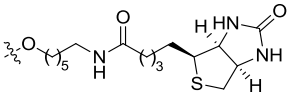
Scheme 6. Reagents and conditions: (a) PBu_3, ADDP, toluene, 60 ºC; (b) CBr_4, PPh_3, DCM, rt, 65%; (c) NaH, TBAI, DMF, rt.

Since ether **3** could not be prepared but ether **20** was obtained instead, we proceeded with its biological evaluation, together with amides **1** and **2**.

Affinity of compounds **1**, **2**, and **20** for CB_1 and CB_2 receptors was evaluated by radioligand competitive binding assays using membranes of HEK-293-EBNA cells transfected with human CB_1R and CB_2R (hCB_1R and hCB_2R), respectively, and [^{3}H]-CP55940 as radioligand. The competitive inhibition assays were first performed at a fixed dose of 1 µM. Then, the complete dose-response curve, at six different concentrations of the ligand, was determined for those compounds that displaced more than the 50% of the radioligand. The affinity constant K_i was calculated from the inhibitory concentration 50 (IC_{50}) value using the Cheng-Prusoff equation[87] and is expressed as the average and standard error (SEM) obtained from two to four independent experiments carried out in triplicate. Table 1 shows the affinity values of probes **1**, **2**, and **20** for both CBRs.

Table 1. Binding affinities of probes **1**, **2**, and **20** for CBRs.

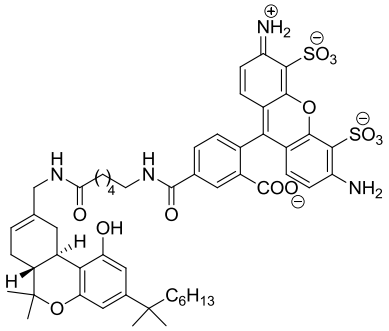


Compound	X	Y	Receptor affinity[a] K_i±SEM (nM)	
			CB_1	CB_2
UCM143		OH	2.4±0.4	1.6±0.4
1		OH	1.6±0.5	0.36±0.02
2		OH	36±12	25±11
20	CH_2OH		40±5	13±3

[a]The values are the mean±SEM obtained from two to four independent experiments performed in triplicate.

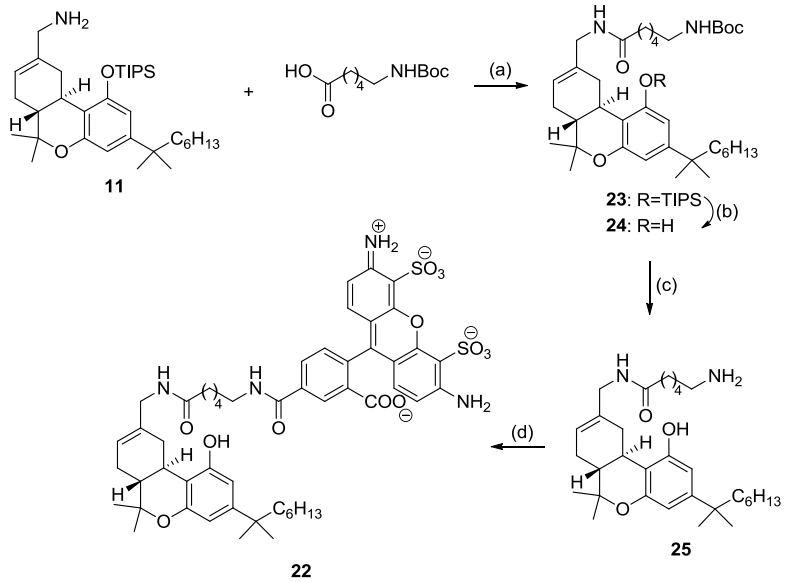
As shown in Table 1, probes **1**, **2**, and **20** displayed high affinity for both CBRs with K_i values in the nanomolar range. In particular, compound **1**, the amide analog of UCM143, presented the best K_i values and was selected for the analysis of CBRs in cellular systems.

With regard to the preparation of the fluorescent derivative, and considering the affinity values represented in Table 1, we selected the amide linker of probe **1** and replaced the biotin subunit by the fluorophore Alexa Fluor 488 in probe **22**.



22

The preparation of **22** was carried out by condensation between the commercially available tetrafluorophenyl (TFP) ester of Alexa Fluor 488, and the HU210 scaffold properly functionalized with the desired spacer. Thus, coupling of amine **11** with commercially available 6-(*N*-Boc-amino)hexanoic acid in the presence of *N,N'*-dicyclohexylcarbodiimide (DCC) provided amide **23** (Scheme 7). Cleavage of the TIPS protecting group of **23** by TBAF and subsequent deprotection of Boc with TFA led to amine **25**. Condensation of amine **25** with Alexa Fluor 488 TFP ester afforded fluorescent derivative **22**.



Scheme 7. Reagents and conditions: (a) DCC, DCM, rt, 74%; (b) TBAF, THF, 0 °C, 81%; (c) TFA, DCM, rt, 84%; (d) Alexa Fluor 488 TFP ester, DCM, DMF, rt, 66%.

Once synthesized, the affinity of **22** for CBRs was evaluated. Interestingly, probe **22** exhibited affinity values in the nanomolar range toward CB_1R while showing almost no affinity for CB_2R (submicromolar range) [K_i (CB_1)=28±4 nM; K_i (CB_2)=0.7±0.2 μM].

Among the different possibilities with therapeutic relevance in the immune system, we have initially focused on allergy. Allergic diseases encompass a number of inflammatory conditions caused by the hypersensitivity of the immune system against innocuous antigens for non-allergic patients, so called human allergenes. It has been suggested that the ECS can play a protective role in some types of allergy,[88,89] including contact allergy in the skin,[90] or allergic asthma.[74] However, studies reporting human data are still scarce.

In this context we aimed to explore the presence of CBRs in human allergic diseases of atopic patients (suffering from food anaphylaxis or allergic rhinitis, atopic dermatitis, and/or asthma) and healthy subjects. Mononuclear cells from peripheral blood (PBMCs) and from lymphoid organs such as tonsils (TMCs), which have also been involved in allergic responses,[91] were selected. The obtained results showed that messenger ribonucleic acid (mRNA) levels of CB_1R were significantly increased in both TMCs and PBMCs of allergic patients when compared to healthy subjects.[92] In order to check whether this expression could be directly observed at the protein level, compound **1** was used in different subsets of TMCs. Thus, purification of B and T lymphocytes, and plasmacytoid and myeloid dendritic cells (pDCs and mDCs, respectively) was carried out and the different cell types were stained with their corresponding marker in red (CD20 for B cells, CD3 for T cells, CD123 for pDCs, and HLA-DR for mDCs) and, simultaneously, with compound **1** in green (Figure 10). The obtained images clearly show that probe **1** can be used to detect the CB_1R in purified subsets of mononuclear cells isolated from human tissues such as tonsils.

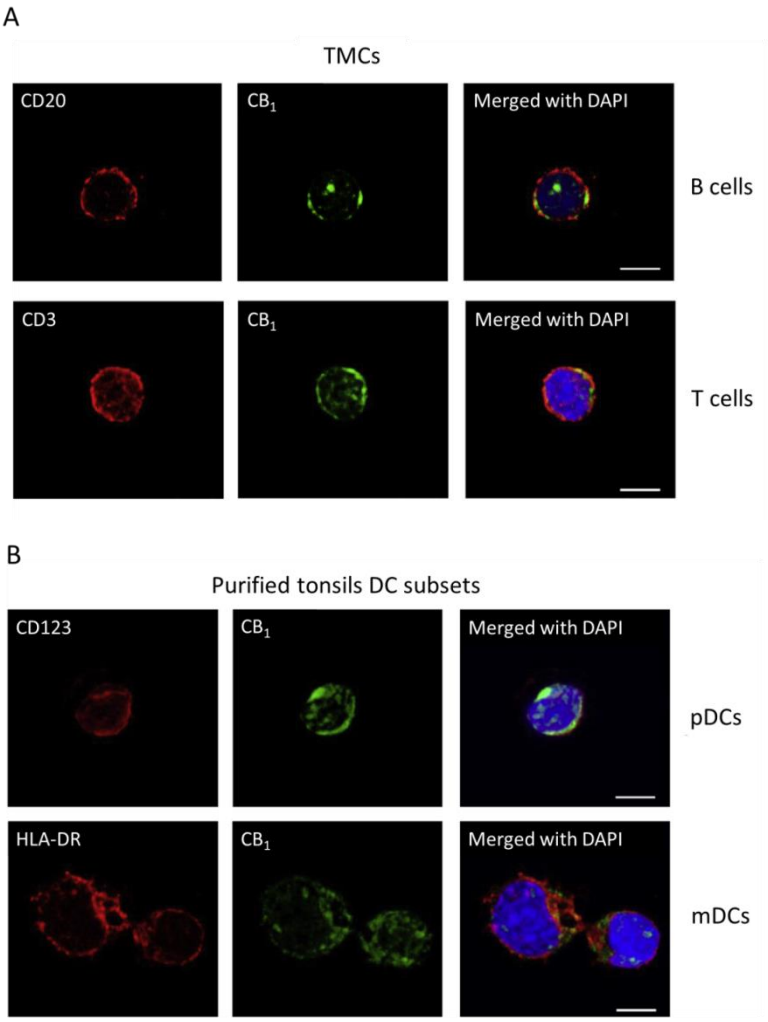


Figure 10. CB_1R expression in tonsil B and T cells (A), and purified pDCs and mDCs (B) from atopic donors. All cell subtypes were stained with probe **1** (0.5 μM, green), the nuclear marker DAPI (blue), and CD20, CD3, CD123 or HLA-DR (red) for B cells, T cells, pDCs and mDCs, respectively. Merged images show the combination of the three types of staining. All preparations were analyzed by confocal microscopy. Data are from 1 of at least 2 different atopic donors with similar results. White bars, 5 μm. DAPI, 4'-6-diamidino-2-phenylindole dihydrochloride.

Taken together, these data demonstrate for the first time that mRNA expression levels of CB_1R are upregulated in tonsils and peripheral blood of patients with different types of allergic diseases (allergic rhinitis, atopic dermatitis, and food allergy). Furthermore, B cells, T cells, pDCs, and mDCs from allergic patients express high protein

levels of CB_1R, indicating that these cell subsets could also contribute to the regulation of the pathogenesis of allergic diseases mediated by the ECS.[92]

Once confirmed that biotinylated probe **1** could be used in human cells to detect the CB_1R using confocal microscopy, we aimed to confirm whether fluorescent probe **22** could allow the direct visualization of this receptor in human PBMCs using flow cytometry. Our results show that probe **22** is able to detect CB_1R in human lymphocytes from PBMCs (54.2% positive cells) and that this signal significantly decreases, as expected, in the presence of HU210 (32.3% positive cells) (Figure 11). In addition, no fluorescent signal was obtained when control experiments using the fluorophore Alexa Fluor 488 alone were carried out.

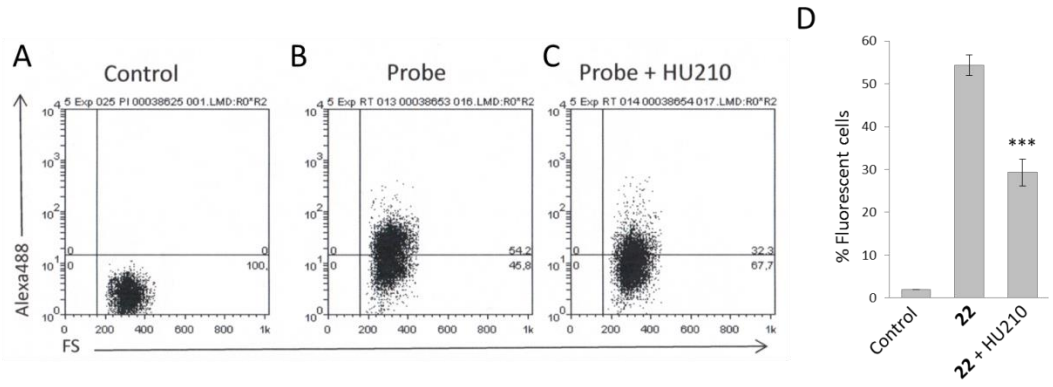


Figure 11. Flow cytometry analysis of PBMCs using probe **22**. Representative dot plots of PBMCs labelled with (A) vehicle, (B) probe **22** (1 μM), or (C) probe **22** (1 μM) plus an excess of HU210 (50 μM). (D) Bar chart displays the percentage of fluorescent PBMCs obtained in dot plots from three different experiments carried out separatedly. Forward scatter (FS) indicates the size of the gated cells. ***, P<0.001 (vs probe-treated cells, Student's t test).

These results indicate that probe **22** can be successfully used to detect and semi-quantify the levels of expression of CB_1R in PBMCs. Hence, this probe stands out as a valuable tool to study the modifications in the expression of this receptor in clinically relevant samples under different physio- or pathological conditions, and to establish its potential as a biomarker.

In summary, we have developed chemical probes **1** and **22** suitable for the detection of CB_1R in complex and clinically relevant systems such as immune cells.

4.2. Design and synthesis of tools to explore the differences between membrane and mitochondrial CB_1Rs

The complexity of the CB_1R-mediated signalling has been recently increased by the identification of a new CB_1R located in the mitochondria, mtCB_1R,[55,57] instead of the plasma membrane, which is the usual cellular location for GPCRs (Figure 12).

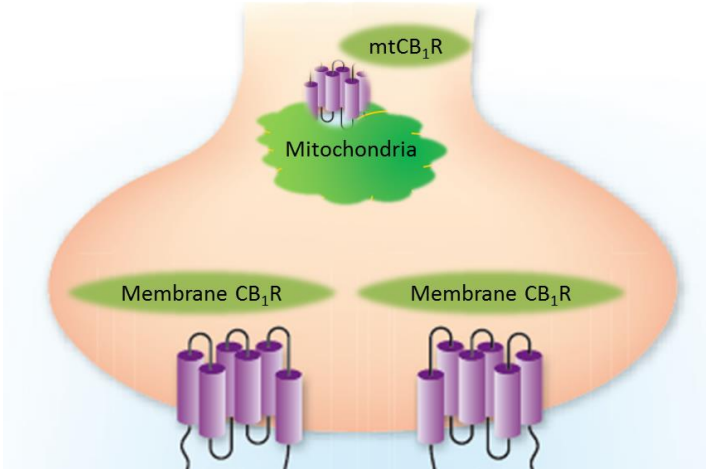
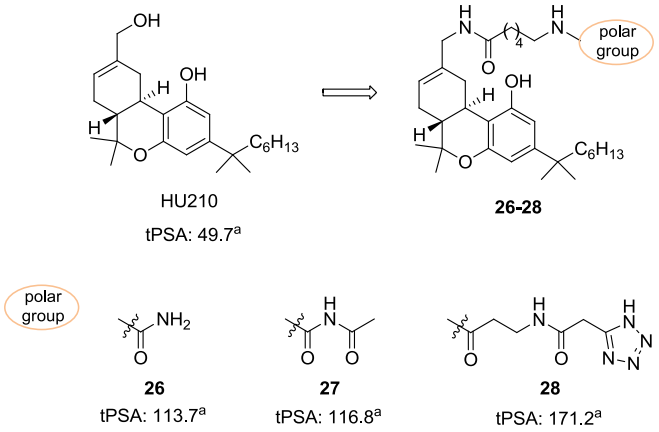


Figure 12. Subcellular locations of the CB_1R.

It has been hypothesized that this receptor could be involved in the regulation of different effects from those mediated by the membrane CB_1R. In particular, recent results suggest that the mtCB_1R mediates important cannabinoid-induced adverse effects such as catalepsy or memory impairment whereas therapeutic properties such as antinociception are associated to the plasma membrane CB_1R.[93,94] However, the described cannabinoid ligands are highly lipophilic (Figures 3 and 4)[28] and therefore, they bind to the membrane CB_1R but they are also able to cross the cell membrane and target the mtCB_1R. This fact makes them unsuitable to study whether these two types of receptors mediate different effects and if this fact is therapeutically relevant. To be able to distinguish between the two receptors, it would be necessary to develop compounds that keep the affinity towards the plasma membrane CB_1R but are unable to cross the cell membrane. In this regard, the topological polar surface area (tPSA) has been recognized as a reliable physicochemical parameter for the prediction of membrane permeability,[95] being generally accepted that the higher the tPSA, the less the cell

membrane penetration. In particular for the intestinal barrier, it has been described that while molecules with tPSA lower than 60 Å^2 are completely permeable,[96] the absortion degree decreases with the rise in the tPSA up to a maximal value of 140 Å^2, when the absortion has been shown to be less than 10%.[97]

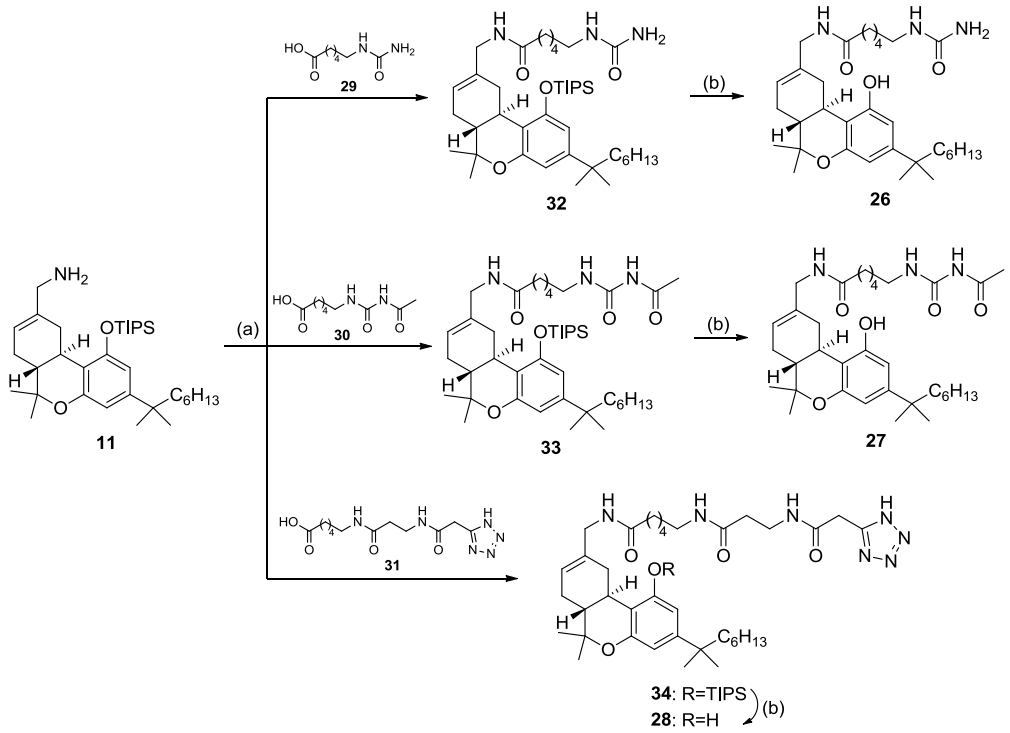
Based on these considerations, and taking into account our previous results, we considered the possibility of introducing highly polar groups to the HU210 scaffold in an attempt to keep the CB_1R affinity, but with high tPSA values to impair plasma membrane crossing. Thus, we designed HU210-based compounds **26-28** with highly polar subunits such as an urea or an acylurea in compounds **26** and **27** respectively, and a tetrazole containing chain in derivative **28**, attached through an amide linker and the optimal length of the spacer as determined before (Figure 13). The polarity of compounds **26-28** (tPSA: 113.7-171.2 Å^2) is highly increased with regard to the lipophilic cannabinoid ligand HU210 (tPSA: 49.7 Å^2).



[a]The tPSA values were predicted using the ACDLabs Percepta software.

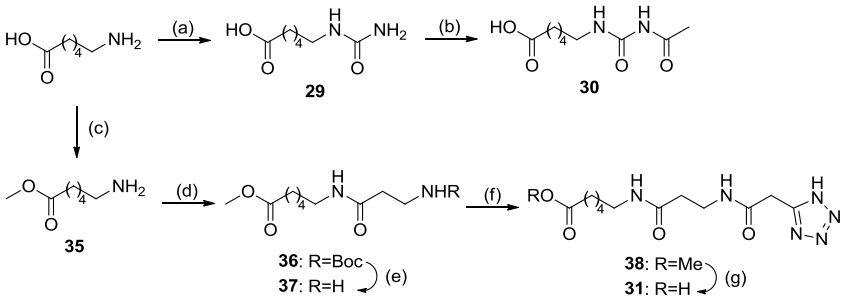
Figure 13. Design of polar derivatives **26-28**.

The synthesis of compounds **26-28** involved the condensation between amine **11** and the carboxylic acids of the corresponding derivatives **29-31**, followed by cleavage of the TIPS protecting group of amides **32-34** by TBAF (Scheme 8).



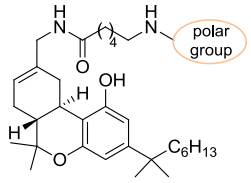
Scheme 8. Reagents and conditions: (a) EDC, HOBt, DMAP, DMF, DCM, rt, 49-75%; (b) TBAF, THF, 0 ºC, 32-93%.

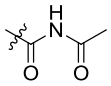
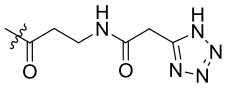
Preparation of polar chains **29-31** started in all cases from commercially available 6-aminohexanoic acid (Scheme 9). Thus, its treatment with potassium cyanate in water provided urea **29** in quantitative yield. Further acylation of urea **29** with potassium acetate in acetic anhydride afforded polar chain **30**. Alternatively, esterification of 6-aminohexanoic acid, followed by condensation of amine **35** with *N*-Boc-β-alanine using EDC and HOBt as coupling reagents led to amide **36**, whose deprotection with TFA yielded amine **37**. Final condensation of **37** with 1*H*-tetrazol-5-ylacetic acid in the presence of bis(2-oxo-3-oxazolidinyl)phosphinic chloride (BOP-Cl) at low temperature and further hydrolysis of the resulting ester **38**, gave the desired carboxylic acid **31**. Then, the affinity of compounds **26-28** for CB_1 and CB_2 receptors was evaluated by radioligand competitive binding assays (Table 2).



Scheme 9. Reagents and conditions: (a) KCN, H_2O, 60 °C, 100%; (b) KOAc, Ac_2O, reflux, 28%; (c) $SOCl_2$, MeOH, 0 °C, 100%; (d) *N*-Boc-β-alanine, EDC, HOBt, Et_3N, DCM, 0 °C to rt, 100%; (e) TFA, DCM, rt, 94%; (f) 1*H*-tetrazol-5-ylacetic acid, Et_3N, BOP-Cl, DCM, 10 °C to rt, 57%; (g) NaOH, MeOH, rt, 100%.

Table 2. Binding affinities of probes **26-28** for CBRs.



Compound	Polar group	Receptor affinity[a] (nM) K_i±SEM	
		CB_1	CB_2
26		4±1	4±1
27		8±3	7±2
28		12.1±0.5	16±4

[a]The values are the mean±SEM obtained from two to four independent experiments performed in triplicate.

These results show that the three compounds displayed good affinities towards both CBRs with K_i values in the nanomolar range. Therefore, compounds **26-28** stand out as promising candidates to study the effects mediated by the plasma membrane CB_1R, as these derivatives are uncapable of crossing the cell membrane and activate the mtCB_1R. This work is currently ongoing in collaboration with Professor Giovanni Marsicano at NeuroCentre Magendie at Université de Bordeaux (France).

4.3. Discovery of the off-targets of the synthetic CBR agonists HU210 and HU308

Among the issues regarding the understanding of the ECS, the identification of cannabinoid binding sites different from the known CBRs is possibly one of the foremost challenges to fully understand the ECS physiology. Although most of the THC-induced effects are mediated through the activation of CB_1R or, albeit with less efficacy, CB_2R, evidence has emerged suggesting that some of its effects are independent of these receptors.[60,98,99] For example, THC has been shown to potentiate glycine receptors,[100] and to activate the deorphanized GPCRs GPR18[101] and GPR55,[28,102] the TRPs of the type 1 ankyrin and type 2 vanilloid channels,[103] and the gamma member of the PPAR family of receptors.[28] Conversely, it has been found that THC blocks the activation of both the serotonin 5-HT_3 receptor and the TRP of the type 8 melastatin channel.[103,104] In addition, there has also been reported that submicromolar concentrations of THC can regulate the activity of some enzymes such as lysophosphaditylcholine acyl transferase or phospholipase C.[105] In a similar manner, some of the effects induced by the endocannabinoids AEA and 2-AG have been described to be CBR-independent.[8,28] Collectively, these findings open new pharmacological strategies regarding (i) the possibility of dissociating therapeutic benefits from adverse effects,[106] or (ii) the existence of alternative targets that may increase the efficacy for treating a particular disorder.[6]

In this context, cancer and immune modulation are two areas that have received a growing attention as non $CB_{1/2}R$-mediated effects have been described.[107-113] Hence, the development of analytical tools specifically tailored to enable the identification of cannabinoid targets would be of great value.

To achieve this objective, we need to develop chemical probes that besides bearing the binding group and the tag, present also an additional element able to “freeze” the possible reversible union between the probe and the protein, as we have previously described for other GPCRs.[114] This additional element is a photocrosslinking group such as benzophenone, which after ultraviolet (UV) irradiation at the appropriate wavelength will form a covalent bond with the probe-target proteins (Figure 14).

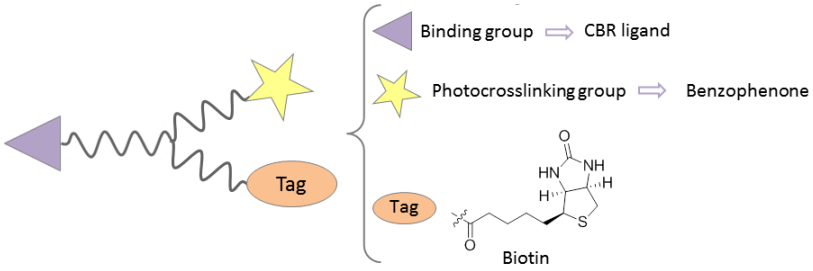


Figure 14. Probe for the isolation and identification of cannabinoid targets.

Thus, after incubation of the probes in the proteome of interest, UV irradiation and enrichment with streptavidin beads, the captured proteins will be digested with trypsin, and the resulting peptides analyzed by multidimensional liquid chromatography altogether with mass espectrometry (LC-MS) detection (Figure 15).

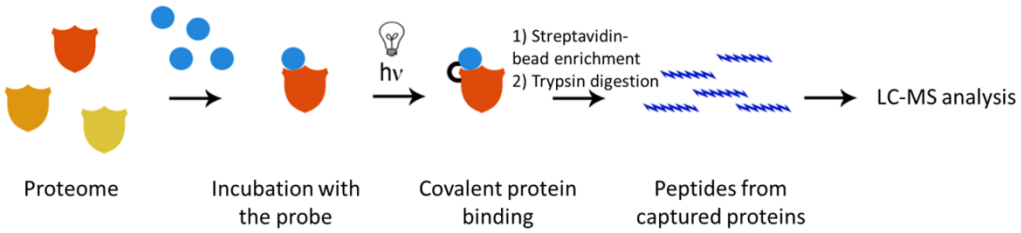


Figure 15. Proteomic platform scheme.

Therefore, design of the new probes was addressed by direct incorporation of a tag bearing the biotin subunit and the benzophenone group into the HU210 scaffold. We also considered the structure of HU308, which is one of the most selective ligands for the CB_2 receptor [K_i (CB_1)>10 µM; K_i (CB_2)=22.7 nM],[115] to study the differences between the two compounds.

Hence, we designed probes **39-41** in which we have incorporated the tag in the allylic alcohol of HU210 and HU308 through an ester in compound **39** or an amide linker in derivatives **40** and **41** (Figure 16).

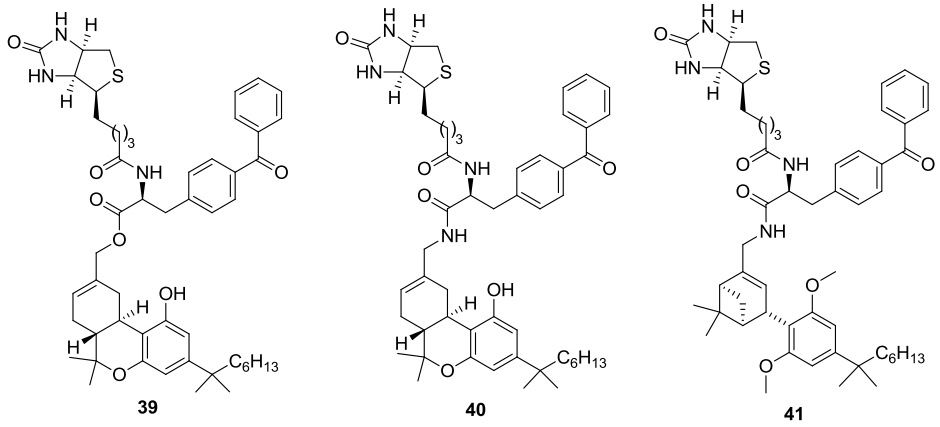
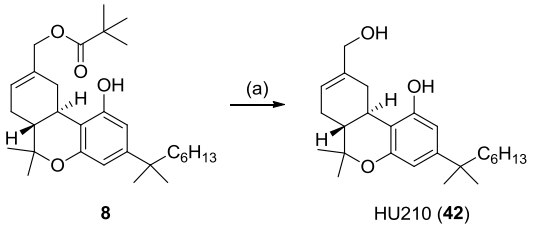


Figure 16. Structure of probes **39-41**.

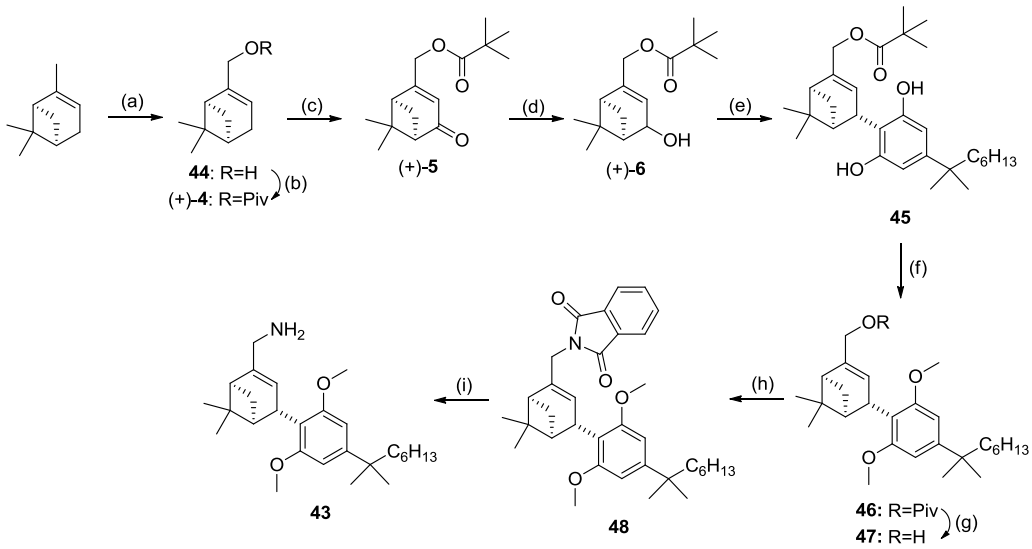
The preparation of compounds **39-41** was achieved by condensation of the HU210 or HU308 scaffolds conveniently functionalized with the carboxylic acid of the tag. Thus, HU210 (**42**) was synthesized from phenol **8**, by cleavage of the pivaloyl group with lithium aluminium hydride (Scheme 10).



Scheme 10. Reagents and conditions: (a) $LiAlH_4$, THF, 0 °C to rt, 100%.

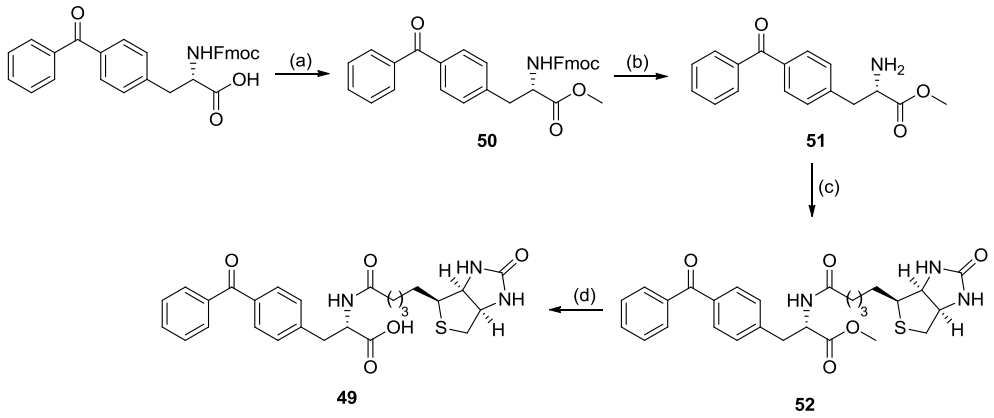
The synthesis of the HU308 amine **43**, necessary for the preparation of probe **41**, started from commercially available (1*R*,5*R*)-2,6,6-trimethylbicyclo[3.1.1]hept-2-ene (α-pinene) (Scheme 11).[31,84] Thus, oxidation of α-pinene with selenium dioxide and *tert*-butyl hydroperoxide led to the corresponding aldehyde which, without further purification, was reduced with lithium aluminium hydride to obtain **44**. Conversion of alcohol **44** into 4-hydroxymyrtenyl pivalate [(+)-**6**] was achieved through the same sequence of reactions previously described for its (-)-enantiomer (Scheme 1, p. 42). Condensation of alcohol (+)-**6** with resorcinol **7** in the presence of *p*-toluenesulfonic acid (*p*-TSA) gave intermediate **45**,[116] whose dimethylation reaction followed by subsequent cleavage of the pivaloyl ester in intermediate **46** with lithium aluminium hydride led to

HU308 (**47**). Mitsunobu reaction of alcohol **47**, followed by deprotection of the resulting phthalimide **48**, gave the desired amine **43**.



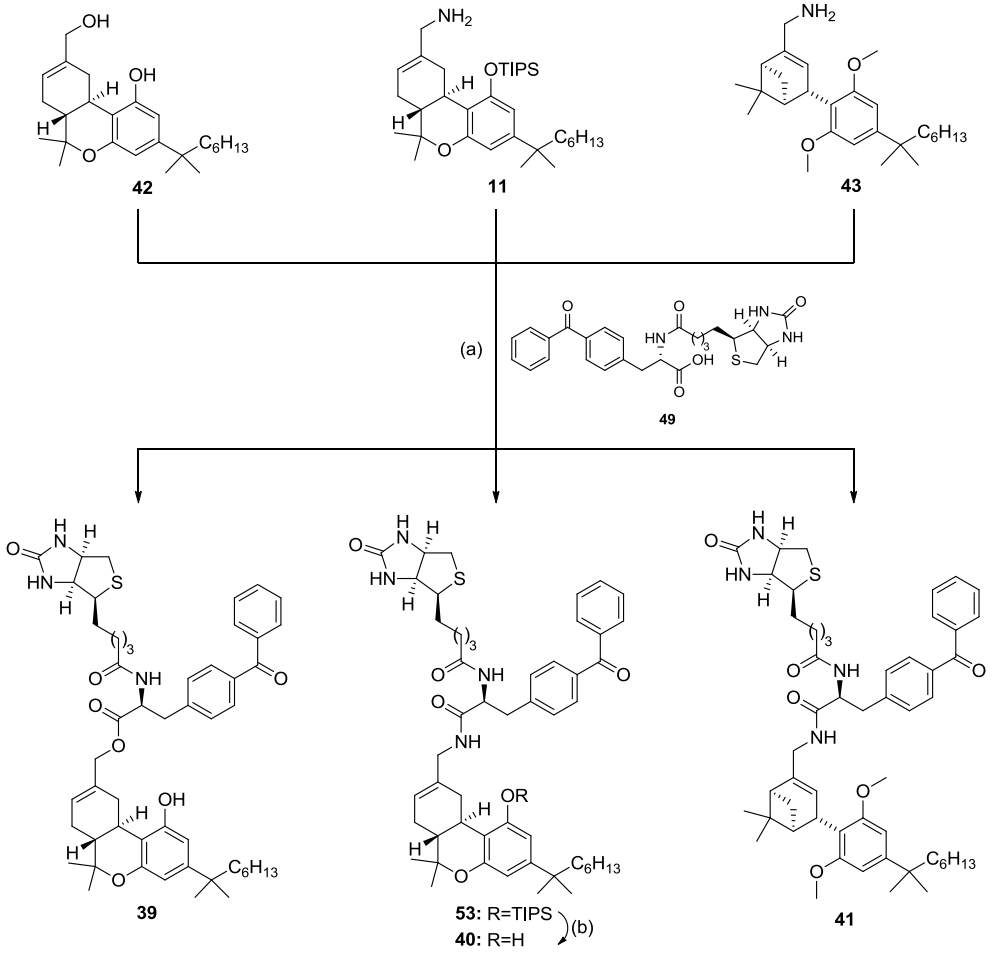
Scheme 11. Reagents and conditions: (a) (i) SeO_2, *t*-BuOOH, DCM, rt; (ii) $LiAlH_4$, Et_2O, 0 °C, 54%; (b) $(CH_3)_3CCOCl$, pyridine, DCM, 0 °C, 100%; (c) CrO_3, 3,5-dimethylpyrazole, DCM, -20 °C, 40%; (d) $LiAlH(t\text{-}BuO)_3$, THF, 0 °C to rt, 63%; (e) **7**, *p*-TSA, DCM, 0 °C to rt, 82%; (f) CH_3I, NaH, DMF, rt, 81%; (g) $LiAlH_4$, Et_2O, reflux, 45%; (h) phthalimide, PPh_3, DEAD, THF, rt, 47%; (i) i) $N_2H_4{\cdot}H_2O$, EtOH, reflux; ii) HCl/H_2O 1:1 reflux to rt, 80%.

Once the scaffolds were synthesized, we proceeded with the preparation of tag **49** from 4-benzoyl-*N*-[(9*H*-fluoren-9-ylmethoxy)carbonyl]-L-phenylalanine (Scheme 12). Its esterification with iodomethane and potassium carbonate yielded methyl ester **50**, whose 9-fluorenylmethoxycarbonyl (Fmoc) deprotection with piperidine afforded free amine **51**.[117] Then, biotin was introduced in the presence of EDC and HOBt as coupling reagents and DMAP as catalyst, leading to methyl ester **52**, which was hydrolyzed with lithium hydroxide and further treated with mineral acid (HCl) to provide carboxylic acid **49**.



Scheme 12. Reagents and conditions: (a) K_2CO_3, CH_3I, DMF, 0 °C to rt, 91%; (b) piperidine, DCM, 0 °C to rt, 88%; (c) biotin, EDC, HOBt, DMAP, DMF, DCM, 77 °C to rt, 59%; (d) (i) $LiOH{\cdot}H_2O$, H_2O, THF, rt; (ii) 1 M HCl, 100%.

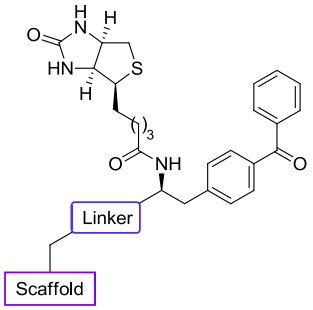
Finally, condensation of alcohol **42** (HU210) or amines **11** and **43** with carboxylic acid **49** in the presence of EDC and HOBt as coupling reagents, followed by TIPS deprotection with TBAF in the case of amide **53**, afforded the desired probes **39-41** (Scheme 13).



Scheme 13. Reagents and conditions: (a) EDC, HOBt, DMF, DCM, 36 ºC to rt, 31-35%; (b) TBAF, THF, 0 ºC, 36%.

Affinity of compounds **39-41** for CB_1 and CB_2 receptors was evaluated. As shown in Table 3, probe **39** presented high affinity towards both receptors, while amide **40** displayed a considerable loss of affinity towards CB_1R. Notably, probe **41** exhibited high K_i value for CB_2R, maintaining the selectivity profile of its parent ligand (HU308).

Table 3. Binding affinities of probes **39-41**.



Compound	Scaffold	Linker	Receptor affinity[a] (nM) K_i±SEM	
			CB_1	CB_2
39	HU210	OCO	25.7±0.4	12.1±0.2
40	HU210	NHCO	268±28	55±17
41	HU308	NHCO	>1000	28±6

[a]The values are the mean±SEM obtained from two to four independent experiments performed in triplicate.

Before carrying out the proteomic experiments, it is necessary to confirm that the designed compounds are able to label proteins in a specific way, which is in a concentration- and UV-dependent manners. In order to do so, we chose probe **39** because of its affinity for CBRs, and replaced the biotin subunit by the fluorophore lissamine (probe **54**, Figure 17A) to enable, after separation by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), the visualization of the labelled proteins by fluorescence scanning (Figure 17B).

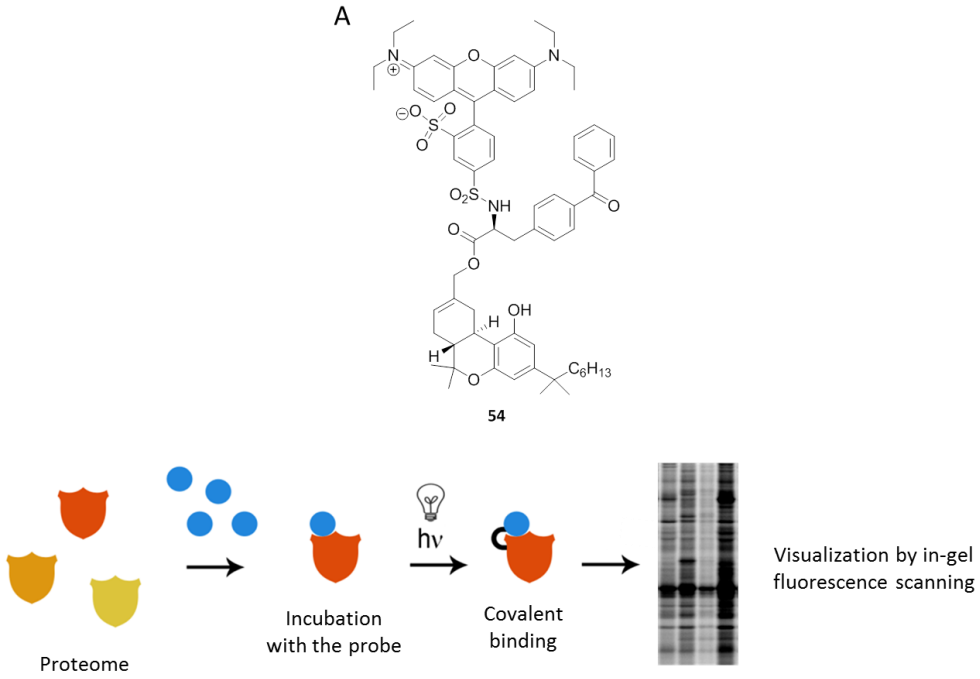
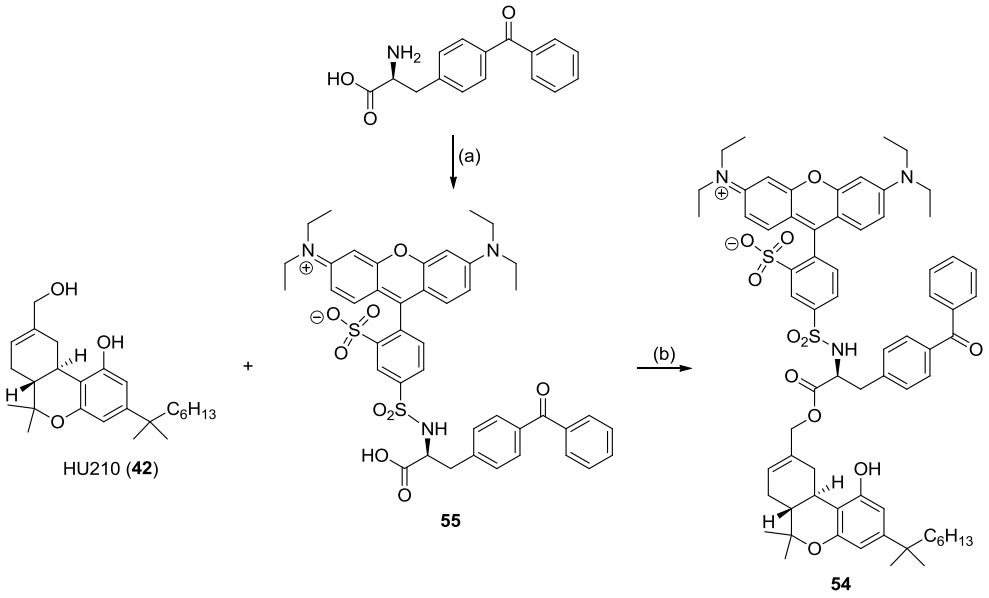


Figure 17. (A) Structure of probe **54**. (B) Proteomic platform for the visualization of probe-labelled proteins by in-gel fluorescence scanning.

Synthesis of probe **54** started with the preparation of fluorescent tag **55** by condensation between commercially available 4-benzoyl-L-phenylalanine and rhodamine B sulfonyl chloride in the presence of *N,N'*-diisopropylethylamine (DIPEA) and sodium hydroxide, followed by its condensation with HU210 (**42**) (Scheme 14).



Scheme 14. Reagents and conditions: (a) rhodamine B sulfonyl chloride, DIPEA, NaOH, acetone, 0 °C to rt, 22%; (b) EDC, HOBt, DMF, DCM, rt, 20%.

In order to test the suitability of probe **54** for labelling proteins, membranes of HEK-293-EBNA cells overexpressing the hCB_2R were treated with different concentrations (5-50 μM) of the probe and subjected to UV irradiation to promote covalent binding between the probe and the target proteins. After that, proteins were separated by SDS-PAGE and the obtained fluorescent bands (corresponding to probe-protein complexes) revealed a concentration- and UV- dependent labelling (data not shown), which highlighted the ability of HU210-based probes to detect the targets of this cannabinoid ligand.

Hence, and as proof of concept, we carried out proteomic experiments (see Figure 15) incubating membranes of HEK-293-EBNA cells overexpressing the hCB_2R with probe **39**, which presented the best affinity values [K_i (CB_1)=25.7 nM; K_i (CB_2)=12.1 nM, Table 3], at 25 μM concentration. Samples were then exposed to UV irradiation at 0 °C for 1 h in order to induce covalent binding between the probe and the target proteins. After that, the labelled proteome was subjected to streptavidin enrichment and on-bead trypsin digestion, and the resulting peptides were analyzed using an Orbitrap. To minimize false positives, only those hits identified in two independent runs and absent from control experiments, carried out in duplicate with DMSO as vehicle instead of the

probe, were considered as significant. Hence, 15 proteins were identified using probe **39** (Table 4). It is important to note that probe **39** was able to engage the CB_2R, thus validating the methodology.

Table 4. Proteins identified with probe **39**.

Accession	Symbol	Description
P34972	CNR2	Cannabinoid receptor 2
P78527	PRKDC	DNA-dependent protein kinase catalytic subunit
P40939	HADHA	Trifunctional enzyme subunit alpha, mitochondrial
P63261	ACTG1	Heat shock 70 kDa protein 1A/1B
P42704	LRPPRC	Leucine-rich PPR motif-containing protein, mitochondrial
P05023	ATP1A1	Sodium/potassium-transporting ATPase subunit alpha-1
P53396	ACLY	ATP-citrate synthase
Q08211	DHX9	ATP-dependent RNA helicase A
P61978	HNRNPK	Heterogeneous nuclear ribonucleoprotein K
P23396	RPS3	40S ribosomal protein S3
Q99623	PHB2	Prohibitin-2
Q12931	TRAP1	Heat shock protein 75 kDa, mitochondrial
P33778	HIST1H2BB	Histone H2B type 1-B
P05141	SLC25A5	ADP/ATP translocase 2
Q9NR30	DDX21	Nucleolar RNA helicase 2

The obtained results were analyzed based on gene ontology (GO) annotations (protein class, cellular localization and biological processes) using the protein analysis through the evolutionary relationships (PANTHER) classification system,[118,119] which includes both experimental data and bioinformatics algorithms. This analysis revealed that the proteins targeted by probe **39** belong to a variety of functional classes (Figure 18A), and participate in different biological processes (Figure 18B). Regarding the cellular component, a great enrichment of membrane proteins (13 out of 15, $p<0.001$) was detected, which deserves special attention considering that the platform was initially designed for the identification of GPCRs.

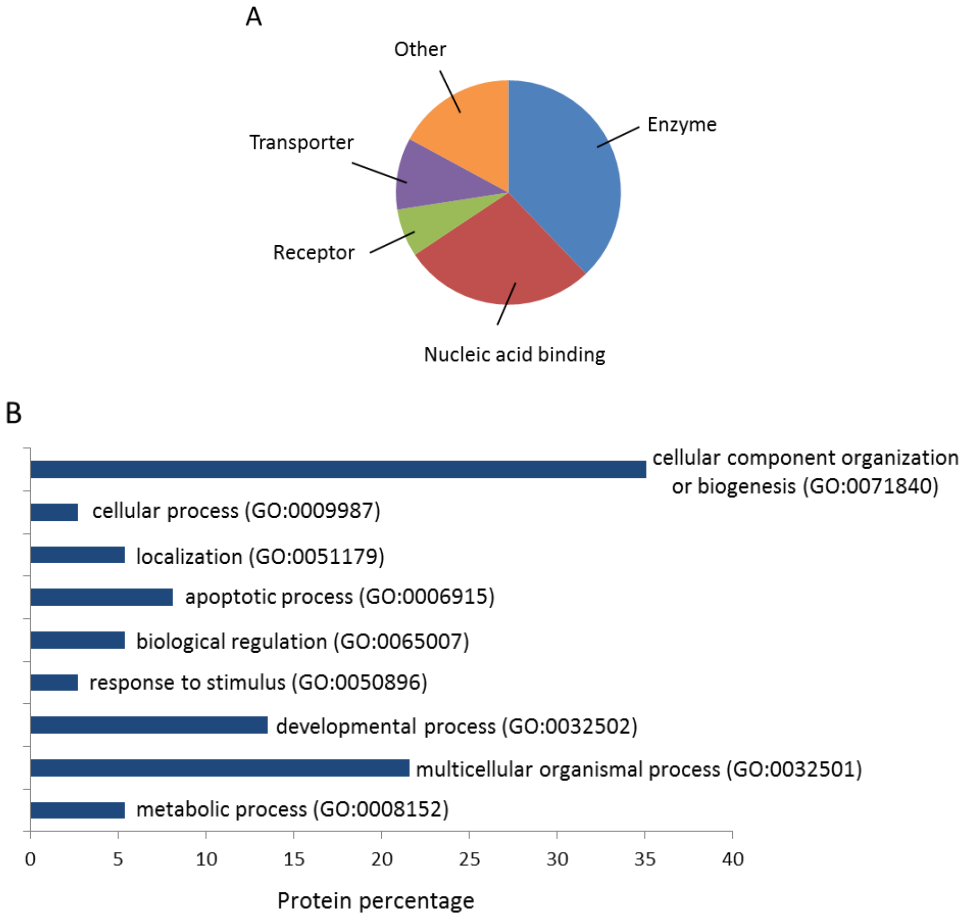


Figure 18. Analysis of gene ontology (GO) terms. (A) Protein classes identified with probe **39**. (B) Involvement of identified proteins in specific biological processes.

Based on these results, and taking into account the reported non $CB_{1/2}R$-mediated effects in immune modulation and cancer,[107-113] we are currently using this probe in the Jurkat T cell line and the breast cancer cell line MDA-MB-231 in order to identify the targets of cannabinoids in these systems.

4.4. Identification of the target proteins of the natural product honokiol

Magnolia plant extracts have been widely used in Asiatic traditional medicine for the treatment of different disorders. The different species of the *Magnolia* family contain several biologically active compounds, being the main ones honokiol, magnolol, and 4'-*O*-methylhonokiol (Figure 19), with therapeutic implications in neuronal, inflammatory and cardiovascular diseases, as well as in different types of cancer.[120]

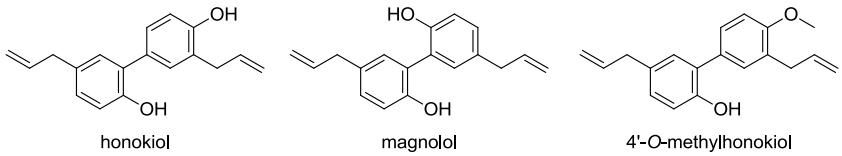


Figure 19. Main constituents of the *Magnolia* family.

In particular, honokiol is known due to its anxiolytic, analgesic, anti-depressant, anti-tumorigenic, and neuroprotective properties among others.[65,66] Interestingly, all these properties are extraordinarily related to those shown by ligands acting at the ECS.[6] Actually, honokiol and its derivatives have been related not only to CB_1 and CB_2 receptors, but also to GPR55, and to the PPAR family of receptors.[63,64,121-123]

Among the different indications reported for honokiol and its derivatives, cancer has received an increasing attention because of the significant activities shown by these compounds in several cancer models of different origins such as lung, breast, ovarian, prostate and gastrointestinal cancers.[65,124] In particular, this family of natural products significantly inhibit the growth of highly aggressive malignancies characterized by a lack of specific treatments and, hence, a poor prognosis, such as some types of breast, pancreas, or brain tumors.[65,125] In order to obtain information about the mechanisms affected by these compounds and to develop more potent derivatives, some structure-activity relationship (SAR) studies have been carried out,[64,126,127] indicating that at least one hydroxyl group and one allyl chain are needed for activity. All these compounds have contributed to elucidate, at least in part, some of the signalling pathways affected by these derivatives. However, the actual targets of honokiol and related compounds are not known, and their identification could lead to the discovery of new therapeutic strategies for highly aggressive type of cancers, an urgent unmet medical need. Hence, we have started a project aimed at the study of the targets of honokiol and its derivatives in order to shed some light on their mechanisms of action. In particular, we

have focused our efforts on the design, synthesis and development of chemical probes based on the synthetic derivative 2-*O*-ethylhonokiol (Figure 20), which is a more potent derivative than honokiol in some assays.[64]

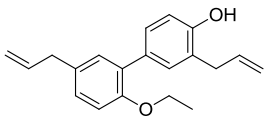


Figure 20. Chemical structure of 2-*O*-ethylhonokiol.

4.4.1. Design and synthesis of probes for the study of the targets of honokiol and its derivatives

In order to be able to identify the targets of these compounds, we introduced in the honokiol scaffold a photocrosslinking group to form the covalent bond with the target proteins, and a biotin subunit to allow their enrichment and identification. Taking into account the previous SAR studies, we kept in all the probes one free phenol and at least one allyl group. Therefore, we designed probes **56-59** (Figure 21, left panel) where the scaffold and the tag are linked by a triazol ring formed through a copper catalyzed cycloaddition of honokiol-based azides (**60-62**, Figure 21, right panel) and the alkyne of the tag, or viceversa (honokiol-based alkyne **63** and the azide of the tag, Figure 21, right panel).

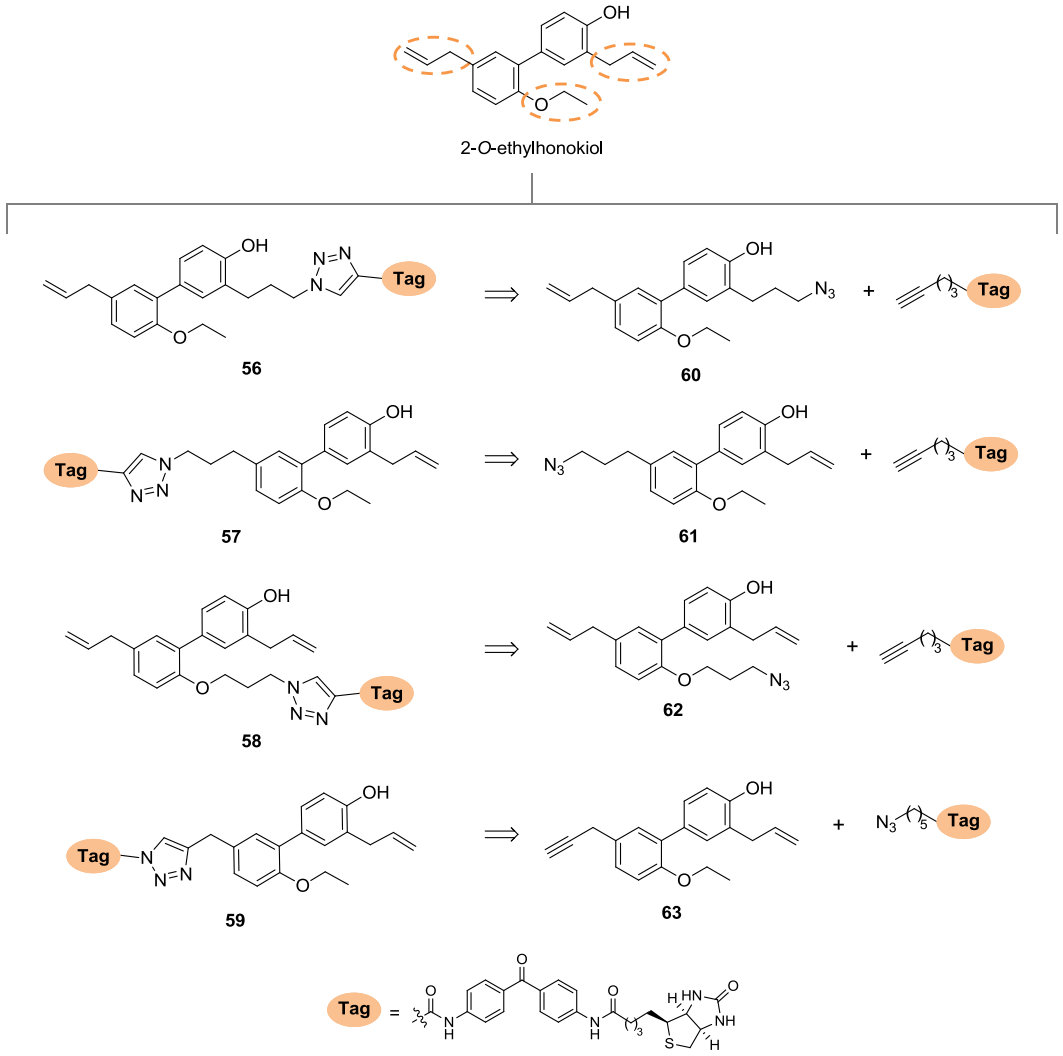


Figure 21. Design of probes **56-59** and the corresponding honokiol-based fragments **60-63**.

In addition, we designed the smaller clickable probes **64** and **65** (Figure 22), where we have replaced the benzophenone by the less bulky diazirine group as the photocrosslinking moiety. Moreover, we have removed the biotin subunit, and introduced instead a terminal alkyne to allow, after incubation with the desired proteome, visualization, enrichment and identification of probe-labelled proteins through a click chemistry reaction with the proper reporter groups (biotin or fluorophore) bearing an azide within their structure.

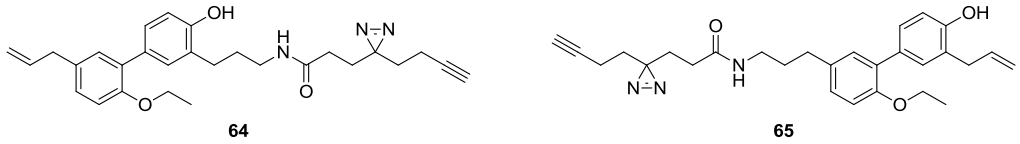


Figure 22. Design of probes **64** and **65**.

The preparation of probes **56-59** required the synthesis of the honokiol-based fragments **60-63**. Starting with azide **60**, the different possibilities of its retrosynthetic analyisis are indicated in Figure 23. Thus, the most convergent synthetic route would entail the preparation of the bromoderivative of one of the phenyl rings and the boronic fragment of the other, which would be attached through a Suzuki coupling in the last step of the synthesis (Figure 23A and B). In addition, a linear approach could be also possible in order to introduce the allyl chain after the Suzuki coupling of both phenyl rings (Figure 23C).

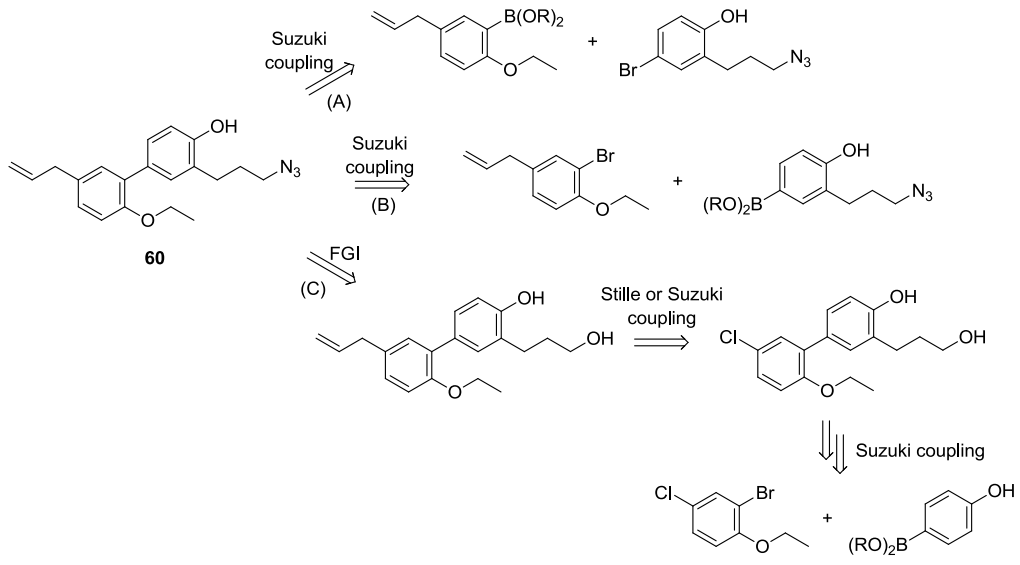
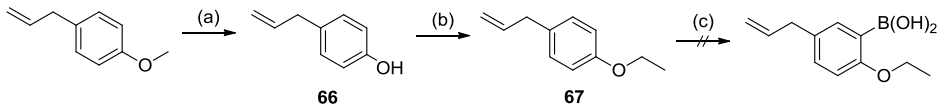


Figure 23. Retrosynthetic analysis of azide **60**.

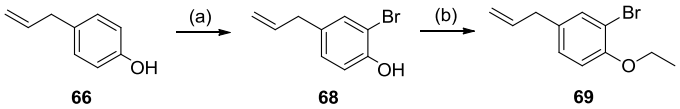
Starting with the first possibility (A), the preparation of the boronic acid derivative started from commercially available 4-allylanisole (Scheme 15). Its deprotection with boron tribromide, followed by Williamson alkylation of phenol **66** with bromoethane under MW irradiation provided ethyl ether **67**. However, organolithiation of **67** followed by treatment with trimethylborate and mineral acid as previously described for 4-

allylanisole[128] was unsuccessful probably due to the higher steric hindrance of the ethoxy group with respect to the methoxy substituent.



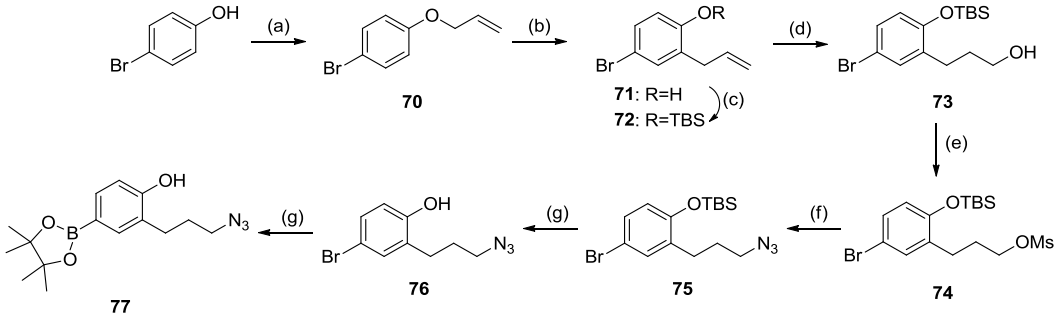
Scheme 15. Reagents and conditions: (a) BBr_3, DCM, -78 ºC to rt, 93%; (b) EtBr, K_2CO_3, acetone, MW, 140 ºC, 98%; (c) (i) *s*-BuLi, TMEDA, -78 ºC, (ii) $B(OMe)_3$, HCl, -78 ºC to rt.

To overcome this obstacle, we decided to perform the Suzuki coupling exchanging the functionality of each partner as depicted in Figure 23B. Hence, bromoderivative **69**[127] was synthesized by bromination of phenol **66** with 1,3-dibromo-5,5-dimethylhydantoin (DBDMH) in the presence of isopropyl magnesium chloride, and further *O*-alkylation of intermediate **68** with bromoethane (Scheme 16).



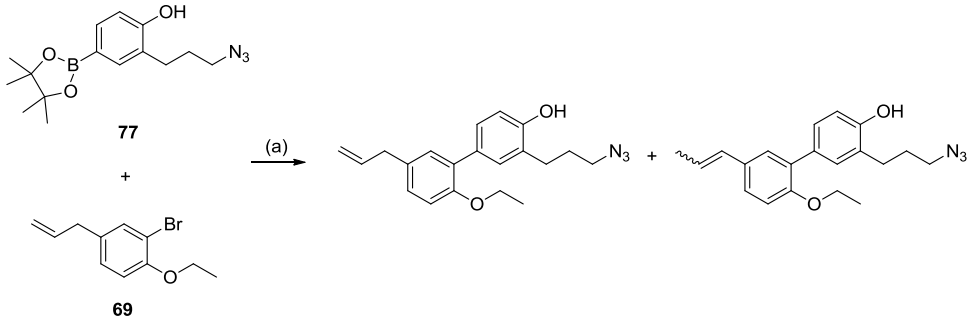
Scheme 16. Reagents and conditions: (a) DBDMH, *i*-PrMgCl, Et_2O, -78 ºC to rt, 33%; (b) EtBr, K_2CO_3, acetone, MW, 140 ºC, 87%.

Boronate **77** was prepared from commercially available 4-bromophenol (Scheme 17). Its Williamson alkylation with allyl bromide, followed by Claisen rearrangement of allyl ether **70** under MW irradiation, and further protection of the resulting phenol **71** with TBS, provided silyl ether **72** in excellent yields. Then, hydroboration-oxidation of **72** afforded alcohol **73**, which was transformed into azide **75** through the nucleophilic substitution of the corresponding mesylate **74** with sodium azide. Finally, cleavage of the TBS group, followed by Suzuki coupling of **76** with bis(pinacolato)diboron in the presence of palladium led to the desired boronate **77**.



Scheme 17. Reagents and conditions: (a) allyl bromide, K_2CO_3, acetone, MW, 140 °C, 97%; (b) 1,2-dichlorobenzene, MW, 250 °C, 73%; (c) TBS-Cl, imidazole, DMF, MW, 100 °C, 97%; (d) BH_3, H_2O_2, THF, 0 °C to rt, 77%; (e) MsCl, Et_3N, DCM, -20 °C to rt, 100%; (f) NaN_3, DMF, 90 °C, 93%; (g) TBAF, THF, 0 °C, 93%; (h) B_2pin_2, $Pd(dppf)Cl_2$, KOAc, 1,4-dioxane, 80 °C, 78%.

The Suzuki coupling of bromoderivative **69** and boronate **77** was carried out in the presence of Pd_2dba_3, 2-dicyclohexylphosphino-2',6'-dimethoxybiphenyl (S-PHOS) and potassium fluoride (Scheme 18). These conditions were selected since Denton *et al.*[128] had previously described the use of these coupling reagents in the presence of aryl halides bearing allyl substituents without observing the isomerization of the allyl chain. However, in our hands the coupling took place with the undesired isomerization and we obtained an inseparable mixture of the expected coupling product together with the conjugated alkene.



Scheme 18. Reagents and conditions: (a) Pd_2dba_3, S-PHOS, KF, 1,4-dioxane, H_2O, 110 °C.

This result prompted us to follow the linear synthetic route showed in Figure 23C where the Suzuki coupling between both phenyl rings would take place before the introduction of the allyl chain in order to avoid its isomerization. This route could start with the coupling between 2-bromo-4-chloro-1-ethoxybenzene and 4-hydroxyphenylboronic acid, followed by alkylation of the free phenol with allyl bromide, Claisen rearrangement, hydroboration-oxidation of the double bond, introduction of the allyl group through the chloro substituent, and transformation of the corresponding alcohol into the desired azide **60** (Figure 24).

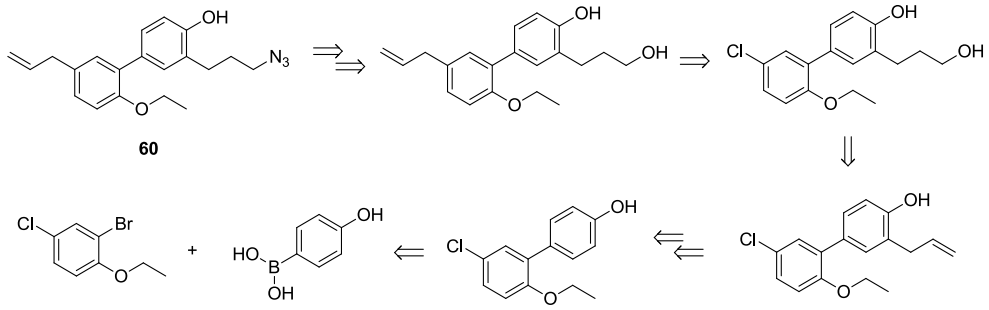
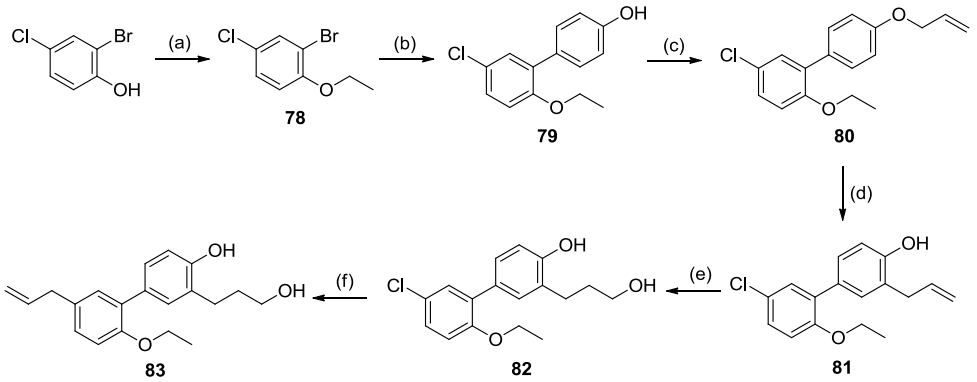


Figure 24. Linear retrosynthetic analysis of azide **60**.

Accordingly, 2-bromo-4-chlorophenol was alkylated with bromoethane to provide 2-bromo-4-chloro-1-ethoxybenzene (**78**) (Scheme 19). MW-assisted Suzuki coupling of **78** with 4-hydroxyphenylboronic acid led to phenol **79**, whose *O*-allylation followed by Claisen rearrangement of intermediate **80** at low temperature afforded phenol **81**. Then, hydroboration-oxidation of **81** gave alcohol **82**. The introduction of the allyl chain through the chlorine substituent of **82** was approached as a Stille coupling using allyltributylstannane in the presence of a palladium source. However, all the assayed conditions provided the desired product **83** with a conversion lower than 60% and always impurified with the starting material **82** (Table 5). Unfortunately, both compounds presented the same retention factor (R_f) and therefore, they could not be separated by column chromatography.



Scheme 19. Reagents and conditions: (a) EtBr, K_2CO_3, acetone, MW, 140 °C, 96%; (b) 4-hydroxyphenylboronic acid, $Pd(PPh_3)_4$, Na_2CO_3, toluene, EtOH, MW, 110 °C, 92%; (c) allyl bromide, K_2CO_3, acetone, MW, 140 °C, 98%; (d) $AlMe_3$, H_2O, DCM, -20 °C, 90%; (e) BH_3, H_2O_2, THF, 0 °C to rt, 84%; (f) allyl$SnBu_3$, **catalyst system**, **base**, 1,4-dioxane, MW (Table 5).

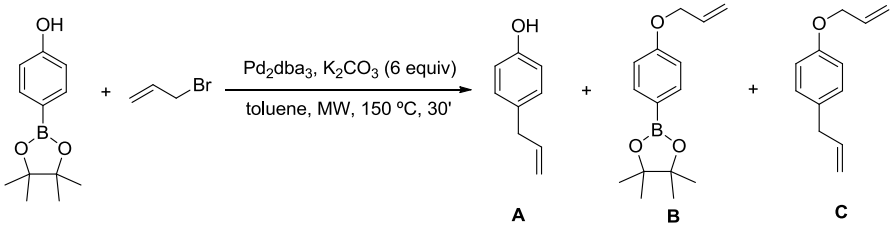
Table 5. Experimental conditions for the synthesis of **83**.

Allyl$SnBu_3$ (equiv)	Catalyst system (equiv)	Base (equiv)	T (°C), t	Conversion[a] (%)
1.1	Pd_2dba_3/P(*t*-Bu)$_3$ (0.06:0.24)	$CsCO_3$ (2.2)	180, 10'	0
1.1	Pd[P(*t*-Bu)$_3$]$_2$ (0.03)	CsF (2.5)	180, 10'	17
1.1	Pd[P(*t*-Bu)$_3$]$_2$ (0.03)	CsF (4)	180, 30'	36
1.1	Pd[P(*t*-Bu)$_3$]$_2$ (0.05)	CsF (4)	200, 1 h	56
1.5	Pd[P(*t*-Bu)$_3$]$_2$ (0.05)	CsF (4)	200, 1.5 h	56
1.5	Pd[P(*t*-Bu)$_3$]$_2$ (0.05)	CsF (4)	110, 48 h[b]	32

[a]Determined by ^{1}H-NMR of the reaction crude.
[b]Carried out under reflux instead of under MW irradiation.

Therefore, we envisioned the synthesis of **83** as a MW-assisted Suzuki coupling between allyl bromide and the corresponding pinacol borane ester of the biphenyl scaffold in the presence of potassium carbonate and Pd_2dba_3 as catalyst. To this end, and taking into account that we are using potassium carbonate and allyl bromide in the presence of a free phenol at high temperature, we optimized the conditions in order to avoid the *O*-alkylation side reaction, using commercially available 4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenol (Table 6).

Table 6. Optimization of the Suzuki coupling.

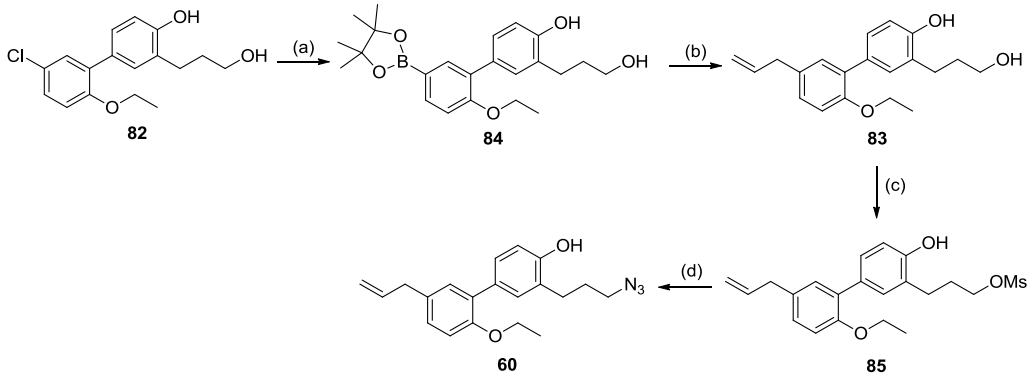


Pd_2dba_3 (equiv)	Allyl bromide (equiv)	Ratio[a] **A**:**B**:**C**
0.1	2.5	1:0.26:0.26
0.1	1.2	1:0.18:0.51
0.2	1.2	1:0.12:0.20

[a]Determined by ^{1}H-NMR of the reaction crude.

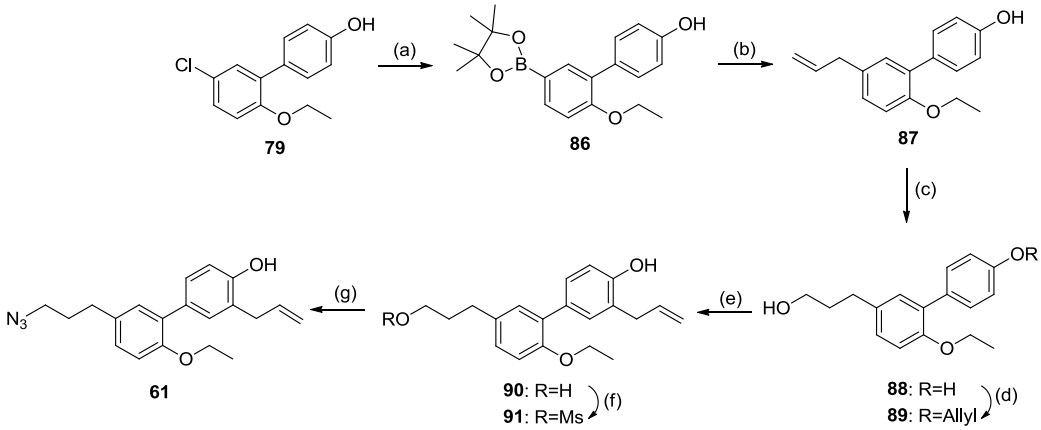
As shown in Table 6, the best results were obtained with 0.2 equiv of catalyst and 1.2 equiv of allyl bromide, since the *O*-alkylation byproducts **B** and **C** were obtained in low proportion. Therefore, we decided to use these conditions for the synthesis of **83**.

Thus, chloroderivative **82** was transformed into boronate **84** in the presence of bis(pinacolato)diboron, Pd_2dba_3, and S-PHOS under MW irradiation (Scheme 20). Suzuki coupling of boronate **84** with allyl bromide under the selected conditions provided pure **83** in 60% yield. Finally, transformation of alcohol **83** into mesylate **85** followed by nucleophilic substitution with sodium azide, provided the desired honokiol-based azide **60**.



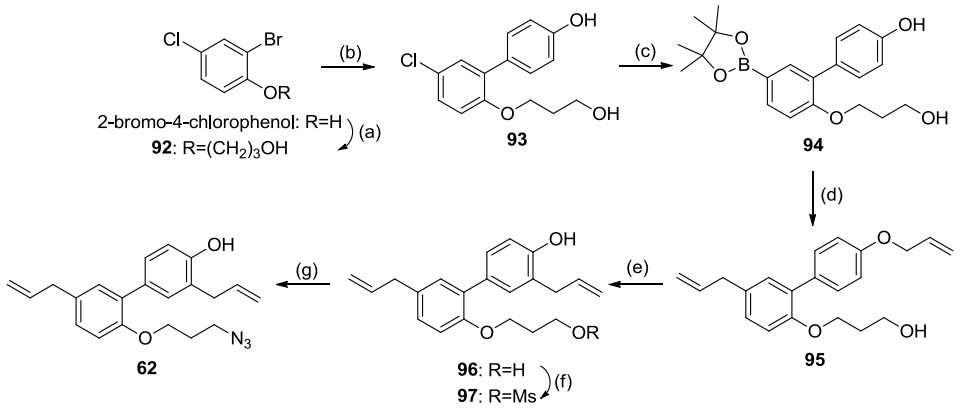
Scheme 20. Reagents and conditions: (a) B_2pin_2, Pd_2dba_3, S-PHOS, KOAc, 1,4-dioxane, MW, 140 ºC, 89%; (b) allyl bromide, Pd_2dba_3, K_2CO_3, toluene, MW, 150 ºC, 60%; (c) MsCl, pyridine, DCM, -20 ºC to rt, 42%; (d) NaN_3, DMF, 60 ºC, 70%.

Following a similar approach (Scheme 21), transformation of chloroderivative **79** into the corresponding boronate **86**, and further Suzuki coupling of **86** with allyl bromide followed by hydroboration-oxidation of the resulting phenol **87**, provided alcohol **88**. *O*-allylation of phenol **88** and Claisen rearrangement of the obtained allyl ether **89** afforded alcohol **90**, which was transformed into the desired azide **61** through the corresponding mesylate **91** (Scheme 21).



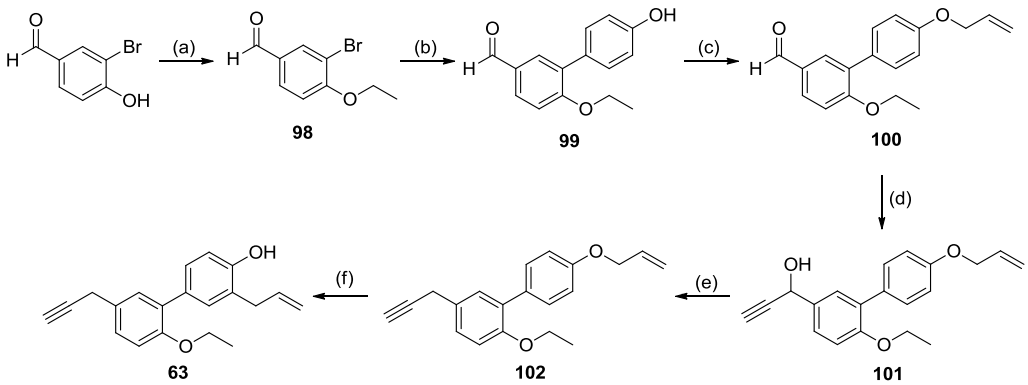
Scheme 21. Reagents and conditions: (a) B_2pin_2, Pd_2dba_3, S-PHOS, KOAc, 1,4-dioxane, MW, 140 °C, 75%; (b) allyl bromide, Pd_2dba_3, K_2CO_3, toluene, MW, 150 °C, 75%; (c) BH_3, H_2O_2, THF, 0 °C to rt, 53%; (d) allyl bromide, K_2CO_3, acetone, MW, 140 °C, 92%; (e) Et_2AlCl, DCM, -20 °C to 0 °C, 97%; (f) MsCl, pyridine, DCM, -20 °C to rt, 77%; (g) NaN_3, DMF, 60 °C, 67%.

Azide **62** was obtained by etherification of 2-bromo-4-chlorophenol with 3-bromopropan-1-ol, followed by subsequent Suzuki couplings, *O*-allylation, Claisen rearrangement and activation of the primary alcohol through the corresponding mesylate (Scheme 22).



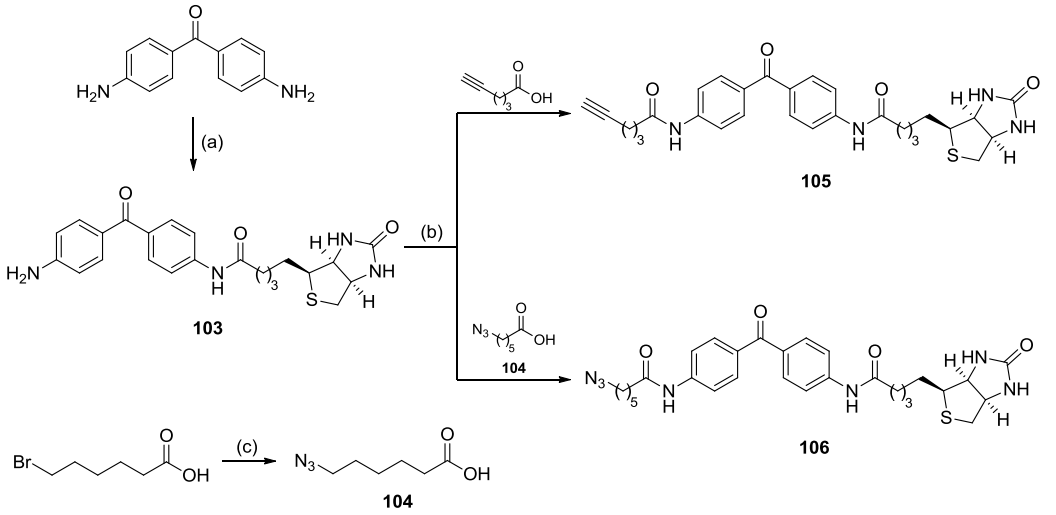
Scheme 22. Reagents and conditions: (a) 3-bromopropan-1-ol, K_2CO_3, acetone, MW, 140 °C, 85%; (b) 4-hydroxyphenylboronic acid, $Pd(PPh_3)_4$, Na_2CO_3, toluene, EtOH, MW, 110 °C, 82%; (c) B_2pin_2, Pd_2dba_3, S-PHOS, KOAc, 1,4-dioxane, MW, 140 °C, 78%; (d) allyl bromide, Pd_2dba_3, K_2CO_3, toluene, MW, 150 °C, 47%; (e) Et_2AlCl, DCM, -20 °C to 0 °C, 70%; (f) MsCl, pyridine, DCM, -20 °C to rt, 53%; (g) NaN_3, DMF, 60 °C, 77%.

Finally, synthesis of alkyne **63** was performed as depicted in Scheme 23. Williamson alkylation of 3-bromo-4-hydroxybenzaldehyde with bromoethane, followed by Suzuki coupling of bromoderivative **98** with 4-hydroxyphenylboronic acid yielded biphenyl derivative **99**. *O*-Allylation of phenol **99**, followed by treatment of the resulting aldehyde **100** with ethynylmagnesium bromide, and subsequent reduction of the propargylic alcohol **101** provided allyl ether **102**, whose Claisen rearrangement led to the desired alkyne **63**.



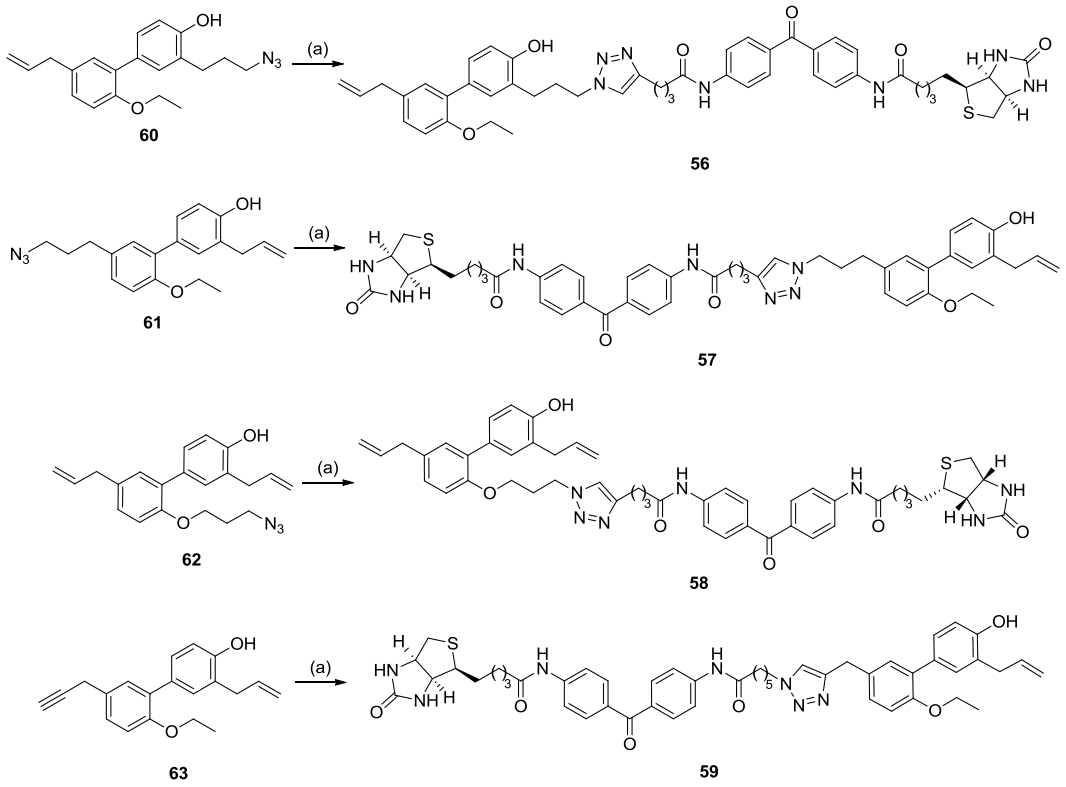
Scheme 23. Reagents and conditions: (a) EtBr, K_2CO_3, acetone, MW, 140 °C, 89%; (b) 4-hydroxyphenylboronic acid, $Pd(PPh_3)_4$, Na_2CO_3, toluene, EtOH, MW, 110 °C, 68%; (c) K_2CO_3, allyl bromide, acetone, MW, 140 °C, 96%; (d) ethynylmagnesium bromide, THF, 0 °C to rt, 97%; (e) TES, TFA, DCM, 0 °C, 33%; (f) Et_2AlCl, DCM, -20 °C to 0 °C, 51%.

Once honokiol-based fragments **60-63** were prepared, we addressed the synthesis of the corresponding tags **105** and **106** by condensation of 4,4'-diaminobenzophenone with biotin using DCC and HOBt in the presence of DMAP, and further coupling of the resulting aniline **103** either with 5-hexynoic acid or 6-azidohexanoic acid (**104**)[129] in the presence of EDC and HOBt (Scheme 24). Acid **104** was prepared as previously described[129] by nucleophilic substitution of 6-bromohexanoic acid with sodium azide.



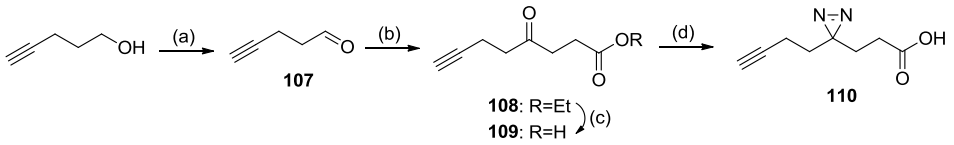
Scheme 24. Reagents and conditions: (a) biotin, DCC, HOBt, DMAP, DCM, DMF, 77 °C to rt, 97%; (b) EDC, HOBt, DCM, DMF, rt, 14-36%; (c) NaN_3, DMF, 50 °C, 76%.

Honokiol derivatives **60-63** and tags **105** and **106** were then assembled via copper-catalyzed cycloadditions as outlined in Scheme 25.



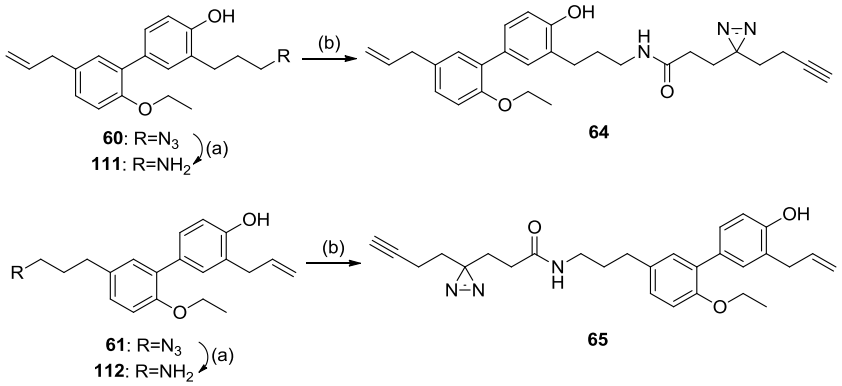
Scheme 25. Reagents and conditions: (a) **105** or **106**, $CuSO_4 \cdot 5H_2O$, sodium ascorbate, DMF, H_2O, rt, 25-64%.

With respect to probes **64** and **65**, their preparation required the synthesis of the corresponding carboxylic acid **110** by oxidation of 4-pentyn-1-ol to aldehyde **107**, followed by treatment with ethyl acrylate, further hydrolysis of the resulting ester **108**, and final transformation of ketone **109** into the diazirine **110** (Scheme 26).



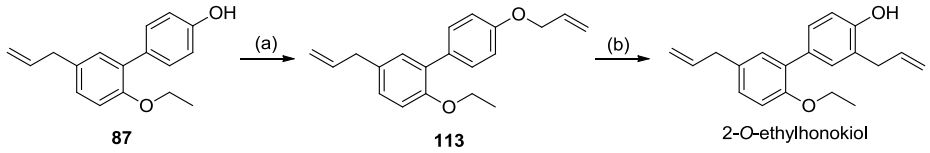
Scheme 26. Reagents and conditions: (a) (i) $(COCl)_2$, DMSO, DCM, -78 °C, (ii) Et_3N, DCM, -78 °C to rt, 85%; (b) ethyl acrylate, 3-benzyl-5-(2-hydroxyethyl)-4-methyl-1,3-thiazolium chloride, Et_3N, 1,4-dioxane, 80 °C, 28%; (c) LiOH, MeOH, rt, 95%; (d) (i) NH_3, MeOH, 0 °C, (ii) NH_2NHSO_3H, MeOH, 0 °C to rt, (iii) I_2, DIPEA, MeOH, 0 °C, 28%.

Finally, the Staudinger reaction of azides **60** and **61** using triphenylphosphine in the presence of water, followed by condensation of the obtained amines **111** and **112** with carboxylic acid **110** yielded the desired probes **64** and **65** (Scheme 27).



Scheme 27. Reagents and conditions: (a) PPh_3, THF, H_2O, reflux, 49-76%; (b) **110**, EDC, HOBt, DIPEA, DCM, rt, 45-62%.

For comparative purposes in the biological experiments, we also synthesized 2-*O*-ethylhonokiol (Scheme 28).



Scheme 28. Reagents and conditions: (a) allyl bromide, K_2CO_3, acetone, MW, 140 °C, 92%; (b) Et_2AlCl, DCM, -20 °C to 0 °C, 97%.

4.4.2. Phenotypic screening of honokiol-based probes

In order to identify the targets of honokiol and its derivatives, responsible for their antitumor effects, we first determined whether the synthesized probes kept their biological activity. Among the different malignancies in which honokiol and related compounds have been shown to be antitumorigenic,[65,124] we focused our efforts on the study of their targets in breast and ovarian cancer cell lines since these types of cancer still show dramatically high incidence and mortality rates.[130,131]

Ovarian cancer represents the leading mortality malignancy within gynaecological cancers.[132] Regarding breast cancer, among the different subtypes of malignancies

based on the expression of estrogen, progesterone, and human epidermal growth factor 2 receptors (ER, PR and HER2, respectively), triple-negative breast cancer (TNBC) - characterized by the absence of the three receptors-[133] deserves special attention. Although TNBC accounts for a low percentage of all breast tumors, it represents a disproportionate number of deaths.[134] For this reason, this group of patients has a very poor prognosis,[135,136] and therefore the discovery of new targets and drugs for the treatment of this disease is an urgent clinical challenge. Hence, the highly aggressive TNBC cell line MDA-MB-231, together with two ovarian cancer cell lines with different invasive potentials, SKOV3 and OVCAR3 (the former one being significantly more invasive than the latter one),[137] were chosen to evaluate the ability of honokiol, 2-*O*-ethylhonokiol and probes **56-59**, **64**, and **65** to inhibit cell proliferation (Table 7).

Table 7. Citotoxicity of honokiol, 2-*O*-ethylhonokiol, and honokiol-based probes **56-59**, **64**, and **65** in MDA-MB-231, SKOV3 and OVCAR3 cancer cells.

Compound	Cell viability (%)[a]		
	MDA-MB-231	SKOV3	OVCAR3
Honokiol	49±3	67±10	74±4
2-*O*-Ethylhonokiol	12±4	41±14	25±5
56	87±11	95±5	90±8
57	96±3	84±11	83±21
58	92±7	79±13	63±2
59	86±19	56±15	72±9
64	7±2	38±15	53±5
65	6±1	26±8	13±3

[a]Cell viability was determined at 50 μM and it is expressed as the average±SEM obtained from two to four independent experiments carried out in triplicate.

The obtained results (Table 7) showed that the introduction of the biotin and benzophenone-containing tags (probes **56-59**) significantly reduced their activity when compared with honokiol and 2-*O*-ethylhonokiol. However, the diazirine-containing probes **64** and **65** showed good cytotoxicity values, specially in the case of MDA-MB-231 cells, and hence were selected for further experiments.

4.4.3. In gel validation of probes **64** and **65**

Before carrying out proteomic experiments, we confirmed that probes **64** and **65** were able to label proteins in a specific way, i.e., in a concentration- and UV-dependent manners. In addition, if, as expected, some of the targets are the same as those of honokiol and 2-*O*-ethylhonokiol, their labelling must be competed by an excess of these compounds. In order to assess all these aspects, we took advantage of the presence of the terminal alkyne group in probes **64** and **65** to introduce, after cell labelling, a fluorophore suitable for in-gel fluorescence scanning (Figure 25A). Briefly, cells were treated with the probes and subjected to UV irradiation to promote covalent binding between the probe and the target proteins. After that, cells were homogenized, the soluble and membrane proteomes separated by centrifugation, and each of these two fractions was reacted with rhodamine-azide (Rh-N_3, Figure 25B) under click chemistry conditions.[138] Finally, proteins were separated by SDS-PAGE and fluorescent bands (corresponding to probe-protein complexes) visualized in a fluorescence scanner.

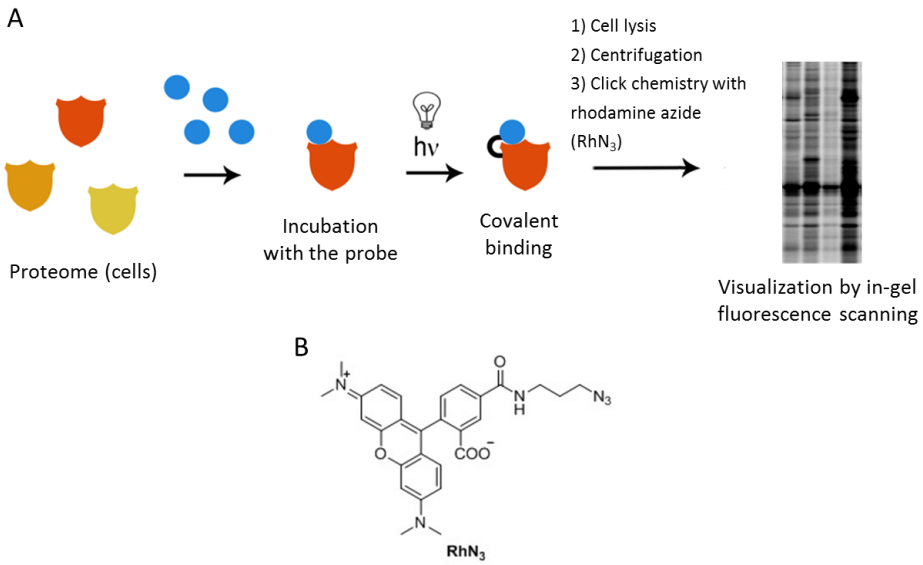


Figure 25. (A) Proteomic platform for the visualization of probe-labelled proteins by in-gel fluorescence scanning after click chemistry with rhodamine azide (RhN_3, B).

Thus, we first evaluated the UV- and concentration-dependent labelling of probes **64** and **65** (0.2-20 µM) in MDA-MB-231 cells. The results shown in Figure 26 confirm that the labelling is concentration-dependent for both probes **64** and **65** as the intensity of

the bands clearly increases with their concentration, ranging from almost no bands for concentrations under 1 μM to intense bands at 10 μM, which seems to reach saturation as as no significant increase is perceived at 20 μM. In addition, we observed that the labelling was UV-dependent since in the absence of irradiation only some non-specific fluorescence was detected (see UV negative lanes in Figure 26).

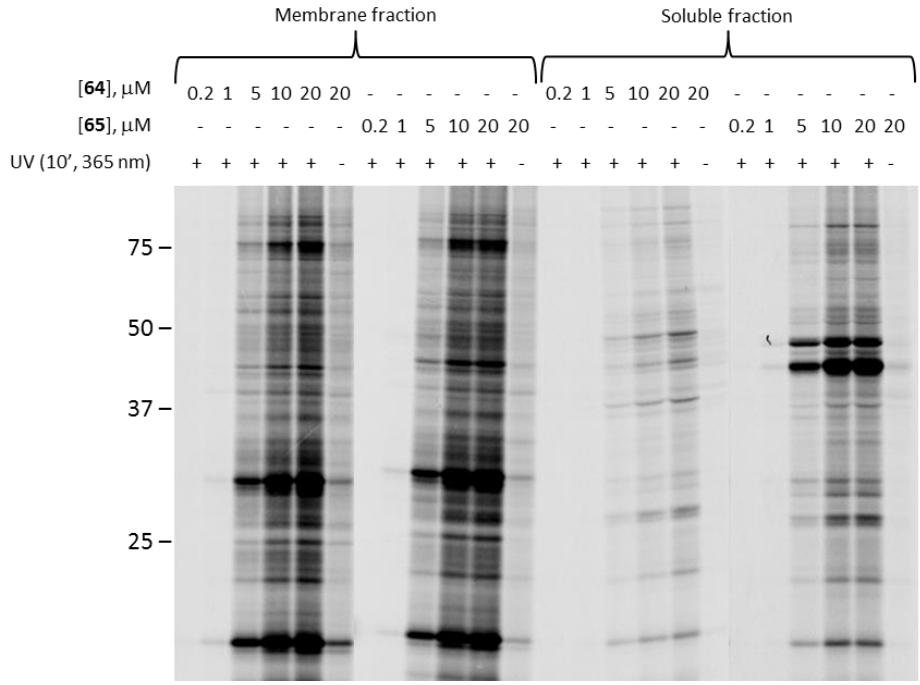


Figure 26. Concentration- and UV-dependent labelling of MDA-MB-231 breast cancer cells by probes **64** and **65**. Numbers in the left side indicate the molecular weights (in kDa) of proteins. Fluorescence is shown in grey scale. All lanes contained 20 μg of total protein and equal loading was confirmed by coomassie brilliant blue staining (see Figure S1 in the Supplementary Information).

Then, we performed competition experiments in the presence of an excess of honokiol and 2-*O*-ethylhonokiol (H and EH, respectively, in Figure 27) to ensure that at least some of the labelled proteins were targets of these compounds. Remarkably, some of the bands disappeared in the presence of an excess of honokiol and 2-*O*-ethylhonokiol both in membrane and soluble fractions (see some examples marked with an orange arrowhead in Figure 27). This result indicates that probes **64** and **65** are indeed labelling some of the targets of the honokiol family in MDA-MB-231 cells.

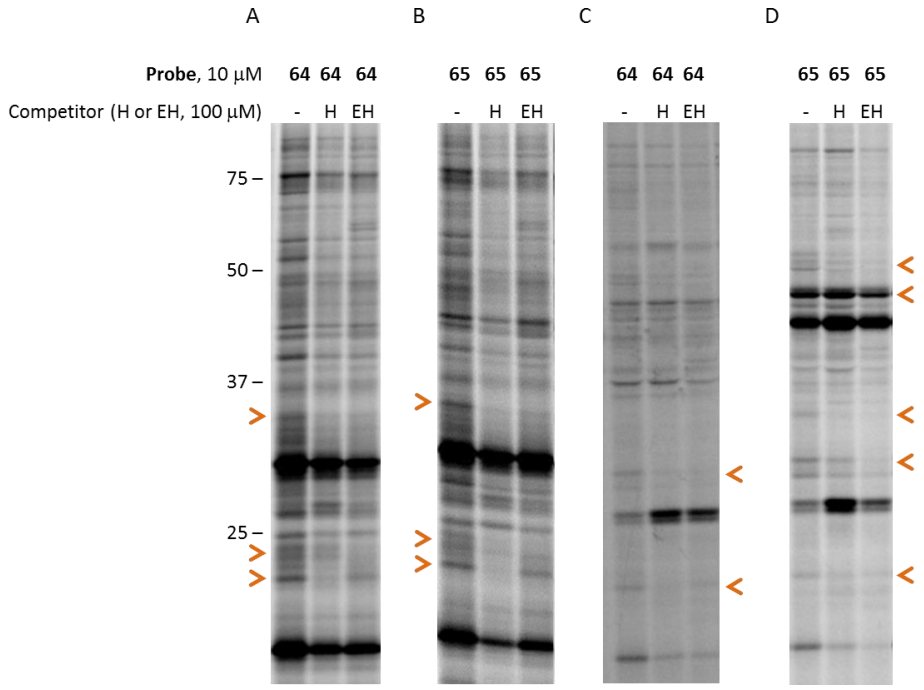


Figure 27. Competition of probes **64** (A, C) and **65** (B, D) at 10 μM by honokiol (H) or 2-*O*-ethylhonokiol (EH) at 100 μM in membrane (A, B) and soluble (C, D) proteomes, respectively, in MDA-MB-231 breast cancer cells. Numbers in the left side indicate the molecular weights (in kDa) of proteins. Fluorescence is shown in grey scale. Arrowheads show some representative competed proteins. All lanes contained 20 μg of total protein and equal loading was confirmed by coomassie brilliant blue staining (see Figure S2 in the Supplementary Information).

Next we wanted to evaluate the potential of the probes to differenciate between SKOV3 and OVCAR3 ovarian cancer cell lines, which have the same origin but different invasive capacity. After confirming that the labelling in these cells was concentration- and UV- dependent, and that this labelling could be competed by honokiol and 2-*O*-ethylhonokiol (data not shown), we used probe **65** to establish the labelling pattern in both cell lines (Figure 28). Probe **65** was selected because of its superior ability to label proteins compared to probe **64**, specially in the soluble proteome (Figure 27).

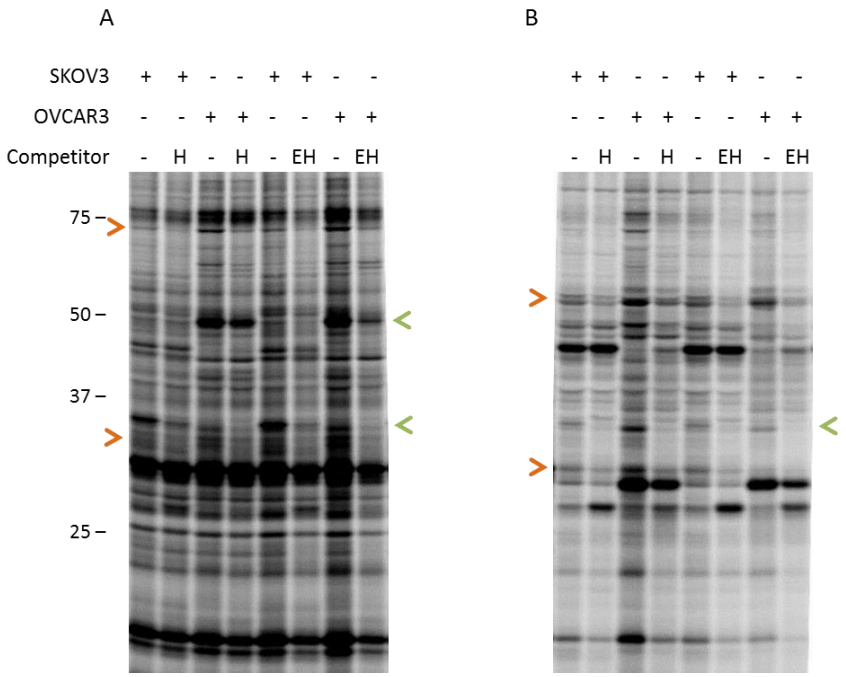


Figure 28. Labelling pattern of probe **65** in the ovarian cancer cell lines SKOV3 and OVCAR3. Competition of probe **65** at 10 μM by honokiol (H) or 2-*O*-ethylhonokiol (EH) at 100 μM in membrane (A) and soluble (B) proteomes. Numbers in the left side indicate the molecular weights (in kDa) of proteins. Fluorescence is shown in grey scale. Arrowheads show some representative bands. All lanes contained 20 μg of total protein and equal loading was confirmed by coomassie brilliant blue staining (see Figure S3 in the Supplementary Information).

The obtained labelling reveals two important findings. Firstly, both honokiol and 2-*O*-ethylhonokiol target specific proteins in these cell lines. These targets are those labelled by probe **65** which disappear in the presence of an excess of honokiol and/or 2-*O*-ethylhonokiol. Some examples are marked with an orange arrowhead around 35 and 75 kDa in the membrane fraction of the proteome (Figure 28A), or around 30 and 50 kDa in the soluble one (Figure 28B). Secondly, there are differences in the obtained profiling between the two cell lines (Figure 28, green arrowheads). For example, in the membrane fraction of the proteome we can observe a clear band around 50 kDa that shows differences in terms of expression, being almost imperceptible in the SKOV3 cell line, while showing an intense fluorescence in the OVCAR3 cells, and which is highly competed by 2-*O*-ethylhonokiol (Figure 28A). Conversely, we observe a band around 37 kDa that is only labelled in the SKOV3 cells but not in the OVCAR3 ones, and that is highly competed by both honokiol and 2-*O*-ethylhonokiol (Figure 28A). As for the soluble

proteome, we observe two bands around 37 kDa, one of them only expressed in the SKOV3 cell line, and the other one in the less aggressive partner (OVCAR3), both of them being competed by honokiol and 2-*O*-ethylhonokiol (Figure 28B).

In summary, the in situ profiling in MDA-MB-231 (Figures 25 and 26) and ovarian (Figure 28) cancer cell lines confirms the suitability of probes **64** and **65** for identifying the targets of honokiol and 2-*O*-ethylhonokiol in these systems.

4.4.4. Proteomic profiling of probe **65**

Proteins targeted by probe **65** were enriched and identified using stable isotope labelling by amino acids in cell culture (SILAC) quantitative MS.[139,140] In SILAC experiments, two groups of cells are grown in parallel using the same culture media but with at least one essential amino acid isotopically labelled in one of the groups (termed heavy), while the same amino acid is the light version in the other group. Since cells are not able to synthesize essential amino acids, they are forced to use only the externally supplied one, eventually incorporating 100% of the given ‘‘light” or ‘‘heavy’’ amino acid in all the proteins. Finally, after lysing the “heavy” and “light” cells, they can be combined and the mixture can be treated as a single sample in all subsequent steps. Hence, once the final sample has been trypsinized, SILAC peptide pairs have the same retention time, but different mass, thus enabling the measurement of the ratio between both peptides, referred to as SILAC ratio, providing an accurate method for quantification of the labelled proteins.

In particular, in these experiments cells are grown in the presence of either the heavy version of Lys ($^{13}C_6$, ^{15}N) and Arg ($^{13}C_6$, $^{15}N_4$) or their light versions ($^{12}C_6$, ^{14}N-Lys; $^{12}C_6$, $^{14}N_4$-Arg). Hence, the same peptide will differentiate in 7 mass units if it possesses only one Lys residue or in 10 mass units if it possesses only one Arg residue.

Hence, MDA-MB-231 cells were cultured for at least six cell doublings to ensure complete incorporation of the light or heavy version of the amino acids in all the proteins. Then, “heavy” cells were incubated with 10 µM of probe **65**, and “light” cells with 10 µM of probe **65** plus 100 µM of honokiol. Both samples were UV-irradiated, homogenized and mixed in a 1:1 ratio (Figure 29A). After that, the sample was coupled to a biotin-azide reporter tag (biotin-N_3, Figure 29B) under click chemistry conditions,[141] and probe-protein complexes were captured by incubation with streptavidin beads.

After digestion with trypsin, the obtained peptides were separated by LC and identified by MS. The resulting SILAC ratios were normalized in a 1-20 scale, where a ratio of 1 indicates no difference between the light and heavy labelled samples, and therefore no competition by honokiol, and 20 is the maximal difference.

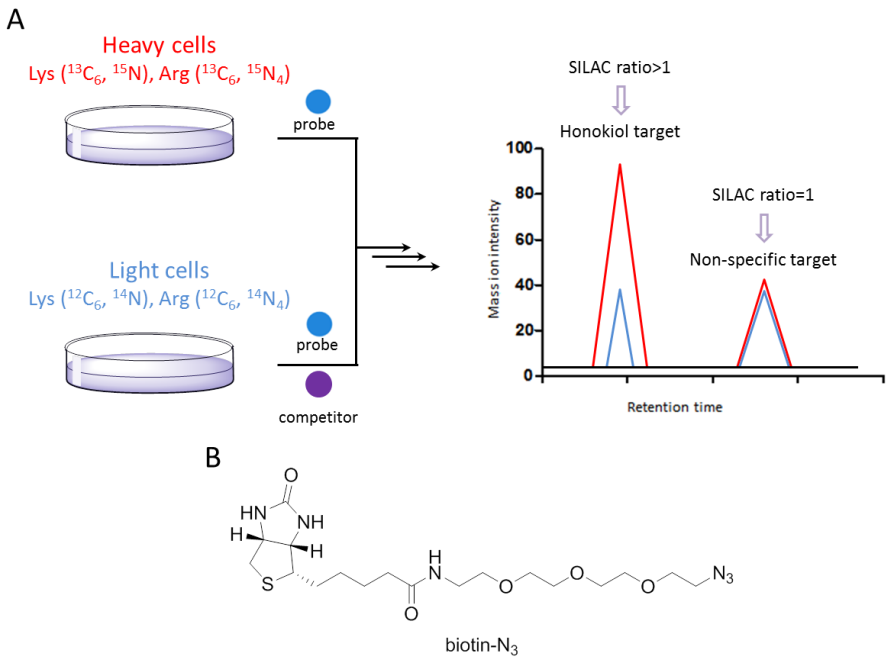


Figure 29. (A) Scheme of the proteomic platform for the preparation of SILAC samples. (B) Structure of the biotin-azide reporter tag.

Figure 30A shows the heavy:light SILAC ratio plot for all the identified proteins using probe **65**. Proteins that exhibited SILAC ratios ≥3 were designated as preferred targets of honokiol (Figure 30B). Therefore, the use of probe **65** enabled the identification of about 40 proteins that were significantly competed by honokiol (Table 8), highlighting the suitability of probe **65** for the study of the targets of this compound in cancer cells.

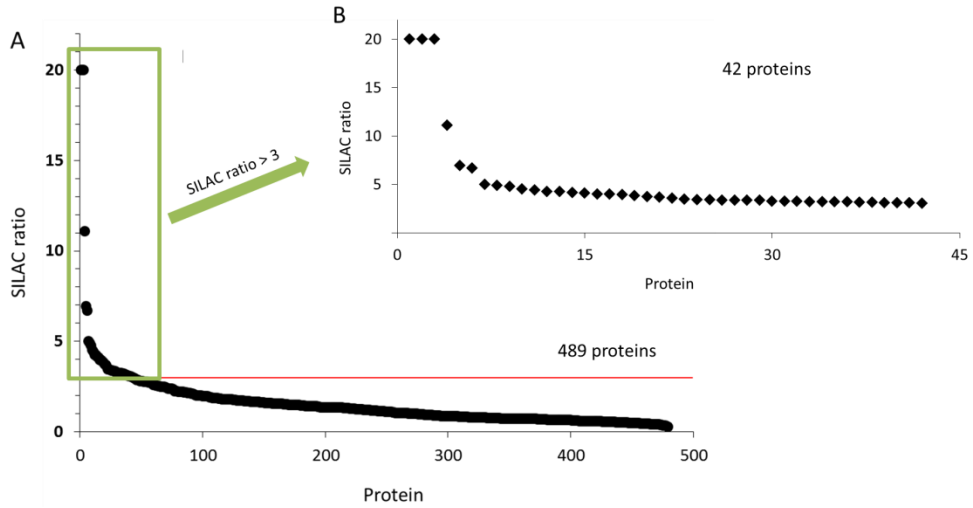


Figure 30. (A) SILAC ratio plots for total proteins or (B) proteins with a SILAC ratio≥3, identified comparing cells treated with probe **65** at 10 µM versus probe **65** at 10 µM plus honokiol at 100 µM.

Among the identified proteins showed in Table 8, some of them deserve particular attention since they are involved in mechanisms related to the viability of cancer cells such as apoptosis, cell proliferation or cell growth. For example, LYRIC protein (pdb number Q86UE4)[142] activates the nuclear factor kappa B (NF-κB), transcription factor which interestingly has been previously related to honokiol.[65]

Although in-depth validation of these hits is ongoing in our laboratory, the results reported herein provide, for the first time, direct insights into the mechanism of action of the natural product honokiol and its derivatives, aiding to the identification of new targets for addressing currently unmet medical needs.

Table 8. Proteins with SILAC ratio≥3

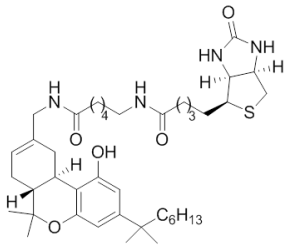
SILAC ratio	Identifier	Description
20	P52209	PGD 6-phosphogluconate dehydrogenase, decarboxylating
20	Q86UE4	MTDH Protein LYRIC
20	P53396	ACLY ATP-citrate synthase
11.1	P07384	CAPN1 Calpain-1 catalytic subunit
6.95	Q9BWD1	ACAT2 Acetyl-CoA acetyltransferase, cytosolic
6.69	P43490	NAMPT Nicotinamide phosphoribosyltransferase
5.02	P29401	TKT Transketolase
4.9	P07339	CTSD Cathepsin D
4.78	P40926	MDH2 Malate dehydrogenase, mitochondrial
4.53	P00367	GLUD1 Glutamate dehydrogenase 1, mitochondrial
4.41	P04181	OAT Ornithine aminotransferase, mitochondrial
4.25	P36776	LONP1 Lon protease homolog, mitochondrial
4.24	P46821	MAP1B Microtubule-associated protein 1B
4.14	P07195	LDHB L-lactate dehydrogenase B chain
4.1	P39687	ANP32A Acidic leucine-rich nuclear phosphoprotein 32 family member A
3.98	P24752	ACAT1 Acetyl-CoA acetyltransferase, mitochondrial
3.97	P62937	PPIA Peptidyl-prolyl cis-trans isomerase A
3.92	P11498	PC Pyruvate carboxylase, mitochondrial
3.84	P10809	HSPD1 60 kDa heat shock protein, mitochondrial
3.72	P49327	FASN Fatty acid synthase
3.7	P14618	PKM Pyruvate kinase isozymes M1/M2
3.56	P26038	MSN Moesin
3.47	P23526	AHCY Adenosylhomocysteinase
3.44	P07900	HSP90AA1 Heat shock protein HSP 90-alpha
3.42	Q01105	SET Protein SET
3.37	Q9NY33	DPP3 Dipeptidyl peptidase 3
3.37	P31948	STIP1 Stress-induced-phosphoprotein 1
3.36	P06733	ENO1 Alpha-enolase
3.34	P06737	PYGL Glycogen phosphorylase, liver form
3.25	P08670	VIM Vimentin
3.24	P47897	QARS Glutamine--tRNA ligase
3.24	P60174	TPI1 Triosephosphate isomerase
3.23	P07737	PFN1 Profilin-1
3.23	Q86VP6	CAND1 Cullin-associated NEDD8-dissociated protein 1
3.22	P15311	EZR Ezrin
3.2	O00299	CLIC1 Chloride intracellular channel protein 1
3.16	P22314	UBA1 Ubiquitin-like modifier-activating enzyme 1
3.15	P22392	NME2 Nucleoside diphosphate kinase B
3.12	O43707	ACTN4 Alpha-actinin-4
3.09	P08238	HSP90AB1 Heat shock protein HSP 90-beta
3.08	P62258	YWHAE 14-3-3 protein epsilon
3.04	Q96TA1	FAM129B Niban-like protein 1

CONCLUSIONS

5. CONCLUSIONS

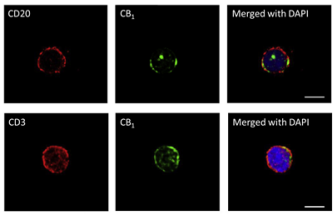
In the present work we have focused our efforts on the development of chemical probes aimed at the study of the ECS. In particular, we were interested in: i) the detection of CBRs in the immune system, ii) studying the functions of the mtCB_1R, iii) the discovery of the off-targets of the cannabinoid agonists HU210 and HU308, and iv) the identification of the targets of the natural product honokiol.

With respect to the first goal, biotinylated probe **1** with high affinity toward both CBRs enabled the visualization of the CB_1R in several cell subsets of immune cells, thus proving the high levels of expression of this receptor in patients with different types of allergic diseases.



1

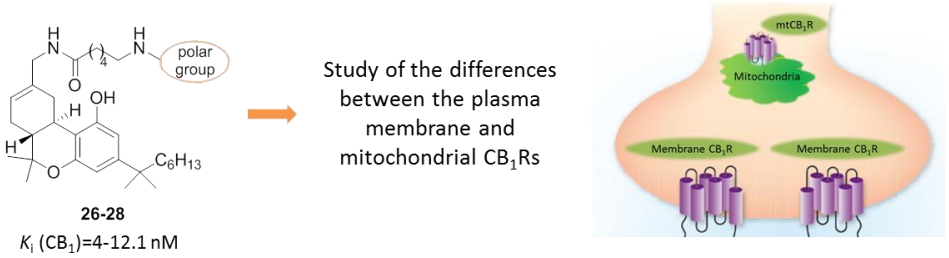
Visualization of CB_1R in the immune system



In addition, the fluorescent CB_1R-selective probe **22** has been successfully used to detect and semi-quantify the levels of expression of CB_1R in PBMCs, standing out as a valuable tool to study possible modifications in such levels in clinically relevant systems, and to establish its potential as a biomarker.

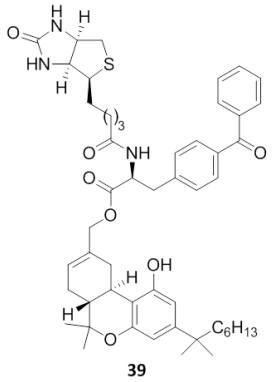
22

Concerning the study of the mtCB$_1$R, polar synthesized compounds **26-28**, with high affinity values for the CB$_1$R (K_i=4-12.1 nM), represent promising tools to differenciate between the effects mediated by the plasma membrane CB$_1$R and the mtCB$_1$R.



26-28
K_i (CB$_1$)=4-12.1 nM

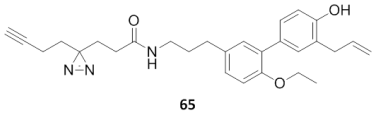
Regarding HU210-based probe **39**, featuring a benzophenone group and a biotin subunit, it displayed high affinity values for CBRs [K_i (CB$_1$)=25.7±0.4 nM; K_i (CB$_2$)=12.1±0.2 nM], and was therefore selected to carry out proteomic experiments. The use of **39** enabled the identification of 15 proteins of different functional classes, which participate in diverse biological processes. Among them, the identification of the CB$_2$R deserves special attention since it supports the solidness of the platform and therefore, validates the methodology.



39

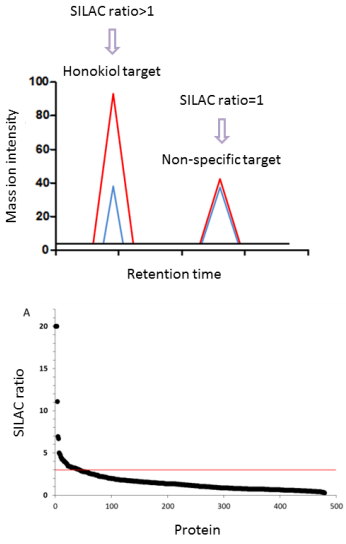
Proteomic platform set up: Identification of the targets of the cannabinoid ligand HU210

Finally, we have developed a series of chemical probes for the identification of the targets of honokiol and its derivatives. Among the synthesized compounds, derivative **65** was used in quantitative SILAC MS experiments to enable the identification of some of the direct targets of honokiol in MDA-MB-231 cells, such as the cancer related protein LYRIC, providing for the first time direct insights into the mechanism of action of this natural product, and aiding to the identification of new targets for addressing currently unmet medical needs.



65

Proteomic platform: Identification of the targets of honokiol by SILAC



EXPERIMENTAL SECTION

6. EXPERIMENTAL SECTION

6.1. Synthesis

Unless otherwise stated, the starting materials, reagents, and solvents were purchased as high-grade commercial products from Sigma-Aldrich, Acros, ABCR, Fluorochem, Bachem, Scharlab, or Panreac. Dry dichloromethane (DCM), tetrahydrofuran (THF), and Et_2O were obtained by passing the previously degassed solvents through activated alumina columns using a Pure Solv™ Micro 100 Liter solvent purification system. Acetone was dried under K_2CO_3. Triethylamine and pyridine were dried over KOH and distilled before using. Reactions under MW irradiation were performed in a Biotage Initiator 2.5 reactor.

Analytical thin-layer chromatography (TLC) was run on Merck silica gel plates (Kieselgel 60 F-254), with detection by UV light (λ=254 nm), ninhydrin solution, 10% phosphomolybdic acid solution in ethanol, or $KMnO_4$ (aqueous, aq). Unless otherwise stated, products were purified by flash chromatography using a VARIAN 971-FP system with cartridges of silica gel (Varian, particle size 50 μm). Alternatively, purification on glass column with silica gel type 60 (particle size 230-400 mesh from Merck) was performed in some indicated cases. For all compounds containing an allyl chain attached to a phenyl ring, silica gel was previously neutralized with triethylamine.

All compounds were obtained as oils, except for those whose melting points (mp) are indicated, which were solids. Mp (uncorrected) were determined on a Stuart Scientific electrothermal apparatus. Optical rotation [α] was measured on a Perkin Elmer 241 polarimeter or an Anton Paar MCP 100 modular circular polarimeter using a

sodium lamp (λ=589 nm) with a 1 dm path length; concentrations (c) are given as g/100 mL. Infrared (IR) spectra were measured on a Bruker Tensor 27 instrument equipped with a Specac ATR accessory of 5200-650 cm^{-1} transmission range; frequencies (ν) are expressed in cm^{-1}. Nuclear magnetic resonance (NMR) spectra were recorded on a Bruker Avance III 700 MHz (^{1}H, 700 MHz; ^{13}C, 175 MHz), Bruker Avance 500 MHz (^{1}H, 500 MHz; ^{13}C, 125 MHz) or Bruker DPX 300 MHz (^{1}H, 300 MHz; ^{13}C, 75 MHz) instrument at room temperature (rt) at the Universidad Complutense de Madrid (UCM) NMR core facility, being the last one the routine equipment, always used unless otherwise stated. Chemical shifts (δ) are expressed in parts per million relative to internal tetramethylsilane; coupling constants (*J*) are in hertz (Hz). The following abbreviations are used to describe peak patterns when appropriate: s (singlet), d (doublet), t (triplet), q (quartet), qt (quintet), sept (septet), m (multiplet), app (apparent), and br (broad). 2D NMR experiments -homonuclear correlation spectroscopy (H,H-COSY), heteronuclear multiple quantum correlation (HMQC) and heteronuclear multiple bond correlation (HMBC)- of representative compounds were acquired to assign protons and carbons of new structures. High resolution mass spectrometry (HRMS) spectra were recorded on a FTMS Bruker APEX Q IV spectrometer in electrospray ionization (ESI) or matrix-assisted laser desorption ionization (MALDI) mode at UCM mass spectrometry core facility.

For all final compounds, purity was determined by high-performance liquid chromatography coupled to mass spectrometry (HPLC-MS) using an Agilent 1200LC-MSD VL instrument, and satisfactory chromatograms confirmed a purity of at least 95% for all tested compounds. LC separation was achieved with a Zorbax SB-C3 column (5 µm, 2.1 mm x 50 mm), together with a guard column (5 µm, 4.6 mm x 12.5 mm). The gradient mobile phase consisted of A (95:5 water/acetonitrile) and B (5:95 water/acetonitrile) with 0.1% ammonium hydroxide and 0.1% formic acid as solvent modifiers, and the gradients are indicated in Table 9. Spectra were acquired in positive or negative ionization mode from 100 to 1000 *m/z* and in UV-mode at four different wavelengths (210, 230, 254, and 280 nm). MS analysis was performed with an ESI source. The capillary voltage was set to 3.0 kV and the fragmentor voltage was set at 25 eV. The drying gas temperature was 350 ºC, the drying gas flow was 10 L/min, and the nebulizer pressure was 20 psi.

Table 9. HPLC gradients.

Method A		Method B	
t (min)	% B	t (min)	% B
0	0	0	0
2	0	2	0
8	80	8	60
12	100	20	100
25	100	25	100
30	0	30	0

IUPAC rules have been followed for naming all organic compounds, except for the radicals {5-[(3a*S*,4*S*,6a*R*)-2-oxohexahydro-1*H*-thieno[3,4-*d*]imidazol-4-yl]pentanoyl}, prop-2-en-1-yl and prop-2-yn-1-yl, whose common names biotinyl, allyl, and propargyl, respectively, have been employed for simplicity.

6.1.1. General procedures

6.1.1.1. Cleavage of silyl ethers

To a solution of the corresponding silyl-protected phenol (1 equiv) in anhydrous THF (50 mL/mmol) at 0 ºC and under an argon atmosphere, a 1 M solution of TBAF (1.3 equiv) in anhydrous THF was added, and the mixture was stirred at this temperature for 3 h. EtOAc and water were added, and the organic layer was separated, washed with brine, dried (Na_2SO_4), filtered, and evaporated under reduced pressure. The crude was purified by chromatography using the appropriate eluent to afford the corresponding phenol.

6.1.1.2. Williamson alkylation of phenols

To a solution of the corresponding phenol (1 equiv) in dry acetone (7.5 mL/mmol) under an argon atmosphere, K_2CO_3 (1.2 equiv) was added at rt and the mixture was stirred for 10 min. The corresponding bromoalkane (1.2 equiv) was then added and the reaction was heated at 140 ºC for 20 min under MW irradiation. Once cooled to rt, water was added and the mixture was extracted with EtOAc (2x), washed with brine, dried (Na_2SO_4), filtered, and evaporated under reduced pressure. The crude was purified by

chromatography using the appropriate eluent to afford the corresponding aryl alkyl ether.

6.1.1.3. Suzuki coupling of bromoderivatives and 4-hydroxyphenylboronic acid

To a solution of the corresponding bromoderivative (1 equiv) and 4-hydroxyphenylboronic acid (1.5 equiv) in a mixture of toluene/EtOH 1:1 (12 mL/mmol) a solution of 0.5 M Na_2CO_3 (aq, 3 equiv) was added and the mixture was degassed with argon. Then, $Pd(PPh_3)_4$ (0.06 equiv) was added and the reaction mixture was heated at 110 ºC for 15 min under MW irradiation. The organic phase was separated, and the aqueous layer was extracted with EtOAc (2x). The combined organic extracts were washed with brine, dried (Na_2SO_4), filtered, and evaporated under reduced pressure. The crude was purified by chromatography using the appropriate eluent to afford the corresponding biphenyl derivative.

6.1.1.4. Hydroboration-oxidation of allyl chains

To a well-stirred solution of the corresponding allyl derivative (1 equiv) in anhydrous THF (2 mL/mmol) at rt, BH_3 (2 equiv, 1 M in THF) was added and the mixture was stirred for 4 h. Water was added (0.2 mL/mmol) followed by 3 M NaOH (aq, 0.3 mL/mmol). Then, 30% H_2O_2 (aq, 3.3 mL/mmol) was slowly added at 0 ºC and the mixture was stirred at rt for 16 h. The reaction was diluted with EtOAc, washed with water and brine, dried (Na_2SO_4), filtered, and evaporated under reduced pressure. The crude was purified by chromatography using the appropriate eluent to afford the corresponding primary alcohol.

6.1.1.5. Transformation of chloroderivatives into boronates

A suspension of the corresponding chloroderivative (1 equiv), B_2pin_2 (1.5 equiv), Pd_2dba_3 (0.05 equiv), S-PHOS (0.2 equiv), and KOAc (3 equiv), in anhydrous 1,4-dioxane (2 mL/mmol) was heated under an argon atmosphere at 140 ºC for 45 min under MW irradiation. Once at rt, the mixture was filtered through a pad of celite, washed with EtOAc and the solvents were evaporated under reduced pressure. The residue was purified by chromatography using the appropriate eluent to afford the corresponding boronate.

6.1.1.6. Suzuki coupling of boronates with allyl bromide

A suspension of the corresponding boronate (1 equiv), allyl bromide (1.2 equiv), Pd_2dba_3 (0.24 equiv), and K_2CO_3 (6 equiv) in anhydrous toluene (9 mL/mmol of boronate) under an argon atmosphere, was heated at 150 ºC for 45 min under MW irradiation. The mixture was filtered through a pad of celite, washed with EtOAc, and the solvents were evaporated under reduced pressure. The residue was purified by chromatography using the appropriate eluent to afford the corresponding allyl arene.

6.1.1.7. Activation of primary alcohols as mesylates

To a well-stirred solution of the corresponding alcohol (1 equiv) in dry DCM (9.5 mL/mmol) at -20 ºC and under an argon atmosphere, methanesulfonyl chloride (MsCl, 1.05 equiv) and anhydrous pyridine (4.2 equiv) were added. The reaction mixture was allowed to warm to rt and stirred for 16 h. Then, concentrated HCl was carefully added and the resulting solution was extracted with EtOAc (2x). The combined organic extracts were sequentially washed with saturated $CuSO_4$ (aq) and brine, dried (Na_2SO_4), filtered, and evaporated under reduced pressure. The residue was purified by chromatography using the appropriate eluent to afford the corresponding mesylate.

6.1.1.8. Nucleophilic substitution of mesylates with NaN_3

To a solution of the corresponding mesylate (1 equiv) in anhydrous DMF (6 mL/mmol) under an argon atmosphere, NaN_3 (2 equiv) was added and the reaction mixture was stirred at 60 ºC for 16 h. The resulting solution was diluted with EtOAc, washed with water and brine, dried (Na_2SO_4), filtered, and evaporated under reduced pressure. The residue was purified by chromatography using the appropriate eluent to afford the corresponding azide.

6.1.1.9. Claisen rearrangement

To a solution of the corresponding allyl phenyl ether (1 equiv) in dry DCM (7 mL/mmol) at -20 ºC and under an argon armosphere, diethylaluminium chloride (2.5 equiv, 1 M in hexane) was slowly added and the mixture was allowed to warm to 0 ºC and stirred at that temperature for 3 h. The reaction was quenched with 1 M HCl (aq) at 0 ºC and the mixture was extracted with EtOAc (2x), washed with brine, dried (Na_2SO_4),

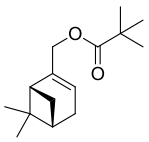
filtered and evaporated under reduced pressure. The residue was purified by chromatography using the appropriate eluent to afford the corresponding phenol.

6.1.2. Synthesis of probes **1**, **2**, **20**, and **22**

- **Synthesis of intermediates 11 and 13**

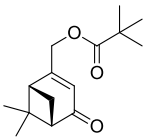
Derivatives **4-6** were obtained following experimental procedures previously described by Liddle *et al*. and their spectroscopic data correspond with those reported.[143]

(-)-[(1*R*,5*S*)-6,6-Dimethylbicyclo[3.1.1]hept-2-en-2-yl]methyl pivalate [(-)-4]. To a solution of (1*R*,5*S*)-myrtenol (5.00 g, 33 mmol) in dry DCM (30 mL) and pyridine (30 mL) at 0 ºC and under an argon atmosphere, trimethylacetyl chloride (5.0 mL, 41 mmol) was added dropwise and the reaction was stirred at 0 ºC for 2.5 h. Et_2O was then added and the mixture was washed with 10% HCl (aq, 6x), saturated $NaHCO_3$ (aq), and brine. The organic layer was dried (Na_2SO_4), filtered, and evaporated under reduced pressure to yield the title compound (-)-**4** (7.12 g, 92%), which was used in the next step without further purification. $[\alpha]_D^{20}$: -14.0 (c=3.2, EtOH). R_f: 0.25 (hexane/DCM, 7:3).



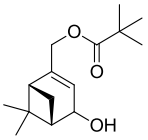
^{1}H-NMR ($CDCl_3$, δ): 0.85 (s, 3H, CH_3), 1.20 (s, 9H, $C(CH_3)_3$), 1.26-1.29 (m, 4H, ½CH_{2cyc}, CH_3), 2.10 (dd, *J*=5.5, 1.2, 2H, 2CH), 2.26-2.29 (m, 2H, CH_2CH=), 2.39 (dt, *J*=8.6, 5.6, 1H, ½CH_{2cyc}), 4.43 (AB system, *J*=12.5, 2H, CH_2O), 5.50-5.53 (m, 1H, CH=).

(-)-[(1*R*,5*S*)-6,6-Dimethyl-4-oxobicyclo[3.1.1]hept-2-en-2-yl]methyl pivalate [(-)-5]. To a suspension of CrO_3 (36.20 g, 362 mmol) in dry DCM (270 mL) at -20 ºC, 3,5-dimethylpyrazole (37.70 g, 362 mmol) was added portionwise. After stirring for 15 min, a solution of compound (-)-**4** (7.12 g, 30 mmol) in dry DCM (50 mL) was added and the mixture was stirred at 0 ºC for 1 h. The organic phase was separated, washed with 10% HCl (aq) and brine, dried (Na_2SO_4), filtered, and evaporated under reduced pressure. The residue was purified by chromatography (hexane/EtOAc, 9:1) to afford ketone (-)-**5** (3.49 g, 46%). $[\alpha]_D^{20}$: -92.3 (c=2.6, EtOH). R_f: 0.33 (chloroform).



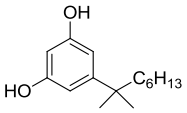
^{1}H-NMR ($CDCl_3$, δ): 1.02 (s, 3H, CH_3), 1.22 (s, 9H, $C(CH_3)_3$), 1.51 (s, 3H, CH_3), 2.12 (d, *J*=9.3, 1H, ½CH_{2cyc}), 2.43 (td, *J*=6.6, 1.3, 1H, CH), 2.69 (td, *J*=6.0, 1.7, 1H, CH), 2.86 (dt, *J*=9.3, 5.5, 1H, ½CH_{2cyc}), 4.70 (AB system, *J*=16.6, 2H, CH_2O), 5.83-5.86 (m, 1H, CH=).

(-)-[(1*R*,5*S*)-4-Hydroxy-6,6-dimethylbicyclo[3.1.1]hept-2-en-2-yl]methyl pivalate [(-)-6]. To a solution of ketone (-)-**5** (3.49 g, 14 mmol) in anhydrous THF (14 mL) at 0 ºC and under an argon atmosphere, lithium tri-*tert*-butoxyaluminium hydride (5.17 g, 20 mmol) was added portionwise. The mixture was allowed to warm to rt and stirred for 4 h. The reaction was quenched with saturated NH_4Cl (aq) and extracted with Et_2O. The combined organic extracts were dried (Na_2SO_4), filtered, and evaporated under reduced pressure. The residue was purified by chromatography (hexane to hexane/ EtOAc, 8:2) to yield alcohol (-)-**6** (2.17 g, 63%). $[\alpha]_D^{20}$: -10.3 (c=2.0, EtOH). R_f: 0.25 (hexane/EtOAc, 7:3).



^{1}H-NMR ($CDCl_3$, δ): 1.10 (s, 3H, CH_3), 1.22 (s, 9H, $C(CH_3)_3$), 1.35-1.38 (m, 4H, CH_3, ½CH_{2cyc}), 1.65 (br s, 1H, OH), 2.11 (td, *J*=5.6, 1.2, 1H, CHC=), 2.32-2.38 (m, 1H, CHCHOH), 2.51 (dt, *J*=9.3, 5.5, 1H, ½CH_{2cyc}), 4.45-4.57 (m, 3H, CH_2O, CHOH), 5.66-5.68 (m, 1H, CH=).

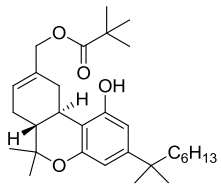
5-(1,1-Dimethylheptyl)benzene-1,3-diol (7). To a solution of 1-(1,1-dimethylheptyl)-3,5-dimethoxybenzene (2.36 g, 8.6 mmol) in dry DCM (9.2 mL) at 0 ºC and under an argon atmosphere, BBr_3 (23 mL, 1 M in DCM) was added dropwise. The reaction mixture was stirred at 0 ºC for 2 h, allowed to warm to rt and stirred for 16 h. The mixture was then cooled to 0 ºC, carefully quenched with water, extracted with 10% NaOH (aq), acidified with concentrated HCl, and extracted with Et_2O (2x). The combined organic extracts were dried (Na_2SO_4), filtered, and evaporated under reduced pressure to yield resorcinol **7** (2.04 g, 100%), which was used in the next step without further purification. Mp: 93-95 ºC (lit.[144] 97-99 ºC). R_f: 0.50 (hexane/EtOAc, 7:3). The spectroscopic data correspond with those previously reported.[144]



^{1}H-NMR ($CDCl_3$, δ): 0.85 (t, *J*=6.5, 3H, $C\underline{H}_3CH_2$), 1.16-1.22 (m, 14H, $2CH_3$, $(CH_2)_4$), 1.48-1.54 (m, 2H, $C\underline{H}_2C(CH_3)_2$), 5.79 (br s, 2H, 2OH), 6.21 (t, *J*=2.1, 1H, CH_{Ar}), 6.41 (d, *J*=2.1, 2H, $2CH_{Ar}$).

(-)-[(6a*R*,10a*R*)-3-(1,1-Dimethylheptyl)-1-hydroxy-6,6-dimethyl-6a,7,10,10a-tetrahydro-6*H*-benzo[*c*]chromen-9-yl]methyl pivalate (8). Benzochromene **8** was obtained following experimental procedures previously described by Mechoulam *et al*.[84]

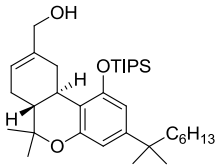
To a stirred solution of resorcinol **7** (2.31 g, 9.8 mmol) and alcohol (-)-**6** (2.46 g, 9.8 mmol) in dry DCM (200 mL) at -20 ºC and under an argon atmosphere, $BF_3{\cdot}Et_2O$ (6.6 mL, 7.4 mmol) was added dropwise. The mixture was allowed to warm to rt and then stirred for additional 20 min. The mixture was carefully washed with saturated $NaHCO_3$ (aq) and extracted with Et_2O. The combined organic extracts were dried (Na_2SO_4), filtered, and evaporated under reduced pressure. The residue was purified by chromatography (hexane to hexane/EtOAc, 9:1) to give ester **8** (2.32 g, 50%). $[\alpha]_D^{20}$: -144.7 (c=2.9, EtOH). R_f: 0.14 (hexane/EtOAc, 95:5).



^{1}H-NMR ($CDCl_3$, δ): 0.85 (t, *J*=6.6, 3H, $C\underline{H}_3CH_2$), 1.13 (s, 3H, CH_3C_{cyc}), 1.17-1.28 (m, 23H, $C(CH_3)_3$, $2CH_3$, $(CH_2)_4$), 1.41 (s, 3H, CH_3C_{cyc}), 1.48-1.54 (m, 2H, $C\underline{H}_2C(CH_3)_2$), 1.84-1.94 (m, 3H, ½$CH_2C$=, ½$C\underline{H}_2CH$=, $C\underline{H}C(CH_3)_2$), 2.27-2.36 (m, 1H, ½$C\underline{H}_2CH$=), 2.71 (td, *J*=11.0, 4.6, 1H, CHC_{Ar}), 3.36 (dd, *J*=16.7, 3.9, 1H, ½CH_2C=), 4.52 (AB system, *J*=13.5, 2H, CH_2O), 4.79 (br s, 1H, OH), 5.77 (d, *J*=4.7, 1H, CH=), 6.24 (d, *J*=1.7, 1H, CH_{Ar}), 6.40 (d, *J*=1.7, 1H, CH_{Ar}).

(-)-{(6a*R*,10a*R*)-3-(1,1-Dimethylheptyl)-6,6-dimethyl-1-[(triisopropylsilyl)oxy]-6a,7,10,10a-tetrahydro-6*H*-benzo[*c*]chromen-9-yl}-methanol (9). A solution of **8** (1.31 g, 3.0 mmol), TIPS-Cl (2.0 mL, 9.2 mmol), and imidazole (832 mg, 12 mmol) in anhydrous DMF (18 mL) under an argon armosphere, was heated at 200 ºC for 45 min under MW irradiation. Once at rt, the solvent was evaporated under reduced pressure and the residue was redissolved in EtOAc, washed with saturated $NaHCO_3$ (aq) and brine. The organic layer was dried (Na_2SO_4), filtered, and evaporated under reduced pressure to yield {(6a*R*,10a*R*)-3-(1,1-dimethylheptyl)-6,6-dimethyl-1-[(triisopropylsilyl)oxy]-6a,7,10,10a-tetrahydro-6*H*-benzo[*c*]chromen-9-yl}methyl pivalate (1.82 g, 95%), which was used in the next step without further purification. ^{1}H-NMR ($CDCl_3$, δ): 0.83 (t, *J*=6.8, 3H, C<u>H</u>$_3$CH$_2$), 1.05-1.30 (m, 47H, CH_3C_{cyc}, $C(CH_3)_3$, $2CH_3$, $3CH(CH_3)_2$, $(CH_2)_4$), 1.38 (s, 3H, CH_3C_{cyc}), 1.46-1.52 (m, 2H, C<u>H</u>$_2$C(CH$_3$)$_2$), 1.81-1.96 (m, 3H, ½CH_2C=, ½C<u>H</u>$_2$CH=, C<u>H</u>C(CH$_3$)$_2$), 2.19-2.27 (m, 1H, ½C<u>H</u>$_2$CH=), 2.57-2.68 (m, 1H, CHC_{Ar}), 3.31 (dd, *J*=16.2, 3.9, 1H, ½CH_2C=), 4.42-4.51 (m, 2H, CH_2O), 5.72 (d, *J*=4.0, 1H, CH=), 6.34 (d, *J*=1.8, 1H, CH_{Ar}), 6.39 (d, *J*=1.8, 1H, CH_{Ar}).

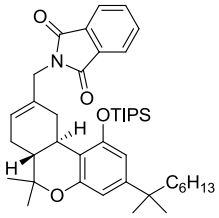
A solution of the silyl ether synthesized above (1.82 g, 2.9 mmol) in anhydrous THF (50 mL) was added dropwise to a suspension of $LiAlH_4$ (441 mg, 12 mmol) in anhydrous THF (50 mL) at 0 ºC and under an argon atmosphere. The reaction was stirred at that temperature for 2 h and allowed to warm to rt. The mixture was then carefully quenched with water and extracted with Et_2O (2x). The organic extracts were washed with brine, dried (Na_2SO_4), filtered, and evaporated under reduced pressure. The residue was purified by chromatography (hexane to hexane/EtOAc, 8:2) to yield alcohol **9** (1.13 g, 72%). $[\alpha]_D^{20}$: -103.2 (c=0.98, chloroform). R_f: 0.17 (hexane/EtOAc, 8:2).



<u>IR (ATR, ν)</u>: 3357 (OH), 1565, 1464 (Ar); <u>^{1}H-NMR ($CDCl_3$, δ)</u>: 0.84 (t, *J*=6.8, 3H, C<u>H</u>$_3$CH$_2$), 1.05-1.33 (m, 38H, CH_3C_{cyc}, $2CH_3$, $3CH(CH_3)_2$, $(CH_2)_4$), 1.39 (s, 3H, CH_3C_{cyc}), 1.40-1.52 (m, 2H, C<u>H</u>$_2$C(CH$_3$)$_2$), 1.79-1.89 (m, 3H, ½CH_2C=, ½C<u>H</u>$_2$CH=, C<u>H</u>C(CH$_3$)$_2$), 2.22-2.26 (m, 1H, ½C<u>H</u>$_2$CH=), 2.63 (td, *J*=10.8, 4.4, 1H, CHC_{Ar}), 3.36 (dd, *J*=16.8, 2.8, 1H, ½CH_2C=), 4.03 (AB system, *J*=12.9, 2H, CH_2O), 5.72 (d, *J*=3.7, 1H, CH=), 6.34 (d, *J*=1.8, 1H, CH_{Ar}), 6.39 (d,

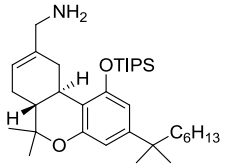
J=1.7, 1H, CH_{Ar}); ^{13}C-NMR ($CDCl_3$, δ): 13.4 (3CHSi), 14.2 ($\underline{C}H_3CH_2$), 18.2 (3CH($\underline{C}H_3$)$_2$), 18.3 ($\underline{C}H_3C_{cyc}$), 22.8, 24.9 ($2CH_2$), 27.6 ($\underline{C}H_3C_{cyc}$), 27.9 ($\underline{C}H_2CH$=), 28.9, 29.1 ($2CH_3$), 30.2, 32.01 ($2CH_2$), 32.09 ($\underline{C}HC_{Ar}$), 32.12 ($\underline{C}H_2C$=), 37.5 (Ar$\underline{C}$(CH_3)$_2$), 44.8 ($\underline{C}H_2C(CH_3)_2$), 45.7 ($\underline{C}HC(CH_3)_2$), 69.9 ($CH_2O$), 76.4 (O$\underline{C}$($CH_3$)$_2$), 108.3, 109.1 ($2CH_{Ar}$), 113.4 ($C_{Ar}$), 120.3 (CH=), 138.7 (C=), 149.4, 154.3, 155.2 ($3C_{Ar}$); MS (ESI, *m/z*): 565.4 $[M+Na]^+$.

(-)-2-({(6a*R*,10a*R*)-3-(1,1-Dimethylheptyl)-6,6-dimethyl-1-[(triisopropylsilyl)oxy]-6a,7,10,10a-tetrahydro-6*H*-benzo[*c*]chromen-9-yl}methyl)-1*H*-isoindole-1,3(2*H*)-dione (10). To a solution of alcohol **9** (300 mg, 0.55 mmol), phthalimide (130 mg, 0.88 mmol), and PPh_3 (216 mg, 0.83 mmol) in anhydrous THF (5 mL) under an argon atmosphere, DEAD (0.4 mL, 0.88 mmol, 40% in toluene) was added dropwise and the mixture was stirred at rt for 16 h. Et_2O was then added, and the reaction was washed with water. The organic layer was dried (Na_2SO_4), filtered, and evaporated under reduced pressure. The crude was purified by chromatography (hexane to hexane/EtOAc, 8:2) to yield phthalimide **10** (343 mg, 93%). $[\alpha]_D^{20}$: -130.0 (c=0.46, chloroform). R_f: 0.29 (hexane/EtOAc, 9:1).



IR (ATR, ν): 1774, 1716, 1612 (CO), 1565, 1464 (Ar); ^{1}H-NMR ($CDCl_3$, δ): 0.83 (t, *J*=6.8, 3H, $C\underline{H}_3CH_2$), 1.00-1.21 (m, 38H, CH_3C_{cyc}, $2CH_3$, 3CH(CH_3)$_2$, (CH_2)$_4$), 1.35 (s, 3H, CH_3C_{cyc}), 1.42-1.50 (m, 2H, $C\underline{H}_2C(CH_3)_2$), 1.76-1.90 (m, 3H, ½$CH_2$C=, ½$C\underline{H}_2$CH=, $C\underline{H}C(CH_3)_2$), 2.15-2.21 (m, 1H, ½$C\underline{H}_2$CH=), 2.65 (td, *J*=10.9, 4.4, 1H, CHC_{Ar}), 3.33 (dd, *J*=15.6, 3.9, 1H, ½CH_2C=), 4.21 (m, 2H, CH_2N), 5.62 (d, *J*=4.7, 1H, CH=), 6.31 (d, *J*=1.6, 1H, CH_{HU}), 6.36 (d, *J*=1.6, 1H, CH_{HU}), 7.69-7.74 (m, 2H, $2CH_{Phth}$), 7.82-7.87 (m, 2H, $2CH_{Phth}$); ^{13}C-NMR ($CDCl_3$, δ): 13.2 (3CHSi), 14.2 ($\underline{C}H_3CH_2$), 18.17 (3CH($\underline{C}H_3$)$_2$), 18.21 ($\underline{C}H_3C_{cyc}$), 22.8, 24.9 ($2CH_2$), 27.6 ($\underline{C}H_3C_{cyc}$), 27.9 ($\underline{C}H_2CH$=), 28.9, 29.2 ($2CH_3$), 30.2, 32.0 ($2CH_2$), 32.1 ($\underline{C}HC_{Ar}$), 32.9 ($\underline{C}H_2C$=), 37.5 (Ar$\underline{C}$(CH_3)$_2$), 43.4 (CH_2N), 44.8 ($\underline{C}H_2C(CH_3)_2$), 45.4 ($\underline{C}HC(CH_3)_2$), 76.4 (O$\underline{C}$($CH_3$)$_2$), 108.2, 109.0 ($2CH_{HU}$), 113.2 ($C_{HU}$), 122.3 (CH=), 123.4 ($2CH_{Phth}$), 132.3 ($2C_{Phth}$), 133.3 (C=), 134.0 ($2CH_{Phth}$), 149.3, 154.3, 155.0 ($3C_{HU}$), 168.2 (2CO); MS (ESI, *m/z*): 672.2 $[M+H]^+$.

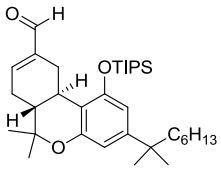
(-)-{(6a*R*,10a*R*)-3-(1,1-Dimethylheptyl)-6,6-dimethyl-1-[(triisopropylsilyl)oxy]-6a,7,10,10a-tetrahydro-6*H*-benzo[*c*]chromen-9-yl}methylamine (11). To a solution of **10** (100 mg, 0.15 mmol) in EtOH (3 mL) under an argon atmosphere, hydrazine (30 μL, 0.44 mmol) was added and the mixture was stirred at reflux for 2 h. Once cooled to rt, a mixture of HCl/water 1:1 (0.3 mL) was added, and the reaction was refluxed for 1 h, and stirred at rt for 16 h. Then, toluene was added and the mixture was filtered off and washed with EtOH. The filtrate was washed with 5% $NaHCO_3$ (aq). The organic layer was dried (Na_2SO_4), filtered, and evaporated under reduced pressure. The crude was purified by chromatography (DCM to DCM/MeOH, 8:2) to yield amine **11** (78 mg, 96%). $[\alpha]_D^{20}$: -0.27 (c=0.18, chloroform). R_f: 0.20 (DCM/MeOH, 95:5).



IR (ATR, ν): 2957, 2928 (NH), 1614, 1566, 1465, 1383 (Ar); ^{1}H-NMR ($CDCl_3$, δ): 0.83 (t, *J*=6.8, 3H, C$\underline{H}_3$CH$_2$), 1.05-1.32 (m, 38H, CH_3C_{cyc}, $2CH_3$, $3CH(CH_3)_2$, $(CH_2)_4$), 1.38 (s, 3H, CH_3C_{cyc}), 1.46-1.51 (m, 2H, C$\underline{H}_2$C(CH$_3$)$_2$), 1.81-1.90 (m, 5H, ½CH$_2$C=, ½C$\underline{H}_2$CH=, C$\underline{H}$C(CH$_3$)$_2$, NH_2), 2.17-2.26 (m, 1H, ½C$\underline{H}_2$CH=), 2.62 (td, *J*=10.9, 4.2, 1H, CHC_{Ar}), 3.22 (m, 2H, CH_2N), 3.28 (dd, *J*=15.9, 4.3, 1H, ½CH$_2$C=), 5.63 (d, *J*=4.3, 1H, CH=), 6.34 (d, *J*=1.8, 1H, CH_{Ar}), 6.39 (d, *J*=1.8, 1H, CH_{Ar}); ^{13}C-NMR ($CDCl_3$, δ): 13.4 (3CHSi), 14.2 (C$\underline{H}_3$CH$_2$), 18.2 (3CH(C$\underline{H}_3$)$_2$), 18.3 ($\underline{C}H_3C_{cyc}$), 22.8, 24.9 ($2CH_2$), 27.6 ($\underline{C}H_3C_{cyc}$), 27.9 ($\underline{C}H_2CH$=), 28.9, 29.1 ($2CH_3$), 30.2, 32.0 ($2CH_2$), 32.2 ($\underline{C}HC_{Ar}$), 33.2 ($\underline{C}H_2C$=), 37.5 (Ar$\underline{C}$(CH$_3$)$_2$), 44.8 ($\underline{C}H_2C(CH_3)_2$), 45.8 ($\underline{C}HC(CH_3)_2$), 48.0 ($CH_2N$), 76.4 (O$\underline{C}$(CH$_3$)$_2$), 108.3, 109.0 ($2CH_{Ar}$), 113.5 ($C_{Ar}$), 118.3 (CH=), 140.3 (C=), 149.3, 154.3, 155.1 ($3C_{Ar}$); HRMS (ESI, *m/z*): calcd for $[M+H]^+$ $C_{34}H_{60}NO_2Si$: 542.4393; found: 542.4388.

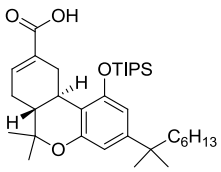
(-)-(6a*R*,10a*R*)-3-(1,1-Dimethylheptyl)-6,6-dimethyl-1-[(triisopropylsilyl)oxy]-6a,7,10,10a-tetrahydro-6*H*-benzo[*c*]chromen-9-carbaldehyde (12). To a solution of alcohol **9** (126 mg, 0.23 mmol) in dry DCM (15 mL), PCC (75 mg, 0.35 mmol) was added and the mixture was stirred at rt for 3 h. The reaction was filtered over silica gel, washing with EtOAc. The solvents were evaporated under reduced pressure and the residue was purified by chromathography (hexane to hexane/EtOAc, 8:2) to yield

aldehyde **12** (87 mg, 85%). $[\alpha]_D^{20}$: -150.2 (c=1.92, chloroform). R_f: 0.36 (hexane/EtOAc, 95:5).



IR (ATR, ν): 2820, 2718, 1688 (CHO), 1613, 1566, 1462 (Ar), 1098 (COSi); ^{1}H-NMR ($CDCl_3$, δ): 0.84 (t, *J*=6.8, 3H, CH_3CH_2), 0.93-1.33 (m, 38H, CH_3C_{cyc}, $2CH_3$, $3CH(CH_3)_2$, $(CH_2)_4$), 1.42 (s, 3H, CH_3C_{cyc}), 1.47-1.52 (m, 2H, $CH_2C(CH_3)_2$), 1.75-1.95 (m, 2H, ½CH_2CH=, $CHC(CH_3)_2$), 2.08-2.20 (m, 1H, ½CH_2CH=), 2.51-2.60 (m, 2H, ½CH_2C=, CHC_{Ar}), 3.46 (dd, *J*=17.7, 2.2, 1H, ½CH_2C=), 6.36 (d, *J*=1.7, 1H, CH_{Ar}), 6.39 (d, *J*=1.7, 1H, CH_{Ar}), 6.80 (m, 1H, CH=), 9.49 (s, 1H, CHO); ^{13}C-NMR ($CDCl_3$, δ): 13.3 (3CHSi), 14.2 (CH_3CH_2), 18.2 (CH_3C_{cyc}, $3CH(CH_3)_2$), 22.8, 24.9 ($2CH_2$), 27.6 (CH_3C_{cyc}), 27.8 (CH_2CH=), 29.0, 29.1 ($2CH_3$), 29.5, 30.2 ($2CH_2$), 31.6 (CHC_{Ar}), 32.0 (CH_2C=), 37.6 ($ArC(CH_3)_2$), 44.8 ($CH_2C(CH_3)_2$), 45.5 ($CHC(CH_3)_2$), 75.9 ($OC(CH_3)_2$), 108.1, 109.2 ($2CH_{Ar}$), 112.5 (C_{Ar}), 142.7 (C=), 148.7 (CH=), 149.7, 154.1, 155.2 ($3C_{Ar}$), 193.4 (CHO); MS (ESI, *m/z*): 541.3 $[M+H]^+$.

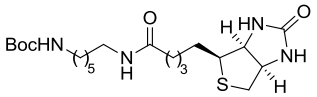
(-)-(6a*R*,10a*R*)-3-(1,1-Dimethylheptyl)-6,6-dimethyl-1-[(triisopropylsilyl)oxy]-6a,7,10,10a-tetrahydro-6*H*-benzo[*c*]chromen-9-carboxylic acid (13). To a vigorously stirred solution of aldehyde **12** (390 mg, 0.72 mmol), 2-methyl-2-butene (1.6 mL, 0.72 mmol), saturated KH_2PO_4 (aq, 0.25 mL) and *tert*-butanol (16 mL, 0.72 mmol), $NaClO_2$ (1.0 mL, 0.72 mmol) was added and the mixture was stirred at rt for 16 h. The reaction was quenched with water, extracted with EtOAc, dried (Na_2SO_4), filtered, and evaporated under reduced pressure. The crude was purified by chromatography (hexane to hexane/EtOAc, 8:2) to yield carboxylic acid **13** (296 mg, 74%). $[\alpha]_D^{20}$: -58.6 (c=0.69, chloroform). R_f: 0.31 (hexane/EtOAc, 9:1).



IR (ATR, ν): 3100 (OH), 1689 (CO), 1612, 1564, 1464 (Ar); ^{1}H-NMR ($CDCl_3$, δ): 0.84 (t, *J*=6.7, 3H, CH_3CH_2), 1.05-1.34 (m, 38H, CH_3C_{cyc}, $2CH_3$, $3CH(CH_3)_2$, $(CH_2)_4$), 1.40 (s, 3H, CH_3C_{cyc}), 1.47-1.52 (m, 2H, $CH_2C(CH_3)_2$), 1.83-2.09 (m, 3H, ½CH_2C=, ½CH_2CH=, $CHC(CH_3)_2$), 2.41-2.47 (m, 1H, ½CH_2CH=), 2.55-2.63 (m, 1H, CHC_{Ar}), 3.87 (dd, *J*=16.8, 3.1, 1H, ½CH_2C=), 6.36 (d, *J*=1.6, 1H, CH_{Ar}), 6.39 (d, *J*=1.4, 1H, CH_{Ar}), 7.14 (d, *J*=2.7, 1H, CH=); ^{13}C-NMR ($CDCl_3$, δ): 13.3 (3CHSi), 14.2 (CH_3CH_2), 18.2 (CH_3C_{cyc}, $3CH(CH_3)_2$), 22.8 (CH_2CH=, CH_2), 24.9 (CH_2), 27.6 (CH_3C_{cyc}), 28.9, 29.0 ($2CH_3$), 30.2, 30.3 ($2CH_2$), 32.0 (CHC_{Ar}, CH_2C=), 37.6 ($ArC(CH_3)_2$), 44.5 ($CHC(CH_3)_2$), 45.9 ($CH_2C(CH_3)_2$), 76.0 ($OC(CH_3)_2$), 108.2, 109.2 ($2CH_{Ar}$), 112.6 (C_{Ar}), 131.1 (C=), 140.3 (CH=), 149.6, 154.2, 155.3 ($3C_{Ar}$), 172.5 (COOH); MS (ESI, *m/z*): 555.2 $[M-H]^-$.

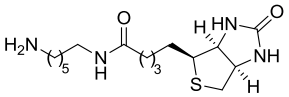
- **Synthesis of the biotin derivative 15**

(+)-*tert*-Butyl 6-(biotinylamino)hexylcarbamate (14). To a solution of biotin (1.00 g, 4.1 mmol) in anhydrous DMF (80 mL) under an argon atmosphere, tributylamine (1.4 mL, 5.9 mmol) was added at rt and the mixture was stirred for 10 min prior to the addition of isobutyl chloroformate (0.6 mL, 5.0 mmol). The resulting mixture was stirred for 30 min and then added dropwise to a solution of *tert*-butyl (6-aminohexyl)carbamate (1.06 g, 4.9 mmol) in anhydrous DMF (80 mL) at 0 ºC. The reaction mixture was stirred at this temperature for 3 h. The solvent was evaporated under reduced pressure and the residue was purified by chromatography (DCM to DCM/MeOH, 8:2) to afford amide **14** (1.45 g, 80%). Mp: 167-168 ºC (lit.[145] 173.7 ºC). $[\alpha]_D^{20}$: +42.6 (c=1.7, MeOH) (lit.[145] +38; c=1.0, MeOH). R_f: 0.28 (DCM/MeOH, 9:1). The spectroscopic data correspond with those previously reported.[145]



^{1}H-NMR ($CDCl_3$/CD_3OD, 9:1, δ): 1.20-1.60 (m, 23H, $C(CH_3)_3$, $(CH_2)_4$, $(CH_2)_3$), 2.08 (t, *J*=7.1, 2H, CH_2CO), 2.62 (d, *J*=12.8, 1H, ½CH_2S), 2.81 (dd, *J*=12.9, 5.0, 1H, ½CH_2S), 2.96 (t, *J*=6.9, 2H, CH_2NBoc), 3.05-3.09 (m, 3H, CHS, CH_2NBiot), 4.19 (dd, *J*=7.6, 4.5, 1H, CHN), 4.40 (dd, *J*=8.1, 4.5, 1H, CHN).

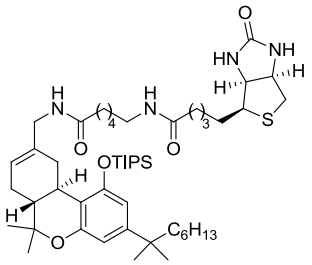
(+)-*N*-(6-Aminohexyl)biotinamide (15). To a solution of **14** (3.20 g, 7.2 mmol) in dry DCM (55 mL) under an argon atmosphere, TFA (17 mL, 221 mmol) was added and the mixture was stirred at rt for 16 h. The solvent was evaporated under reduced pressure and the residue was redissolved in the minimum amount of DCM. Triethylamine was then added until basic pH. The insoluble product was isolated by filtration and washed with cold Et_2O to yield amine **15** (1.98 g, 81%), which was used in the next step without further purification. Mp: 77-79 ºC. $[\alpha]_D^{20}$: +34.5 (c=1.6, MeOH). R_f: 0.17 (DCM/MeOH, 85:15). The spectroscopic data correspond with those previously reported.[145]



^{1}H-NMR (CD_3OD, δ): 1.39-1.80 (m, 14H, $(CH_2)_4$, $(CH_2)_3$), 2.20 (t, *J*=7.3, 2H, CH_2CO), 2.70 (d, *J*=12.7, 1H, ½CH_2S), 2.88-2.96 (m, 3H, ½CH_2S, CH_2NH_2), 3.13-3.24 (m, 3H, CHS, CH_2NBiot), 4.30 (dd, *J*=7.8, 4.4, 1H, CHN), 4.50 (dd, *J*=7.7, 4.7, 1H, CHN).

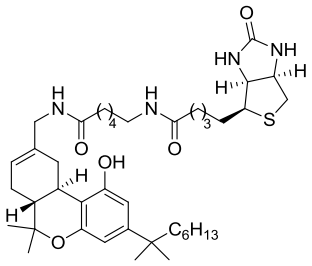
- **Synthesis of final compounds 1 and 2**

(-)-*N*-({(6a*R*,10a*R*)-3-(1,1-Dimethylheptyl)-6,6-dimethyl-1-[(triisopropylsilyl)oxy]-6a,7,10,10a-tetrahydro-6*H*-benzo[*c*]chromen-9-yl}methyl)-6-(biotinylamino)-hexanamide (16). A suspension of *N*-(+)-biotinyl-6-aminohexanoic acid (132 mg, 0.37 mmol), HOBt (50 mg, 0.37 mmol) and activated 4 Å molecular sieves in anhydrous DMF (8 mL) was heated at 77 ºC until a clear solution was obtained. Once cooled to rt, a solution of EDC (78 mg, 0.41 mmol) in dry DCM (3 mL) was added dropwise and the mixture was stirred at rt for 3 h. Then, a solution of amine **11** (100 mg, 0.18 mmol) and DMAP (4 mg, 37 μmol) in DCM (3 mL) was added and the reaction was stirred at rt for 24 h. The mixture was filtered, diluted with DCM, washed with saturated $NaHCO_3$ (aq) and brine, dried (Na_2SO_4), filtered, and evaporated under reduced pressure. The crude was purified by chromatography (DCM to DCM/MeOH, 9:1) to afford amide **16** (98 mg, 62%). $[\alpha]_D^{20}$: -21.6 (c=0.32, chloroform). R_f: 0.32 (DCM/EtOH, 9:1).



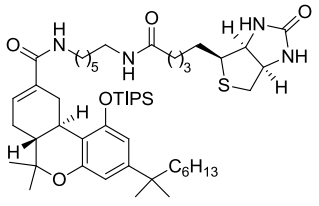
IR (ATR, ν): 3292 (NH), 1703, 1643 (CO), 1561, 1464 (Ar); ^{1}H-NMR ($CDCl_3$, δ): 0.82 (t, *J*=6.8, 3H, CH_3CH_2), 1.04-1.51 (m, 53H, $2CH_3C_{cyc}$, $2CH_3$, $3CH(CH_3)_2$, $(CH_2)_4$, $2(CH_2)_3$), 1.61-1.83 (m, 5H, $CH_2C(CH_3)_2$, ½CH_2C=, ½CH_2CH=, $CHC(CH_3)_2$), 2.15-2.20 (m, 5H, ½CH_2CH=, $2CH_2CO$), 2.62 (td, *J*=10.8, 4.1, 1H, CHC_{Ar}), 2.70 (d, *J*=12.8, 1H, ½CH_2S), 2.86 (dd, *J*=12.8, 4.7, 1H, ½CH_2S), 3.07-3.29 (m, 4H, ½CH_2C=, CHS, CH_2NBiot), 3.75 (d, *J*=5.0, 2H, CH_2N_{HU}), 4.27 (dd, *J*=7.3, 4.9, 1H, CHN), 4.47 (dd, *J*=7.3, 5.0, 1H, CHN), 5.57 (d, *J*=3.6, 1H, CH=), 5.85 (br s, 1H, NH), 5.88 (t, *J*=6.0, 1H, NH), 6.32 (d, *J*=1.5, 1H, CH_{Ar}), 6.36 (d, *J*=1.4, 1H, CH_{Ar}), 6.53 (t, *J*=5.6, 1H, NH), 6.76 (br s, 1H, NH); ^{13}C-NMR ($CDCl_3$, δ): 13.3 (3CHSi), 14.2 (CH_3CH_2), 18.2 (CH_3C_{cyc}, $3CH(CH_3)_2$), 22.8, 24.8, 25.3, 26.0, 26.6 ($5CH_2$), 27.6 (CH_3C_{cyc}), 27.9, 28.2, 28.3 ($2CH_2$, CH_2CH=), 28.8, 29.16 ($2CH_3$), 29.23, 30.2 ($2CH_2$), 32.0 (CH_2C=), 32.1 (CHC_{Ar}), 33.3 (CH_2), 36.0, 36.5 ($2CH_2CO$), 37.5 ($ArC(CH_3)_2$), 39.2 (CH_2NBiot), 40.6 (CH_2S), 44.8 ($CH_2C(CH_3)_2$), 45.0 (CH_2N_{HU}), 45.6 ($CHC(CH_3)_2$), 55.9 (CHS), 60.4, 61.8 (2CHN), 76.4 ($OC(CH_3)_2$), 108.3, 109.1 ($2CH_{Ar}$), 113.2 (C_{Ar}), 120.8 (CH=), 135.2 (C=), 149.4, 154.3, 155.1 ($3C_{Ar}$), 164.3 (NCON), 173.1, 173.5 (2CON); MS (ESI, *m/z*): 903.6 $[M+Na]^+$.

(+)-*N*-{[(6a*R*,10a*R*)-3-(1,1-Dimethylheptyl)-1-hydroxy-6,6-dimethyl-6a,7,10,10a-tetrahydro-6*H*-benzo[*c*]chromen-9-yl]methyl}-6-(biotinylamino)hexanamide (1). Following the general procedure 6.1.1.1, probe **1** was obtained from **16** (19 mg, 22 μmol) in 80% yield (13 mg). Chromatography: DCM to DCM/MeOH, 9:1. Mp: 124-125 ºC. $[\alpha]_D^{20}$: +11.8 (c=0.28, chloroform). R_f: 0.15 (DCM/EtOH, 95:5).



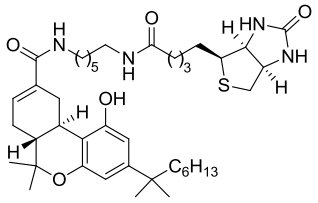
IR (ATR, ν): 3284 (NH, OH), 1696, 1646 (CO), 1552, 1460 (Ar); ^{1}H-NMR ($CDCl_3$, δ): 0.83 (t, *J*=7.0, 3H, CH_3CH_2), 1.05-1.84 (m, 37H, $CHC(CH_3)_2$, $CH_2C(CH_3)_2$, $(CH_2)_4$, $2(CH_2)_3$, ½CH_2C=, ½CH_2CH=), 2.17-2.30 (m, 5H, ½CH_2CH=, $2CH_2CO$), 2.64 (td, *J*=10.8, 4.2, 1H, CHC_{Ar}), 2.71 (d, *J*=12.8, 1H, ½CH_2S), 2.86 (dd, *J*=12.8, 4.8, 1H, ½CH_2S), 3.08 (dd, *J*=11.7, 7.0, 1H, CHS), 3.19-3.24 (m, 1H, ½CH_2NBiot), 3.31-3.37 (m, 1H, ½CH_2NBiot), 3.48 (dd, *J*=17.1, 3.6, 1H, ½CH_2C=), 3.61 (dd, *J*=14.1, 2.7, 1H, ½CH_2N_{HU}), 3.98 (dd, *J*=14.3, 6.7, 1H, ½CH_2N_{HU}), 4.22 (dd, *J*=7.6, 4.5, 1H, CHN), 4.44 (dd, *J*=7.4, 4.9, 1H, CHN), 5.26 (br s, 1H, OH), 5.64 (d, *J*=1.6, 1H, CH=), 6.22 (br s, 1H, NH), 6.32 (d, *J*=1.6, 1H, CH_{Ar}), 6.37 (br s, 1H, NH), 6.54 (d, *J*=1.6, 1H, CH_{Ar}), 6.58 (br s, 1H, NH); ^{13}C-NMR ($CDCl_3$, δ): 14.3 (CH_3CH_2), 18.6 (CH_3C_{cyc}), 22.9, 24.8, 25.1, 25.4, 26.1 ($5CH_2$), 27.7 (CH_3C_{cyc}), 28.06, 28.14, 28.3 ($2CH_2$, CH_2CH=), 28.9 ($2CH_3$), 29.4, 30.2 ($2CH_2$), 31.69 (CH_2C=), 31.72 (CHC_{Ar}), 32.0 (CH_2), 35.8, 36.4 ($2CH_2$CO), 37.5 ($ArC(CH_3)_2$), 39.5 (CH_2NBiot), 40.8 (CH_2S), 44.7 ($CH_2C(CH_3)_2$), 45.2 ($CHC(CH_3)_2$), 45.8 (CH_2N_{HU}), 55.8 (CHS), 60.3, 62.1 (2CHN), 76.4 ($OC(CH_3)_2$), 106.0, 107.0 ($2CH_{Ar}$), 109.6 (C_{Ar}), 123.3 (CH=), 135.5 (C=), 150.1, 154.3, 155.9 ($3C_{Ar}$), 163.8 (NCON), 173.6, 174.2 (2CON); HRMS (MALDI, *m/z*): calcd for $[M+H]^+$ $C_{41}H_{65}N_4O_5S$: 725.4676; found: 725.4688; HPLC-MS (ESI, *m/z*): 725.3 $[M+H]^+$; t_R (method A): 13.29 min.

(-)-(6a*R*,10a*R*)-3-(1,1-Dimethylheptyl)-6,6-dimethyl-*N*-[6-(biotinylamino)hexyl]-1-[(triisopropylsilyl)oxy]-6a,7,10,10a-tetrahydro-6*H*-benzo[*c*]chromen-9-carboxamide (17). To a solution of carboxylic acid **13** (198 mg, 0.36 mmol) and HOBt (53 mg, 0.39 mmol) in dry DCM (10 mL) under an argon atmosphere, EDC (75 mg, 0.39 mmol) was added and the mixture was stirred at rt for 40 min. Then, a solution of amine **15** (188 mg, 0.55 mmol) and DMAP (14 mg, 0.11 mmol) in DMF (1 mL) was added, and the mixture was stirred at rt for 48 h. The reaction was diluted with EtOAc, washed with saturated $NaHCO_3$ (aq), dried (Na_2SO_4), filtered, and evaporated under reduced pressure. The crude was purified by chromatography (DCM to DCM/MeOH, 8:2) to yield amide **17** (151 mg, 48%). Mp: 143-145 ºC. $[\alpha]_D^{20}$: -120.2 (c=5.1, chloroform). R_f: 0.44 (DCM/MeOH, 9:1).



IR (ATR, ν): 3295 (NH), 1702, 1639 (CO), 1559, 1464 (Ar); ^{1}H-NMR ($CDCl_3$, δ): 0.82 (t, *J*=6.8, 3H, CH_3CH_2), 1.02-1.51 (m, 55H, $2CH_3C_{cyc}$, $2CH_3$, $3CH(CH_3)_2$, $2(CH_2)_4$, $(CH_2)_3$) 1.63-2.02 (m, 5H, $CH_2C(CH_3)_2$, ½CH_2C=, ½CH_2CH=, $CHC(CH_3)_2$), 2.19 (t, *J*=7.4, 2H, CH_2CO), 2.33-2.40 (m, 1H, ½CH_2CH=), 2.62 (td, *J*=10.9, 4.2, 1H, CHC_{Ar}), 2.71 (d, *J*=12.6, 1H, ½CH_2S), 2.87 (dd, *J*=13.4, 5.0, 1H, ½CH_2S), 3.11-3.21 (m, 3H, CHS, CH_2N), 3.27 (q app, *J*=6.5, 2H, CH_2N), 3.61-3.67 (m, 1H, ½CH_2C=), 4.28 (dd, *J*=7.6, 4.6, 1H, CHN), 4.48 (dd, *J*=7.6, 4.8, 1H, CHN), 5.78 (br s, 2H, 2NH), 6.36 (d, *J*=1.5, 1H, CH_{Ar}), 6.39 (d, *J*=1.4, 1H, CH_{Ar}), 6.46 (m, 1H, NH), 6.63 (br s, 1H, NH), 6.80 (d, *J*=4.9, 1H, CH=); ^{13}C-NMR ($CDCl_3$, δ): 13.3 (3CHSi), 14.2 (CH_3CH_2), 18.1 (CH_3C_{cyc}), 18.2 ($3CH(CH_3)_2$), 22.8 ($2CH_2$), 24.9, 26.0, 26.4 ($3CH_2$), 27.6 (CH_3C_{cyc}), 28.2, 28.3, 28.5 ($2CH_2$, CH_2CH=), 28.8, 29.2 ($2CH_3$), 29.5, 29.8, 30.2, 30.6 ($4CH_2$), 32.0 (CH_2C=), 32.1 (CHC_{Ar}), 36.1 (CH_2CO), 37.5 ($ArC(CH_3)_2$), 39.2, 39.4 ($2CH_2$N), 40.6 (CH_2S), 44.7 ($CH_2C(CH_3)_2$), 45.0 ($CHC(CH_3)_2$), 55.8 (CHS), 60.3, 61.9 (2CHN), 76.2 ($OC(CH_3)_2$), 108.5, 109.2 ($2CH_{Ar}$), 112.7 (C_{Ar}), 133.0 (C=), 133.2 (CH=), 149.8, 154.3, 155.0 ($3C_{Ar}$), 164.1 (NCON), 167.7, 172.5 (2CON); MS (ESI, *m/z*): 881.6 $[M+H]^+$.

(-)-(6a*R*,10a*R*)-3-(1,1-Dimethylheptyl)-1-hydroxy-6,6-dimethyl-*N*-[6-(biotinylamino)hexyl]-6a,7,10,10a-tetrahydro-6*H*-benzo[*c*]chromen-9-carboxamide (2). Following the general procedure 6.1.1.1, probe **2** was obtained from **17** (49 mg, 56 μmol) in 84% yield (34 mg). Chromatography: DCM to DCM/MeOH, 8:2. Mp: 116-119 ºC. $[\alpha]_D^{20}$: -92.1 (c=1.28, MeOH). R_f: 0.33 (DCM/MeOH, 9:1).



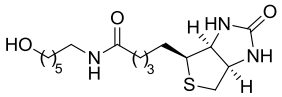
IR (ATR, ν): 3290 (NH), 1693, 1623 (CO), 1539, 1459 (Ar); ^{1}H-NMR (CD_3OD, δ): 0.86 (t, *J*=6.8, 3H, CH_3CH_2), 1.10-1.82 (m, 37H, $CHC(CH_3)_2$, $CH_2C(CH_3)_2$, $2(CH_2)_4$, $(CH_2)_3$), 1.92-2.02 (m, 2H, ½CH_2C=, ½CH_2CH=), 2.20 (t, *J*=7.3, 2H, CH_2CO), 2.35-2.44 (m, 1H, ½CH_2CH=), 2.64 (td, *J*=11.1, 4.5, 1H, CHC_{Ar}), 2.70 (d, *J*=12.8, 1H, ½CH_2S), 2.91 (dd, *J*=12.8, 5.6, 1H, ½CH_2S), 3.15-3.26 (m, 5H, CHS, $2CH_2$N), 3.75-3.81 (m, 1H, ½CH_2C=), 4.29 (dd, *J*=8.0, 4.4, 1H, CHN), 4.48 (dd, *J*=7.8, 4.8, 1H, CHN), 6.24 (d, *J*=1.8, 1H, CH_{Ar}), 6.36 (d, *J*=1.9, 1H, CH_{Ar}), 6.58 (m, 1H, CH=); ^{13}C-NMR (CD_3OD, δ): 14.4 (CH_3CH_2), 18.5 (CH_3C_{cyc}), 23.7 ($2CH_2$), 25.8, 26.9, 27.6 ($3CH_2$), 27.9 (CH_3C_{cyc}), 29.2 (CH_2), 29.4 ($2CH_3$), 29.5, 29.8, 30.3, 30.4, 31.1, 31.7 ($5CH_2$,

$\underline{C}H_2CH=$), 32.8 ($\underline{C}HC_{Ar}$), 32.9 ($\underline{C}H_2C=$), 36.8 ($\underline{C}H_2CO$), 38.2 ($Ar\underline{C}(CH_3)_2$), 40.2, 40.4 ($2CH_2N$), 41.0 (CH_2S), 45.6 ($\underline{C}H_2C(CH_3)_2$), 46.2 ($\underline{C}HC(CH_3)_2$), 57.0 (CHS), 61.6, 63.4 (2CHN), 77.1 ($O\underline{C}(CH_3)_2$), 106.3, 107.6 ($2CH_{Ar}$), 110.6 (C_{Ar}), 132.6 (CH=), 135.6 (C=), 150.8, 155.5, 157.6 ($3C_{Ar}$), 166.1 (NCON), 171.5, 176.0 (2CO); HRMS (MALDI, *m/z*): calcd for $[M+H]^+$ $C_{41}H_{65}N_4O_5S$: 725.4676; found: 725.4697; HPLC-MS (ESI, *m/z*): 725.5 $[M+H]^+$; t_R (method A): 12.02 min.

- **Synthesis of probe 20**

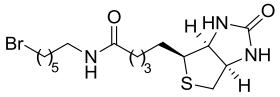
Compounds **18** and **19** were obtained following the experimental procedures previously described by Takenishi *et al.*[85]

(+)-*N*-(6-Hydroxyhexyl)biotinylamide (18). Amide **18** was prepared from biotin (1.00 g, 4.1 mmol) and 6-amino-1-hexanol (590 mg, 5.0 mmol) following the procedure described for **14** in 97% yield (1.37 g). Chromatography: DCM to DCM/MeOH, 8:2. Mp: 182-185 ºC (lit.[146] 159-160 ºC). $[\alpha]_D^{20}$: +35.8 (c=1.11, DMSO). R_f: 0.13 (DCM/MeOH, 9:1). The spectroscopic data correspond with those previously reported.[146]



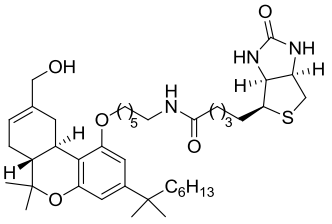
^{1}H-NMR ($(CD_3)_2SO$, δ): 1.24-1.67 (m, 14H, $(CH_2)_4$, $(CH_2)_3$), 2.04 (t, *J*=7.3, 2H, CH_2CO), 2.57 (d, *J*=12.4, 1H, ½CH_2S), 2.82 (dd, *J*=12.4, 5.1, 1H, ½CH_2S), 3.00 (q, *J*=6.4, 2H, CH_2N), 3.06-3.12 (m, 1H, CHS), 3.37 (q app, *J*=6.0, 2H, CH_2O), 4.10-4.14 (m, 1H, CHN), 4.28-4.33 (m, 2H, CHN, OH), 6.35 (br s, 1H, NH), 6.41 (br s, 1H, NH), 7.71 (t, *J*=5.5, 1H, CONH).

(+)-*N*-(6-Bromohexyl)biotinylamide (19). To a solution of alcohol **18** (2.79 g, 8.1 mmol) in anhydrous DMF (200 mL) and under an argon atmosphere, PPh_3 (2.56 g, 9.8 mmol) and carbon tetrabromide (4.04 g, 12 mmol) were added and the mixture was stirred at rt for 2 h. The solvent was evaporated under reduced pressure and the residue was redissolved in EtOAc, washed with saturated $NaHCO_3$ (aq) and brine, dried (Na_2SO_4), filtered, and evaporated under reduced pressure. The crude was purified by chromatography (DCM to DCM/MeOH, 8:2) to afford bromoderivative **19** (2.45 g, 74%). Mp: 147-150 ºC. $[\alpha]_D^{20}$: +41.3 (c=0.63, MeOH). R_f: 0.38 (DCM/MeOH, 9:1).



IR (ATR, ν): 3294 (NH), 1702, 1640 (CO); ^{1}H-NMR (CD_3OD, δ): 1.30-1.88 (m, 14H, $(CH_2)_4$, $(CH_2)_3$), 2.20 (t, *J*=7.3, 2H, CH_2CO), 2.70 (d, *J*=12.7, 1H, ½CH_2S), 2.93 (dd, *J*=12.8, 5.0, 1H, ½CH_2S), 3.15-3.25 (m, 3H, CH_2N, CHS), 3.45 (t, *J*=6.8, 2H, CH_2Br), 4.30 (dd, *J*=7.8, 4.6, 1H, CHN), 4.50 (dd, *J*=7.8, 4.4, 1H, CHN); ^{13}C-NMR (CD_3OD, δ): 26.9, 27.1, 28.9, 29.5, 29.8, 30.3, 33.9 ($(CH_2)_4$, $(CH_2)_3$), 34.3 (CH_2Br), 36.8 ($\underline{C}H_2CO$), 40.2 (CH_2N), 41.0 (CH_2S), 57.0 (CHS), 61.6, 63.4 (2CHN), 166.1 (NCON), 176.0 (CON); MS (ESI, *m/z*): 405.6 [M(^{79}Br)+H]$^+$, 407.6 [M(^{81}Br)+H]$^+$.

(-)-*N*-(6-{[(6a*R*,10a*R*)-3-(1,1-Dimethylheptyl)-9-(hydroxymethyl)-6,6-dimethyl-6a,7,10,10a-tetrahydro-6*H*-benzo[*c*]chromen-1-yl]oxy}hexyl)biotinamide (20). To a solution of alcohol **9** (85 mg, 0.15 mmol) in anhydrous THF (2 mL) at 0 ºC and under an argon atmosphere, NaH (3.8 mg, 0.16 mmol, 60% in mineral oil) was added and the mixture was stirred at that temperature for 30 min. Then, TBAI (7.4 mg, 20 μmol) and bromoderivative **19** (85 mg, 0.21 mmol) were added and the mixture was stirred at reflux for 16 h. Once at rt, the reaction was quenched with saturated NH_4Cl (aq) and extracted with EtOAc (2x). The organic layer was washed with brine, dried (Na_2SO_4), filtered, and evaporated under reduced pressure. The residue was purified by chromatography (DCM/MeOH, 85:15) to give **20** (80 mg, 75%). Mp: 137-140 ºC. $[\alpha]_D^{20}$: -141.4 (c=0.74, chloroform). R_f: 0.41 (DCM/MeOH, 85:15).



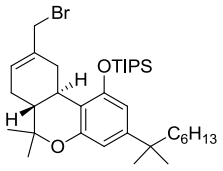
IR (ATR, ν): 3296 (NH, OH), 1696, 1646 (CO), 1568, 1463 (Ar); ^{1}H-NMR ($CDCl_3$, δ): 0.84 (t, *J*=6.7, 3H, $C\underline{H}_3CH_2$), 1.07 (s, 3H, CH_3C_{cyc}), 1.19-1.25 (m, 14H, $2CH_3$, $(CH_2)_4$), 1.38 (s, 3H, CH_3C_{cyc}), 1.38-1.85 (m, 19H, $(CH_2)_4$, $(CH_2)_3$, $C\underline{H}_2C(CH_3)_2$, ½$CH_2C$=, ½$C\underline{H}_2CH$=, $C\underline{H}C(CH_3)_2$), 2.15-2.26 (m, 3H, CH_2CO, ½$C\underline{H}_2CH$=), 2.65 (td, *J*=12.8, 4.8, 1H, CHC_{Ar}), 2.71 (d, *J*=13.0, 1H, ½CH_2S), 2.89 (dd, *J*=12.8, 4.8, 1H, ½CH_2S), 3.13-3.26 (m, 4H, CH_2N, CH_2O), 3.45 (dd, *J*=16.6, 3.7, 1H, ½CH_2C=), 3.88-4.08 (m, 3H, $C\underline{H}_2OH$, CHS), 4.32 (m, 1H, CHN), 4.48 (m, 1H,

CHN), 5.39 (br s, 1H, OH), 5.72 (d, *J*=4.2, 1H, CH=), 6.31-6.35 (m, 2H, CH_{Ar}, NH), 6.41 (d, *J*=1.3, 1H, CH_{Ar}); ^{13}C-NMR ($CDCl_3$, δ): 14.3 (CH_3CH_2), 18.6 (CH_3C_{cyc}), 22.8, 24.8, 25.8, 26.6, 27.0 ($5CH_2$), 27.8 (CH_3C_{cyc}), 28.0, 28.2, 28.3 ($2CH_2$, CH_2CH=), 29.0 ($2CH_3$), 29.7, 29.8, 30.2 ($3CH_2$), 31.7 (CHC_{Ar}), 31.9, 32.2 (CH_2C=, CH_2), 35.6 (CH_2CO), 37.8 ($ArC(CH_3)_2$), 39.8 (CH_2N), 40.6 (CH_2S), 44.6 ($CH_2C(CH_3)_2$), 45.5 ($CHC(CH_3)_2$), 55.8 (CHS), 60.6, 62.2 (2CHN), 66.7 (CH_2OH), 67.9 (CH_2O), 76.5 ($OC(CH_3)_2$), 101.4, 108.2 ($2CH_{Ar}$), 111.3 (C_{Ar}), 120.9 (CH=), 138.5 (C=), 149.9, 154.1, 158.2 ($3C_{Ar}$), 164.2 (NCON), 174.3 (CON); HRMS (MALDI, *m/z*): calcd for $[M+H]^+$ $C_{41}H_{66}N_3O_5S$: 712.4723; found: 712.4691; HPLC-MS (ESI, *m/z*): 712.5 $[M+H]^+$; t_R (method A): 14.41 min.

- **Attempts of synthesis of compound 3**

Mitsunobu reaction of alcohols 9 and 18. To a solution of ADDP (45 mg, 0.18 mmol) in anhydrous toluene (10 mL) at rt and under an argon atmosphere, PBu_3 (0.36 mL, 0.18 mmol) and biotinylated alcohol **18** (63 mg, 0.18 mmol) were added. The mixture was stirred at this temperature for 30 min and then alcohol **9** (50 mg, 92 μmol) was added, and the reaction mixture was stirred at 60 ºC for 16 h. Once cooled to rt, the solvent was evaporated under reduced pressure and the residue was redissolved in EtOAc and washed with water and brine. The organic layer was dried (Na_2SO_4), filtered, and evaporated under reduced pressure to yield a mixture of both starting materials, and the Mitsunobu byproduct, but not the desired allyl ether **3**.

{[(6a*R*,10a*R*)-9-(Bromomethyl)-3-(1,1-dimethylheptyl)-6,6-dimethyl-6a,7,10,10a-tetrahydro-6*H*-benzo[*c*]chromen-1-yl]oxy}(triisopropyl)silane (21). To a solution of alcohol **9** (56 mg, 0.10 mmol) in dry DCM (10 mL) under an argon atmosphere, PPh_3 (32 mg, 0.12 mmol) and carbon tetrabromide (51 mg, 0.16 mmol) were added and the mixture was stirred at rt for 1 h. The solvent was evaporated under reduced pressure and the reaction crude was purified by chromatography (hexane to hexane/EtOAc, 8:2) to provide bromoderivative **21** (41 mg, 65%). R_f: 0.34 (hexane/EtOAc, 95:5).

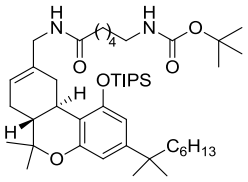


^{1}H-NMR ($CDCl_3$, δ): 0.84 (t, *J*=6.8, 3H, CH_3CH_2), 1.07-1.34 (m, 38H, CH_3C_{cyc}, $2CH_3$, $3CH(CH_3)_2$, $(CH_2)_4$), 1.38 (s, 3H, CH_3C_{cyc}), 1.47-1.52 (m, 2H, $CH_2C(CH_3)_2$), 1.77-2.08 (m, 3H, ½CH_2C=, ½CH_2CH=, CHC$(CH_3)_2$), 2.22-2.27 (m, 1H, ½CH_2CH=), 2.67 (td, *J*=10.9, 4.5, 1H, CHC_{Ar}), 3.46 (dd, *J*=17.0, 4.0, 1H, ½CH_2C=), 3.95 (AB system, *J*=10.4, 2H, CH_2Br), 5.90 (d, *J*=5.0, 1H, CH=), 6.36 (d, *J*=1.8, 1H, CH_{Ar}), 6.38 (d, *J*=1.7, 1H, CH_{Ar}); ^{13}C-NMR ($CDCl_3$, δ): 13.4 (3CHSi), 14.2 (CH_3CH_2), 18.3 (CH_3C_{cyc}, $3CH(CH_3)_2$), 22.8, 24.9 ($2CH_2$), 27.6 (CH_3C_{cy}), 28.3 (CH_2CH=), 28.9, 29.1 ($2CH_3$), 30.2 (CH_2), 31.99 (CHC_{Ar}), 32.01, 33.1 (CH_2C=, CH_2), 37.5 (Ar$C(CH_3)_2$), 38.6 (CH_2Br), 44.8 ($CH_2C(CH_3)_2$), 45.0 ($CHC(CH_3)_2$), 76.2 (O$C(CH_3)_2$), 108.2, 109.0 ($2CH_{Ar}$), 113.0 (C_{Ar}), 126.3 (CH=), 135.8 (C=), 149.5, 154.2, 155.2 ($3C_{Ar}$).

Nucleophilic substitution of bromoderivative 21 with alcohol 18. To a solution of alcohol **18** (206 mg, 0.60 mmol) in anhydrous DMF (5 mL) at rt and under an argon atmosphere, NaH (64 mg, 1.6 mmol) was added and the reaction mixture was stirred for 1 h. Then, a solution of bromoderivative **21** (180 mg, 0.40 mmol) in anhydrous DMF (5 mL) was added, and the mixture was stirred at rt for 16 h. The reaction was quenched with saturated NH_4Cl (aq), extracted with EtOAc, and washed with water and brine. The combined organic extracts were dried (Na_2SO_4), filtered, and evaporated under reduced pressure to yield a complex mixture of products which did not contain the desired allyl ether **3**.

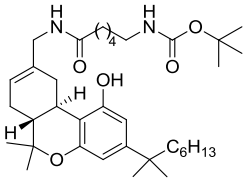
- **Synthesis of fluorescent probe 22**

(-)-*tert*-Butyl {6-[({(6a*R*,10a*R*)-3-(1,1-dimethylheptyl)-6,6-dimethyl-1-[(triisopropylsilyl)oxy]-6a,7,10,10a-tetrahydro-6*H*-benzo[*c*]chromen-9-yl}methyl)-amino]-6-oxohexyl}carbamate (23). To a solution of 6-[(*tert*-butoxycarbonyl)amino]hexanoic acid (77 mg, 0.33 mmol) in dry DCM (0.7 mL), DCC (125 mg, 0.60 mmol) was added under an argon atmosphere, and the mixture was stirred at rt for 10 min. Then, a solution of amine **11** (164 mg, 0.30 mmol) in dry DCM (2 mL) was added, and the mixture was stirred at rt for 16 h. The reaction mixture was filtered, washing with EtOAc. The filtrate was washed with water, dried (Na_2SO_4), filtered, and evaporated under reduced pressure. The crude was purified by chromatography (DCM to DCM/EtOAc, 9:1) to give compound **23** (169 mg, 74%). Mp: 53 ºC. $[\alpha]_D^{20}$: -87.9 (c=0.96, chloroform). R_f: 0.25 (DCM/EtOAc, 9:1).



IR (ATR, ν): 3319 (NH), 1692, 1649 (CO), 1564, 1463 (Ar); ^{1}H-NMR ($CDCl_3$, δ): 0.84 (t, *J*=6.8, 3H, CH_3CH_2), 1.05-1.71 (m, 61H, $C(CH_3)_3$, $3CH(CH_3)_2$, $CHC(CH_3)_2$, $CH_2C(CH_3)_2$, $(CH_2)_4$, $(CH_2)_3$, ½CH_2C=, ½CH_2CH=), 2.15-2.23 (m, 3H, ½CH_2CH=, CH_2CO), 2.64 (td, *J*=10.8, 4.4, 1H, CHC_{Ar}), 3.07-3.13 (m, 2H, CH_2NBoc), 3.28 (dd, *J*=16.1, 3.7, 1H, ½CH_2C=), 3.79 (d, *J*=16.1, 2H, CH_2N_{HU}), 4.53 (br s, 1H, NH), 5.43 (t, *J*=4.9, 1H, NH), 5.60 (d, *J*=4.5, 1H, CH=), 6.34 (d, *J*=1.4, 1H, CH_{Ar}), 6.38 (d, *J*=1.6, 1H, CH_{Ar}); ^{13}C-NMR ($CDCl_3$, δ): 13.4 (3CHSi), 14.2 (CH_3CH_2), 18.3 (CH_3C_{cyc}, $3CH(CH_3)_2$), 22.8, 24.9, 25.5, 26.6 ($4CH_2$), 27.6 (CH_3C_{cyc}), 28.0 (CH_2CH=), 28.6 ($C(CH_3)_3$), 28.9, 29.2 ($2CH_3$), 30.2, 31.1, 32.0 ($3CH_2$), 32.2 (CHC_{Ar}), 33.4 (CH_2C=), 34.1 (CH_2NBoc), 36.8 (CH_2CO), 37.6 ($ArC(CH_3)_2$), 44.8 ($CH_2C(CH_3)_2$), 45.1 (CH_2N_{HU}), 45.6 ($CHC(CH_3)_2$), 76.4 ($OC(CH_3)_2$), 77.4 ($C(CH_3)_3$), 108.4, 109.1 ($2CH_{Ar}$), 113.2 (C_{Ar}), 121.2 (CH=), 135.4 (C=), 149.5, 154.3, 155.1 ($3C_{Ar}$), 156.1 (NCOO), 172.7 (CON); MS (ESI, *m/z*): 753.6 $[M-H]^-$.

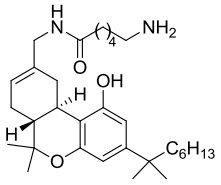
(-)-*tert*-Butyl [6-({[(6a*R*,10a*R*)-3-(1,1-dimethylheptyl)-1-hydroxy-6,6-dimethyl-6a,7,10,10a-tetrahydro-6*H*-benzo[*c*]chromen-9-yl]methyl}amino)-6-oxohexyl]-carbamate (24). Following the general procedure 6.1.1.1, phenol **24** was obtained from **23** (62 mg, 83 μmol) in 81% yield (40 mg). Chromatography: DCM to DCM/EtOAc, 8:2. Mp: 83-85 ºC. $[\alpha]_D^{20}$: -101.5 (c=0.54, chloroform). R_f: 0.35 (DCM/MeOH, 95:5).



IR (ATR, ν): 3308 (NH, OH), 1686, 1649 (CO), 1575, 1523, 1457 (Ar); ^{1}H-NMR ($CDCl_3$, δ): 0.84 (t, *J*=6.8, 3H, CH_3CH_2), 1.04-1.86 (m, 40H, $C(CH_3)_3$, $CHC(CH_3)_2$, $CH_2C(CH_3)_2$, $(CH_2)_4$, $(CH_2)_3$, ½CH_2C=, ½CH_2CH=), 2.17-2.24 (m, 3H, ½CH_2CH=, CH_2CO), 2.68 (td, *J*=10.9, 4.6, 1H, CHC_{Ar}), 3.15 (q, *J*=6.8, 2H, CH_2NBoc), 3.48 (d, *J*=17.2, 1H, ½CH_2C=), 3.69 (d, *J*=13.2, 1H, ½CH_2N_{HU}), 3.99 (dd, *J*=13.8, 7.3, 1H, ½CH_2N_{HU}), 4.68 (t, *J*=5.4, 1H, NH), 5.47 (br s, 1H, NH), 5.68 (d, *J*=4.3, 1H, CH=), 6.32 (d, *J*=1.7, 1H, CH_{Ar}), 6.44 (m, 1H, CH_{Ar}), 7.80 (br s, 1H, OH);

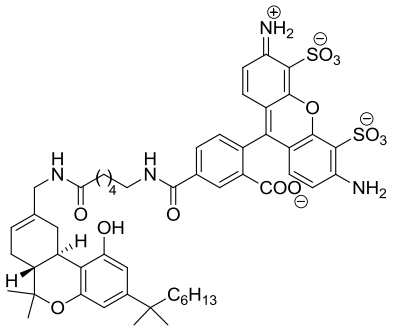
^{13}C-NMR ($CDCl_3$, δ): 14.2 ($\underline{C}H_3CH_2$), 18.6 ($\underline{C}H_3C_{cyc}$), 22.8, 24.8, 25.5, 26.1 ($4CH_2$), 27.8 ($\underline{C}H_3C_{cyc}$), 28.0 ($\underline{C}H_2CH$=), 28.6 ($C(\underline{C}H_3)_3$), 28.7, 29.0 ($2CH_3$), 30.2 ($2CH_2$), 31.7 ($\underline{C}HC_{Ar}$), 31.8 ($\underline{C}H_2C$=), 32.0 (CH_2), 36.8 ($\underline{C}H_2CO$), 37.5 ($Ar\underline{C}(CH_3)_2$), 40.5 ($\underline{C}H_2NBoc$), 44.7 ($\underline{C}H_2C(CH_3)_2$), 45.2 ($\underline{C}HC(CH_3)_2$), 45.7 ($CH_2N_{HU}$), 76.4 ($O\underline{C}(CH_3)_2$), 77.2 ($\underline{C}(CH_3)_3$), 105.6, 107.0 ($2CH_{Ar}$), 109.7 ($C_{Ar}$), 123.5 (CH=), 135.7 (C=), 150.0, 154.3, 156.1 ($3C_{Ar}$), 156.8 (NCOO), 172.9 (CON); MS (ESI, *m/z*): 599.4 $[M+H]^+$.

(-)-6-Amino-*N*-{[(6a*R*,10a*R*)-3-(1,1-dimethylheptyl)-1-hydroxy-6,6-dimethyl-6a,7,10,10a-tetrahydro-6*H*-benzo[*c*]chromen-9-yl]methyl}hexanamide (25). Phenol **24** (32 mg, 53 μmol) was treated with a mixture of TFA/DCM 1:1 (0.1 mL) under an argon atmosphere, and the mixture was stirred at rt for 1 h. The solvent was evaporated under reduced pressure and the residue was redissolved in DCM, washed with saturated $NaHCO_3$ (aq) and brine. The organic layer was dried (Na_2SO_4), filtered, and evaporated under reduced pressure to yield amine **25** (22 mg, 84), which was used in the next step without further purification. $[\alpha]_D^{20}$: -145.1 (c=0.46, chloroform). R_f: 0.10 (DCM/MeOH, 95:5).



IR (ATR, ν): 3302 (NH, OH), 1646 (CO), 1568, 1449 (Ar); ^{1}H-NMR ($CDCl_3$, δ): 0.84 (t, *J*=6.7, 3H, $C\underline{H}_3CH_2$), 1.06-1.82 (m, 31H, $CHC(CH_3)_2$, $CH_2C(CH_3)_2$, $(CH_2)_4$, $(CH_2)_3$, ½CH_2C=, ½$C\underline{H}_2CH$=), 2.08-2.21 (m, 2H, CH_2CO), 2.31-2.37 (m, 1H, ½$C\underline{H}_2CH$=), 2.62 (td, *J*=10.8, 4.5, 1H, CHC_{Ar}), 2.58-2.65 (m, 2H, $C\underline{H}_2NH_2$), 3.54 (dd, *J*=14.0, 3.2, 1H, ½$C\underline{H}_2NH$), 3.60 (dd, *J*=17.7, 3.6, 1H, ½CH_2C=), 4.19 (dd, *J*=13.9, 8.3, 1H, ½$C\underline{H}_2NH$), 5.42-5.45 (m, 1H, NH), 5.62 (d, *J*=3.6, 1H, CH=), 6.12 (d, *J*=1.6, 1H, CH_{Ar}), 6.28 (d, *J*=1.5, 1H, CH_{Ar}); ^{13}C-NMR ($CDCl_3$, δ): 14.2 ($\underline{C}H_3CH_2$), 18.5 ($\underline{C}H_3C_{cyc}$), 22.8, 24.8, 25.6, 26.2 ($4CH_2$), 27.7 ($\underline{C}H_3C_{cyc}$), 28.0 ($\underline{C}H_2CH$=), 28.8, 29.0 ($2CH_3$), 30.2, 31.2, 31.4 ($3CH_2$), 31.7 ($\underline{C}HC_{Ar}$), 32.0 ($\underline{C}H_2C$=), 36.5 ($\underline{C}H_2CO$), 37.3 ($Ar\underline{C}(CH_3)_2$), 41.6 (CH_2NH_2), 44.7 ($\underline{C}H_2C(CH_3)_2$), 45.2 ($\underline{C}HC(CH_3)_2$), 45.4 ($CH_2NH$), 76.4 ($O\underline{C}(CH_3)_2$), 105.1, 106.3 ($2CH_{Ar}$), 110.2 ($C_{Ar}$), 123.2 (CH=), 136.3 (C=), 149.7, 154.3, 156.6 ($3C_{Ar}$), 172.8 (CON); MS (ESI, *m/z*): 499.3 $[M+H]^+$.

2-(6-Amino-3-imino-4,5-disulfo-3*H*-xanthen-9-yl)-5-({[6-({[3-(1,1-dimethylheptyl)-1-hydroxy-6,6-dimethyl-6a,7,10,10a-tetrahydro-6*H*-benzo[*c*]chromen-9-yl]methyl}-amino)-6-oxohexyl]amino}carbonyl)benzoic acid (22). To a solution of Alexa Fluor 488 TFP ester (12.5 mg, 18 μmol) in anhydrous DMF (0.2 mL) protected from light and under an argon atmosphere, a solution of amine **25** (11.7 mg, 23 μmol) in dry DCM (0.26 mL) was added and the resulting mixture was stirred at rt for 30 min. The solvent was evaporated under reduced pressure and the crude was purified by chromatography (glass column, DCM to DCM/MeOH/ammonia, 1:1:0.02) to yield probe **22** (12 mg, 66%).



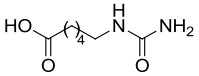
HRMS (ESI, *m/z*): calcd for $[M+H]^+$ $C_{52}H_{61}N_4O_{13}S_2$: 1013.3688; found: 1013.3682.

6.1.3. Synthesis of polar compounds **26-28**

- **Synthesis of polar chains 29-31**

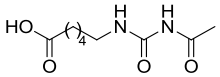
6-[(Aminocarbonyl)amino]hexanoic acid (29). Carboxylic acid **29** was obtained following the experimental procedure previously described by Huryn *et al.*[147]

To a solution of 6-aminohexanoic acid (555 mg, 4.2 mmol) in water (9 mL) at rt and under an argon atmosphere, potassium cyanate (1.00 g, 12 mmol) was added portionwise for 10 min. The reaction mixture was stirred at 60 ºC for 3 h. The solution was then cooled to 0 ºC and acidified with 1 M HCl (aq) until pH 1. The obtained precipitate was filtered, washed with cold water, and dried under high vacuum to obtain **29** (725 mg, 100%), which was used in the next step without further purification. Mp: 175-178 ºC (lit.[148] 175-176 ºC).



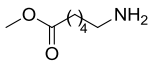
IR (ATR, ν): 3404, 3342, 3206 (NH, OH), 1707, 1656 (CO); ^{1}H-NMR ($(CD_3)_2SO$, δ): 1.18-1.38 (m, 4H, $2CH_2$), 1.43-1.53 (qt, *J*=7.4, 2H, CH_2), 2.19 (t, *J*=7.3, 2H, CH_2CO), 2.92 (t, *J*=6.6, 2H, CH_2N), 5.91 (br s, 1H, NH); ^{13}C-NMR ($(CD_3)_2SO$, δ): 24.3, 26.0, 29.8 ($(CH_2)_3$), 33.7 (CH_2CO), 39.1 (CH_2N), 158.7 (NCON), 174.5 (COO); MS (ESI, *m/z*): 175.1 $[M+H]^+$.

6-{[(Acetylamino)carbonyl]amino}hexanoic acid (30). To a refluxing suspension of KOAc (41 mg, 0.42 mmol) in acetic anhydride (1.7 mL) under an argon atmosphere, urea **29** was added (100 mg, 0.57 mmol). After stirring for 15 min under reflux, the reaction mixture was poured into ice and stirred for 30 min to remove the excess of acetic anhydride. Then, the solvent was evaporated under reduced pressure. Toluene was then added and further evaporated. The crude was purified by chromatography (DCM to MeOH) to obtain **30** (35 mg, 28%). Mp: 115-117 ºC.



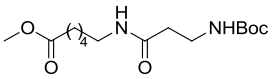
IR (ATR, ν): 3318, 3235, 3118 (NH, OH), 1700, 1549 (CO); ^{1}H-NMR (CD_3OD, δ): 1.33-1.43 (m, 2H, CH_2), 1.52-1.68 (m, 4H, $2CH_2$), 2.06 (s, 3H, CH_3), 2.30 (t, *J*=7.4, 2H, CH_2CO), 3.25 (t, *J*=7.0, 2H, CH_2N); ^{13}C-NMR (CD_3OD, δ): 23.6 (CH_3), 25.7, 27.4, 30.4 ($(CH_2)_3$), 34.8 (CH_2CO), 40.3 (CH_2N), 155.7 (NCON), 174.2 (CON), 177.4 (COO); MS (ESI, *m/z*): 217.1 $[M+H]^+$.

Methyl 6-aminohexanoate (35). A solution of $SOCl_2$ (1.0 mL, 15 mmol) in anhydrous MeOH (5 mL) under an argon atmosphere was stirred at 0 ºC for 10 min. Then, 6-aminohexanoic acid (525 mg, 4.0 mmol) was added and the mixture was stirred at that temperature for 20 min. The solvent was evaporated under reduced pressure to yield **35** (580 mg, 100%), which was used in the next step without further purification, mp: 93-96 ºC (lit.[149] 80-85 ºC). R_f: 0.17 (DCM/MeOH, 85:15).



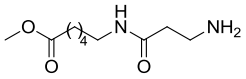
IR (ATR, ν): 1729 (CO); ^{1}H-NMR (CD_3OD, δ): 1.37-1.47 (m, 2H, CH_2), 1.62-1.71 (m, 4H, $2CH_2$), 2.37 (t, *J*=7.3, 2H, CH_2CO), 2.92 (t, *J*=7.6, 2H, CH_2N), 3.66 (s, 3H, CH_3); ^{13}C-NMR (CD_3OD, δ): 25.4, 27.0, 28.3 ($(CH_2)_3$), 34.5 (CH_2CO), 41.1 (CH_2N), 52.3 (CH_3), 175.4 (CO); MS (ESI, *m/z*): 146.0 $[M+H]^+$.

Methyl 6-{[*N*-(*tert*-butoxycarbonyl)-β-alanyl]amino}hexanoate (36). To a solution of *N*-(*tert*-butoxycarbonyl)-β-alanine (0.76 g, 4.0 mmol) in dry DCM at 0 ºC and under an argon atmosphere, EDC (1.0 g, 5.2 mmol) and HOBt (0.71 g, 5.2 mmol) were added, and the mixture was stirred at 0 ºC for 30 min. Then, amine **35** (0.64 g, 4.4 mmol) and triethylamine (2.8 mL, 20 mmol) were added and the resulting mixture was allowed to warm to rt and stirred for 8 h. The reaction was quenched with water and the organic layer was separated, dried (Na_2SO_4), filtered, and evaporated under reduced pressure. The residue was purified by chromatography (DCM to DCM/MeOH, 8:2) to afford **36** (1.27 g, 100%). R_f: 0.21 (DCM/MeOH, 8:2).



IR (ATR, ν): 3316 (NH), 1707, 1649 (CO); ^{1}H-NMR ($CDCl_3$, δ): 1.34-1.69 (m, 15H, $(CH_2)_3$, $C(CH_3)_3$), 2.31 (t, *J*=7.4, 2H, CH_2COO), 2.38 (t, *J*=5.8, 2H, CH_2CON), 3.25 (q app, *J*=6.6, 2H, CH_2NCO), 3.36-3.42 (m, 2H, CH_2NBoc), 3.66 (s, 3H, OCH_3), 5.18 (br s, 1H, NH), 5.80 (br s, 1H, NH); ^{13}C-NMR ($CDCl_3$, δ): 24.5, 26.4 ($2CH_2$), 28.5 ($C(CH_3)_3$) 29.3 (CH_2), 34.0 (CH_2COO), 36.5, 36.8 ($2CH_2N$), 39.3 (CH_2CON), 51.7 (OCH_3), 79.5 ($C(CH_3)_3$), 156.3 (NCOO), 171.4 (CON), 174.2 (COO).

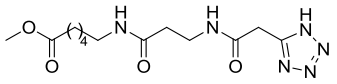
Methyl 6-(β-alanylamino)hexanoate (37). To a solution of **36** (512 mg, 1.6 mmol) in dry DCM (8 mL) under an argon atmosphere, TFA (2.5 mL, 32 mmol) was added dropwise and the reaction mixture was stirred at rt for 8 h. The solvent was evaporated under reduced pressure and the residue was redissolved in the minimum amount of DCM. Triethylamine was then added until basic pH was achieved and the solvent was evaporated under reduced pressure. The residue was purified by chromatography (DCM to DCM/MeOH, 8:2) to yield amine **37** (329 mg, 94%). R_f: 0.26 (DCM/MeOH, 85:15).



IR (ATR, ν): 3304, 3110 (NH), 1679 (CO); ^{1}H-NMR ($CDCl_3$, δ): 1.32-1.35 (m, 2H, CH_2), 1.51 (qt, *J*=7.3, 2H, CH_2), 1.60 (qt, *J*=7.5, 2H, CH_2), 2.32 (t, *J*=7.2, 2H, CH_2COO), 2.68 (t, *J*=6.4, 2H, CH_2CON), 3.21 (q app, *J*=6.4, 2H, CH_2NH), 3.31 (m, 2H, CH_2NH_2), 3.66 (s, 3H, CH_3), 6.87 (br s, 1H, NH), 7.89 (br s, 2H, NH_2); ^{13}C-NMR ($CDCl_3$, δ): 24.2, 26.1, 28.5

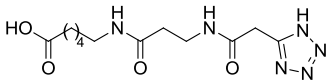
($(CH_2)_3$), 31.6 (CH_2NH_2), 33.8 ($\underline{C}H_2COO$), 36.8 (CH_2NH), 39.6 ($\underline{C}H_2CON$), 51.8 (CH_3), 171.4 (CON), 174.9 (COO); MS (ESI, *m/z*): 217.1 $[M+H]^+$.

Methyl 6-{[*N*-(1*H*-tetrazol-5-ylacetyl)-β-alanyl]amino}hexanoate (38). To a solution of 1*H*-tetrazol-5-ylacetic acid (223 mg, 1.7 mmol), **37** (377 mg, 1.7 mmol) and triethylamine (0.5 mL, 3.5 mmol) in dry DCM (3.5 mL) at 10 ºC and under an argon atmosphere, BOP-Cl (444 mg, 1.7 mmol) was added and the reaction mixture was stirred at rt for 1 h. The reaction was quenched with water and 2 M HCl (aq) was added until pH 1-2. The obtained precipitate was filtered and washed with DCM to obtain **38** (254 mg, 57%), which was used in the next step without further purification. R_f: 0.29 (DCM/MeOH/ammonia, 8:2:0.3).



^{1}H-NMR ($CD_3OD/(CD_3)_2CO$, 9:1, δ): 1.26-1.39 (m, 2H, CH_2), 1.50 (qt, *J*=7.2, 2H, CH_2), 1.60 (qt, *J*=7.5, 2H, CH_2), 2.33 (t, *J*=7.4, 2H, CH_2COO), 2.41 (t, *J*=6.7, 2H, CH_2CON), 3.17 (t, *J*=7.0, 2H, CH_2N), 3.48 (t, *J*=6.7, 2H, CH_2N), 3.65 (s, 3H, CH_3), 3.95 (s, 2H, CH_2CN).

6-{[*N*-(1*H*-Tetrazol-5-ylacetyl)-β-alanyl]amino}hexanoic acid (31). To a solution of methyl ester **38** (224 mg, 0.69 mmol) in MeOH (2.5 mL) under an argon atmosphere, 1 M NaOH (aq, 2.1 mL, 2.1 mmol) was added and the mixture was stirred at rt for 3 h. The solvent was evaporated under reduced pressure and the residue was acidified with 1 M HCl (aq) until an acid pH was achieved. The obtained precipitate was isolated by filtration and washed with water to afford acid **31** (214 mg, 100%), which was used in the next step without further purification. Mp: 190-193 ºC. R_f: 0.28 (DCM/MeOH, 1:1).

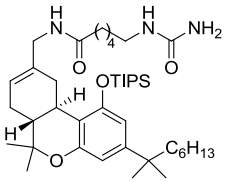


IR (ATR, ν): 3314 (NH), 2143 (NN), 1729, 1644, 1551 (CO); ^{1}H-NMR ($(CD_3)_2SO$, δ): 1.19-1.29 (m, 2H, CH_2), 1.38 (qt, *J*=7.2, 2H, CH_2), 1.48 (qt, *J*=7.4, 2H, CH_2), 2.19 (t, *J*=7.5, 2H, CH_2COO), 2.24 (t, *J*=7.0, 2H, CH_2CON), 3.02 (q app, *J*=6.3, 2H, CH_2N), 3.27 (q app, *J*=6.5, 2H, CH_2N), 3.85 (s, 2H, CH_2CN), 4.05 (br s, 1H, NH), 7.85 (t, *J*=5.4, 1H, NH), 8.37 (t, *J*=5.5, 1H, NH); ^{13}C-NMR ($(CD_3)_2SO$, δ): 24.2, 26.0, 28.8 ($(CH_2)_3$), 30.4 (CH_2N), 33.6, 35.1

($\underline{C}H_2CN$, CH_2COO), 35.6 (CH_2N), 38.3 ($\underline{C}H_2CON$), 154.2 (CN), 166.0, 169.9 (2CON), 174.4 (COO); MS (ESI, *m/z*): 313.1 $[M+H]^+$.

General procedure for the synthesis of intermediates 32-34. To a suspension of the corresponding carboxylic acid (1-2 equiv), HOBt (1.2-2.2 equiv), and activated 4 Å molecular sieves in anhydrous DMF (11 mL/mmol of acid) under an argon armosphere, a solution of EDC (1.2-2.2 equiv) in dry DCM (18 mL/mmol of acid) was added dropwise and the mixture was stirred at rt for 3 h. Then, a solution of the corresponding amine (1 equiv) and DMAP (0.2 equiv) in dry DCM (46 mL/mmol of amine) was added and the reaction was stirred at rt for 16 h. The mixture was filtered, diluted with DCM, washed with saturated $NaHCO_3$ (aq) and brine. The organic layer was dried (Na_2SO_4), filtered, and evaporated under reduced pressure. The crude was purified by chromatography using the appropriate eluent to afford the corresponding amide.

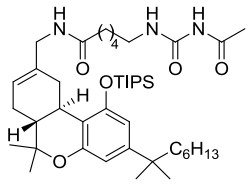
(-)-6-[(Aminocarbonyl)amino]-*N*-({(6a*R*,10a*R*)-3-(1,1-dimethylheptyl)-6,6-dimethyl-1-[(triisopropylsilyl)oxy]-6a,7,10,10a-tetrahydro-6*H*-benzo[*c*]chromen-9-yl}methyl)-hexanamide (32). Obtained from acid **29** (68 mg, 0.28 mmol) and amine **11** (146 mg, 0.27 mmol) in 75% yield (141 mg). Chromatography: DCM to DCM/MeOH, 95:5. Mp: 86-88 ºC. $[\alpha]_D^{20}$: -120.2 (c=1.03, chloroform). R_f: 0.20 (DCM/MeOH, 95:5).



IR (ATR, ν): 3312 (NH), 1647, 1610 (CO), 1564, 1463 (Ar); ^{1}H-NMR ($CDCl_3$, δ): 0.83 (t, *J*=7.2, 3H, $C\underline{H}_3CH_2$), 1.05-1.37 (m, 43H, $3CH(CH_3)_2$, $2CH_3C_{cyc}$, $2CH_3$, $(CH_2)_4$, CH_2), 1.46-1.54 (m, 4H, $C\underline{H}_2C(CH_3)_2$, CH_2), 1.59-1.69 (m, 2H, CH_2), 1.74-1.88 (m, 3H, ½CH_2C=, ½$C\underline{H}_2$CH=, $C\underline{H}C(CH_3)_2$), 2.16-2.21 (m, 3H, ½$C\underline{H}_2$CH=, CH_2CO), 2.62 (td, *J*=10.7, 4.1, 1H, CHC_{Ar}), 3.15 (m, 2H, CH_2NCON), 3.28 (dd, *J*=16.1, 3.6, 1H, ½CH_2C=), 3.71-3.85 (m, 2H, CH_2N_{HU}), 5.10 (br s, 1H, NH), 5.59 (d, *J*=4.2, 1H, CH=), 5.64 (t, *J*=5.4, 1H, NH), 6.34 (d, *J*=1.4, 1H, CH_{Ar}), 6.38 (d, *J*=1.4, 1H, CH_{Ar}); ^{13}C-NMR ($CDCl_3$, δ): 13.3 (3CHSi), 14.2 ($\underline{C}H_3CH_2$), 18.22 ($3CH(\underline{C}H_3)_2$), 18.25 ($\underline{C}H_3C_{cyc}$), 22.8, 24.8, 25.3, 26.5 ($4CH_2$), 27.6 ($\underline{C}H_3C_{cyc}$), 27.9 ($\underline{C}H_2CH$=), 28.8, 29.2 ($2CH_3$), 29.8, 30.2, 32.0 ($3CH_2$), 32.1 ($\underline{C}HC_{Ar}$), 33.3 ($\underline{C}H_2C$=), 36.5 ($\underline{C}H_2CO$), 37.5 ($Ar\underline{C}(CH_3)_2$), 40.2 ($\underline{C}H_2NCON$), 44.8 ($\underline{C}H_2C(CH_3)_2$), 45.1 ($CH_2N_{HU}$), 45.5 ($\underline{C}HC(CH_3)_2$), 76.4

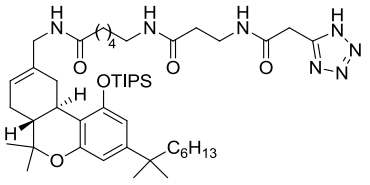
($O\underline{C}(CH_3)_2$), 108.3, 109.1 ($2CH_{Ar}$), 113.2 (C_{Ar}), 121.1 (CH=), 135.2 (C=), 149.5, 154.2, 155.1 ($3C_{Ar}$), 159.4 (NCON), 173.2 (CON); MS (ESI): 696.5 $[M-H]^-$.

(-)-6-{[(Acetylamino)carbonyl]amino}-*N*-({(6a*R*,10a*R*)-3-(1,1-dimethylheptyl)-6,6-dimethyl-1-[(triisopropylsilyl)oxy]-6a,7,10,10a-tetrahydro-6*H*-benzo[*c*]chromen-9-yl}methyl)hexanamide (33). Obtained from acid **30** (35 mg, 0.16 mmol) and amine **11** (87 mg, 0.16 mmol) in 51% yield (60 mg). Chromatography: DCM to DCM/MeOH, 95:5. $[\alpha]_D^{20}$: -101.9 (c=0.97, chloroform). R_f: 0.42 (DCM/MeOH, 95:5).



IR (ATR, ν): 3306, 3104 (NH), 1696, 1649 (CO), 1560, 1463 (Ar); ^{1}H-NMR ($CDCl_3$, δ): 0.83 (t, *J*=6.8, 3H, $C\underline{H}_3CH_2$), 1.04-1.41 (m, 43H, $3CH(CH_3)_2$, $2CH_3C_{cyc}$, $2CH_3$, $(CH_2)_4$, CH_2), 1.45-1.51 (m, 2H, $C\underline{H}_2C(CH_3)_2$), 1.53-1.71 (m, 4H, $2CH_2$), 1.74-1.85 (m, 3H, ½CH_2C=, ½$C\underline{H}_2$CH=, $C\underline{H}C(CH_3)_2$), 2.10 (s, 3H, CH_3CO), 2.15-2.20 (m, 3H, ½$C\underline{H}_2$CH=, CH_2CO), 2.62 (td, *J*=10.7, 4.3, 1H, CHC_{Ar}), 3.23-3.29 (m, 3H, CH_2NCON, ½CH_2C=), 3.78 (d, *J*=5.0, 2H, CH_2N_{HU}), 5.47 (t, *J*=5.2, 1H, NH), 5.58 (d, *J*=3.7, 1H, CH=), 6.32 (d, *J*=1.4, 1H, CH_{Ar}), 6.37 (d, *J*=1.4, 1H, CH_{Ar}), 8.46 (t, *J*=5.3, 1H, NH), 9.80 (br s, 1H, NH); ^{13}C-NMR ($CDCl_3$, δ): 13.3 (3CHSi), 14.2 ($\underline{C}H_3CH_2$), 18.22 ($3CH(\underline{C}H_3)_2$), 18.25 ($\underline{C}H_3C_{cyc}$), 22.8 (CH_2), 24.1 ($\underline{C}H_3CO$), 24.8, 25.4, 26.7 ($3CH_2$), 27.6 ($\underline{C}H_3C_{cyc}$), 27.9 ($\underline{C}H_2CH$=), 28.8, 29.1 ($2CH_3$), 29.4, 30.2, 32.0 ($3CH_2$), 32.1 ($\underline{C}HC_{Ar}$), 33.3 ($\underline{C}H_2C$=), 36.7 ($\underline{C}H_2CO$), 37.5 ($Ar\underline{C}(CH_3)_2$), 39.5 ($\underline{C}H_2NCON$), 44.8 ($\underline{C}H_2C(CH_3)_2$), 45.0 ($CH_2N_{HU}$), 45.5 ($\underline{C}HC(CH_3)_2$), 76.3 ($O\underline{C}(CH_3)_2$), 108.3, 109.0 ($2CH_{Ar}$), 113.2 ($C_{Ar}$), 121.1 (CH=), 135.3 (C=), 149.4, 154.3 ($2C_{Ar}$), 154.7 (NCON), 155.1 (C_{Ar}), 172.5, 172.6 (2CON); MS (ESI, *m/z*): 738.5 $[M-H]^-$.

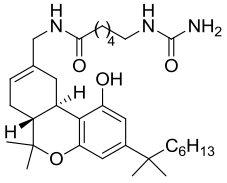
(-)-*N*-({(6a*R*,10a*R*)-3-(1,1-Dimethylheptyl)-6,6-dimethyl-1-[(triisopropylsilyl)oxy]-6a,7,10,10a-tetrahydro-6*H*-benzo[*c*]chromen-9-yl}methyl)-6-{[*N*-(1*H*-tetrazol-5-ylacetyl)-β-alanyl]amino}hexanamide (34). Obtained from acid **31** (28 mg, 89 μmol) and amine **11** (24 mg, 44 μmol) in 49% yield (16 mg). Chromatography: DCM to DCM/MeOH, 8:2. Mp: 86-87 °C. $[\alpha]_D^{20}$: -20.0 (c=0.11, chloroform). R_f: 0.35 (DCM/MeOH, 8:2).



IR (ATR, ν): 3286 (NH), 1647, 1561 (CO), 1464 (Ar); ^{1}H-NMR (CD_3OD, δ): 0.86 (t, *J*=6.8, 3H, CH_3CH_2), 1.06-1.64 (m, 49H, $3CH(CH_3)_2$, $2CH_3C_{cyc}$, $CH_2C(CH_3)_2$, $(CH_2)_4$, $(CH_2)_3$), 1.73-1.88 (m, 3H, ½CH_2C=, ½CH_2CH=, $CHC(CH_3)_2$), 2.16-2.23 (m, 3H, ½CH_2CH=, CH_2CO), 2.39 (t, *J*=6.8, 2H, CH_2CO), 2.66 (td, *J*=10.9, 4.3, 1H, CHC_{Ar}), 3.14 (t, *J*=6.9, 2H, CH_2N), 3.32-3.38 (m, 1H, ½CH_2C=), 3.45 (t, *J*=6.6, 2H, CH_2N), 3.67 (d, *J*=15.4, 1H, ½CH_2N_{HU}), 3.80 (d, *J*=14.7, 1H, ½CH_2N_{HU}), 3.90 (m, 2H, CH_2CN), 5.61 (m, 1H, CH=), 6.34-6.35 (m, 2H, $2CH_{Ar}$); ^{13}C-NMR ($CDCl_3$, δ): 13.3 (3CHSi), 14.3 (CH_3CH_2), 18.2 ($3CH(CH_3)_2$), 18.3 (CH_3C_{cyc}), 22.8, 24.9, 25.2, 26.2 ($4CH_2$), 27.6 (CH_3C_{cyc}), 27.9 (CH_2CH=), 28.83 (CH_3), 28.85 (CH_2), 29.2 (CH_3), 29.8, 30.2 ($2CH_2$), 32.0 (CH_2CN), 32.1 (CHC_{Ar}), 33.3 (CH_2CN), 35.8, 36.3 ($2CH_2CO$), 36.6 (CH_2N), 37.6 ($ArC(CH_3)_2$), 39.2 (CH_2N), 44.8 ($CH_2C(CH_3)_2$), 45.46 (CH_2N_{HU}), 45.51 ($CHC(CH_3)_2$), 76.4 ($OC(CH_3)_2$), 108.4, 109.1 ($2CH_{Ar}$), 113.2 (C_{Ar}), 121.8 (CH=), 134.7 (C=), 149.6 (C_{Ar}), 154.3 (C_{Ar}, CN), 155.4 (C_{Ar}), 171.6, 171.8, 174.1 (3CON); MS (ESI, *m/z*): 834.6 $[M-H]^-$.

- **Synthesis of final compounds 26-28**

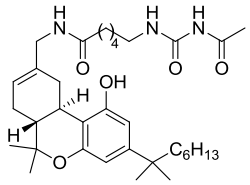
(-)-6-[(Aminocarbonyl)amino]-*N*-{[(6a*R*,10a*R*)-3-(1,1-dimethylheptyl)-1-hydroxy-6,6-dimethyl-6a,7,10,10a-tetrahydro-6*H*-benzo[*c*]chromen-9-yl]methyl}hexanamide (26). Following the general procedure 6.1.1.1, phenol **26** was obtained from amide **32** (110 mg, 0.16 mmol) in 93% yield (81 mg). Chromatography: DCM to DCM/MeOH, 8:2. Mp: 104-106 ºC. $[\alpha]_D^{20}$: -121.4 (c=0.63, MeOH). R_f: 0.10 (DCM/MeOH, 97:3).



IR (ATR, ν): 3330 (NH, OH), 1648 (CO), 1568, 1457 (Ar); ^{1}H-NMR (CD_3OD, δ): 0.86 (t, *J*=6.7, 3H, CH_3CH_2), 1.01-1.41 (m, 22H, $2CH_3C_{cyc}$, $2CH_3$, $(CH_2)_4$, CH_2), 1.45-1.55 (m, 4H, $CH_2C(CH_3)_2$, CH_2), 1.60-1.92 (m, 5H, CH_2, ½CH_2C=, ½CH_2CH=, $CHC(CH_3)_2$), 2.20-2.25 (m, 3H, ½CH_2CH=, CH_2CO), 2.64 (td, *J*=11.0, 4.4, 1H, CHC_{Ar}), 3.08 (t, *J*=7.0, 2H, CH_2NCON),

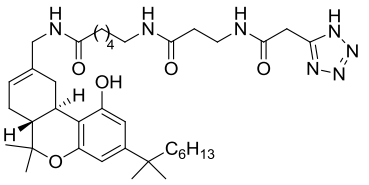
3.40 (dd, *J*=17.9, 4.1, 1H, ½CH_2C=), 3.68-3.79 (m, 2H, CH_2N_{HU}), 5.63 (d, *J*=3.2, 1H, CH=), 6.22 (d, *J*=1.6, 1H, CH_{Ar}), 6.34 (d, *J*=1.8, 1H, CH_{Ar}); ^{13}C-NMR (CD_3OD, δ): 14.4 (CH_3CH_2), 18.6 (CH_3C_{cyc}), 23.6, 25.8, 26.8, 27.5 (4CH_2), 28.0 (CH_3C_{cyc}), 28.8 (CH_2CH=), 29.4, 29.5 (2CH_3), 30.9, 31.1, 32.9 (3CH_2), 33.0 (CHC_{Ar}), 33.7 (CH_2C=), 37.0 (CH_2CO), 38.2 (Ar$C(CH_3)_2$), 40.9 (CH_2NCON), 45.5 ($CH_2C(CH_3)_2$), 46.0 (CH_2N_{HU}), 46.8 ($CHC(CH_3)_2$), 77.3 ($OC(CH_3)_2$), 106.4, 107.6 (2CH_{Ar}), 111.2 (C_{Ar}), 122.2 (CH=), 136.6 (C=), 150.6, 155.5, 157.6 (3C_{Ar}), 162.3 (NCON), 176.1 (CON); HRMS (MALDI, *m/z*): calcd for $[M+H]^+$ $C_{32}H_{52}N_3O_4$: 542.3958; found: 542.3945; HPLC-MS (ESI, *m/z*): 542.3 $[M+H]^+$; t_R (method A): 18.50 min.

(-)-6-{[(Acetylamino)carbonyl]amino}-*N*-{[(6a*R*,10a*R*)-3-(1,1-dimethylheptyl)-1-hydroxy-6,6-dimethyl-6a,7,10,10a-tetrahydro-6*H*-benzo[*c*]chromen-9-yl]methyl}-hexanamide (27). Following the general procedure 6.1.1.1, phenol **27** was obtained from amide **33** (46 mg, 62 μmol) in 89% yield (32 mg). Chromatography: DCM to DCM/MeOH, 98:2. Mp: 97-99 ºC. $[\alpha]_D^{20}$: -132.9 (c=0.55, MeOH). R_f: 0.20 (DCM/MeOH, 97:3).



IR (ATR, ν): 3291 (NH), 1695, 1651 (CO), 1552, 1462 (Ar); ^{1}H-NMR (CD_3OD, δ): 0.86 (t, *J*=6.7, 3H, CH_3CH_2), 1.08-1.42 (m, 22H, 2CH_3C_{cyc}, 2CH_3, $(CH_2)_4$, CH_2), 1.49-1.88 (m, 9H, $CH_2C(CH_3)_2$, 2CH_2, ½CH_2C=, ½CH_2CH=, $CHC(CH_3)_2$), 2.05 (s, 3H, CH_3CO), 2.20-2.25 (m, 3H, ½CH_2CH=, CH_2CO), 2.64 (td, *J*=11.0, 4.5, 1H, CHC_{Ar}), 3.23 (t, *J*=6.9, 2H, CH_2NCON), 3.40 (dd, *J*=17.8, 3.2, 1H, ½CH_2C=), 3.68-3.79 (m, 2H, CH_2N_{HU}), 5.62 (m, 1H, CH=), 6.22 (d, *J*=1.6, 1H, CH_{Ar}), 6.32 (d, *J*=1.7, 1H, CH_{Ar}); ^{13}C-NMR (CD_3OD, δ): 14.4 (CH_3CH_2), 18.6 (CH_3C_{cyc}), 23.61 (CH_3CO), 23.64, 25.8, 26.7, 27.5 (4CH_2), 28.0 (CH_3C_{cyc}), 28.8 (CH_2CH=), 29.4, 29.5 (2CH_3), 30.4, 31.1, 32.9 (3CH_2), 33.0 (CHC_{Ar}), 33.7 (CH_2C=), 37.0 (CH_2CO), 38.2 (Ar$C(CH_3)_2$), 40.3 (CH_2NCON), 45.5 ($CH_2C(CH_3)_2$), 46.0 (CH_2N_{HU}), 46.8 ($CHC(CH_3)_2$), 77.3 ($OC(CH_3)_2$), 106.3, 107.6 (2CH_{Ar}), 111.2 (C_{Ar}), 122.2 (CH=), 136.7 (C=), 150.6, 155.5 (2C_{Ar}), 155.7 (NCON), 157.6 (C_{Ar}), 174.2, 176.0 (2CON); HRMS (MALDI, *m/z*): calcd for $[M+H]^+$ $C_{34}H_{54}N_3O_5$: 584.4063; found: 584.4079; HPLC-MS (ESI, *m/z*): 584.3 $[M+H]^+$; t_R (method A): 13.10 min.

(-)-*N*-{[(6a*R*,10a*R*)-3-(1,1-Dimethylheptyl)-1-hydroxy-6,6-dimethyl-6a,7,10,10a-tetrahydro-6*H*-benzo[*c*]chromen-9-yl]methyl}-6-{[*N*-(1*H*-tetrazol-5-ylacetyl)-β-alanyl]-amino}hexanamide (28). Following the general procedure 6.1.1.1, phenol **28** was obtained from amide **34** (58 mg, 69 μmol) in 32% yield (15 mg). Chromatography: DCM to DCM/MeOH, 8:2. Mp: 140-143 ºC. $[\alpha]_D^{20}$: -78.2 (c=0.22, MeOH). R_f: 0.38 (DCM/MeOH, 8:2).

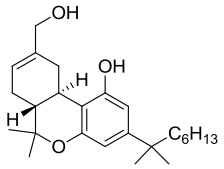


IR (ATR, ν): 3289, 3083 (NH, OH), 1647, 1560 (CO), 1457 (Ar); ^{1}H-NMR (500 MHz, $CD_3OD/CDCl_3$ 9:1, δ): 0.85 (t, *J*=6.9, 3H, CH_3CH_2), 1.08 (s, 3H, CH_3C_{cyc}), 1.19-1.37 (m, 19H, CH_3C_{cyc}, $2CH_3$, $(CH_2)_4$, CH_2), 1.42-1.53 (m, 4H, $CH_2C(CH_3)_2$, CH_2), 1.59-1.65 (m, 2H, CH_2), 1.70-1.90 (m, 3H, ½CH_2C=, ½CH_2CH=, $CHC(CH_3)_2$), 2.19-2.22 (m, 3H, ½CH_2CH=, CH_2CO), 2.40 (m, 2H, CH_2CO), 2.64 (td, *J*=10.7, 4.3, 1H, CHC_{Ar}), 3.14 (app t, *J*=6.9, 2H, CH_2N), 3.37-3.42 (m, 1H, ½CH_2C=), 3.50 (m, 2H, CH_2N), 3.70 (d, *J*=15.9, 1H, ½CH_2N_{HU}), 3.76 (d, *J*=15.8, 1H, ½CH_2N_{HU}), 3.84 (m, 2H, CH_2CN), 5.61 (m, 1H, CH=), 6.23 (d, *J*=1.5, 1H, CH_{Ar}), 6.34 (d, *J*=1.4, 1H, CH_{Ar}); ^{13}C-NMR (125 MHz, $CD_3OD/CDCl_3$ 9:1, δ): 14.4 (CH_3CH_2), 18.6 (CH_3C_{cyc}), 23.6, 25.7, 26.6, 27.4 ($4CH_2$), 28.0 (CH_3C_{cyc}), 28.7 (CH_2CH=), 29.4 ($2CH_3$), 29.9, 30.7, 31.1 ($3CH_2$), 32.8 (CH_2CN), 32.9 (CHC_{Ar}), 33.6 (CH_2C=), 36.7, 36.9 ($2CH_2CO$), 37.3 (CH_2N), 38.1 ($ArC(CH_3)_2$), 40.2 (CH_2N), 45.5 ($CH_2C(CH_3)_2$), 45.9 (CH_2N_{HU}), 46.7 ($CHC(CH_3)_2$), 77.3 ($OC(CH_3)_2$), 106.3, 107.5 ($2CH_{Ar}$), 111.1 (C_{Ar}), 122.1 (CH=), 136.5 (C=), 150.5 (C_{Ar}), 155.4 (C_{Ar}, CN), 157.5 (C_{Ar}), 173.4, 173.7, 176.0 (3CON); HRMS (MALDI, *m/z*): calcd for $[M+H]^+$ $C_{37}H_{58}N_7O_5$: 680.4499; found: 680.4470; HPLC-MS (ESI, *m/z*): 680.4 $[M+H]^+$; t_R (method A): 15.20 min.

6.1.4. Synthesis of probes **39-41**, and **54**

(-)-(6a*R*,10a*R*)-3-(1,1-Dimethylheptyl)-9-(hydroxymethyl)-6,6-dimethyl-6a,7,10,10a-tetrahydro-6*H*-benzo[*c*]chromen-1-ol (HU210, 42). Phenol **42** was obtained following the experimental procedure previously described by Mechoulam *et al*.[84]

A solution of pivaloyl ester **8** (611 mg, 1.3 mmol)in anhydrous THF (25 mL) was added dropwise to a suspension of $LiAlH_4$ (198 mg, 5.4 mmol) in anhydrous THF (25 mL) at 0 ºC and under an argon atmosphere. The reaction was stirred at that temperature for 2 h and allowed to warm to rt. The mixture was then carefully quenched with water and extracted with Et_2O (2x). The organic extracts were washed with brine, dried (Na_2SO_4), filtered, and evaporated under reduced pressure. The residue was purified by chromatography (hexane to hexane/EtOAc, 9:1) to yield alcohol **42** (400 mg, 100%). Mp: 120-123 ºC. $[\alpha]_D^{20}$: -16.7 (c=1.0, EtOH). R_f: 0.15 (hexane/EtOAc, 7:3).



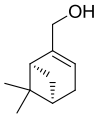
^{1}H-NMR ($CDCl_3$, δ): 0.87 (t, *J*=6.8, 3H, C<u>H</u>$_3$CH$_2$), 1.15 (s, 3H, CH_3C_{cyc}), 1.21-1.25 (m, 8H, $(CH_2)_4$), 1.28 (s, 6H, $2CH_3$), 1.42 (s, 3H, CH_3C_{cyc}), 1.47-1.52 (m, 2H, C<u>H</u>$_2$C(CH$_3$)$_2$), 1.82-1.98 (m, 3H, ½CH$_2$C=, ½C<u>H</u>$_2$CH=, C<u>H</u>C(CH$_3$)$_2$), 2.19-2.27 (m, 1H, ½C<u>H</u>$_2$CH=), 2.75 (td, *J*=11.0, 6.1, 1H, CHC_{Ar}), 3.40 (dd, *J*=15.7, 4.5, 1H, ½CH$_2$C=), 4.09 (AB system, *J*=13.0, 2H, CH_2O), 4.70 (br s, 2H, 2OH), 5.77 (d, *J*=5.1, 1H, CH=), 6.25 (d, *J*=1.7, 1H, CH_{Ar}), 6.42 (d, *J*=1.7, 1H, CH_{Ar}).

- **Synthesis of amine 43**

(+)-[(1*S*,5*R*)-6,6-Dimethylbicyclo[3.1.1]hept-2-en-2-yl]methanol (44). Alcohol **44** was obtained following the experimental procedure previously described by Harwood *et al.*[150] The spectroscopic data correspond with those reported.[151]

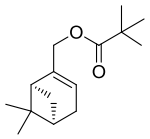
A solution of (+)-α-pinene (13.00 g, 95 mmol) in dry DCM (50 mL) was stirred at rt with SeO_2 (5.29 g, 48 mmol) and *t*-butylhydroperoxide (24 mL, 80%, 191 mmol) for 24 h. The mixture was washed with 10% KOH (aq, 4x). The organic phase was dried (Na_2SO_4), filtered, and evaporated under reduced pressure. The residue was dissolved in Et_2O (100

mL) and $LiAlH_4$ (3.80 g, 100 mmol) was added portionwise to the stirred solution at 0 °C and under an argon atmosphere. After 4 h, the excess of hydride was carefully destroyed with water and the organic phase was separated, dried (Na_2SO_4), filtered, and evaporated under reduced pressure. The residue was purified by chromatography (hexane/EtOAc, 9:1) to give alcohol **44** (7.86 g, 54%). $[\alpha]_D^{20}$: +35.0 (c=3.2, EtOH), lit.[150] +44.3 (c=3.2, chloroform). R_f: 0.56 (hexane/EtOAc, 7:3).



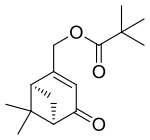
^{1}H-NMR ($CDCl_3$, δ): 0.82 (s, 3H, CH_3), 1.17 (d, *J*=8.6, 1H, ½CH_{2cyc}), 1.28 (s, 3H, CH_3), 2.07-2.20 (m, 2H, 2CH), 2.24-2.28 (m, 2H, CH_2CH=), 2.39 (dt, *J*=8.6, 5.5, 1H, ½CH_{2cyc}), 3.96 (dd, *J*=3.4, 1.7, 2H, CH_2O), 5.45-5.49 (m, 1H, CH=).

(+)-[(1*S*,5*R*)-6,6-Dimethylbicyclo[3.1.1]hept-2-en-2-yl]methyl pivalate [(+)-4)]. Pivaloyl ester (+)-**4** was prepared from alcohol **44** (7.80 g, 51 mmol), following the procedure described for (-)-**4** in 100% yield (12.12 g). $[\alpha]_D^{20}$: +26.8 (c=3.8, EtOH).



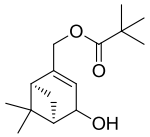
The spectroscopic data of (+)-**4** were identical to those obtained for (-)-**4**.

(+)-[(1*S*,5*R*)-6,6-Dimethyl-4-oxobicyclo[3.1.1]hept-2-en-2-yl]methyl pivalate [(+)-5)]. Ketone (+)-**5** was prepared from compound (+)-**4** (12.11 g, 51 mmol), following the procedure described for (-)-**5** in 40% yield (5.10 g). $[\alpha]_D^{20}$: +135.3 (c=1.8, EtOH).



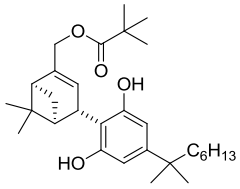
The spectroscopic data of (+)-**5** were identical to those obtained for (-)-**5**.

(+)-[(1*S*,5*R*)-4-Hydroxy-6,6-dimethylbicyclo[3.1.1]hept-2-en-2-yl]methyl pivalate [(+)-6)]. Alcohol (+)-**6** was prepared from ketone (+)-**5** (4.41 g, 18 mmol), following the procedure described for (-)-**6** in 63% yield (2.80 g). $[\alpha]_D^{20}$: +10.0 (c=2.0, EtOH).



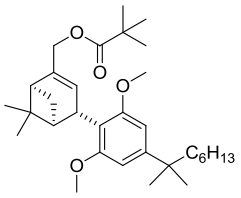
The spectroscopic data of (+)-**6** were identical to those obtained for (-)-**6**.

(+)-{(1*S*,4*S*,5*S*)-4-[4-(1,1-Dimethylheptyl)-2,6-dihydroxyphenyl]-6,6-dimethyl-bicyclo[3.1.1]hept-2-en-2-yl}methyl pivalate (45). To a solution of resorcinol **7** (1.64 g, 7.0 mmol) and anhydrous *p*-TSA (383 mg, 2.2 mmol) in dry DCM (255 mL) at 0 ºC and under an argon atmosphere, a solution of alcohol (+)-**6** in dry DCM (50 mL) was added over a period of 30 min. The reaction was stirred at rt for 1 h and then washed with saturated $NaHCO_3$ (aq). The aqueous layer was extracted with DCM and the combined organic extracts were dried (Na_2SO_4), filtered, and evaporated under reduced pressure. The residue was purified by chromatography (hexane to hexane/EtOAc, 9:1) to give resorcinol **45** (2.69 g, 82%). $[\alpha]_D^{20}$: +66.5 (c=0.75, EtOH). R_f: 0.11 (hexane/DCM, 1:1).



IR (ATR, ν): 3402 (OH), 1730 (CO), 1546, 1454 (Ar); ^{1}H-NMR ($CDCl_3$, δ): 0.86 (t, *J*=6.6, 3H, C<u>H</u>$_3$CH$_2$), 0.99 (s, 3H, CH$_3$C$_{cyc}$), 1.05-1.29 (m, 23H, 2CH$_3$, C(CH$_3$)$_3$, (CH$_2$)$_4$), 1.35 (s, 3H, CH$_3$C$_{cyc}$), 1.48-1.53 (m, 3H, C<u>H</u>$_2$C(CH$_3$)$_2$, ½CH$_{2cyc}$), 2.30-2.42 (m, 3H, C<u>H</u>CHC$_{Ar}$, CHC=, ½CH$_{2cyc}$), 4.01 (m, 1H, CHC$_{Ar}$), 4.58 (AB system, *J*=13.5, 2H, CH$_2$O), 5.70 (br s, 2H, 2OH), 6.01-6.02 (m, 1H, CH=), 6.35 (s, 2H, 2CH$_{Ar}$); ^{13}C-NMR ($CDCl_3$, δ): 14.1 (<u>C</u>H$_3$CH$_2$), 20.8 (<u>C</u>H$_3$C$_{cyc}$), 22.7, 24.7 (2CH$_2$), 25.9 (<u>C</u>H$_3$C$_{cyc}$), 27.3 (C(<u>C</u>H$_3$)$_3$), 28.1 (CH$_{2cyc}$), 28.7 (2CH$_3$), 30.1, 31.8 (2CH$_2$), 37.4 (<u>C</u>HC$_{Ar}$), 37.8 (Ar<u>C</u>(CH$_3$)$_2$), 39.0 (<u>C</u>(CH$_3$)$_3$), 40.9 (<u>C</u>$_{cyc}$(CH$_3$)$_2$), 44.1 (<u>C</u>HC=), 44.4 (<u>C</u>H$_2$C(CH$_3$)$_2$), 47.3 (<u>C</u>HCHC$_{Ar}$), 66.4 (CH$_2$O), 106.4 (2CH$_{Ar}$), 111.5 (C$_{Ar}$), 120.2 (CH=), 149.5 (C$_{Ar}$, C=), 150.6, 154.9 (2C$_{Ar}$), 178.5 (CO).

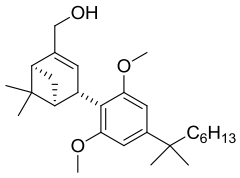
(+)-{(1*S*,4*S*,5*S*)-4-[4-(1,1-Dimethylheptyl)-2,6-dimethoxyphenyl]-6,6-dimethyl-bicyclo[3.1.1]hept-2-en-2-yl}methyl pivalate (46). To a solution of **45** (2.90 g, 6.2 mmol) in anhydrous DMF (55 mL) under an argon atmosphere, NaH (60% in mineral oil, 542 mg, 14 mmol) was added portionwise. Iodomethane (2.21 g, 15 mmol) was then added dropwise and the reaction was stirred at rt for 3 h. The mixture was poured into water and extracted with Et_2O (4x). The combined organic extracts were separated, washed with water, dried (Na_2SO_4), filtered, and evaporated under reduced pressure. The residue was purified by chromatography (hexane/EtOAc, 8:2) to give the title compound **46** (2.49 g, 81%). $[\alpha]_D^{20}$: +78.7 (c=0.7, EtOH). R_f: 0.28 (hexane/DCM, 1:1).



IR (ATR, ν): 1730 (CO), 1556, 1460 (Ar); ^{1}H-NMR ($CDCl_3$, δ): 0.86 (t, *J*=6.6, 3H, CH_3CH_2), 0.98 (s, 3H, CH_3C_{cyc}), 1.10-1.23 (m, 17H, $C(CH_3)_3$, $(CH_2)_4$), 1.28 (s, 6H, $2CH_3$), 1.30 (s, 3H, CH_3C_{cyc}), 1.54-1.60 (m, 2H, $CH_2C(CH_3)_2$), 1.73 (d, *J*=7.9, 1H, ½CH_{2cyc}), 2.05-2.08 (m, 1H, $CHCHC_{Ar}$), 2.16-2.20 (m, 2H, CHC=, ½CH_{2cyc}), 3.75 (s, 6H, $2CH_3O$), 4.00 (m, 1H, CHC_{Ar}), 4.55 (AB system, *J*=13.5, 2H, CH_2O), 5.79 (m, 1H, CH=), 6.49 (s, 2H, $2CH_{Ar}$); ^{13}C-NMR ($CDCl_3$, δ): 14.1 (CH_3CH_2), 20.1 (CH_3C_{cyc}), 22.7, 24.7 ($2CH_2$), 26.3 (CH_3C_{cyc}), 27.3 ($C(CH_3)_3$), 27.6 (CH_{2cyc}), 28.9, 29.0 ($2CH_3$), 30.1, 31.8 ($2CH_2$), 37.6 (CHC_{Ar}), 38.0 ($ArC(CH_3)_2$), 39.0 ($C(CH_3)_3$), 40.9 ($C_{cyc}(CH_3)_2$), 43.8 (CHC=), 44.6 ($CH_2C(CH_3)_2$), 47.4 ($CHCHC_{Ar}$), 55.7 ($2CH_3O$), 67.5 (CH_2O), 102.7 ($2CH_{Ar}$), 117.5 (C_{Ar}), 126.4 (CH=), 137.2 (C=), 149.5 (C_{Ar}), 158.4 ($2C_{Ar}$), 178.5 (CO).

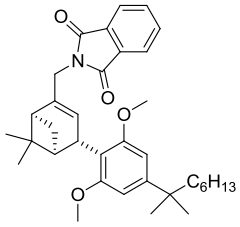
(+)-{(1*S*,4*S*,5*S*)-4-[4-(1,1-Dimethylheptyl)-2,6-dimethoxyphenyl]-6,6-dimethyl-bicyclo[3.1.1]hept-2-en-2-yl}methanol (47). To a suspension of $LiAlH_4$ (171 mg, 4.5 mmol) in anhydrous Et_2O (12 mL) under an argon atmosphere, a solution of pivaloyl ester **46** (900 mg, 1.8 mmol) in anhydrous Et_2O (20 mL) was added dropwise. The mixture was refluxed for 3 h. The reaction was carefully quenched with water. The organic layer was separated, dried (Na_2SO_4), filtered, and evaporated under reduced pressure. The residue was purified by chromatography (hexane to hexane/EtOAc, 8:2) to

give alcohol **47** (346 mg, 45%). $[\alpha]_D^{20}$: +95.0 (c=0.5, EtOH); lit.[31] +127.0 (c=0.3, chloroform). R_f: 0.13 (hexane/DCM, 1:9).



^{1}H-NMR ($CDCl_3$, δ): 0.86 (t, *J*=6.7, 3H, C<u>H</u>$_3$CH$_2$), 0.98 (s, 3H, CH_3C_{cyc}), 1.10-1.28 (m, 14H, 2CH_3, $(CH_2)_4$), 1.32 (s, 3H, CH_3C_{cyc}), 1.54-1.60 (m, 2H, C<u>H</u>$_2$C$(CH_3)_2$), 1.72 (d, *J*=7.8, 1H, ½CH_{2cyc}), 2.06-2.10 (m, 1H, C<u>H</u>CHC$_{Ar}$), 2.17-2.24 (m, 2H, CHC=, ½CH_{2cyc}), 3.75 (s, 6H, 2CH_3O), 4.01 (m, 1H, CHC_{Ar}), 4.01-4.09 (m, 2H, CH_2O), 5.71 (m, 1H, CH=), 6.49 (s, 2H, 2CH_{Ar}); ^{13}C-NMR ($CDCl_3$, δ): 14.1 (<u>C</u>H$_3$CH$_2$), 21.1 (<u>C</u>H$_3C_{cyc}$), 22.7, 24.7 (2CH_2), 26.3 (<u>C</u>H$_3C_{cyc}$), 27.9 (CH_{2cyc}), 28.9, 29.0 (2CH_3), 30.1, 31.8 (2CH_2), 37.5 (<u>C</u>HC$_{Ar}$), 38.0 (Ar<u>C</u>$(CH_3)_2$), 40.8 (<u>C</u>$_{cyc}(CH_3)_2$), 43.8 (<u>C</u>HC=), 44.6 (<u>C</u>H$_2$C$(CH_3)_2$), 47.4 (<u>C</u>HCHC$_{Ar}$), 55.8 (2CH_3O), 66.7 (CH_2O), 102.7 (2CH_{Ar}), 117.6 (C_{Ar}), 123.8 (CH=), 141.5, 141.9 (C_{Ar}, C=), 158.4 (2C_{Ar}).

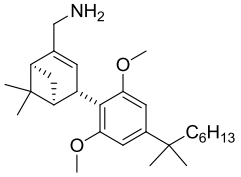
(+)-2-({(1*S*,4*S*,5*S*)-4-[4-(1,1-Dimethylheptyl)-2,6-dimethoxyphenyl]-6,6-dimethylbicyclo[3.1.1]hept-2-en-2-yl}methyl)-1*H*-isoindole-1,3(2*H*)-dione (48). Phthalimide **48** was prepared from alcohol **47** (307 mg, 0.74 mmol), following the procedure described for **10**, in 47% yield (189 mg). Chromatography: hexane to hexane/EtOAc, 8:2. $[\alpha]_D^{20}$: +62.2 (c=1.27, chloroform). R_f: 0.30 (hexane/EtOAc, 8:2).



<u>IR (ATR, ν)</u>: 1773 (CO), 1606, 1572, 1460 (Ar); <u>^{1}H-NMR ($CDCl_3$, δ)</u>: 0.85 (t, *J*=6.7, 3H, C<u>H</u>$_3$CH$_2$), 0.96 (s, 3H, CH_3C_{cyc}), 1.21-1.28 (m, 17H, CH_3C_{cyc}, 2CH_3, $(CH_2)_4$), 1.53-1.59 (m, 2H, C<u>H</u>$_2$C$(CH_3)_2$), 1.76 (d, *J*=7.7, 1H, ½CH_{2cyc}), 2.00-2.06 (m, 1H, C<u>H</u>CHC$_{Ar}$), 2.13-2.21 (m, 2H, CHC=, ½CH_{2cyc}), 3.74 (s, 6H, 2CH_3O), 3.97 (m, 1H, CHC_{Ar}), 4.29 (AB system, *J*=15.4, 2H, CH_2N), 5.58 (m, 1H, CH=), 6.46 (s, 2H, 2CH_{HU}), 7.69-7.71 (m, 2H, 2CH_{Phth}), 7.84-7.87 (m, 2H, 2CH_{Phth}); <u>^{13}C-NMR ($CDCl_3$, δ)</u>: 14.2 (<u>C</u>H$_3$CH$_2$), 20.9 (<u>C</u>H$_3C_{cyc}$), 22.8, 24.7 (2CH_2), 26.4

($\underline{C}H_3C_{cyc}$), 27.6 (CH_{2cyc}), 29.0 ($2CH_3$), 30.1, 31.8 ($2CH_2$), 37.4 ($\underline{C}HC_{Ar}$), 38.0 ($Ar\underline{C}(CH_3)_2$), 41.1 ($\underline{C}_{cyc}(CH_3)_2$), 42.5 ($CH_2N$), 44.2 ($\underline{C}HC=$), 44.6 ($\underline{C}H_2C(CH_3)_2$), 47.5 ($\underline{C}HCHC_{Ar}$), 55.7 ($2CH_3O$), 102.5 ($2CH_{HU}$), 117.4 ($C_{HU}$), 123.2 ($2CH_{Phth}$), 123.6 (CH=), 132.3 ($2C_{Phth}$), 133.8 ($2CH_{Phth}$), 135.9 (C=), 149.4 ($C_{HU}$), 158.5 ($2C_{HU}$), 168.2 (2CO); MS (ESI, *m/z*): 566.4 $[M+Na]^+$.

(+)-{(1*S*,4*S*,5*S*)-4-[4-(1,1-Dimethylheptyl)-2,6-dimethoxyphenyl]-6,6-dimethylbicyclo[3.1.1]hept-2-en-2-yl}methylamine (43). Amine **43** was prepared from phtalimide **48** (84 mg, 0.15 mmol), following the procedure described for **11** in 80% yield (50 mg). This compound was used in the next step without further purification. $[\alpha]_D^{20}$: +86.0 (c=1.04, chloroform). R_f: 0.21 (DCM/MeOH, 95:5).

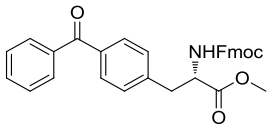


IR (ATR, ν): 2926 (NH_2), 1605, 1571, 1459 (Ar); ^{1}H-NMR ($CDCl_3$, δ): 0.85 (t, *J*=6.6, 3H, $C\underline{H}_3CH_2$), 0.96 (s, 3H, CH_3C_{cyc}), 1.06-1.27 (m, 14H, $2CH_3$, $(CH_2)_4$), 1.30 (s, 3H, CH_3C_{cyc}), 1.54-1.59 (m, 2H, $C\underline{H}_2C(CH_3)_2$), 1.72 (d, *J*=8.2, 1H, ½CH_{2cyc}), 1.72 (br s, 2H, NH_2), 2.05-2.22 (m, 3H, $C\underline{H}CHC_{Ar}$, CHC=, ½CH_{2cyc}), 3.23 (m, 2H, CH_2N), 3.74 (s, 6H, $2CH_3O$), 3.99 (m, 1H, CHC_{Ar}), 5.55 (m, 1H, CH=), 6.49 (s, 2H, $2CH_{Ar}$); ^{13}C-NMR ($CDCl_3$, δ): 14.2 ($\underline{C}H_3CH_2$), 21.2 ($\underline{C}H_3C_{cyc}$), 22.8, 24.7 ($2CH_2$), 26.4 ($\underline{C}H_3C_{cyc}$), 28.1 (CH_{2cyc}), 29.0 ($2CH_3$), 30.1, 31.8 ($2CH_2$), 37.5 ($\underline{C}HC_{Ar}$), 38.0 ($Ar\underline{C}(CH_3)_2$), 40.9 ($\underline{C}_{cyc}(CH_3)_2$), 44.58 ($\underline{C}HC=$), 44.61 ($\underline{C}H_2C(CH_3)_2$), 47.52 ($\underline{C}HCHC_{Ar}$), 47.55 ($CH_2N$), 55.9 ($2CH_3O$), 102.8 ($2CH_{Ar}$), 118.0 ($C_{Ar}$), 121.0 (CH=), 143.4 (C=), 149.4 (C_{Ar}), 158.5 ($2C_{Ar}$); MS (ESI, *m/z*): 397.4 $[M-CH_3+H]^+$.

- **Synthesis of tag 49**

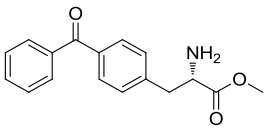
(+)-Methyl 4-benzoyl-*N*-[(9*H*-fluoren-9-ylmethoxy)carbonyl]-L-phenylalaninate (50). A suspension of 4-benzoyl-*N*-[(9*H*-fluoren-9-ylmethoxy)carbonyl]-L-phenylalanine (263 mg, 0.54 mmol) and K_2CO_3 (89 mg, 0.64 mmol) in anhydrous DMF (4.8 mL), under an argon atmosphere, was stirred at rt for 30 min. Iodomethane (100 µL, 1.6 mmol) was then added at 0 ºC and the reaction was stirred at rt for 90 min. The mixture was diluted with water, extracted with Et_2O (3x), and the combined organic extracts were dried (Na_2SO_4), filtered, and evaporated under reduced pressure. The residue was purified by

chromatography (hexane to hexane/EtOAc, 1:1) to afford methyl ester **50** (246 mg, 91%), mp: 58-62 ºC. $[\alpha]_D^{20}$: +43.0 (c=1.0, chloroform). R_f: 0.30 (hexane/EtOAc, 7:3).



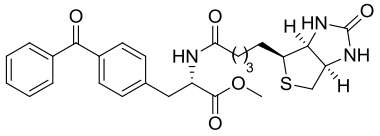
IR (ATR, ν): 1723, 1656 (CO), 1605, 1526, 1445 (Ar); ^{1}H-NMR ($CDCl_3$, δ): 3.22 (AB system, *J*=13.8, 5.8, 2H, $C\underline{H}_2$Bzph), 3.77 (s, 3H, CH_3), 4.22 (t, *J*=6.7, 1H, CH_{Fmoc}), 4.40 (dd, *J*=10.5, 6.8, 1H, ½CH_2O), 4.52 (dd, *J*=10.6, 7.0, 1H, ½CH_2O), 4.71-4.78 (m, 1H, CHN), 5.36 (d, *J*=8.1, 1H, NH), 7.21 (d, *J*=7.9, 2H, $2CH_{Bzph}$), 7.32 (t, *J*=7.4, 2H, $2CH_{Fmoc}$), 7.41 (t, *J*=7.5, 2H, $2CH_{Fmoc}$), 7.48 (t, *J*=7.5, 2H, $2CH_{Bzph}$), 7.57-7.63 (m, 3H, $2CH_{Fmoc}$, CH_{Bzph}), 7.74-7.80 (m, 6H, $2CH_{Fmoc}$, $4CH_{Bzph}$); ^{13}C-NMR ($CDCl_3$, δ): 38.6 ($\underline{C}H_2$Bzph), 47.6 (CH_{Fmoc}), 52.9 (CH_3), 55.0 (CHN), 67.3 (CH_2O), 120.4 ($2CH_{Fmoc}$), 125.4, 125.5 ($2CH_{Fmoc}$), 127.5 ($2CH_{Fmoc}$), 128.2 ($2CH_{Fmoc}$), 128.7 ($2CH_{Bzph}$), 129.7 ($2CH_{Bzph}$), 130.4 ($2CH_{Bzph}$), 130.8 ($2CH_{Bzph}$), 132.8 (CH_{Bzph}), 136.8 ($2C_{Bzph}$), 138.0, 141.2, 141.8, 144.1, 144.2 (C_{Bzph}, $4C_{Fmoc}$), 155.9 (NCOO), 172.0 (COO), 196.7 (CO); MS (ESI, *m/z*): 284.1 $[M\text{-Fmoc}]^+$, 506.1 $[M+H]^+$, 528.1 $[M+Na]^+$.

(+)-Methyl 4-benzoyl-L-phenylalaninate (51). To a solution of **50** (240 mg, 0.48 mmol) in dry DCM (0.3 mL) at 0 ºC and under an argon atmosphere, a solution of piperidine (0.24 mL, 2.4 mmol) in dry DCM (4.5 mL) was added dropwise. The reaction was stirred at rt for 1 h. The mixture was then quenched with 5% citric acid, washed with water and extracted with EtOAc (3x). The organic layers were dried (Na_2SO_4), filtered, and evaporated under reduced pressure. The residue was purified by chromatography (DCM to DCM/MeOH, 98:2) to afford the title compound **51** (118 mg, 88%). $[\alpha]_D^{20}$: +6.9 (c=1.0, chloroform). R_f: 0.21 (DCM/MeOH, 95:5). The spectroscopic data correspond with those previously reported.[117]



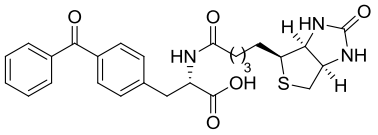
^{1}H-NMR ($CDCl_3$, δ): 1.56 (br s, 2H, NH_2), 2.95 (dd, *J*=13.5, 7.9, 1H, ½CH_2), 3.18 (dd, *J*=13.5, 5.2, 1H, ½CH_2), 3.74 (s, 3H, CH_3), 3.79 (dd, *J*=7.9, 5.2, 1H, CHN), 7.32 (d, *J*=8.2, 2H, $2CH_{Ar}$), 7.45-7.50 (m, 2H, $2CH_{Ar}$), 7.59 (t, *J*=7.5, 1H, CH_{Ar}), 7.75-7.81 (m, 4H, $4CH_{Ar}$).

(+)-Methyl 4-benzoyl-*N*-biotinyl-L-phenylalaninate (52). Following the procedure described for the synthesis of biotin derivative **16**, amide **52** was obtained from biotin (204 mg, 0.84 mmol) and amine **51** (118 mg, 0.42 mmol) in 59% yield (126 mg). Chromatography: DCM to DCM/MeOH, 95:5. Mp: 85-86 ºC. $[\alpha]_D^{20}$: +63.6 (c=0.59, chloroform). R_f: 0.30 (DCM/MeOH, 95:5).



IR (ATR, ν): 3272 (NH), 1738, 1698, 1656 (CO), 1606, 1539, 1448 (Ar); ^{1}H-NMR ($CDCl_3$, δ): 1.31-1.40 (m, 2H, CH_2), 1.54-1.72 (m, 4H, $2CH_2$), 2.18 (t, *J*=7.2, 2H, CH_2CO), 2.26 (d, *J*=12.8, 1H, ½CH_2S), 2.81 (dd, *J*=12.8, 4.9, 1H, ½CH_2S), 3.04-3.15 (m, 2H, CHS, ½CH_2Ar), 3.24 (dd, *J*=13.9, 5.5, 1H, ½CH_2Ar), 3.72 (s, 3H, CH_3), 4.26 (dd, *J*=7.6, 4.7, 1H, CHN_{Biot}), 4.47 (dd, *J*=7.6, 4.9, 1H, CHN_{Biot}), 4.85-4.92 (m, 1H, CHN), 6.00 (br s, 1H, NH), 6.77 (br s, 1H, NH), 7.23 (d, *J*=7.9, 1H, NH), 7.28 (d, *J*=8.2, 2H, $2CH_{Ar}$), 7.46 (t, *J*=7.4, 2H, $2CH_{Ar}$), 7.59 (t, *J*=7.5, 1H, CH_{Ar}), 7.73-7.77 (m, 4H, $4CH_{Ar}$); ^{13}C-NMR ($CDCl_3$, δ): 25.5, 28.1, 28.5 ($(CH_2)_3$), 35.7, 37.7 (CH_2CO, CH_2Ar), 40.6 (CH_2S), 52.6 (CH_3), 53.1 (CHN), 56.0 (CHS), 60.3, 62.0 ($2CHN_{Biot}$), 128.4 ($2CH_{Ar}$), 129.3 ($2CH_{Ar}$), 130.1 ($2CH_{Ar}$), 130.5 ($2CH_{Ar}$), 132.6 (CH_{Ar}), 136.3, 137.6, 141.6 ($3C_{Ar}$), 164.5 (NCON), 173.1, 173.5 (CON, COO), 196.5 (CO); MS (ESI, *m/z*): 510.5 $[M+H]^+$.

(+)-4-Benzoyl-*N*-biotinyl-L-phenylalanine (49). To a solution of ester **52** (40 mg, 78 μmol) in anhydrous THF (0.2 mL) under an argon atmosphere, a solution of $LiOH{\cdot}H_2O$ (6 mg, 0.16 mmol) in water (0.2 mL) was added dropwise. The reaction was stirred at rt for 1 h and THF was evaporated under reduced pressure. The mixture was acidified with 1 M HCl (aq). The resulting precipitate was filtered, washed with cold water and dried under high vacuum at 35 ºC to afford acid **49** (39 mg, 100%), which was used in the next step without further purification. Mp: 129-133 ºC. $[\alpha]_D^{20}$: +61.3 (c=0.69, DCM/MeOH 1:1). R_f: 0.35 (DCM/MeOH, 1:1).

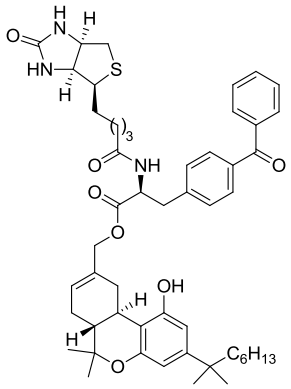


IR (ATR, ν): 3420 (NH, OH), 1696, 1651 (CO), 1540, 1453 (Ar); ^{1}H-NMR ($(CD_3)_2SO$, δ): 1.14-1.22 (m, 2H, CH_2), 1.37-1.61 (m, 4H, $2CH_2$), 2.06 (t, *J*=7.2, 2H, CH_2CO), 2.49-2.55 (m, 1H, ½CH_2S), 2.73 (dd, *J*=12.4, 5.0, 1H, ½CH_2S), 2.91-3.02 (m, 2H, CHS, ½CH_2Ar), 3.18 (dd, *J*=13.7, 4.5, 1H, ½CH_2Ar), 4.03-4.07 (m, 1H, CHN_{Biot}), 4.22-4.26 (m, 1H, CHN_{Biot}), 4.47-4.55 (m, 1H, CHN), 6.35 (br s, 1H, NH), 6.39 (br s, 1H, NH), 7.42 (d, *J*=8.2, 2H, $2CH_{Ar}$), 7.56 (t, *J*=7.3, 2H, $2CH_{Ar}$), 7.65-7.72 (m, 5H, $5CH_{Ar}$), 8.21 (d, *J*=8.3, 1H, NH), 12.77 (br s, 1H, OH); ^{13}C-NMR ($(CD_3)_2SO$, δ): 25.1 (CH_2), 27.9 ($2CH_2$), 34.8, 36.7 (CH_2CO, CH_2Ar), 39.8 (CH_2S), 52.9 (CHN), 55.4 (CHS), 59.1, 61.0 ($2CHN_{Biot}$), 128.5 ($2CH_{Ar}$), 129.3 ($2CH_{Ar}$), 129.5 ($2CH_{Ar}$), 129.6 ($2CH_{Ar}$), 132.6 (CH_{Ar}), 135.1, 137.2, 143.1 ($3C_{Ar}$), 162.6 (NCON), 172.1, 172.9 (CON, COO), 195.4 (CO); MS (ESI, *m/z*): 494.0 $[M-H]^-$.

- **Synthesis of probes 39-41**

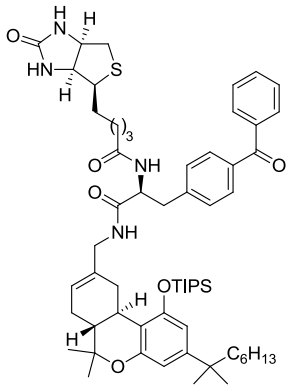
General procedure. A suspension of carboxylic acid **49** (1.5 equiv), HOBt (1.5 equiv), EDC (1.5 equiv) and 4 Å molecular sieves in anhydrous DMF and dry DCM (1:1, 16 mL/mmol) was stirred at 36 ºC for 1 h under an argon atmosphere. Then, a solution of the corresponding alcohol or amine (1 equiv) in the minimum amount of dry DCM was added, and the mixture was stirred at rt for 14 h. The mixture was filtered and washed with DCM/MeOH (1:1). The solvents were removed under reduced pressure and the residue was purified by chromatography using the appropriate eluent to afford the corresponding ester or amide.

(-)-[(6a*R*,10a*R*)-3-(1,1-Dimethylheptyl)-1-hydroxy-6,6-dimethyl-6a,7,10,10a-tetrahydro-6*H*-benzo[*c*]chromen-9-yl]methyl 4-benzoyl-*N*-biotinylphenylalaninate (39). Obtained from carboxylic acid **49** (42 mg, 85 μmol) and alcohol **42** (27 mg, 70 μmol) in 32% yield (19 mg). Chromatography: DCM/MeOH, 9:1. Mp: 122-125 ºC. $[\alpha]_D^{20}$:-26.3 (c=0.9, EtOH). R_f: 0.36 (DCM/MeOH, 9:1).



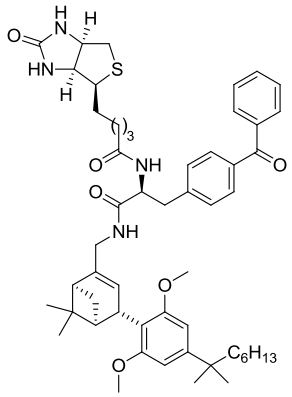
IR (ATR, ν): 3265 (NH, OH), 1695, 1657 (CO), 1574, 1454 (Ar); ^{1}H-NMR ($(CD_3)_2CO$, δ): 0.83 (t, *J*=6.6, 3H, CH_3CH_2), 1.07 (s, 3H, CH_3C_{cyc}), 1.19-1.41 (m, 17H, CH_3C_{cyc}, $2CH_3$, $(CH_2)_4$), 1.50-1.61 (m, 6H, $CH_2C(CH_3)_2$, $(CH_2)_2$), 1.70-1.77 (m, 3H, CH_2, $CHC(CH_3)_2$), 1.84-1.90 (m, 2H, ½CH_2CH=, ½CH_2C=), 2.21-2.28 (m, 3H, ½CH_2CH=, CH_2CO), 2.63-2.73 (m, 2H, CHC_{Ar}, ½CH_2S), 2.91 (dd, *J*=12.6, 4.9, 1H, ½CH_2S), 3.09-3.17 (m, 2H, CHS, ½CH_2Bzph), 3.30-3.37 (m, 1H, ½CH_2Bzph), 3.50-3.61 (m, 1H, ½CH_2C=), 4.25-4.32 (m, 1H, CHN_{Biot}), 4.46-4.51 (m, 1H, CHN_{Biot}), 4.56-4.66 (m, 2H, CH_2O), 4.82-4.90 (m, 1H, CHN), 5.80-5.83 (m, 2H, CH=, NH), 6.02 (br s, 1H, NH), 6.17 (br s, 1H, NH), 6.25 (s, 1H, CH_{HU}), 6.44 (dd, *J*=8.7, 1.8, 1H, CH_{HU}), 7.46 (t, *J*=8.3, 2H, $2CH_{Bzph}$), 7.52-7.59 (m, 2H, $2CH_{Bzph}$), 7.63-7.66 (m, 1H, CH_{Bzph}), 7.71-7.79 (m, 4H, $4CH_{Bzph}$); ^{13}C-NMR ($(CD_3)_2CO$, δ): 14.3 (CH_3CH_2), 18.7 (CH_3C_{cyc}), 23.3, 25.4, 26.3 ($3CH_2$), 27.9 (CH_3C_{cyc}), 28.4, 28.9, 29.0 (CH_2CH=, $2CH_2$), 29.2, 29.3 ($2CH_3$), 30.8 (CH_2), 32.3 (CHC_{Ar}), 32.5 (CH_2), 35.6, 35.9 (CH_2C=, CH_2CO), 37.8 ($ArC(CH_3)_2$), 38.2 (CH_2Bzph), 40.9 (CH_2S), 45.1 ($CH_2C(CH_3)_2$), 45.9 ($CHC(CH_3)_2$), 54.1 (CHN), 56.3 (CHS), 60.9, 62.3 ($2CHN_{Biot}$), 69.4 (CH_2O), 76.6 ($OC(CH_3)_2$), 106.0, 107.2 ($2CH_{HU}$), 110.4 (C_{HU}), 125.4 (CH=), 129.3 ($2CH_{Bzph}$), 130.3 ($2CH_{Bzph}$), 130.5 ($2CH_{Bzph}$), 130.8 ($2CH_{Bzph}$), 133.2 (CH_{Bzph}), 134.5, 136.8 (C_{Bzph}), 138.6 (C=), 143.5 (C_{Bzph}), 150.1, 155.3, 157.2 ($3C_{HU}$), 164.0 (NCON), 172.3, 173.4 (2CON), 196.2 (CO); HRMS (ESI, *m/z*): calcd for $[M+Na]^+$ $C_{51}H_{65}N_3NaO_7S$: 886.4435; found: 886.4415; HPLC-MS (ESI, *m/z*): 864.4 $[M+H]^+$; t_R (method A): 15.65 min.

(-)-4-Benzoyl-*N*-biotinyl-*N*-({(6a*R*,10a*R*)-3-(1,1-dimethylheptyl)-6,6-dimethyl-1-[(triisopropylsilyl)-oxy]-6a,7,10,10a-tetrahydro-6*H*-benzo[*c*]chromen-9-yl}-methyl)-L-phenyl-alaninamide (53). Obtained from carboxylic acid **49** (50 mg, 0.11 mmol) and amine **11** (50 mg, 92 μmol) in 31% yield (28 mg). Chromatography: DCM/MeOH, 95:5. Mp: 128-130 °C. $[\alpha]_D^{20}$:-51.4 (c=0.46, chloroform). R_f: 0.32 (DCM/MeOH 9:1).



IR (ATR, ν): 3271 (NH), 1696, 1652 (CO), 1563, 1462 (Ar); ^{1}H-NMR ($CD_3OD/CDCl_3$ 9:1, δ): mixture of rotamers (1:1), 0.85 (t, *J*=6.8, 3H, CH_3CH_2), 1.02-1.33 (m, 41H, $2CH_3C_{cyc}$, $2CH_3$, $3CH(CH_3)_2$, $(CH_2)_4$), 1.47-1.82 (m, 11H, $(CH_2)_3$, $CH_2C(CH_3)_2$, ½CH_2C=, ½CH_2CH=, $CHC(CH_3)_2$), 2.13-2.23 (m, 3H, ½CH_2CH=, CH_2CO), 2.58 (td, *J*=11.1, 4.2, 1H, CHC_{Ar}), 2.65 (d, *J*=13.0, 1H, ½CH_2S rotamer), 2.66 (d, *J*=12.7, 1H, ½CH_2S rotamer), 2.87 (dd, *J*=12.7, 4.9, 1H, ½CH_2S), 2.95-3.26 (m, 3H, CHS, CH_2Bzph), 3.49-3.56 (m, 1H, ½CH_2C=), 3.64-3.92 (m, 2H, CH_2N), 4.19 (dd, *J*=7.9, 4.5, 1H, CHN_{Biot} rotamer), 4.26 (dd, *J*=7.8, 4.5, 1H, CHN_{Biot} rotamer), 4.43 (dd, *J*=8.3, 4.7, 1H, CHN_{Biot} rotamer), 4.46 (dd, *J*=8.2, 4.8, 1H, CHN_{Biot} rotamer), 4.69-4.76 (m, 1H, CHN), 5.37 (m, 1H, CH=), 6.22 (d, *J*=1.6, 1H, CH_{HU} rotamer), 6.34 (m, 3H, $3CH_{HU}$ rotamers), 7.38 (d, *J*=8.2, 1H, CH_{Bzph}), 7.44 (d, *J*= 8.2, 1H, CH_{Bzph}), 7.52 (td, *J*=7.4, 3.0, 2H, $2CH_{Bzph}$), 7.61-7.67 (m, 2H, $2CH_{Bzph}$), 7.71-7.77 (m, 3H, $3CH_{Bzph}$); ^{13}C-NMR ($CD_3OD/CDCl_3$ 9:1, δ): mixture of rotamers, 14.3 (3CHSi), 14.4 (CH_3CH_2), 18.4 (CH_3C_{cyc}), 18.6 ($3CH(CH_3)_2$), 23.6, 25.8, 26.6, 26.7 ($4CH_2$), 28.0 (CH_3C_{cyc}), 28.8 (CH_2CH=), 29.3 (CH_2), 29.4, 29.5, 29.6, 29.7 ($2CH_3$ rotamers), 31.1, 32.9 ($2CH_2$), 33.3 (CHC_{Ar}), 34.5 (CH_2C=), 36.3, 36.4 (CH_2CO rotamers), 38.2 ($ArC(CH_3)_2$), 39.0, 39.2 (CH_2Bzph rotamers), 41.0 (CH_2S), 45.5, 45.6, 45.7 ($CH_2C(CH_3)_2$, CH_2N rotamers), 46.8, 46.9 ($CHC(CH_3)_2$ rotamers), 55.5 (CHN), 56.7 (CHS), 61.5, 63.1 ($2CHN_{Biot}$), 77.3 ($OC(CH_3)_2$), 109.4 (CH_{HU}), 109.8, 109.9 (CH_{HU} rotamers), 114.5 (C_{HU}), 121.5 (CH=), 129.4 ($2CH_{Bzph}$), 130.4, 130.5, 130.9, 131.0 ($4CH_{Bzph}$), 131.2 ($2CH_{Bzph}$), 133.7 (CH_{Bzph}), 135.6, 135.8 (C_{Bzph} rotamers), 137.0 (C_{Bzph}), 138.7 (C=), 143.7, 144.0 (C_{Bzph} rotamers), 150.1, 155.5, 156.1 ($3C_{HU}$), 165.8 (NCON), 172.8, 175.6 (2CON), 198.0 (CO); MS (ESI, *m/z*): 1017.6 $[M-H]^-$.

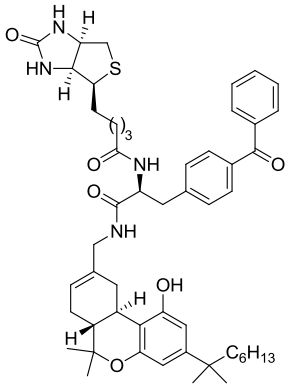
(+)-4-Benzoyl-*N*-biotinyl-*N*-({(1*S*,4*S*,5*S*)-4-[2,6-dimethoxy-4-(1,1-dimethylheptyl)-phenyl]-6,6-dimethylbicyclo[3.1.1]hept-2-en-2-yl}methyl)-L-phenylalaninamide (41). Obtained from carboxylic acid **49** (43 mg, 86 μmol) and amine **43** (32 mg, 78 μmol) in 35% yield (25 mg). Chromatography: DCM/MeOH, 95:5. Mp: 139-143 ºC. $[\alpha]_D^{20}$: +69.0 (c=0.30, chloroform). R_f: 0.16 (DCM/MeOH 95:5).



IR (ATR, ν): 3289 (NH), 1649, 1607 (CO), 1572, 1449, 1411 (Ar); ^{1}H-NMR ($CDCl_3$/CD_3OD 9:1, δ): 0.71 (t, *J*=6.6, 3H, CH_3CH_2), 0.78 (s, 3H, CH_3C_{cyc}), 0.93-1.21 (m, 19H, CH_3C_{cyc}, $2CH_3$, $(CH_2)_4$, CH_2), 1.39-1.55 (m, 6H, $CH_2C(CH_3)_2$, $(CH_2)_2$), 1.80-2.07 (m, 6H, $CHCHC_{Ar}$, CHC=, CH_{2cyc}, CH_2CO), 2.56 (d, *J*=12.8, 1H, ½CH_2S), 2.72-2.79 (m, 1H, ½CH_2S), 2.83-3.11 (m, 3H, CH_2Bzph, CHS), 3.59-3.70 (m, 8H, CH_2N, $2CH_3O$), 3.79-3.81 (m, 1H, CHC_{Ar}), 4.12 (td, *J*=13.2, 4.5, 1H, CHN_{Biot}), 4.34 (td, *J*=6.0, 0.9, 1H, CHN_{Biot}), 4.57 (t, *J*=7.4, 1H, CHN), 5.42 (d, *J*=11.9, 1H, CH=), 6.34 (s, 2H, $2CH_{HU}$), 7.23 (dd, *J*=8.3, 2.0, 2H, $2CH_{Bzph}$), 7.35 (t, *J*=7.0, 2H, $2CH_{Bzph}$), 7.47 (t, *J*=6.9, 1H, CH_{Bzph}), 7.58 (t, *J*=8.2, 2H, $2CH_{Bzph}$), 7.93 (d, *J*=7.9, 2H, $2CH_{Bzph}$); ^{13}C-NMR (125 MHz, $CDCl_3$/CD_3OD 9:1, δ): mixture of rotamers, 14.1 (CH_3CH_2), 20.9, 21.0 (CH_3C_{cyc} rotamers), 22.8, 24.8 ($2CH_2$), 25.4, 25.5 (CH_2 rotamers), 26.3 (CH_3C_{cyc}), 27.8 (CH_{2cyc}), 28.2, 28.4 ($2CH_2$), 29.0 ($2CH_3$), 29.8, 30.1 (CH_2 rotamers), 31.9 (CH_2), 35.6, 35.8 (CH_2CO rotamers), 37.6 (CHC_{Ar}), 38.1 ($ArC(CH_3)_2$), 38.3 (CH_2Bzph), 40.4, 40.5 (CH_2S rotamers), 40.9 ($C_{cyc}(CH_3)_2$), 44.3 (CHC=), 44.5 (CH_2N), 44.6 ($CH_2C(CH_3)_2$), 47.4 ($CHCHC_{Ar}$), 54.1 (CHN), 55.5, 55.6 (CHS rotamers), 55.8, 55.9 ($2CH_3O$), 60.3, 62.0 ($2CHN_{Biot}$), 102.9, 103.0 ($2CH_{HU}$), 117.5 (C_{HU}), 124.0, 124.2 (CH= rotamers), 128.5 ($2CH_{Bzph}$), 129.4 ($2CH_{Bzph}$), 130.1 ($2CH_{Bzph}$), 130.5 ($2CH_{Bzph}$), 132.7 (CH_{Bzph}), 136.0, 137.6 ($2C_{Bzph}$), 137.8, 137.9 (C= rotamers), 142.4, 142.5 (C_{Bzph} rotamers), 149.7 (C_{HU}), 158.5 ($2C_{HU}$), 164.3 (NCON), 171.4, 174.0 (2CON), 197.2 (CO); HRMS (MALDI, *m/z*): calcd for

$[M+H]^+$ $C_{53}H_{71}N_4O_6S$: 891.5094; found: 891.5102; HPLC-MS (ESI, *m/z*): 891.4 $[M+H]^+$; t_R (method A): 19.55 min.

(-)-4-Benzoyl-*N*-biotinyl-*N*-{[(6a*R*,10a*R*)-3-(1,1-dimethylheptyl)-1-hydroxy-6,6-dimethyl-6a,7,10,10a-tetrahydro-6*H*-benzo[*c*]chromen-9-yl]methyl}-L-phenylalaninamide (40). Following the general procedure 6.1.1.1, phenol **40** was obtained from amide **53** (24 mg, 25 μmol) in 36% yield (6 mg). Chromatography: DCM to DCM/MeOH, 9:1. Mp: 138-140 ºC. $[\alpha]_D^{20}$: -39.1 (c=0.19, chloroform). R_f: 0.28 (DCM/MeOH, 9:1).

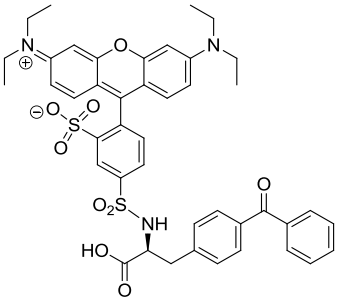


IR (ATR, ν): 3264 (NH, OH), 1693, 1654 (CO), 1576, 1458, 1416 (Ar); ^{1}H-NMR (500 MHz, $CD_3OD/CDCl_3$ 9:1, δ): mixture of rotamers (1:1), 0.85 (t, *J*=7.0, 3H, CH_3CH_2), 1.03-1.33 (m, 20H, $2CH_3C_{cyc}$, $2CH_3$, $(CH_2)_4$), 1.48-1.83 (m, 11H, $(CH_2)_3$, $CH_2C(CH_3)_2$, ½CH_2C=, ½CH_2CH=, $CHC(CH_3)_2$), 2.12-2.23 (m, 3H, ½CH_2CH=, CH_2CO), 2.58 (td, *J*=11.2, 4.7, 1H, CHC_{Ar} rotamer), 2.62 (dd, *J*=11.2, 4.6, 1H, CHC_{Ar} rotamer), 2.65 (d, *J*=12.5, 1H, ½CH_2S rotamer), 2.66 (d, *J*=12.7, 1H, ½CH_2S rotamer), 2.86 (dd, *J*=12.9, 5.0, 1H, ½CH_2S), 2.98-3.12 (m, 2H, CH_2Bzph), 3.20-3.27 (m, 1H, CHS), 3.35-3.40 (m, 1H, ½CH_2C=), 3.62 (d, *J*=14.7, 1H, ½CH_2N rotamer), 3.67 (d, *J*=14.7, 1H, ½CH_2N rotamer), 3.78 (d, *J*=14.6, 1H, ½CH_2N rotamer), 3.82 (d, *J*=14.3, 1H, ½CH_2N rotamer), 4.19 (dd, *J*=7.9, 4.4, 1H, CHN_{Biot} rotamer), 4.25 (dd, *J*=7.9, 4.5, 1H, CHN_{Biot} rotamer), 4.42 (dd, *J*=7.8, 4.4, 1H, CHN_{Biot} rotamer), 4.45 (dd, *J*=8.0, 4.5, 1H, CHN_{Biot} rotamer), 4.74 (t, *J*=6.3, 1H, CHN rotamer), 4.76 (t, *J*=6.8, 1H, CHN rotamer), 5.43-5.46 (m, 1H, CH=), 6.15 (d, *J*=1.8, 1H, CH_{HU} rotamer), 6.22 (d, *J*=1.8, 1H, CH_{HU} rotamer), 6.32-6.33 (m, 2H, $2CH_{HU}$ rotamers), 7.41 (d, *J*=8.2, 1H, CH_{Bzph}), 7.46 (d, *J*=8.2, 1H, CH_{Bzph}), 7.50-7.54 (m, 2H, $2CH_{Bzph}$), 7.62-7.66 (m, 2H, $2CH_{Bzph}$), 7.72-7.77 (m, 3H, $3CH_{Bzph}$); ^{13}C-NMR (125 MHz, $CD_3OD/CDCl_3$ 9:1, δ): mixture of

rotamers, 14.4 ($\underline{C}H_3CH_2$), 18.6 ($\underline{C}H_3C_{cyc}$), 23.7, 25.8 ($2CH_2$), 26.6, 26.7 (CH_2 rotamers), 28.0 ($\underline{C}H_3C_{cyc}$), 28.8 ($\underline{C}H_2CH=$), 29.35 (CH_2), 29.41, 29.5 ($2CH_3$), 29.6, 31.1 ($2CH_2$), 32.8 ($\underline{C}HC_{Ar}$), 32.9 (CH_2), 33.7 ($\underline{C}H_2C=$), 36.4, 36.5 ($\underline{C}H_2CO$ rotamers), 38.2 ($Ar\underline{C}(CH_3)_2$), 39.2 ($\underline{C}H_2Bzph$), 41.0 (CH_2S), 45.6 ($\underline{C}H_2C(CH_3)_2$), 45.8, 46.0 ($CH_2N$ rotamers), 46.6, 46.8 ($\underline{C}HC(CH_3)_2$ rotamers), 55.7 (CHN), 56.8, 56.9 (CHS rotamers), 61.6 (CHN_{Biot}), 63.2, 63.3 (CHN_{Biot} rotamers), 77.3 ($O\underline{C}(CH_3)_2$), 106.3, 107.6 ($2CH_{HU}$), 111.1 (C_{HU}), 122.1 (CH=), 129.5, 129.6, 130.5, 130.6 ($4CH_{Bzph}$), 131.0 ($2CH_{Bzph}$), 131.3 ($2CH_{Bzph}$), 133.8 (CH_{Bzph}), 136.2, 136.3 (C_{Bzph} rotamers), 137.2 (C_{Bzph}), 138.9 (C=), 144.0, 144.3 (C_{Bzph} rotamers), 150.5, 150.6 (C_{HU} rotamers), 155.5 (C_{HU}), 157.5, 157.6 (C_{HU} rotamers), 166.1 (NCON), 173.1, 173.2 (CON rotamers), 175.8 (CON), 198.2 (CO); HRMS (MALDI, *m/z*): calcd for $[M+H]^+$ $C_{51}H_{67}N_4O_6S$: 863.4781; found: 863.4747; HPLC-MS (ESI, *m/z*): 863.5 $[M+H]^+$; t_R (method A): 13.52 min.

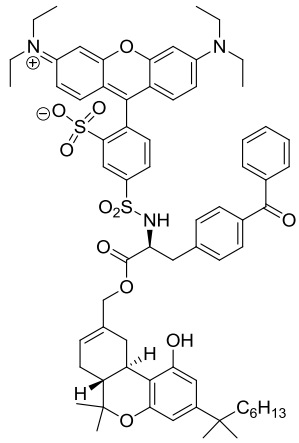
- **Synthesis of fluorescent probe 54**

5-({[(1*S*)-2-(4-Benzoylphenyl)-1-carboxyethyl]amino}sulfonyl)-2-[6-(diethylamino)-3-(diethyliminio)-3*H*-xanthen-9-yl]benzenesulfonate (55). To a solution 4-benzoyl-L-phenylalanine (200 mg, 0.74 mmol) in 2 M NaOH (aq, 0.8 mL) at 0 °C and under an argon atmosphere, a solution of sulforhodamine B acid chloride (lissamine chloride, 471 mg, 0.82 mmol) and DIPEA (106 mg, 0.82 mmol) in acetone (0.8 mL) was added and the reaction mixture was stirred for 10 min at this temperature. Then, the mixture was allowed to warm to rt and stirred for 18 h. The crude was diluted with water and washed with Et_2O (3x). The aqueous layer was acidified with 1 M HCl (aq) and extracted with EtOAc (3x). The combined organic layers were dried (Na_2SO_4), filtered, and evaporated under reduced pressure. The residue was purified by chromatography (DCM to DCM/MeOH, 8:2) to afford sulfonamide **55** (133 mg, 22%). Mp: 266-267 °C. R_f: 0.45 (DCM/MeOH, 8:2).



IR (ATR, ν): 3320 (NH, OH), 1653 (CO), 1450, 1413 (Ar), 1114, 1015 (SO_2); ^{1}H-NMR (500 MHz, CD_3OD, δ): 1.29 (t, *J*=6.4, 12H, $4CH_3$), 3.07 (dd, *J*=13.6, 7.8, 1H, ½CH_2Bzph), 3.26 (dd, *J*=13.7, 4.6, 1H, ½CH_2Bzph), 3.61-3.68 (m, 8H, $4CH_2N$), 4.08 (dd, *J*=7.5, 4.7, 1H, CHN), 6.90 (m, 2H, $2CH_{Liss}$), 6.96 (dd, *J*=9.6, 2.2, 2H, $2CH_{Liss}$), 7.08 (d, *J*=9.4, 2H, $2CH_{Liss}$), 7.33 (d, *J*=8.0, 1H, CH_{Liss}), 7.44-7.49 (m, 4H, $4CH_{Bzph}$), 7.60 (t, *J*=7.4, 1H, CH_{Bzph}), 7.68 (t, *J*=7.6, 4H, $4CH_{Bzph}$), 7.88 (dd, *J*=8.0, 1.8, 1H, CH_{Liss}), 8.64 (d, *J*=1.7, 1H, CH_{Liss}); ^{13}C-NMR (125 MHz, CD_3OD, δ): 12.8 ($4CH_3$), 40.6 (CH_2Bzph), 46.8 ($4CH_2N$), 60.6 (CHN), 96.9 ($2CH_{Liss}$), 115.1 ($2CH_{Liss}$), 115.2, 115.3 ($2C_{Liss}$), 127.9, 129.2 ($2CH_{Liss}$), 129.4 ($2CH_{Bzph}$), 130.9 ($2CH_{Bzph}$), 131.0 ($2CH_{Bzph}$), 131.1 ($2CH_{Bzph}$), 132.2 (CH_{Liss}), 133.6 (CH_{Bzph}), 133.8 ($2CH_{Liss}$), 135.1 (C_{Liss}), 137.0, 138.9 ($2C_{Bzph}$), 144.0 (C_{Liss}), 144.8 (C_{Bzph}), 146.8 (C_{Liss}), 157.2 ($2C_{Liss}$), 158.0, 159.3, 159.4 ($3C_{Liss}$), 176.7 (COOH), 198.5 (CO); MS (ESI, *m/z*): 891.4 $[M+H]^+$.

5-({[(1*S*)-2-(4-Benzoylphenyl)-1-({[(6a*R*,10a*R*)-3-(1,1-dimethylheptyl)-1-hydroxy-6,6-dimethyl-6a,7,10,10a-tetrahydro-6*H*-benzo[*c*]chromen-9-yl]methoxy}carbonyl)-ethyl]amino}sulfonyl)-2-[6-(diethylamino)-3-(diethyliminio)-3*H*-xanthen-9-yl]-benzenesulfonate (54). To a solution of carboxylic acid **55** (98 mg, 0.12 mmol) in dry DCM (2 mL), anhydrous DMF (3 mL) and activated 4 Å molecular sieves under an argon atmosphere, EDC (26 mg, 0.14 mmol) and HOBt (18 mg, 0.14 mmol) were added. The reaction was stirred at rt for 1 h before a solution of alcohol **42** (40 mg, 0.10 mmol) in dry DCM (2 mL) was added. The mixture was stirred at rt for 48 h and the solvents were evaporated under reduced pressure. The residue was resuspended in DCM, filtered and washed with saturated $NaHCO_3$ (aq). The aqueous layer was extracted with DCM (2x) and the combined organic layers were dried (Na_2SO_4), filtered, and evaporated under reduced pressure. The residue was purified by chromatography (DCM to DCM/MeOH, 95:5) to yield ester **54** (24 mg, 20%). Mp: 148-149 ºC. R_f: 0.42 (DCM/MeOH, 95:5).

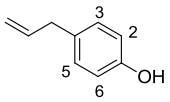


IR (ATR, ν): 3332 (NH, OH), 1649 (CO), 1592, 1467 (Ar), 1340, 1182 (SO_2); ^{1}H-NMR (700 MHz, CD_3OD, δ): 0.83 (t, *J*=7.1, 3H, CH_3CH_2), 1.00 (s, 3H, CH_3C_{cyc}), 1.16-1.29 (m, 29H, CH_3C_{cyc}, $2CH_3$, $4CH_3CH_2N$, $(CH_2)_4$), 1.48-1.51 (m, 2H, $CH_2C(CH_3)_2$), 1.57-1.62 (m, 2H, ½CH_2C=, $CHC(CH_3)_2$), 1.78-1.82 (m, 1H, ½CH_2CH=), 2.13-2.16 (m, 1H, ½CH_2CH=), 2.58 (td, *J*=11.3, 4.7, 1H, CHC_{Ar}), 3.10 (dd, *J*=13.8, 8.7, 1H, ½CH_2Bzph), 3.26 (dd, *J*=13.7, 5.9, 1H, ½CH_2Bzph), 3.39-3.41 (m, 1H, ½CH_2C=), 3.57-3.65 (m, 8H, $4CH_2N$), 4.40 (dd, *J*=8.4, 6.0, 1H, CHN), 4.44 (AB system, *J*=12.1, 2H, CH_2O), 5.68 (m, 1H, CH=), 6.20 (d, *J*=1.8, 1H, CH_{HU}), 6.32 (d, *J*=1.8, 1H, CH_{HU}), 6.85 (d, *J*=2.4, 1H, CH_{Liss}), 6.88 (dd, *J*=9.5, 2.4, 1H, CH_{Liss}), 6.90-6.93 (m, 2H, $2CH_{Liss}$), 7.08 (d, *J*=5.6, 1H, CH_{Liss}), 7.09 (d, *J*=5.4, 1H, CH_{Liss}), 7.30 (d, *J*=7.9, 1H, CH_{Liss}), 7.44 (d, *J*=8.2, 2H, $2CH_{Bzph}$), 7.47 (t, *J*=7.8, 2H, $2CH_{Bzph}$), 7.60 (t, *J*=7.4, 1H, CH_{Bzph}), 7.69-7.72 (m, 4H, $4CH_{Bzph}$), 7.90 (dd, *J*=7.9, 1.9, 1H, CH_{Liss}), 8.62 (d, *J*=1.9, 1H, CH_{Liss}); ^{13}C-NMR (175 MHz, CD_3OD, δ): 12.9 ($4CH_3CH_2N$), 14.4 (CH_3CH_2), 18.6 (CH_3C_{cyc}), 23.7, 25.8, 27.1 ($3CH_2$), 27.9 (CH_3C_{cyc}), 28.7 (CH_2CH=), 29.4, 29.5 ($2CH_3$), 31.2 (CH_2), 32.7 (CHC_{Ar}), 33.0 (CH_2C=), 38.2 ($ArC(CH_3)_2$), 39.9 (CH_2Bzph), 45.5 ($CH_2C(CH_3)_2$), 46.5 ($CHC(CH_3)_2$), 46.8 ($4CH_2N$), 58.9 (CHN), 69.8 (CH_2O), 77.2 ($OC(CH_3)_2$), 96.9, 97.0 ($2CH_{Liss}$), 106.4, 107.6 ($2CH_{HU}$), 110.8 (C_{HU}), 114.9, 115.0 ($2CH_{Liss}$), 115.1, 115.2 ($2C_{Liss}$), 125.5 (CH=), 127.8, 129.2 ($2CH_{Liss}$), 129.5 ($2CH_{Bzph}$), 130.8 ($2CH_{Bzph}$), 131.0 ($2CH_{Bzph}$), 131.4 ($2CH_{Bzph}$), 132.3, 133.6 ($2CH_{Liss}$), 133.8 (CH_{Bzph}, CH_{Liss}), 134.5 (C_{Liss}), 135.4 (C=), 137.5, 138.7 ($2C_{Bzph}$), 143.1 (C_{Liss}), 143.9 (C_{Bzph}), 147.1 (C_{Liss}), 150.7, 155.5 ($2C_{HU}$), 157.1 ($2C_{Liss}$), 157.6 (C_{Liss}), 157.8 (C_{Bzph}), 159.3, 159.4 ($2C_{Liss}$), 172.0 (COO), 198.2 (CO); HRMS (MALDI, *m/z*): calcd for $[M+H]^+$ $C_{68}H_{80}N_3O_{11}S_2$: 1178.5234; found: 1178.5193.

6.1.5. Synthesis of honokiol-based probes **56-59**

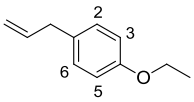
- **First attempts of synthesis of azide 60**

4-Allylphenol (66). To a solution of 4-allylanisole (10 mL, 65 mmol) in dry dochloromethane (200 mL) at -78 ºC and under an argon atmosphere, BBr_3 (70 mL, 1 M in DCM) was added dropwise and the mixture was allowed to warm to rt and stirred for 50 min. The reaction was carefully quenched with water and the organic layer was separated, washed with brine, dried (Na_2SO_4), filtered, and evaporated under reduced pressure to give phenol **66** (9.91 g, 93%), which was used in the next step without further purification. R_f: 0.36 (hexane/EtOAc, 85:15). The spectroscopic data correspond with those previously reported.[152]



^{1}H-NMR ($CDCl_3$, δ): 3.32 (d, *J*=6.7, 2H, CH_2), 4.69 (br s, 1H, OH), 5.02-5.10 (m, 2H, CH_2=), 5.95 (ddt, *J*=15.9, 10.8, 6.6, 1H, CH=), 6.77 (d, *J*=8.5, 2H, H_2, H_6), 7.06 (d, *J*=9.2, 2H, H_3, H_5).

1-Allyl-4-ethoxybenzene (67). Following the general procedure 6.1.1.2, ether **67** was obtained from phenol **66** (500 mg, 3.7 mmol) and bromoethane (0.33 mL, 4.5 mmol) in 98% yield (593 mg), and used in the next step without further purification. R_f: 0.46 (hexane/EtOAc 95:5).

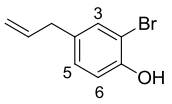


^{1}H-NMR ($CDCl_3$, δ): 1.40 (t, *J*=7.0, 3H, CH_3), 3.33 (d, *J*=6.6, 2H, CH_2C_1), 4.01 (q, *J*=7.0, 2H, CH_2O), 5.02-5.09 (m, 2H, CH_2=), 5.96 (ddt, *J*=16.9, 10.3, 6.7, 1H, CH=), 6.83 (d, *J*=8.6, 2H, H_3, H_5), 7.09 (d, *J*=8.6, 2H, H_2, H_6).

Reaction of 67 with trimethylborate under basic conditions. To a solution of *sec*-butillithium (5.5 mL, 1.4 M in cyclohexane) in anhydrous THF (40 mL) at -78 ºC and under an argon atmosphere, tetramethylethylenediamine (TMEDA, 1.2 mL, 7.7 mmol) was added. After stirring at that temperature for 30 min, a solution of **67** (1.00 g, 6.4 mmol) in anhydrous THF (20 mL) was added dropwise and the mixture was stirred at -78 ºC for 2 h. Trimethyl borate (1.4 mL, 13 mmol) was then added and the reaction was allowed to warm to rt and stirred for an additional 1 h. 2 M HCl (aq) was added and the aqueous phase was extracted with EtOAc (2x). The organic layer was washed with 2 M HCl (aq) and brine, dried (Na_2SO_4), filtered, and evaporated under reduced pressure to yield a complex mixture of products which did not contain the desired boronic acid.

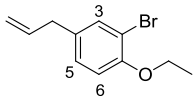
4-Allyl-2-bromophenol (68). Derivative **68** was obtained following the experimental procedure previously described by Lin *et al.* and the spectroscopic data correspond with those reported.[127]

To a solution of **66** (1.20 g, 9.2 mmol) in anhydrous Et_2O (120 mL) at -78 ºC and under an argon atmosphere, isopropyl magnesium chloride (4.6 mL, 2 M in THF) was added dropwise and the mixture was stirred at -78 ºC for 1 h before DBDMH (1.30 g, 4.6 mmol) was added. The reaction was stirred at -78 ºC for 20 h, and then warmed up to rt over 1 h. The reaction was quenched with 10% $NaHSO_3$ (aq) and extracted with Et_2O (3x). The organic extracts were dried (Na_2SO_4), filtered, and evaporated under reduced pressure. The residue was purified by chromatography (hexane to hexane/DCM, 7:3) to afford bromoderivative **68** (650 mg, 33%). R_f: 0.59 (hexane/DCM, 2:8).



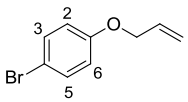
^{1}H-NMR ($CDCl_3$, δ): 3.30 (d, *J*=6.7, 2H, CH_2), 5.03-5.10 (m, 2H, CH_2=), 5.37 (br s, 1H, OH), 5.91 (ddt, *J*=18.0, 9.2, 6.7, 1H, CH=), 6.95 (d, *J*=8.3, 1H, H_6), 7.04 (dd, *J*=8.3, 1.9, 1H, H_5), 7.28 (d, *J*=2.0, 1H, H_3).

4-Allyl-2-bromo-1-ethoxybenzene (69). Following the general procedure 6.1.1.2, ethoxy derivative **69** was obtained from phenol **68** (500 mg, 2.3 mmol) and bromoethane (210 μL, 2.8 mmol) in 87% yield (482 mg), and used in the next step without further purification. R_f: 0.62 (hexane/EtOAc, 9:1).



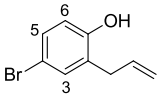
IR (ATR, ν): 1639, 1603, 1494 (Ar), 1279, 1248 (COC); ^{1}H-NMR ($CDCl_3$, δ): 1.46 (t, *J*=7.0, 3H, CH_3), 3.30 (d, *J*=6.7, 2H, CH_2C_4), 4.08 (q, *J*=7.0, 2H, CH_2O), 5.03-5.10 (m, 2H, CH_2=), 5.85-5.99 (m, 1H, CH=), 6.82 (d, *J*=8.4, 1H, H_6), 7.05 (dd, *J*=8.3, 2.2, 1H, H_5), 7.37 (d, *J*=2.1, 1H, H_3); ^{13}C-NMR ($CDCl_3$, δ): 14.9 (CH_3), 39.1 (CH_2C_4), 65.1 (CH_2O), 112.2 (C_2), 113.5 (C_6), 116.2 (CH_2=), 128.5 (C_5), 133.5 (C_3), 133.7 (C_4), 137.2 (CH=), 158.8 (C_1); MS (ESI, *m/z*): 262.9 [M(^{79}Br)+Na]$^+$, 264.9 [M(^{81}Br)+Na]$^+$.

1-(Allyloxy)-4-bromobenzene (70). Following the general procedure 6.1.1.2, bromoderivative **70** was obtained from 4-bromophenol (450 mg, 2.6 mmol) and allyl bromide (270 μL, 3.1 mmol) in 97% yield (537 mg), and used in the next step without further purification. R_f: 0.32 (hexane/DCM, 9:1). The spectroscopic data correspond with those previously reported.[153]



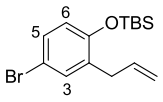
^{1}H-NMR ($CDCl_3$, δ): 4.51 (dt, *J*=5.3, 1.5, 2H, CH_2O), 5.30 (dq, *J*=10.5, 1.4, 1H, ½CH_2=), 5.40 (dq, *J*=17.2, 1.6, 1H, ½CH_2=), 6.03 (ddt, *J*=17.3, 10.5, 5.3, 1H, CH=), 6.80 (d, *J*=9.1, 2H, H_2, H_6), 7.37 (d, *J*=9.0, 2H, H_3, H_5).

2-Allyl-4-bromophenol (71). A solution of **70** (1.00 g, 4.7 mmol) in 1,2-dichlorobenzene under an argon atmosphere was heated at 250 ºC for 1 h under MW irradiation. The solvent was removed under reduced pressure and the residue was purified by chromatography (hexane to hexane/DCM 9:1) to obtain phenol **71** (729 mg, 73%). Mp: 58-59 ºC (lit.[154] 58.1-59.6 ºC). R_f: 0.45 (hexane/EtOAc, 85:15). The spectroscopic data correspond with those previously reported.[153]



^{1}H-NMR ($CDCl_3$, δ): 3.39 (d, *J*=6.3, 2H, CH_2), 5.15-5.23 (m, 2H, CH_2=), 6.00 (ddt, *J*=16.7, 10.4, 6.3, 1H, CH=), 6.71 (d, *J*=8.4, 1H, H_6), 7.22- 7.27 (m, 2H, H_3, H_5).

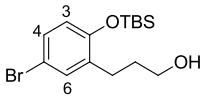
(2-Allyl-4-bromophenoxy)(*tert*-butyl)dimethylsilane (72). A solution of **71** (1.60 g, 7.6 mmol), imidazole (1.50 g, 23 mmol), and TBS-Cl (2.90 g, 19 mmol) in anhydrous DMF (16 mL), and under an argon atmosphere, was heated at 100 ºC for 15 min under MW irradiation. Then, 1 M H_3PO_4 (aq) was added and the mixture was extracted with DCM. The organic phase was washed with saturated $NaHCO_3$ (aq) and brine, dried (Na_2SO_4), filtered, and evaporated under reduced pressure to afford silyl ether **72** (2.40 g, 97%), which was used in the next step without further purification. R_f: 0.78 (hexane/DCM, 8:2). The spectroscopic data correspond with those previously reported.[155]



^{1}H-NMR ($CDCl_3$, δ): 0.22 (s, 6H, $2CH_3Si$), 1.00 (s, 9H, $C(CH_3)_3$), 3.32 (d, *J*=6.6, 2H, CH_2), 5.02-5.11 (m, 2H, CH_2=), 5.92 (ddt, *J*=16.9, 10.3, 6.7, 1H, CH=), 6.66 (d, *J*=8.5, 1H, H_6), 7.18 (dd, *J*=8.6, 2.6, 1H, H_5), 7.28 (d, *J*=2.6, 1H, H_3).

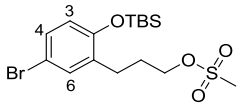
Derivatives **73-75** were obtained following experimental procedures previously described by Liang *et al*. and their spectroscopic data correspond with those reported.[155]

3-(5-Bromo-2-{[*tert*-butyl(dimethyl)silyl]oxy}phenyl)propan-1-ol (73). To a well-stirred solution of **72** (2.40 g, 7.4 mmol) in anhydrous THF (16 mL) at 0 ºC and under an argon atmosphere, BH_3 (15 mL, 1 M in THF) was added. After 2 h, 30% H_2O_2 (aq, 15 mL, 481 mmol) and saturated $NaHCO_3$ (aq, 15 mL) were slowly added, and the mixture was allowed to warm to rt and stirred for 16 h. The reaction was quenched with saturated NH_4Cl (aq) and extracted with EtOAc (3x). The combined organic extracts were sequentially washed with saturated NH_4Cl (aq, 2x) and brine, dried (Na_2SO_4), filtered, and evaporated under reduced pressure. The residue was purified by chromatography (hexane to hexane/DCM, 1:1) to yield alcohol **73** (1.97 g, 77%). R_f: 0.30 (hexane/EtOAc, 85:15).



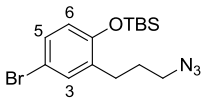
^{1}H-NMR ($CDCl_3$, δ): 0.22 (s, 6H, $2CH_3Si$), 1.00 (s, 9H, $C(CH_3)_3$), 1.78-1.87 (m, 2H, CH_2), 2.64 (dd, *J*=8.3, 6.7, 2H, CH_2C_1), 3.62 (q app, *J*=5.4, 2H, CH_2O), 5.30 (br s, 1H, OH), 6.66 (d, *J*=8.6, 1H, H_3), 7.17 (dd, *J*=8.5, 2.6, 1H, H_4), 7.26 (d, *J*=2.8, 1H, H_6).

3-(5-Bromo-2-{[*tert*-butyl(dimethyl)silyl]oxy}phenyl)propyl methanesulfonate (74). To a well-stirred solution of alcohol **73** (455 mg, 1.3 mmol) in dry DCM (5 mL) at -20 ºC and under an argon atmosphere, MsCl (123 µL, 1.6 mmol) and triethylamine (278 µL, 2.0 mmol) were added. Then, the solution became cloudy and the mixture was allowed to warm to rt and stirred for 16 h. The reaction was cooled to 0 °C, quenched with saturated NH_4Cl (aq) and extracted with EtOAc (3x). The combined organic extracts were sequentially washed with saturated NH_4Cl (aq, 2x) and brine, dried (Na_2SO_4), filtered, and evaporated under reduced pressure to obtain **74** (562 mg, 100%), which was used in the next step without further purification. R_f: 0.51 (hexane/DCM, 2:8).



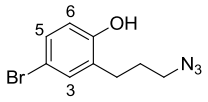
^{1}H-NMR ($CDCl_3$, δ): 0.23 (s, 6H, $2CH_3Si$), 1.00 (s, 9H, $C(CH_3)_3$), 1.98-2.07 (m, 2H, CH_2), 2.67 (dd, *J*=8.3, 6.7, 2H, CH_2C_1), 3.00 (s, 3H, CH_3S), 4.22 (t, *J*=6.3, 2H, CH_2O), 6.66 (d, *J*=8.5, 1H, H_3), 7.19 (dd, *J*=8.5, 2.6, 1H, H_4), 7.24 (d, *J*=2.6, 1H, H_6).

[2-(3-Azidopropyl)-4-bromophenoxy](*tert*-butyl)dimethylsilane (75). To a solution of mesylate **74** (1.60 g, 3.7 mmol) in anhydrous DMF (8.3 mL), NaN_3 (362 mg, 5.6 mmol) was added and the mixture was stirred at 90 ºC for 2 h. The reaction was cooled to 0 ºC and water was added. The mixture was extracted with EtOAc (2x) and the organic extracts were dried (Na_2SO_4), filtered, and evaporated under reduced pressure to yield azide **75** (1.28 g, 93%), which was used in the next step without further purification. R_f: 0.65 (hexane/DCM, 1:1).



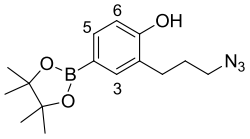
^{1}H-NMR ($CDCl_3$, δ): 0.23 (s, 6H, $2CH_3Si$), 1.01 (s, 9H, $C(CH_3)_3$), 1.83-1.90 (m, 2H, CH_2), 2.62 (dd, *J*=8.5, 6.7, 2H, CH_2C_2), 3.28 (t, *J*=6.9, 2H, CH_2N_3), 6.66 (d, *J*=8.6, 1H, H_6), 7.18 (dd, *J*=8.6, 2.6, 1H, H_5), 7.24 (d, *J*=2.5, 1H, H_3).

2-(3-Azidopropyl)-4-bromophenol (76). Following the general procedure 6.1.1.1, phenol **76** was obtained from **75** (1.28 g, 3.5 mmol) in 93% yield (824 mg). Chromatography: hexane to hexane/DCM, 2:8. R_f: 0.11 (hexane/DCM, 2:8).



IR (ATR, ν): 3373 (OH), 2098 (N_3), 1655, 1583, 1490 (Ar); ^{1}H-NMR ($CDCl_3$, δ): 1.85-1.94 (m, 2H, CH_2), 2.68 (t, *J*=7.4, 2H, CH_2C_2), 3.32 (t, *J*=6.6, 2H, CH_2N_3), 5.11 (br s, 1H, OH), 6.66 (d, *J*=8.3, 1H, H_6), 7.20 (dd, *J*=8.3, 2.4, 1H, H_5), 7.22 (d, *J*=2.4, 1H, H_3); ^{13}C-NMR ($CDCl_3$, δ): 26.8 ($\underline{C}H_2C_2$), 28.9 (CH_2), 50.7 (CH_2N_3), 113.0 (C_4), 117.4 (C_6), 129.4 (C_2), 130.4 (C_5), 133.1 (C_3), 153.0 (C_1); MS (ESI, *m/z*): 227.7 [M(^{79}Br)-N_2+H]$^+$, 229.8 [M(^{81}Br)-N_2+H]$^+$.

2-(3-Azidopropyl)-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenol (77). Bromoderivative **76** (68 mg, 0.26 mmol), Pd(dppf)Cl_2 (10 mg, 13 μmol), and KOAc (52 mg, 0.53 mmol) were introduced into a flask previously dried under vacuum and flushed with argon. Anhydrous 1,4-dioxane (1.8 mL) was added and the mixture was stirred at 80 ºC for 30 min. Then, a solution of B_2pin_2 (74 mg, 0.29 mmol) in anhydrous 1,4-dioxane (0.8 mL) was added and the reaction was stirred at 80 ºC for 16 h. Once cooled to rt, the suspension was filtered through activated carbon and celite and evaporated under reduced pressure. The residue was redissolved in EtOAc, washed with water and brine, dried (Na_2SO_4), filtered, and evaporated under reduced pressure. The crude was purified by chromatography (hexane to DCM) to afford boronate **77** (62 mg, 78%). R_f: 0.11 (DCM).

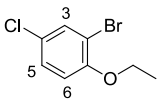


IR (ATR, ν): 3338 (OH), 2094 (N_3), 1604 (Ar), 1352 (BO), 1268 (COC), 1144, 1117 (BC); ^{1}H-NMR ($CDCl_3$, δ): 1.33 (s, 12H, 2C(CH_3)$_2$), 1.87-1.98 (m, 2H, CH_2), 2.71 (t, *J*=7.4, 2H, CH_2C_2), 3.31 (t, *J*=6.7, 2H, CH_2N_3), 5.33 (br s, 1H, OH), 6.76 (d, *J*=8.3, 1H, H_6), 7.571 (dd, *J*=8.2, 1.6, 1H, H_5), 7.573 (d, *J*=1.4, 1H, H_3); ^{13}C-NMR ($CDCl_3$, δ): 25.0 (2C($\underline{C}H_3$)$_2$), 26.9 ($\underline{C}H_2C_2$), 29.2 (CH_2), 51.0 (CH_2N_3), 83.7 (2$\underline{C}$(CH_3)$_2$), 100.1 (C_4), 115.2 (C_6), 126.4 (C_2), 134.9, 137.5 (C_3, C_5), 156.7 (C_1); MS (ESI, *m/z*): 302.1 [M-H]$^-$.

Suzuki coupling of bromoderivative 69 and boronate 77. Boronate **77** (93 mg, 0.39 mmol), KF (112 mg, 1.9 mmol) and bromoderivative **69** (130 mg, 0.39 mmol) were introduced in a Shlenk falsk previously dried under vacuum and refilled with argon. Then, a 10:1 mixture of 1,4-dioxane/water (8.5 mL) was added and the mixture was stirred in an ultrasound bath for 1 min. Pd_2dba_3 (20.0 mg, 19 μmol) and S-PHOS (51 mg, 0.12 mmol) were added and the reaction mixture was stirred at 110 ºC for 16 h. Once cooled to rt, the resulting suspension was filtered through a pad of celite, washing with EtOAc, dried (Na_2SO_4), filtered, and evaporated under reduced pressure. The crude was analyzed by NMR, observing a mixture of the desired coupling product, together with the product resulting from the double bond isomerization, which could not be separated by chromatography.

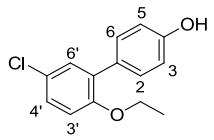
- **Linear synthesis of azide 60**

2-Bromo-4-chloro-1-ethoxybenzene (78). Following the general procedure 6.1.1.2, compound **78** was obtained from 2-bromo-4-chlorophenol (2.00 g, 9.6 mmol) and bromoethane (0.9 mL, 12 mmol) in 96% yield (2.20 g). Chromatography: hexane. R_f: 0.49 (hexane/EtOAc, 96:4).



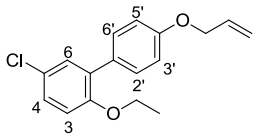
IR (ATR, ν): 1585, 1470 (Ar), 1285, 1253 (COC); ^{1}H-NMR ($CDCl_3$, δ): 1.46 (t, *J*=7.0, 3H, CH_3), 4.07 (q, *J*=7.0, 2H, CH_2), 6.80 (d, *J*=8.8, 1H, H_6), 7.21 (dd, *J*=8.8, 2.5, 1H, H_5), 7.53 (d, *J*=2.6, 1H, H_3); ^{13}C-NMR ($CDCl_3$, δ): 14.8 (CH_3), 63.4 (CH_2), 112.8 (C_2), 114.0 (C_6), 126.0 (C_4), 128.4 (C_5), 133.0 (C_3), 154.4 (C_1); MS (ESI, *m/z*): 232.6 [M(^{35}Cl, ^{79}Br)-H]$^-$, 234.6 [M(^{37}Cl, ^{79}Br)-H]$^-$ and [M(^{35}Cl, ^{81}Br)-H]$^-$, 236.6 [M(^{37}Cl, ^{81}Br)-H]$^-$.

5'-Chloro-2'-ethoxybiphenyl-4-ol (79). Following the general procedure 6.1.1.3, biphenyl **79** was obtained from bromoderivative **78** (100 mg, 0.42 mmol) and 4-hydroxyphenylboronic acid (88 mg, 0.64 mmol) in 92% yield (96 mg). Chromatography: hexane to hexane/EtOAc, 9:1. Mp: 123.0-123.8 ºC. R_f: 0.16 (hexane/EtOAc, 9:1).



IR (ATR, ν): 3288, 3258 (OH), 1610, 1515, 1485 (Ar), 1262, 1232 (COC); ^{1}H-NMR ($CDCl_3$, δ): 1.34 (t, *J*=7.0, 3H, CH_3), 4.00 (q, *J*=7.0, 2H, CH_2), 4.76 (br s, 1H, OH), 6.87 (d, *J*=8.6, 3H, H_3, H_5, $H_{3'}$), 7.20 (dd, *J*=8.7, 2.7, 1H, $H_{4'}$), 7.27 (d, *J*=2.7, 1H, $H_{6'}$), 7.43 (d, *J*=8.6, 2H, H_2, H_6); ^{13}C-NMR ($CDCl_3$, δ): 14.8 (CH_3), 64.6 (CH_2), 114.1 ($C_{3'}$), 115.1 (C_3, C_5), 125.8 ($C_{5'}$), 127.7 ($C_{4'}$), 130.1 (C_1), 130.4 ($C_{6'}$), 130.9 (C_2, C_6), 132.2 ($C_{1'}$), 154.6 ($C_{2'}$), 155.0 (C_4); MS (ESI, *m/z*): 247.1 [M(^{35}Cl)-H]$^-$, 249.1 [M(^{37}Cl)-H]$^-$.

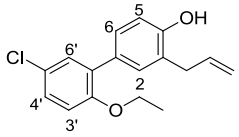
4'-(Allyloxy)-5-chloro-2-ethoxybiphenyl (80). Following the general procedure 6.1.1.2, allyl ether **80** was obtained from phenol **79** (157 mg, 0.63 mmol) and allyl bromide (60 µL, 0.70 mmol) in 98% yield (178 mg), and was used in the next step without further purification. R_f: 0.36 (hexane/EtOAc, 95:5).



IR (ATR, ν): 1609, 1515, 1484 (Ar), 1292, 1240 (COC); ^{1}H-NMR ($CDCl_3$, δ): 1.35 (t, *J*=7.0, 3H, CH_3), 4.00 (q, *J*=7.0, 2H, CH_2CH_3), 4.58 (dt, *J*=5.2, 1.3, 2H, CH_2CH=), 5.29-5.33 (m, 1H, ½CH_2=), 5.44 (dq, *J*=17.3, 1.4, 1H, ½CH_2=), 6.09 (ddt, *J*=17.1, 10.5, 5.3, 1H, CH=), 6.87 (d, *J*=8.7, 1H, H_3), 6.96 (d, *J*=8.8, 2H, $H_{3'}$, $H_{5'}$), 7.20 (dd, *J*=8.7, 2.7, 1H, H_4), 7.28 (d, *J*=2.7, 1H, H_6), 7.47 (d, *J*=8.8, 2H, $H_{2'}$, $H_{6'}$); ^{13}C-NMR ($CDCl_3$, δ): 14.8 (CH_3), 64.6 (CH_2CH_3), 69.0 (CH_2CH=), 114.0 (C_3), 114.4 ($C_{3'}$, $C_{5'}$), 117.9 (CH_2=), 125.8 (C_5), 127.7 (C_4), 130.0 ($C_{1'}$), 130.4 (C_6), 130.6 ($C_{2'}$, $C_{6'}$), 132.2 (C_1), 133.4 (CH=), 154.6 (C_2), 158.1 ($C_{4'}$); MS (ESI, *m/z*): 289.2 [M(^{35}Cl)+H]$^+$, 291.0 [M(^{37}Cl)+H]$^+$.

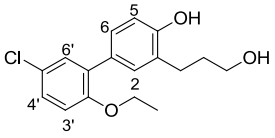
3-Allyl-5'-chloro-2'-ethoxybiphenyl-4-ol (81). Trimethyl aluminium (0.6 mL, 2 M in hexanes) was introduced in a flask and cooled at -20 °C. Then, water (5.3 µL, 0.29 mmol) was added and the mixture was allowed to warm to rt. The resulting solution was then cooled to -20 °C prior to the addition of a solution of **80** (85 mg, 0.29 mmol) in dry DCM (0.5 mL) and the reaction was stirred at that temperature for 3 h. The crude was diluted with DCM, washed with water and brine, dried (Na_2SO_4), filtered, and evaporated under

reduced pressure. The residue was purified by chromatography (hexane to hexane/EtOAc 9:1) to yield phenol **81** (75 mg, 90%). Mp: 69.3-70.3 ºC. R_f: 0.19 (hexane/EtOAc, 9:1).



IR (ATR, ν): 3422 (OH), 1609, 1509, 1436 (Ar), 1270, 1235 (COC); ^{1}H-NMR ($CDCl_3$, δ): 1.34 (t, *J*=7.0, 3H, CH_3), 3.45 (d, *J*=6.4, 2H, CH_2C_3), 4.00 (q, *J*=7.0, 2H, CH_2O), 5.15-5.24 (m, 2H, CH_2=), 6.05 (ddt, *J*=16.5, 10.1, 6.4, 1H, CH=), 6.84 (d, *J*=8.9, 1H, H_5), 6.86 (d, *J*=8.7, 1H, $H_{3'}$), 7.19 (dd, *J*=8.7, 2.7, 1H, $H_{4'}$), 7.28 (d, *J*=2.7, 1H, $H_{6'}$), 7.320 (d, *J*=2.1, 1H, H_2), 7.323 (dd, *J*=8.8, 2.3, 1H, H_6); ^{13}C-NMR ($CDCl_3$, δ): 14.8 (CH_3), 35.3 (CH_2C_3), 64.6 (CH_2O), 114.0 ($C_{3'}$), 115.6 (C_5), 116.8 (CH_2=), 125.0 (C_3), 125.8 ($C_{5'}$), 127.6 ($C_{4'}$), 129.1 (C_6), 130.1 (C_1), 130.4 ($C_{6'}$), 131.7 (C_2), 132.2 ($C_{1'}$), 136.5 (CH=), 153.6 (C_4), 154.6 ($C_{2'}$); MS (ESI, *m/z*): 287.0 [M(^{35}Cl)-H]$^-$, 289.1 [M(^{37}Cl)-H]$^-$.

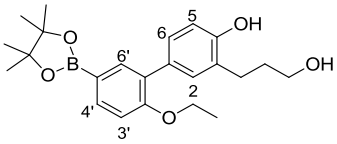
5'-Chloro-2'-ethoxy-3-(3-hydroxypropyl)biphenyl-4-ol (82). Following the general procedure 6.1.1.4, primary alcohol **82** was obtained from **81** (1.19 g, 4.1 mmol) in 84% yield (1.06 g). Chromatography: hexane to hexane/EtOAc, 7:3. Mp: 89.4-90.8 ºC. R_f: 0.12 (hexane/EtOAc, 7:3).



IR (ATR, ν): 3300 (OH), 1608, 1509, 1471 (Ar), 1259, 1234 (COC); ^{1}H-NMR ($CDCl_3$, δ): 1.34 (t, *J*=7.0, 3H, CH_3), 1.87-1.96 (m, 2H, CH_2), 2.81 (t, *J*=6.7, 2H, CH_2C_3), 3.69 (t, *J*=5.8, 2H, CH_2OH), 4.00 (q, *J*=7.0, 2H, CH_2O), 6.86 (d, *J*=8.7, 1H, H_5), 6.88 (d, *J*=8.8, 1H, $H_{3'}$), 7.18 (dd, *J*=8.7, 2.7, 1H, $H_{4'}$), 7.28 (d, *J*=2.7, 1H, $H_{6'}$), 7.28-7.31 (m, 2H, H_2, H_6); ^{13}C-NMR ($CDCl_3$, δ): 14.9 (CH_3), 25.4 (CH_2C_3), 32.3 (CH_2), 60.9 (CH_2OH), 64.5 (CH_2O), 114.0 ($C_{3'}$), 116.0 (C_5), 125.8 ($C_{5'}$), 126.8 (C_3), 127.5 ($C_{4'}$), 128.8 (C_6), 129.8 (C_1), 130.3 ($C_{6'}$), 131.3 (C_2), 132.3 ($C_{1'}$), 154.2 (C_4), 154.5 ($C_{2'}$); MS (ESI, *m/z*): 305.1 [M(^{35}Cl)-H]$^-$, 307.1 [M(^{37}Cl)-H]$^-$.

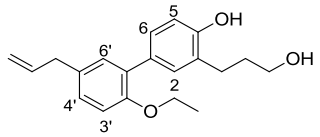
Representative example of the Stille coupling of 82 with allyltributylstannane. $Pd[P(t\text{-}Bu)_3]_2$ (2 mg, 4 μmol) and CsF (52 mg, 0.34 mmol) were introduced in a MW vial. The vial was evacuated and refilled with argon twice. Then, a solution of chloroderivative **82** (26 mg, 85 μmol) in anhydrous 1,4-dioxane (3.8 mL) was added followed by allyltributylstannane (40 μL, 0.13 mmol). The mixture was heated at 200 ºC for 90 min under MW irradiation. Once cooled to rt, the reaction was filtered through celite, washing with EtOAc, and evaporated under reduced pressure to yield a mixture of the coupling product **83** and starting material **82** (analyzed by NMR of the reaction crude).

2'-Ethoxy-3-(3-hydroxypropyl)-5'-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)biphenyl-4-ol (84). Following the general procedure 6.1.1.5, boronate **84** was obtained from chloroderivative **82** (800 mg, 2.6 mmol) in 89% yield (929 mg). Chromatography: DCM to DCM/EtOAc, 9:1. Mp: 69.7-70.5 ºC. R_f: 0.30 (DCM/EtOAc, 9:1).



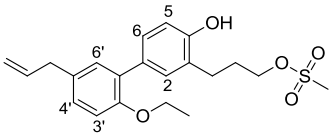
IR (ATR, ν): 3319 (OH), 1601, 1484 (Ar), 1262 (COC), 1097, 1031 (BC); ^{1}H-NMR ($CDCl_3$, δ): 1.34 (s, 12H, $2C(CH_3)_2$), 1.36 (t, *J*=7.0, 3H, CH_3), 1.78 (br s, 1H, CH_2OH), 1.90 (qt, *J*=6.3, 2H, CH_2), 2.80 (t, *J*=6.7, 2H, CH_2C_3), 3.67 (t, *J*=5.8, 2H, CH_2OH), 4.00 (q, *J*=7.0, 2H, CH_2O), 6.85 (d, *J*=8.0, 1H, H_5), 6.94 (d, *J*=8.2, 1H, $H_{3'}$), 7.32 (dd, *J*=8.1, 2.3, 1H, H_6), 7.34 (d, *J*=2.2, 1H, H_2), 7.72 (dd, *J*=8.1, 1.7, 1H, $H_{4'}$), 7.75 (d, *J*=1.6, 1H, $H_{6'}$); ^{13}C-NMR ($CDCl_3$, δ): 14.8 (CH_3), 25.0 ($2C(CH_3)_2$), 25.4 (CH_2C_3), 32.4 (CH_2), 61.0 (CH_2OH), 63.9 (CH_2O), 83.8 ($2C(CH_3)_2$), 111.7 ($C_{3'}$), 115.7 (C_5), 121.0 ($C_{5'}$), 126.5 (C_3), 129.0 (C_6), 130.0 ($C_{1'}$), 131.0 (C_1), 132.0 (C_2), 135.3 ($C_{4'}$), 137.4 ($C_{6'}$), 153.7 (C_4), 158.5 ($C_{2'}$); MS (ESI, *m/z*): 397.2 $[M\text{-}H]^-$.

5'-Allyl-2'-ethoxy-3-(3-hydroxypropyl)biphenyl-4-ol (83). Following the general procedure 6.1.1.6, allyl derivative **83** was obtained from boronate **84** (247 mg, 0.62 mmol) in 60% yield (116 mg). Chromatography: DCM to DCM/EtOAc, 9:1. R_f: 0.26 (DCM/EtOAc, 85:15).



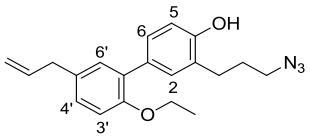
IR (ATR, ν): 3357 (OH), 1606, 1492 (Ar), 1269, 1236 (COC); ^{1}H-NMR ($CDCl_3$, δ): 1.34 (t, *J*=7.0, 3H, CH_3), 1.87-1.96 (m, 2H, CH_2), 2.66 (br s, 1H, CH_2OH), 2.82 (t, *J*=6.8, 2H, CH_2C_3), 3.37 (d, *J*=6.7, 2H, $CH_2C_{5'}$), 3.69 (t, *J*=5.8, 2H, CH_2OH), 4.00 (q, *J*=7.0, 2H, CH_2O), 5.03-5.13 (m, 2H, CH_2=), 5.99 (ddt, *J*=16.9, 10.1, 6.8, 1H, CH=), 6.87 (d, *J*=8.2, 1H, H_5), 6.89 (d, *J*=8.3, 1H, $H_{3'}$), 7.07 (dd, *J*=8.3, 2.3, 1H, $H_{4'}$), 7.14 (d, *J*=2.2, 1H, $H_{6'}$), 7.31 (dd, *J*=8.2, 2.3, 1H, H_6), 7.35 (d, *J*=2.2, 1H, H_2); ^{13}C-NMR ($CDCl_3$, δ): 15.0 (CH_3), 25.4 (CH_2C_3), 32.3 (CH_2), 39.6 ($CH_2C_{5'}$), 60.9 (CH_2OH), 64.3 (CH_2O), 113.0 ($C_{3'}$), 115.6 (CH_2=), 115.9 (C_5), 126.4 (C_3), 128.0 ($C_{4'}$), 128.9 (C_6), 130.6 ($C_{1'}$), 131.0 ($C_{6'}$), 132.00 (C_2), 132.04 (C_1), 132.4 ($C_{5'}$), 138.0 (CH=), 153.8 (C_4), 154.3 ($C_{2'}$); MS (ESI, *m/z*): 311.0 $[M-H]^-$.

3-(5'-Allyl-2'-ethoxy-4-hydroxybiphenyl-3-yl)propyl methanesulfonate (85). Following the general procedure 6.1.1.7, mesylate **85** was obtained from primary alcohol **83** (210 mg, 0.67 mmol) in 42% yield (110 mg). Chromatography: DCM to DCM/EtOAc, 99:1. R_f: 0.35 (DCM/EtOAc, 98:2).



IR (ATR, ν): 3420 (OH), 1608, 1494 (Ar), 1265, 1234 (COC), 1171 (SO_2); ^{1}H-NMR ($CDCl_3$, δ): 1.34 (t, *J*=7.0, 3H, CH_3CH_2), 2.12 (qt, *J*=6.9, 2H, CH_2), 2.79 (t, *J*=7.4, 2H, CH_2C_3), 2.99 (s, 3H, CH_3S), 3.36 (d, *J*=6.7, 2H, $CH_2C_{5'}$), 4.01 (q, *J*=7.0, 2H, CH_2O), 4.30 (t, *J*=6.3, 2H, CH_2OMs), 4.97 (br s, 1H, OH), 5.03-5.12 (m, 2H, CH_2=), 5.98 (ddt, *J*=16.9, 10.1, 6.8, 1H, CH=), 6.77 (d, *J*=8.1, 1H, H_5), 6.89 (d, *J*=8.3, 1H, $H_{3'}$), 7.07 (dd, *J*=8.3, 2.1, 1H, $H_{4'}$), 7.12 (d, *J*=2.1, 1H, $H_{6'}$), 7.30 (dd, *J*=8.2, 2.2, 1H, H_6), 7.34 (d, *J*=1.9, 1H, H_2); ^{13}C-NMR ($CDCl_3$, δ): 15.0 (CH_3CH_2), 26.2 (CH_2C_3), 29.4 (CH_2), 37.6 (CH_3S), 39.6 ($CH_2C_{5'}$), 64.3 (CH_2O), 69.9 (CH_2OMs), 113.0 ($C_{3'}$), 115.2 (C_5), 115.7 (CH_2=), 126.0 (C_3), 128.2 ($C_{4'}$), 128.9 (C_6), 130.4 ($C_{1'}$), 131.0 ($C_{6'}$), 131.6 (C_1), 131.8 (C_2), 132.5 ($C_{5'}$), 138.0 (CH=), 152.7 (C_4), 154.3 ($C_{2'}$); MS (ESI, *m/z*): 413.1 $[M+Na]^+$.

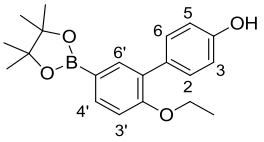
5'-Allyl-3-(3-azidopropyl)-2'-ethoxybiphenyl-4-ol (60). Following the general procedure 6.1.1.8, azide **60** was obtained from mesylate **85** (12 mg, 31 μmol) in 70% yield (7 mg). Chromatography: DCM to DCM/EtOAc, 95:5. R_f: 0.54 (DCM/EtOAc, 99:1).



IR (ATR, ν): 2930 (OH), 2096 (N_3), 1638, 1580, 1493 (Ar), 1264, 1231 (COC); ^{1}H-NMR (500 MHz, $CDCl_3$, δ): 1.34 (t, *J*=7.0, 3H, CH_3), 1.96 (qt, *J*=7.0, 2H, CH_2), 2.75 (t, *J*=7.3, 2H, CH_2C_3), 3.357 (t, *J*=6.9, 2H, CH_2N_3), 3.364 (d, *J*=6.9, 2H, $CH_2C_{5'}$), 4.01 (q, *J*=7.0, 2H, CH_2O), 4.99 (br s, 1H, OH), 5.05-5.11 (m, 2H, CH_2=), 5.98 (ddt, *J*=16.9, 10.1, 6.8, 1H, CH=), 6.79 (d, *J*=8.2, 1H, H_5), 6.89 (d, *J*=8.3, 1H, $H_{3'}$), 7.07 (dd, *J*=8.3, 2.0, 1H, $H_{4'}$), 7.12 (d, *J*=1.9, 1H, $H_{6'}$), 7.30 (dd, *J*=8.2, 2.1, 1H, H_6), 7.34 (d, *J*=1.8, 1H, H_2); ^{13}C-NMR (125 MHz, $CDCl_3$, δ): 15.0 (CH_3), 27.1 (CH_2C_3), 29.1 (CH_2), 39.6 ($CH_2C_{5'}$), 50.9 (CH_2N_3), 64.3 (CH_2O), 113.0 ($C_{3'}$), 115.2 (C_5), 115.7 (CH_2=), 126.3 (C_3), 128.1 ($C_{4'}$), 128.8 (C_6), 130.5 ($C_{1'}$), 131.0 ($C_{6'}$), 131.5 (C_1), 131.8 (C_2), 132.5 ($C_{5'}$), 138.0 (CH=), 152.8 (C_4), 154.3 ($C_{2'}$); HPLC-MS (ESI, *m/z*): 335.7 $[M-H]^-$; t_R (method A): 11.91 min.

- **Synthesis of azide 61**

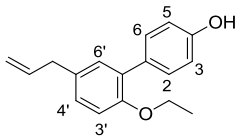
2'-Ethoxy-5'-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)biphenyl-4-ol (86). Following the general procedure 6.1.1.5, boronate **86** was obtained from chloroderivative **79** (460 mg, 1.8 mmol) in 75% yield (472 mg). Chromatography: hexane to hexane/EtOAc, 8:2. Mp: 168.2-169.1 ºC. R_f: 0.08 (hexane/EtOAc, 9:1).



IR (ATR, ν): 3370 (OH), 1599, 1515, 1471 (Ar), 1353 (BO), 1262, 1231 (COC), 1139 (BC); ^{1}H-NMR ($CDCl_3$, δ): 1.34 (s, 12H, $2C(CH_3)_2$), 1.36 (t, *J*=7.0, 3H, CH_3), 4.08 (q, *J*=7.0, 2H, CH_2), 4.91 (br s, 1H, OH), 6.84 (d, *J*=8.7, 2H, H_3, H_5), 6.94 (d, *J*=8.1, 1H, $H_{3'}$), 7.45 (d, *J*=8.7, 2H, H_2, H_6), 7.73 (dd, *J*=8.2, 1.7, 1H, $H_{4'}$), 7.75 (d, *J*=1.5, 1H, $H_{6'}$); ^{13}C-NMR ($CDCl_3$,

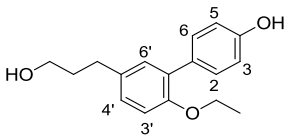
δ): 14.8 (CH_3), 25.0 (2C($\underline{C}H_3)_2$), 63.9 (CH_2), 83.8 (2$\underline{C}(CH_3)_2$), 111.7 ($C_{3'}$), 114.8 (C_3, C_5), 120.5 ($C_{5'}$), 129.9 ($C_{1'}$), 131.0 (C_2, C_6), 131.2 (C_1), 135.5 ($C_{4'}$), 137.6 ($C_{6'}$), 154.6 (C_4), 158.5 ($C_{2'}$); MS (ESI, *m/z*): 338.8 $[M-H]^-$.

5'-Allyl-2'-ethoxybiphenyl-4-ol (87). Following the general procedure 6.1.1.6, allyl derivative **87** was obtained from boronate **86** (536 mg, 1.6 mmol) in 75% yield (301 mg). Chromatography: hexane to hexane/EtOAc, 9:1. R_f: 0.21 (hexane/EtOAc, 9:1).



IR (ATR, ν): 3404 (OH), 1609, 1515, 1495, 1394 (Ar), 1264, 1234 (COC); ^{1}H-NMR ($CDCl_3$, δ): 1.33 (t, *J*=7.0, 3H, CH_3), 3.36 (d, *J*=6.7, 2H, $CH_2C_{5'}$), 4.00 (q, *J*=7.0, 2H, CH_2O), 4.73 (br s, 1H, OH), 5.03-5.12 (m, 2H, CH_2=), 5.98 (ddt, *J*=16.9, 10.1, 6.8, 1H, CH=), 6.86 (d, *J*=8.8, 2H, H_3, H_5), 6.89 (d, *J*=8.9, 1H, $H_{3'}$), 7.07 (dd, *J*=8.3, 2.2, 1H, $H_{4'}$), 7.12 (d, *J*=2.2, 1H, $H_{6'}$), 7.45 (d, *J*=8.7, 2H, H_2, H_6); ^{13}C-NMR ($CDCl_3$, δ): 15.0 (CH_3), 39.6 ($\underline{C}H_2C_5$), 64.4 (CH_2O), 113.1 ($C_{3'}$), 114.9 (C_3, C_5), 115.7 (CH_2=), 128.1 ($C_{4'}$), 130.5 (C_1), 130.9 (C_2, C_6), 131.0 ($C_{6'}$), 131.4 ($C_{1'}$), 132.5 ($C_{5'}$), 138.0 (CH=), 154.4 ($C_{2'}$), 154.6 (C_4); MS (ESI, *m/z*): 252.8 $[M-H]^-$.

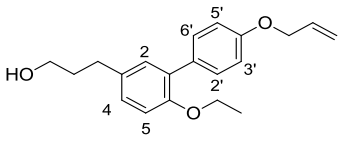
2'-Ethoxy-5'-(3-hydroxypropyl)biphenyl-4-ol (88). Following the general procedure 6.1.1.4, primary alcohol **88** was obtained from **87** (400 mg, 1.6 mmol) in 53% yield (227 mg). Chromatography: hexane to hexane/EtOAc, 6:4. Mp: 88.1-88.8 °C. R_f: 0.15 (hexane/EtOAc, 6:4).



IR (ATR, ν): 3314 (OH), 1610, 1516, 1495, 1446 (Ar), 1262, 1234 (COC); ^{1}H-NMR ($CDCl_3$, δ): 1.33 (t, *J*=7.0, 3H, CH_3), 1.62 (br s, 1H, $CH_2O\underline{H}$), 1.86-1.96 (m, 2H, CH_2), 2.69 (t, *J*=7.6, 2H, $CH_2C_{5'}$), 3.71 (t, *J*=6.4, 2H, $C\underline{H}_2OH$), 4.00 (q, *J*=7.0, 2H, CH_2O), 5.51 (br s, 1H, OH), 6.85 (d, *J*=8.7, 2H, H_3, H_5), 6.88 (d, *J*=8.4, 1H, $H_{3'}$), 7.08 (dd, *J*=8.3, 2.3, 1H, $H_{4'}$), 7.14 (d, *J*=2.3, 1H, $H_{6'}$), 7.44 (d, *J*=8.7, 2H, H_2, H_6); ^{13}C-NMR ($CDCl_3$, δ): 15.0 (CH_3), 31.4 ($\underline{C}H_2C_{5'}$),

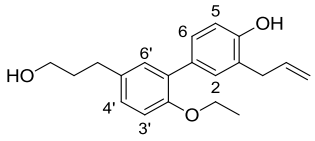
34.4 (CH_2), 62.6 (CH_2OH), 64.4 (CH_2O), 113.2 ($C_{3'}$), 115.0 (C_3, C_5), 127.9 ($C_{4'}$), 130.6 ($C_{1'}$), 130.8 ($C_{6'}$), 130.9 (C_2, C_6), 131.2 (C_1), 134.1 ($C_{5'}$), 154.2 ($C_{2'}$), 154.8 (C_4); MS (ESI, *m/z*): 270.8 $[M-H]^-$.

3-[4'-(Allyloxy)-6-ethoxybiphenyl-3-yl]propan-1-ol (89). Following the general procedure 6.1.1.2, allyl ether **89** was obtained from phenol **88** (220 mg, 0.81 mmol) and allyl bromide (84 µL, 0.97 mmol) in 92% yield (235 mg) and was used in the next step without further purification. Mp: 55.9-56.3 ºC. R_f: 0.39 (hexane/EtOAc, 6:4).



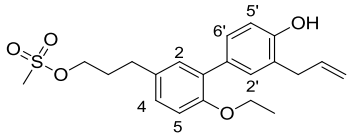
IR (ATR, ν): 3357 (OH), 1608, 1515, 1494 (Ar), 1239 (COC); ^{1}H-NMR ($CDCl_3$, δ): 1.33 (t, *J*=7.0, 3H, CH_3), 1.85-1.95 (m, 2H, CH_2), 2.69 (t, *J*=7.7, 2H, CH_2C_3), 3.69 (t, *J*=6.4, 2H, CH_2OH), 4.00 (q, *J*=7.0, 2H, CH_2CH_3), 4.58 (dt, *J*=5.3, 1.5, 2H, CH_2CH=), 5.30 (dq, *J*=10.5, 1.4, 1H, ½CH_2=), 5.44 (dq, *J*=17.3, 1.6, 1H, ½CH_2=), 6.09 (ddt, *J*=17.2, 10.6, 5.3, 1H, CH=), 6.88 (d, *J*=8.3, 1H, H_5), 6.95 (d, *J*=8.8, 2H, $H_{3'}$, $H_{5'}$), 7.08 (dd, *J*=8.3, 2.2, 1H, H_4), 7.15 (d, *J*=2.2, 1H, H_2), 7.49 (d, *J*=8.9, 2H, $H_{2'}$, $H_{6'}$); ^{13}C-NMR ($CDCl_3$, δ): 15.0 (CH_3), 31.4 (CH_2C_3), 34.6 (CH_2), 62.5 (CH_2OH), 64.4 (CH_2CH_3), 69.0 (CH_2CH=), 113.1 (C_5), 114.3 ($C_{3'}$, $C_{5'}$), 117.8 (CH_2=), 127.9 (C_4), 130.5 (C_1), 130.7 ($C_{2'}$, $C_{6'}$), 130.9 (C_2), 131.4 ($C_{1'}$), 133.6 (CH=), 134.2 (C_3), 154.2 (C_6), 157.7 ($C_{4'}$); MS (ESI, *m/z*): 313.1 $[M+H]^+$.

3-Allyl-2'-ethoxy-5'-(3-hydroxypropyl)biphenyl-4-ol (90). Following the general procedure 6.1.1.9, phenol **90** was obtained from allyl ether **89** (30 mg, 96 µmol) in 97% yield (29 mg), and was used in the next step without further purification. R_f: 0.27 (hexane/EtOAc, 6:4).



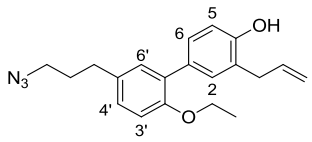
IR (ATR, ν): 3316 (OH), 1608, 1510, 1491, 1441 (Ar), 1268, 1236 (COC); ^{1}H-NMR ($CDCl_3$, δ): 1.34 (t, *J*=7.0, 3H, CH_3), 1.86-1.95 (m, 2H, CH_2), 2.69 (app t, *J*=7.7, 2H, $CH_2C_{5'}$), 3.45 (d, *J*=6.4, 2H, CH_2C_3), 3.70 (t, *J*=6.4, 2H, CH_2OH), 4.00 (q, *J*=7.0, 2H, CH_2O), 5.08 (br s, 1H, OH), 5.14-5.24 (m, 2H, CH_2=), 6.06 (ddt, *J*=16.9, 10.3, 6.5, 1H, CH=), 6.84 (d, *J*=8.9, 1H, H_5), 6.88 (d, *J*=8.3, 1H, $H_{3'}$), 7.08 (dd, *J*=8.3, 2.2, 1H, $H_{4'}$), 7.14 (d, *J*=2.2, 1H, $H_{6'}$), 7.32-7.35 (m, 2H, H_2, H_6); ^{13}C-NMR ($CDCl_3$, δ): 15.0 (CH_3), 31.4 ($CH_2C_{5'}$), 34.6 (CH_2), 35.4 (CH_2C_3), 62.5 (CH_2OH), 64.4 (CH_2O), 113.1 ($C_{3'}$), 115.4 (C_5), 116.6 (CH_2=), 124.8 (C_3), 127.9 ($C_{4'}$), 129.1 (C_6), 130.6 ($C_{1'}$), 130.8 ($C_{6'}$), 131.4 (C_1), 131.8 (C_2), 134.2 ($C_{5'}$), 136.7 (CH=), 153.2 (C_4), 154.2 ($C_{2'}$); MS (ESI, *m/z*): 311.1 $[M-H]^-$.

3-(3'-Allyl-6-ethoxy-4'-hydroxybiphenyl-3-yl)propyl methanesulfonate (91). Following the general procedure 6.1.1.7, mesylate **91** was obtained from primary alcohol **90** (330 mg, 1.1 mmol) in 77% yield (319 mg). Chromatography: DCM/EtOAc, 99:1. R_f: 0.16 (hexane/EtOAc, 7:3).



IR (ATR, ν): 3468 (OH), 1608, 1494 (Ar), 1350, 1171 (SO_2), 1269, 1237 (COC); ^{1}H-NMR ($CDCl_3$, δ): 1.34 (t, *J*=7.0, 3H, CH_3CH_2), 2.03-2.12 (m, 2H, CH_2), 2.72 (t, *J*=7.5, 2H, CH_2C_3), 2.99 (s, 3H, CH_3S), 3.46 (d, *J*=6.4, 2H, $CH_2C_{3'}$), 4.00 (q, *J*=7.0, 2H, CH_2O), 4.24 (t, *J*=6.3, 2H, CH_2OMs), 4.95 (br s, 1H, OH), 5.15-5.24 (m, 2H, CH_2=), 6.06 (ddt, *J*=16.9, 10.3, 6.5, 1H, CH=), 6.84 (d, *J*=8.9, 1H, $H_{5'}$), 6.88 (d, *J*=8.3, 1H, H_5), 7.06 (dd, *J*=8.3, 2.2, 1H, H_4), 7.12 (d, *J*=2.2, 1H, H_2), 7.33 (dd, *J*=8.7, 2.2, 1H, $H_{6'}$), 7.34 (d, *J*=2.1, 1H, $H_{2'}$); ^{13}C-NMR ($CDCl_3$, δ): 14.9 (CH_3CH_2), 30.8 (CH_2C_3), 30.9 (CH_2), 35.2 ($CH_2C_{3'}$), 37.4 (CH_3S), 64.3 (CH_2O), 69.4 (CH_2OMs), 113.0 (C_5), 115.4 ($C_{5'}$), 116.5 (CH_2=), 124.9 ($C_{3'}$), 127.9 (C_4), 128.9 ($C_{6'}$), 130.7 (C_1), 130.8 (C_2), 131.1 ($C_{1'}$), 131.7 ($C_{2'}$), 132.6 (C_3), 136.6 (CH=), 153.3 ($C_{4'}$), 154.4 (C_6); MS (ESI, *m/z*): 389.1 $[M-H]^-$.

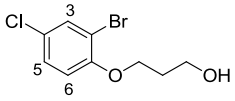
3-Allyl-5'-(3-azidopropyl)-2'-ethoxybiphenyl-4-ol (61). Following the general procedure 6.1.1.8, azide **61** was obtained from mesylate **91** (260 mg, 0.67 mmol) in 67% yield (152 mg). Chromatography: hexane to hexane/EtOAc, 9:1. R_f: 0.36 (hexane/EtOAc, 8:2).



IR (ATR, ν): 3444 (OH), 2098 (N_3), 1609, 1495 (Ar), 1266, 1238 (COC); ^{1}H-NMR (500 MHz, $CDCl_3$, δ): 1.34 (t, *J*=7.0, 3H, CH_3), 1.91 (qt, *J*=7.2, 2H, CH_2), 2.68 (t, *J*=7.5, 2H, $CH_2C_{5'}$), 3.30 (t, *J*=6.8, 2H, CH_2N_3), 3.46 (d, *J*=6.3, 2H, CH_2C_3), 4.00 (q, *J*=7.0, 2H, CH_2O), 5.16-5.23 (m, 2H, CH_2=), 6.06 (ddt, *J*=16.9, 10.3, 6.5, 1H, CH=), 6.84 (d, *J*=8.9, 1H, H_5), 6.88 (d, *J*=8.3, 1H, $H_{3'}$), 7.06 (dd, *J*=8.3, 2.2, 1H, $H_{4'}$), 7.12 (d, *J*=2.2, 1H, $H_{6'}$), 7.33 (dd, *J*=6.8, 2.0, 1H, H_6), 7.34 (br s, 1H, H_2); ^{13}C-NMR (125 MHz, $CDCl_3$, δ): 15.0 (CH_3), 30.8 (CH_2), 32.1 ($\underline{C}H_2C_{5'}$), 35.4 ($\underline{C}H_2C_3$), 50.8 (CH_2N_3), 64.3 (CH_2O), 113.1 ($C_{3'}$), 115.5 (C_5), 116.7 (CH_2=), 124.8 (C_3), 127.9 ($C_{4'}$), 129.1 (C_6), 130.7 ($C_{1'}$), 130.8 ($C_{6'}$), 131.4 (C_1), 131.8 (C_2), 133.2 ($C_{5'}$), 136.6 (CH=), 153.3 (C_4), 154.4 ($C_{2'}$); HRMS (ESI): calcd for $[M+Na]^+$ $C_{20}H_{23}N_3NaO_2$: 360.1688; found: 360.1682; HPLC-MS (ESI, *m/z*): 336.1 $[M-H]^-$; t_R (method A): 13.24 min.

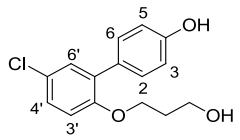
- **Synthesis of azide 62**

3-(2-Bromo-4-chlorophenoxy)propan-1-ol (92). Following the general procedure 6.1.1.2, ether **92** was obtained from 2-bromo-4-chlorophenol (2.00 g, 9.6 mmol) and 3-bromopropan-1-ol (1.0 mL, 12 mmol) in 85% yield (2.20 g). Chromatography: hexane to hexane/EtOAc, 7:3. R_f: 0.16 (hexane/EtOAc, 7:3).



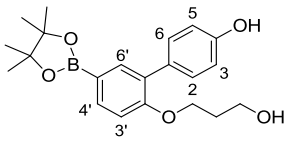
IR (ATR, ν): 3362 (OH), 1585, 1471, (Ar), 1286, 1258 (COC); ^{1}H-NMR ($CDCl_3$, δ): 2.00 (t, *J*=4.8, 1H, OH), 2.10 (qt, *J*=5.7, 2H, CH_2), 3.91 (q app, *J*=5.1, 2H, $C\underline{H}_2OH$), 4.17 (t, *J*=5.8, 2H, CH_2O), 6.84 (d, *J*=8.8, 1H, H_6), 7.23 (dd, *J*=8.8, 2.5, 1H, H_5), 7.53 (d, *J*=2.5, 1H, H_3); ^{13}C-NMR ($CDCl_3$, δ): 31.8 (CH_2), 60.8 (CH_2OH), 67.9 (CH_2O), 112.7 (C_2), 113.7 (C_6), 126.4 (C_4), 128.5 (C_5), 132.9 (C_3), 154.1 (C_1); MS (ESI, *m/z*): 264.6 $[M(^{35}Cl, ^{79}Br)+H]^+$, 266.6 $[M(^{35}Cl, ^{81}Br)+H]^+$ and $[M(^{37}Cl, ^{79}Br)+H]^+$, 268.6 $[M(^{37}Cl, ^{81}Br)+H]^+$.

5'-Chloro-2'-(3-hydroxypropoxy)biphenyl-4-ol (93). Following the general procedure 6.1.1.3, biphenyl derivative **93** was obtained from bromoderivative **92** (262 mg, 0.99 mmol) and 4-hydroxyphenylboronic acid (204 mg, 1.5 mmol) in 82% yield (226 mg). Chromatography: DCM to DCM/EtOAc, 8:2. Mp: 114.3-115.0 ºC. R_f: 0.19 (DCM/EtOAc, 8:2).



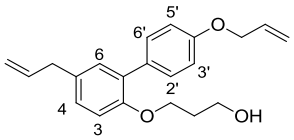
IR (ATR, ν): 3316 (OH), 1611, 1516, 1486, 1466 (Ar), 1263, 1232 (COC); ^{1}H-NMR ($CDCl_3$, δ): 2.02 (qt, *J*=5.6, 2H, CH_2), 2.23 (t, *J*=5.6, 1H, $CH_2O\underline{H}$), 3.76 (q app, *J*=5.3, 2H, $C\underline{H}_2OH$), 4.14 (t, *J*=5.6, 2H, CH_2O), 6.63 (s, 1H, OH), 6.84 (d, *J*=8.6, 2H, H_3, H_5), 6.89 (d, *J*=8.9, 1H, $H_{3'}$), 7.23-7.27 (m, 2H, $H_{4'}$, $H_{6'}$), 7.31 (d, *J*=8.6, 2H, H_2, H_6); ^{13}C-NMR ($CDCl_3$, δ): 31.6 (CH_2), 61.8 (CH_2OH), 68.5 (CH_2O), 113.3 ($C_{3'}$), 115.5 (C_3, C_5), 126.3 ($C_{5'}$), 127.8 ($C_{4'}$), 128.9 (C_1), 130.6 (C_2, C_6, $C_{6'}$), 132.7 ($C_{1'}$), 154.2 ($C_{2'}$), 156.2 (C_4); MS (ESI, *m/z*): 276.7 [M(^{35}Cl)-H]$^-$, 278.7 [M(^{37}Cl)-H]$^-$.

2'-(3-Hydroxypropoxy)-5'-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)biphenyl-4-ol (94). Following the general procedure 6.1.1.5, boronate **94** was obtained from chloroderivative **93** (625 mg, 2.5 mmol) in 78% yield (710 mg). Chromatography: DCM to DCM/EtOH, 98:2. R_f: 0.15 (DCM/EtOH, 98:2).



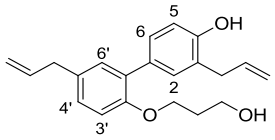
IR (ATR, ν): 3358 (OH), 1600, 1518, 1469 (Ar), 1355 (BO), 1264, 1234 (COC), 1140 (BC); ^{1}H-NMR ($CDCl_3$, δ): 1.34 (s, 12H, 2C(CH_3)$_2$), 1.92 (br s, 1H, $CH_2O\underline{H}$), 1.97 (qt, *J*=5.6, 2H, CH_2), 3.74 (q app, *J*=4.8, 2H, $C\underline{H}_2OH$), 4.17 (t, *J*=5.7, 2H, CH_2O), 5.66 (br s, 1H, OH), 6.83 (d, *J*=8.6, 2H, H_3, H_5), 6.96 (d, *J*=8.0, 1H, $H_{3'}$), 7.35 (d, *J*=8.7, 2H, H_2, H_6), 7.73-7.76 (m, 2H, $H_{4'}$, $H_{6'}$); ^{13}C-NMR ($CDCl_3$, δ): 24.9 (2C($\underline{C}H_3$)$_2$), 31.5 (CH_2), 61.4 (CH_2OH), 67.5 (CH_2O), 83.8 (2$\underline{C}$(CH_3)$_2$), 111.2 ($C_{3'}$), 115.3 (C_3, C_5), 121.1 ($C_{5'}$), 129.7 (C_1), 130.4 ($C_{1'}$), 130.6 (C_2, C_6), 135.4 ($C_{4'}$), 137.5 ($C_{6'}$), 155.8 (C_4), 158.1 ($C_{2'}$); MS (ESI, *m/z*): 368.7 [M-H]$^-$.

3-{[5-Allyl-4'-(allyloxy)biphenyl-2-yl]oxy}propan-1-ol (95). A suspension of boronate **94** (100 mg, 0.27 mmol), allyl bromide (70 μL, 0.69 mmol), Pd_2dba_3 (67 mg, 61 μmol), and K_2CO_3 (224 mg, 1.6 mmol) in anhydrous toluene (2 mL) was heated at 150 ºC for 45 min under MW irradiation. The mixture was filtered through a pad of celite, washed with EtOAc and evaporated under reduced pressure. The residue was purified by chromatography (DCM to DCM/EtOAc 98:2) to obtain **95** (41 mg, 47%). R_f: 0.29 (DCM/EtOAc 98:2).



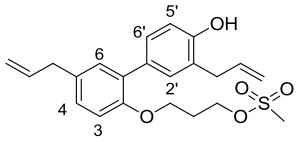
IR (ATR, ν): 3388 (OH), 1608, 1513, 1494 (Ar), 1238 (COC); ^{1}H-NMR ($CDCl_3$, δ): 1.64 (t, *J*=5.7, 1H, $CH_2O\underline{H}$), 1.96 (qt, *J*=5.7, 2H, CH_2), 3.36 (d, *J*=6.7, 2H, CH_2C_5), 3.72 (q, *J*=5.7, 2H, $C\underline{H}_2OH$), 4.08 (t, *J*=5.8, 2H, CH_2OC_2), 4.57 (dt, *J*=5.3, 1.5, 2H, $CH_2OC_{4'}$), 5.03-5.12 (m, 2H, $C\underline{H}_2$=$CHCH_2C_5$), 5.30 (dq, *J*=10.5, 1.4, 1H, ½$C\underline{H}_2$=$CHCH_2O$), 5.44 (dq, *J*=17.2, 1.5, 1H, ½$C\underline{H}_2$=$CHCH_2O$), 5.98 (ddt, *J*=16.9, 10.1, 6.6, 1H, $C\underline{H}CH_2C_5$), 6.04-6.15 (m, 1H, $C\underline{H}CH_2O$), 6.92 (d, *J*=8.2, 1H, H_3), 6.96 (d, *J*=8.8, 2H, $H_{3'}$, $H_{5'}$), 7.07-7.12 (m, 2H, H_4, H_6), 7.42 (d, *J*=8.8, 2H, $H_{2'}$, $H_{6'}$); ^{13}C-NMR ($CDCl_3$, δ): 32.1 (CH_2), 39.5 ($\underline{C}H_2C_5$), 61.1 (CH_2OH), 67.3 ($\underline{C}H_2OC_2$), 68.9 ($\underline{C}H_2OC_{4'}$), 112.8 (C_3), 114.4 ($C_{3'}$, $C_{5'}$), 115.8 ($\underline{C}H_2$=$CHCH_2C_5$), 117.8 ($\underline{C}H_2$=$CHCH_2O$), 128.2 (C_4), 130.6 ($C_{2'}$, $C_{6'}$), 130.8 ($C_{1'}$), 131.10 (C_6), 131.11 (C_1), 132.8 (C_5), 133.5 ($\underline{C}HCH_2O$), 137.8 ($\underline{C}HCH_2C_5$), 154.2 (C_2), 157.8 ($C_{4'}$); MS (ESI, *m/z*): 324.8 $[M+H]^+$.

3,5'-Diallyl-2'-(3-hydroxypropoxy)biphenyl-4-ol (96). Following the general procedure 6.1.1.9, phenol **96** was obtained from allyl ether **95** (26 mg, 80 μmol) in 70% yield (18 mg). Chromatography: hexane to hexane/EtOAc, 6:4. R_f: 0.26 (hexane/EtOAc, 6:4).



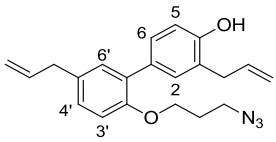
IR (ATR, ν): 3319 (OH), 1640 ($CH=CH_2$), 1606, 1492, 1427 (Ar), 1269, 1235 (COC); ^{1}H-NMR ($CDCl_3$, δ): 2.00 (qt, *J*=5.3, 2H, CH_2), 3.39 (d, *J*=6.7, 2H, $CH_2C_{5'}$), 3.47 (d, *J*=6.5, 2H, CH_2C_3), 3.77 (t, *J*=5.2, 2H, CH_2OH), 4.14 (t, *J*=5.6, 2H, CH_2O), 5.09-5.19 (m, 4H, $2CH_2=$), 6.01 (ddt, *J*=16.7, 9.8, 6.7, 1H, $CHCH_2C_{5'}$), 6.09 (ddt, *J*=16.8, 10.1, 6.6, 1H, $CHCH_2C_3$), 6.74 (d, *J*=8.1, 1H, H_5), 6.91 (d, *J*=8.1, 1H, $H_{3'}$), 7.10-7.14 (m, 2H, $H_{4'}$, $H_{6'}$), 7.21 (dd, *J*=8.1, 2.2, 1H, H_6), 7.24 (d, *J*=2.1, 1H, H_2); ^{13}C-NMR ($CDCl_3$, δ): 31.5 (CH_2), 34.6 (CH_2C_3), 39.5 ($CH_2C_{5'}$), 61.9 (CH_2OH), 68.2 (CH_2O), 112.1 ($C_{3'}$), 115.2 (C_5), 115.7, 115.9 ($2CH_2=$), 126.1 (C_3), 128.0, 128.3 ($C_{4'}$, C_6), 130.0 (C_1), 131.1 (C_2/$C_{6'}$), 131.2 (C_2/$C_{6'}$, $C_{1'}$), 132.9 ($C_{5'}$), 137.0 ($CHCH_2C_3$), 137.8 ($CHCH_2C_{5'}$), 153.9, 154.0 ($C_{2'}$, C_4); MS (ESI, *m/z*): 323.1 $[M-H]^-$.

3-[(3',5-Diallyl-4'-hydroxybiphenyl-2-yl)oxy]propyl methanesulfonate (97). Following the general procedure 6.1.1.7, mesylate **97** was obtained from primary alcohol **96** (58 mg, 0.18 mmol) in 53% yield (38 mg). Chromatography: DCM/EtOAc, 99:1. R_f: 0.18 (hexane/EtOAc, 7:3).



IR (ATR, ν): 3478 (OH), 1640 ($CH=CH_2$), 1608, 1493, 1470 (Ar), 1353, 1173 (SO_2), 1270, 1236 (COC); ^{1}H-NMR ($CDCl_3$, δ): 2.11 (qt, *J*=5.9, 2H, CH_2), 2.80 (s, 3H, CH_3), 3.35 (d, *J*=6.7, 2H, CH_2C_5), 3.44 (d, *J*=6.4, 2H, $CH_2C_{3'}$), 4.03 (t, *J*=5.7, 2H, CH_2O), 4.25 (t, *J*=6.1, 2H, CH_2OMs), 5.03-5.17 (m, 4H, $2CH_2=$), 5.97 (ddt, *J*=16.9, 10.1, 6.8, 1H, $CHCH_2C_5$), 6.04 (ddt, *J*=17.0, 10.3, 6.6, 1H, $CHCH_2C_{3'}$), 6.86 (d, *J*=8.1, 1H, $H_{5'}$), 6.88 (d, *J*=8.2, 1H, H_3), 7.07 (dd, *J*=8.3, 2.2, 1H, H_4), 7.11 (d, *J*=2.2, 1H, H_6), 7.23 (dd, *J*=8.2, 2.2, 1H, $H_{6'}$), 7.26 (d, *J*=1.9, 1H, $H_{2'}$); ^{13}C-NMR ($CDCl_3$, δ): 29.1 (CH_2), 34.9 ($CH_2C_{3'}$), 36.8 (CH_3), 39.5 (CH_2C_5), 63.9 (CH_2O), 67.0 (CH_2OMs), 112.9 (C_3), 115.2 ($C_{5'}$), 115.7 ($CH_2=CHCH_2C_5$), 116.1 ($CH_2=CHCH_2C_{3'}$), 125.4 ($C_{3'}$), 128.1 (C_4), 128.8 ($C_{6'}$), 130.7, 130.9 (C_1, $C_{1'}$), 131.0 (C_6), 131.5 ($C_{2'}$), 133.0 (C_5), 136.8 ($CHCH_2C_{3'}$), 137.8 ($CHCH_2C_5$), 153.6, 153.7 (C_2, $C_{4'}$); MS (ESI, *m/z*): 401.1 $[M-H]^-$.

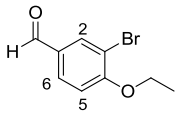
3,5'-Diallyl-2'-(3-azidopropoxy)biphenyl-4-ol (62). Following the general procedure 6.1.1.8, azide **62** was obtained from mesylate **97** (41 mg, 0.10 mmol) in 77% yield (26 mg). Chromatography: hexane to hexane/EtOAc, 9:1. R_f: 0.14 (hexane/EtOAc, 9:1).



IR (ATR, ν): 3388 (OH), 2098 (N_3), 1640 (CH=CH_2), 1607, 1493, 1398 (Ar), 1268, 1235 (COC); ^{1}H-NMR ($CDCl_3$, δ): 1.95 (qt, *J*=6.3, 2H, CH_2), 3.371 (d, *J*=6.6, 2H, $CH_2C_{5'}$), 3.373 (t, *J*=6.6, 2H, CH_2N_3), 3.46 (d, *J*=6.3, 2H, CH_2C_3), 4.00 (t, *J*=5.8, 2H, CH_2O), 5.04-5.13 (m, 2H, CH_2=$CHCH_2C_{5'}$) 5.15-5.23 (m, 2H, CH_2=$CHCH_2C_3$), 5.98 (ddt, *J*=16.9, 10.1, 6.8, 1H, $CHCH_2C_{5'}$), 6.06 (ddt, *J*=16.9, 10.3, 6.5, 1H, $CHCH_2C_3$), 6.85 (d, *J*=8.3, 1H, H_5), 6.89 (d, *J*=8.3, 1H, $H_{3'}$), 7.08 (dd, *J*=8.3, 2.2, 1H, $H_{4'}$), 7.13 (d, *J*=2.2, 1H, $H_{6'}$), 7.25-7.28 (m, 2H, H_2, H_6); ^{13}C-NMR ($CDCl_3$, δ): 28.9 (CH_2), 35.3 (CH_2C_3), 39.6 ($CH_2C_{5'}$), 48.4 (CH_2N_3), 65.3 (CH_2O), 113.0 ($C_{3'}$), 115.4 (C_5), 115.7 (CH_2=$CHCH_2C_{5'}$), 116.6 (CH_2=$CHCH_2C_3$), 124.8 (C_3), 128.1 ($C_{4'}$), 129.0 (C_6), 130.9 (C_1/$C_{1'}$), 131.1 ($C_{6'}$), 131.2 (C_1/$C_{1'}$), 131.7 (C_2), 132.9 ($C_{5'}$), 136.6 ($CHCH_2C_3$), 137.9 ($CHCH_2C_{5'}$), 153.4 (C_4), 154.0 ($C_{2'}$); HRMS (ESI, *m/z*): calcd for $[M-H]^-$ $C_{21}H_{22}N_3O_2$: 348.17175; found: 348.17175; HPLC-MS (ESI, *m/z*): 348.1 $[M-H]^-$; t_R (method A): 12.53 min.

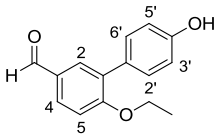
- **Synthesis of alkyne 63**

3-Bromo-4-ethoxybenzaldehyde (98). Following the general procedure 6.1.1.2, bromoderivative **98** was obtained from 3-bromo-4-hydroxybenzaldehyde (600 mg, 3.0 mmol) and bromoethane (0.3 mL, 3.6 mmol) in 89% yield (612 mg). Chromatography: hexane to hexane/EtOAc, 9:1. Mp: 69-72 ºC (lit.[156] 64.5 ºC). R_f: 0.30 (hexane/EtOAc, 8:2).



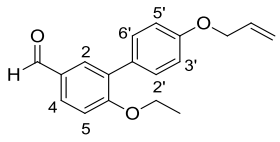
IR (ATR, ν): 2835, 2729, 1693 (CHO), 1593, 1567, 1494 (Ar), 1271 (COC); ^{1}H-NMR ($CDCl_3$, δ): 1.52 (t, *J*=7.0, 3H, CH_3), 4.21 (q, *J*=7.0, 2H, CH_2), 6.98 (d, *J*=8.5, 1H, H_5), 7.80 (dd, *J*=8.5, 2.0, 1H, H_6), 8.09 (d, *J*=2.0, 1H, H_2), 9.84 (s, 1H, CHO); ^{13}C-NMR ($CDCl_3$, δ): 14.6 (CH_3), 65.4 (CH_2), 112.4 (C_5), 113.0 (C_3), 130.6 (C_1), 131.2 (C_6), 134.8 (C_2), 160.3 (C_4), 189.8 (CHO); MS (ESI, *m/z*): 250.8 [M(^{79}Br)+Na]$^+$, 252.9 [M(^{81}Br)+Na]$^+$.

6-Ethoxy-4'-hydroxy-1,1'-biphenyl-3-carbaldehyde (99). Following the general procedure 6.1.1.3, biphenyl derivative **99** was obtained from bromoderivative **98** (750 mg, 3.3 mmol) and 4-hydroxyphenylboronic acid (677 mg, 4.9 mmol) in 68% yield (544 mg). Chromatography: DCM to DCM/EtOAc, 9:1. Mp: 122-125 ºC. R_f: 0.18 (hexane/EtOAc, 7:3).



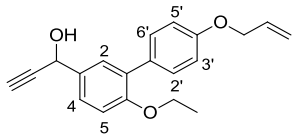
IR (ATR, ν): 3344 (OH), 2853, 1670 (CHO), 1592, 1515, 1496 (Ar), 1262 (COC); ^{1}H-NMR ($CDCl_3$, δ): 1.41 (t, *J*=7.0, 3H, CH_3), 4.16 (q, *J*=7.0, 2H, CH_2), 5.36 (br s, 1H, OH), 6.90 (d, *J*=8.6, 2H, $H_{3'}$, $H_{5'}$), 7.05 (d, *J*=8.4, 1H, H_5), 7.46 (d, *J*=8.6, 2H, $H_{2'}$, $H_{6'}$), 7.81 (dd, *J*=8.4, 2.1, 1H, H_4), 7.84 (d, *J*=2.0, 1H, H_2), 9.91 (s, 1H, CHO); ^{13}C-NMR ($CDCl_3$, δ): 14.7 (CH_3), 64.5 (CH_2), 112.0 (C_5), 115.2 ($C_{3'}$, $C_{5'}$), 129.7 ($C_{1'}$), 129.8 (C_3), 130.9 ($C_{2'}$, $C_{6'}$), 131.1 (C_1), 131.2 (C_4), 132.3 (C_2), 155.3 ($C_{4'}$), 161.1 (C_6), 191.4 (CHO); MS (ESI, *m/z*): 241.1 [M-H]$^-$.

4'-(Allyloxy)-6-ethoxy-1,1'-biphenyl-3-carbaldehyde (100). Following the general procedure 6.1.1.2, allyl ether **100** was obtained from phenol **99** (134 mg, 0.55 mmol) and allyl bromide (72 µL, 0.83 mmol) in 96% yield (149 mg). Chromatography: hexane to hexane/EtOAc, 8:2. Mp: 60.5-61.2 ºC. R_f: 0.35 (hexane/EtOAc, 8:2).



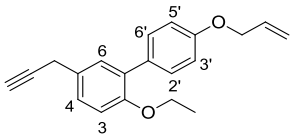
IR (ATR, ν): 2729, 1690 (CHO), 1597, 1513, 1496, 1471 (Ar), 1263, 1244 (COC); ^{1}H-NMR ($CDCl_3$, δ): 1.42 (t, *J*=7.0, 3H, CH_3), 4.16 (q, *J*=7.0, 2H, CH_2CH_3), 4.59 (dt, *J*=5.3, 1.5, 2H, CH_2CH=), 5.32 (dq, *J*=10.5, 1.4, 1H, ½CH_2=), 5.45 (dq, *J*=17.3, 1.6, 1H, ½CH_2=), 6.10 (ddt, *J*=17.2, 10.5, 5.3, 1H, CH=), 6.98 (d, *J*=8.9, 2H, $H_{3'}$, $H_{5'}$), 7.05 (d, *J*=8.4, 1H, H_5), 7.50 (d, *J*=8.8, 2H, $H_{2'}$, $H_{6'}$), 7.81 (dd, *J*=8.4, 2.1, 1H, H_4), 7.85 (d, *J*=2.1, 1H, H_2), 9.92 (s, 1H, CHO); ^{13}C-NMR ($CDCl_3$, δ): 14.7 (CH_3), 64.5 (CH_2CH_3), 69.0 (CH_2CH=), 112.0 (C_5), 114.5 ($C_{3'}$, $C_{5'}$), 117.9 (CH_2=), 129.8 ($C_{1'}$), 129.9 (C_3), 130.7 ($C_{2'}$, $C_{6'}$), 131.0 (C_4), 131.1 (C_1), 132.3 (C_2), 133.4 (CH=), 158.2 ($C_{4'}$), 161.0 (C_6), 191.2 (CHO); MS (ESI, *m/z*): 283.1 $[M+H]^+$.

1-[4'-(Allyloxy)-6-ethoxy-1,1'-biphenyl-3-yl]propargyl-1-ol (101). To a solution of aldehyde **100** (390 mg, 1.4 mmol) in anhydrous THF (1 mL) at 0 °C and under an argon atmosphere, ethynylmagnesium bromide (8.3 mL, 0.5 M in THF) was added dropwise and the mixture was stirred at rt for 2.5 h. The reaction was quenched with saturated NH_4Cl (aq) at 0 °C, followed by Et_2O and 1 M HCl (aq). The organic phase was separated and the aqueous phase was extracted with Et_2O. The combined organic extracts were washed with water and brine, dried (Na_2SO_4), filtered and evaporated under reduced pressure to yield pure **101** (412 mg, 97%), which was used in the next step without further purification. R_f: 0.26 (hexane/EtOAc, 7:3).



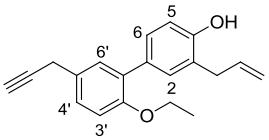
IR (ATR, ν): 3407, 3286 (OH), 2117 (C≡C), 1607, 1513, 1493 (Ar); ^{1}H-NMR ($CDCl_3$, δ): 1.36 (t, *J*=7.0, 3H, CH_3), 2.25 (br s, 1H, OH), 2.66 (d, *J*=2.2, 1H, CH≡), 4.05 (q, *J*=7.0, 2H, CH_2CH_3), 4.58 (dt, *J*=5.3, 1.5, 2H, CH_2CH=), 5.31 (dq, *J*=10.5, 1.4, 1H, ½CH_2=), 5.45 (dq, *J*=17.2, 1.6, 1H, ½CH_2=), 5.46 (d, *J*=1.9, 1H, CHOH), 6.10 (ddt, *J*=17.2, 10.5, 5.3, 1H, CH=), 6.96 (d, *J*=8.2, 1H, H_5), 6.97 (d, *J*=8.9, 2H, $H_{3'}$, $H_{5'}$), 7.45 (dd, *J*=8.4, 2.4, 1H, H_4), 7.50 (d, *J*=2.4, 1H, H_2), 7.51 (d, *J*=8.9, 2H, $H_{2'}$, $H_{6'}$); ^{13}C-NMR ($CDCl_3$, δ): 14.9 (CH_3), 64.29 (CHOH), 64.30 (CH_2CH_3), 69.0 (CH_2CH=), 74.8 (CH≡), 83.8 (C≡), 112.7 (C_5), 114.3 ($C_{3'}$, $C_{5'}$), 117.8 (CH_2=), 126.6 (C_4), 129.4 (C_2), 130.7 ($C_{2'}$, $C_{6'}$), 130.8, 130.9 (C_1, $C_{1'}$), 132.5 (C_3), 133.5 (CH=), 156.2 (C_6), 157.9 ($C_{4'}$); MS (ESI, *m/z*): 331.1 $[M+Na]^+$.

2-Ethoxy-4'-(allyloxy)-5-(propargyl)biphenyl (102). To a solution of alcohol **101** (50 mg, 0.16 mmol) in dry DCM (0.4 mL) at 0 ºC and under an argon atmosphere, triethylsilane (TES, 39 µL, 0.24 mmol) and TFA (50 µL, 0.65 mmol) were added and the mixture was stirred at that temperature for 10 min. The reaction was quenched with saturated $NaHCO_3$ (aq), extracted with DCM, dried (Na_2SO_4), filtered, and evaporated under reduced pressure. The crude was purified by chromatography (glass column, hexane/EtOAc 8:2 to hexane/EtOAc 7:3) to afford **102** (16 mg, 33%). R_f: 0.17 (hexane/DCM, 7:3).



IR (ATR, ν): 1646 (C=C), 1608, 1574, 1515, 1495 (Ar), 1268, 1242 (COC); ^{1}H-NMR ($CDCl_3$, δ): 1.34 (t, *J*=7.0, 3H, CH_3), 2.18 (t, *J*=2.7, 1H, CH≡), 3.58 (d, *J*=2.7, 2H, CH_2C≡), 4.02 (q, *J*=7.0, 2H, CH_2CH_3), 4.58 (dt, *J*=5.3, 1.5, 2H, CH_2CH=), 5.31 (dq, *J*=10.5, 1.4, 1H, ½CH_2=), 5.45 (dq, *J*=17.3, 1.6, 1H, ½CH_2=), 6.10 (ddt, *J*=17.2, 10.5, 5.3, 1H, CH=), 6.92 (d, *J*=8.4, 1H, H_3), 6.96 (d, *J*=8.9, 2H, $H_{3'}$, $H_{5'}$), 7.24 (dd, *J*=8.3, 2.4, 1H, H_4), 7.28 (d, *J*=2.3, 1H, H_6), 7.50 (d, *J*=8.9, 2H, $H_{2'}$, $H_{6'}$); ^{13}C-NMR ($CDCl_3$, δ): 15.0 (CH_3), 24.2 (CH_2C≡), 64.4 (CH_2CH_3), 69.0 (CH_2CH=), 70.4 (CH≡), 82.6 (C≡), 113.1 (C_3), 114.3 ($C_{3'}$, $C_{5'}$), 117.8 (CH_2=), 127.4 (C_4), 128.1 (C_5), 130.3 (C_6), 130.7 ($C_{2'}$, $C_{6'}$, C_1), 131.1 ($C_{1'}$), 133.6 (CH=), 154.8 (C_2), 157.8 ($C_{4'}$); MS (ESI, *m/z*): 293.2 $[M+H]^+$.

2'-Ethoxy-3-(allyl)-5'-(propargyl)biphenyl-4-ol (63). Following the general procedure 6.1.1.9, phenol **63** was obtained from allyl ether **102** (63 mg, 0.22 mmol) in 51% yield (33 mg). Chromatography: glass column, hexane/EtOAc, 9:1. R_f: 0.23 (hexane/EtOAc, 8:2).

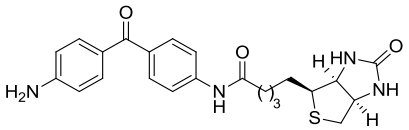


IR (ATR, ν): 3307, 3291 (OH), 2253 (C≡C), 1511, 1493, 1475, 1438 (Ar), 1270, 1237 (COC); ^{1}H-NMR (500 MHz, $CDCl_3$, δ): 1.34 (t, *J*=7.0, 3H, CH_3), 2.17 (t, *J*=2.7, 1H, CH≡), 3.46 (d, *J*=6.4, 2H, CH_2C=), 3.58 (d, *J*=2.7, 2H, CH_2C≡), 4.00 (q, *J*=7.0, 2H, CH_2O), 4.94 (br s, 1H, OH), 5.17 (dq, *J*=10.1, 1.5, 1H, ½CH_2=), 5.21 (dq, *J*=17.2, 1.7, 1H, ½CH_2=), 6.06 (ddt,

J=16.9, 10.2, 6.5, 1H, CH=), 6.84 (d, *J*=8.9, 1H, H_5), 6.90 (d, *J*=8.3, 1H, $H_{3'}$), 7.23 (dd, *J*=8.3, 2.3, 1H, $H_{4'}$), 7.27 (d, *J*=2.3, 1H, $H_{6'}$), 7.34 (dd, *J*=6.9, 2.0, 1H, H_6), 7.35 (m, 1H, H_2); ^{13}C-NMR (125 MHz, $CDCl_3$, δ): 15.0 (CH_3), 24.2 ($CH_2C\equiv$), 35.4 (CH_2C_3), 64.4 (CH_2O), 70.4 (CH≡), 82.6 (C≡), 113.1 ($C_{3'}$), 115.5 (C_5), 116.7 (CH_2=), 124.8 (C_3), 127.4 ($C_{4'}$), 128.4 ($C_{5'}$), 129.1 (C_6), 130.3 ($C_{6'}$), 130.8 ($C_{1'}$), 131.2 (C_1), 131.8 (C_2), 136.6 (CH=), 153.3 (C_4), 154.8 ($C_{2'}$); HPLC-MS (ESI, *m/z*): 291.1 $[M-H]^-$; t_R (method A): 10.60 min.

- **Synthesis of probes 56-59**

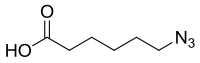
(+)-*N*-[4-(4-Aminobenzoyl)phenylbiotinamide (103). A suspension of biotin (293 mg, 1.2 mmol), HOBt (163 mg, 1.2 mmol), and activated 4 Å molecular sieves in anhydrous DMF (10 mL) under an argon atmosphere, was heated at 77 ºC for 50 min until dissolution of biotin. After cooling to rt, a solution of DCC (272 mg, 1.3 mmol) in dry DCM (2 mL) was added dropwise. The mixture was stirred at rt for 3 h before a solution of 4,4'-diaminobenzophenone (500 mg, 2.4 mmol) and DMAP (15 mg, 0.12 mmol) in anhydrous DMF (2 mL) was added. The resulting mixture was heated at 60 ºC for 4 h and allowed to stir at rt for 16 h. The mixture was filtered, washed with DCM/MeOH 1:1, and evaporated under reduced pressure. The crude was purified by chromatography (glass column, DCM to DCM/MeOH 7:3) to afford amine **103** (500 mg, 97%). Mp: 272-275 ºC (lit.[157] 269-270 ºC). $[\alpha]_D^{20}$= +41.0 (c=0.9, DMSO). The spectroscopic data correspond with those previously reported.[157]



^{1}H-NMR ($(CD_3)_2SO$, δ): 1.32-1.67 (m, 6H, $(CH_2)_3$), 2.36 (t, *J*=7.3, 2H, CH_2CO), 2.58 (d, *J*=12.4, 1H, ½CH_2S), 2.83 (dd, *J*=12.4, 5.1, 1H, ½CH_2S), 3.10-3.16 (m, 1H, CHS), 4.12-4.16 (m, 1H, CHN), 4.29-4.33 (m, 1H, CHN), 6.09 (br s, 2H, NH_2), 6.37 (br s, 1H, NH), 6.45 (br s, 1H, NH), 6.59 (d, *J*=8.7, 2H, 2CH_{Ar}CNH_2), 7.50 (d, *J*=8.7, 2H, 2CH_{Ar}CCO), 7.59 (d, *J*=8.7, 2H, 2CH_{Ar}CCO), 7.71 (d, *J*=8.7, 2H, 2CH_{Ar}CNH), 10.20 (br s, 1H, NHC_{Ar}).

6-Azidohexanoic acid (104). Compound **104** was obtained following a previously described procedure, and the spectroscopic data correspond with those reported.[129]

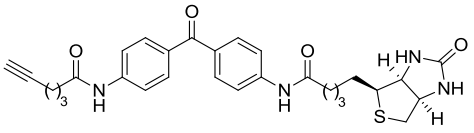
A solution of 6-bromohexanoic acid (500 mg, 2.6 mmol) and NaN_3 (500 mg, 7.7 mmol) in anhydrous DMF (2.5 mL) was heated at 50 ºC for 3 h. The solvent was evaporated under reduced pressure and the residue was treated with EtOAc and 10% HCl (aq) and the organic phase was separated, washed with brine, dried (Na_2SO_4), filtered and evaporated under reduced pressure to yield **104** (308 mg, 76%), which was used in the next step without further purification.



^{1}H-NMR ($CDCl_3$, δ): 1.38-1.49 (m, 2H, CH_2), 1.65 (sept, *J*=7.3, 4H, CH_2CH_2CO, CH_2CH_2N), 2.38 (t, *J* =7.4, 2H, CH_2COO), 3.28 (t, *J*=6.8, 2H, CH_2N_3).

General procedure for the synthesis of amides 105 and 106. To a solution of the corresponding carboxylic acid (1 equiv) and HOBt (1.1 equiv) in dry DCM (28 mL/mmol of acid) under an argon atmosphere, EDC (1.1 equiv) was added and the mixture was stirred at rt for 40 min. Then, a solution of the corresponding amine (1.5 equiv) in DMF (4.5 mL/mmol) was added, and the mixture was stirred at rt for 48 h. The reaction was diluted with EtOAc, washed with saturated $NaHCO_3$ (aq), dried (Na_2SO_4), filtered, and evaporated under reduced pressure. The crude was purified by chromatography (DCM to DCM/MeOH, 8:2) to afford the corresponding amide.

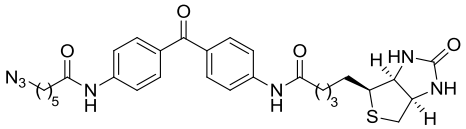
(+)-*N*-{4-[4-(Biotinylamino)benzoyl]phenyl}hex-5-ynamide (105). Obtained from 5-hexynoic acid (0.25 mL, 2.1 mmol) and amine **103** (578 mg, 1.1 mmol) in 36% yield (211 mg). Mp: 149-152 ºC. $[\alpha]_D^{20}$:+25.7 (c=0.53, DMSO). R_f: 0.30 (DCM/MeOH, 9:1).



IR (ATR, ν): 3424, 3299 (NH), 1689, 1641 (CO, CON), 1593, 1526, 1457 (Ar); ^{1}H-NMR ($(CD_3)_2SO$, δ): 1.27-1.69 (m, 6H, $(CH_2)_3CHS$), 1.77 (qt, *J*=7.2, 2H, $CH_2CH_2C\equiv$), 2.23 (td, *J*=6.9, 2.4, 2H, $CH_2C\equiv$), 2.37 (t, *J*=7.1, 2H, CH_2CO), 2.48 (t, *J*=7.5, 2H, CH_2CO), 2.58 (d, *J*=12.5, 1H, ½CH_2S), 2.79-2.85 (m, 2H, ½CH_2S, CH≡), 3.09-3.15 (m, 1H, CHS), 4.12-4.16 (m,

1H, CHN), 4.29-4.33 (m, 1H, CHN), 6.40 (br s, 1H, NH), 6.48 (br s, 1H, NH), 7.69 (d, *J*=8.7, 4H, $4CH_{Ar}CCO$), 7.76 (d, *J*=8.6, 4H, $4CH_{Ar}CN$), 10.26 (br s, 1H, NHC_{Ar}), 10.31 (br s, 1H, NHC_{Ar}); ^{13}C-NMR ($(CD_3)_2SO$, δ): 17.4 ($CH_2C\equiv$), 23.8 ($CH_2CH_2C\equiv$), 25.1, 28.2, 28.3 ($(CH_2)_3CHS$), 35.3, 36.4 ($2CH_2CO$), 39.9 (CH_2S), 55.5 (CHS), 59.3, 61.1 (2CHN), 71.8 (CH≡), 84.0 (C≡), 118.2, 118.3 ($4CH_{Ar}CN$), 131.0 ($4CH_{Ar}CCO$), 131.7, 131.8 ($2C_{Ar}N$), 143.1, 143.2 ($2C_{Ar}CO$), 162.9 (NCON), 171.3, 171.9 (2CON), 193.4 (CO); MS (ESI, *m/z*): 530.9 $[M-H]^-$.

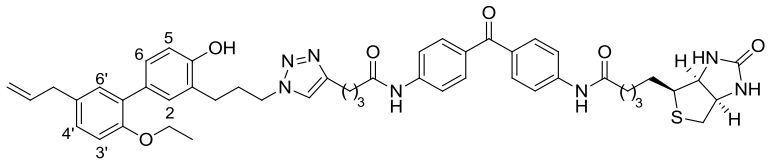
6-Azido-*N*-{[(4-biotinylamino)benzoyl]phenyl}hexanamide (106). Obtained from acid **104** (181 mg, 1.2 mmol) and amine **103** (420 mg, 0.96 mmol) in 14% yield (78 mg). Mp: 169-172 ºC. R_f: 0.23 (DCM/MeOH, 95:5).



IR (ATR, ν): 3311 (NH), 2096 (N_3), 1691, 1593 (CO), 1527, 1458, 1407 (Ar); ^{1}H-NMR (700 MHz, $(CD_3)_2SO$, δ): 1.35-1.41 (m, 4H, CH_2CH_2CHS, $CH_2(CH_2)_2CHS$), 1.48-1.67 (m, 8H, CH_2CHS, $(CH_2)_3CH_2N_3$), 2.37 (t, *J*=7.3, 4H, $2CH_2CO$), 2.58 (d, *J*=12.4, 1H, ½CH_2S), 2.82 (dd, *J*=12.4, 5.1, 1H, ½CH_2S), 3.13 (ddd, *J*=8.4, 5.9, 4.4, 1H, CHS), 3.34 (t, *J*=6.9, 2H, CH_2N_3), 4.13-4.15 (m, 1H, CHN), 4.31 (dd, *J*=7.3, 5.5, 1H, CHN), 6.37 (s, 1H, NH), 6.45 (s, 1H, NH), 7.70 (d, *J*=8.6, 4H, $4CH_{Ar}CCO$), 7.76 (d, *J*=8.7, 4H, $4CH_{Ar}CN$), 10.25 (br s, 2H, $2NHC_{Ar}$); ^{13}C-NMR (175 MHz, $(CD_3)_2SO$, δ): 24.5, 25.0, 25.8, 28.0 ($CH_2(CH_2)_2CHS$, $(CH_2)_3CH_2N_3$), 28.1, 28.2 ($(CH_2)_2CHS$), 36.4 ($2CH_2CO$), 39.9 (CH_2S), 50.5 (CH_2N_3), 55.4 (CHS), 59.2, 61.0 (2CHN), 118.2 ($4CH_{Ar}CN$), 130.9 ($4CH_{Ar}CCO$), 131.7 ($2C_{Ar}N$), 143.1 ($2C_{Ar}CO$), 162.7 (NCON), 171.7, 171.8 (2CON), 193.4 (CO); MS (ESI, *m/z*): 576.2 $[M-H]^-$.

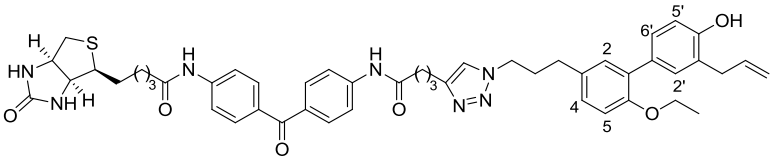
General procedure for the synthesis of probes 56-59 To a mixture of the corresponding biotinylated alkyne or azide **105** or **106** (1 equiv), $CuSO_4 \cdot 5H_2O$ (1.1 equiv) and sodium ascorbate (1.1 equiv) in water (11 mL/mmol), a solution of the corresponding honokiol-based azide or alkyne **60-63** (1 equiv) in DMF (9 mL/mmmol) was added, and the mixture was stirred at rt for 16 h. Then, EtOAc and water were added and the organic phase was separated, washed with brine, dried (Na_2SO_4), filtered and evaporated under reduced pressure. The resulting solid was triturated and washed with Et_2O to afford the corresponding pure 1,2,3-triazol.

(+)-*N*-(4-{4-[(4-{1-[3-(5'-Allyl-2'-ethoxy-4-hydroxybiphenyl-3-yl)propyl]-1*H*-1,2,3-triazol-4-yl}butanoyl)amino]benzoyl}phenyl)biotinamide (56). Obtained from alkyne **105** (24 mg, 45 μmol) and azide **60** (15 mg, 45 μmol) in 64% yield (25 mg). Mp: 198.6-199.3 ºC. $[\alpha]_D^{20}$:+25.6 (c=0.18, DMSO). R_f: 0.15 (DCM/MeOH, 9:1).



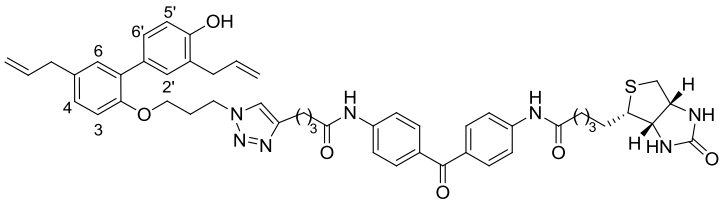
IR (ATR, ν): 3300 (OH, NH), 1676 (CO), 1592, 1524, 1474, 1456 (Ar), 1255 (COC); ^{1}H-NMR (700 MHz, $(CD_3)_2SO$, δ): 1.22 (t, *J*=6.9, 3H, CH_3), 1.34-1.43 (m, 2H, CH_2CH_2CHS), 1.48-1.54 (m, 1H, ½CH_2CHS), 1.60-1.68 (m, 3H, ½CH_2CHS, $CH_2(CH_2)_2CHS$), 1.93 (qt, *J*=7.5, 2H, CH_2CH_2C=), 2.09 (qt, *J*=7.3, 2H, $CH_2CH_2C_3$), 2.37 (td, *J*=7.4, 2.6, 2H, CH_2CO), 2.42 (t, *J*=7.5, 2H, CH_2CO), 2.55 (app t, *J*=7.5, 2H, CH_2C_3), 2.58 (d, *J*=12.6, 1H, ½CH_2S), 2.67 (t, *J*=7.5, 2H, CH_2C=), 2.83 (dd, *J*=12.5, 5.1, 1H, ½CH_2S), 3.11-3.14 (m, 1H, CHS), 3.31 (d, *J*=6.7, 2H, $CH_2C_{5'}$), 3.96 (q, *J*=6.9, 2H, CH_2O), 4.14-4.15 (m, 1H, CHN), 4.31 (dd, *J*=7.2, 5.5, 1H, CHN), 4.34 (t, *J*=7.1, 2H, CH_2N), 5.00-5.02 (m, 1H, ½CH_2=), 5.06 (ddd, *J*=17.0, 3.2, 1.4, 1H, ½CH_2=), 5.95 (ddt, *J*=17.0, 10.1, 6.9, 1H, CH=), 6.37 (br s, 1H, NH), 6.45 (br s, 1H, NH), 6.81 (d, *J*=8.2, 1H, H_5), 6.95 (d, *J*=8.4, 1H, $H_{3'}$), 7.03 (dd, *J*=8.2, 2.0, 1H, $H_{4'}$), 7.05 (d, *J*=2.0, 1H, $H_{6'}$), 7.15 (dd, *J*=8.2, 2.1, 1H, H_6), 7.23 (d, *J*=2.0, 1H, H_2), 7.69 (dd, *J*=8.7, 2.0, 4H, $4CH_{Ar}CCO$), 7.75 (dd, *J*=8.6, 2.8, 4H, $4CH_{Ar}CN$), 7.92 (s, 1H, NCH=), 9.41 (br s, 1H, OH), 10.25 (br s, 1H, NHC_{Ar}), 10.26 (br s, 1H, NHC_{Ar}); ^{13}C-NMR (175 MHz, $(CD_3)_2SO$, δ): 14.7 (CH_3), 24.6 (CH_2C=), 24.8 (CH_2CH_2C=), 25.0 ($CH_2(CH_2)_2CHS$), 26.8 (CH_2C_3), 28.1, 28.2 (($CH_2)_2CHS$), 30.0 ($CH_2CH_2C_3$), 35.9, 36.4 ($2CH_2CO$), 38.7 ($CH_2C_{5'}$), 39.9 (CH_2S), 49.1 (CH_2N), 55.4 (CHS), 59.2, 61.1 (2CHN), 63.6 (CH_2O), 112.9 ($C_{3'}$), 114.5 (C_5), 115.5 (CH_2=), 118.2 ($4CH_{Ar}CN$), 121.8 (NCH=), 126.2 (C_3), 127.6 ($C_{4'}$), 127.9 (C_6), 128.9 (C_1), 129.9 ($C_{1'}$), 130.2 ($C_{6'}$), 130.88 (C_2), 130.91 ($4CH_{Ar}CCO$), 131.7 ($2C_{Ar}N$), 131.8 ($C_{5'}$), 138.2 (CH=), 143.1 ($2C_{Ar}CO$), 146.3 (NC=), 153.7 ($C_{2'}$), 154.1 (C_4), 162.7 (NCON), 171.6, 171.8 (2CON), 193.4 (CO); HRMS (MALDI, *m/z*): calcd for $[M+H]^+$ $C_{49}H_{56}N_7O_6S$: 870.4013; found: 870.4024; HPLC-MS (ESI, *m/z*): 868.4 $[M-H]^-$; t_R (method B): 12.04 min.

(+)-*N*-(4-{4-[(4-{1-[3-(3'-Allyl-6-ethoxy-4'-hydroxybiphenyl-3-yl)propyl]-1*H*-1,2,3-triazol-4-yl}butanoyl)amino]benzoyl}phenyl)biotinamide (57). Obtained from alkyne **105** (79 mg, 0.15 mmol) and azide **61** (50 mg, 0.15 mmol) in 25% yield (33 mg). Mp: 180.9-181.7 ºC. $[\alpha]_D^{20}$:+11.8 (c=0.39, DMSO). R_f: 0.17 (DCM/MeOH, 9:1).



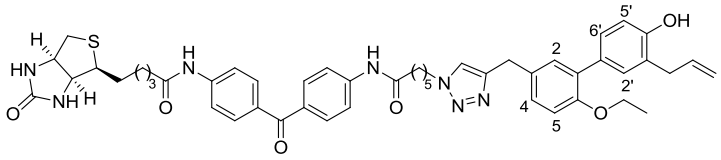
IR (ATR, ν): 3334 (OH, NH), 1685 (CO), 1596, 1456 (Ar), 1262 (COC); ^{1}H-NMR (700 MHz, $(CD_3)_2SO$, δ): 1.24 (t, *J*=6.9, 3H, CH_3), 1.24-1.41 (m, 2H, CH_2CH_2CHS), 1.48-1.54 (m, 1H, ½CH_2CHS), 1.60-1.68 (m, 3H, ½CH_2CHS, $CH_2(CH_2)_2CHS$), 1.93 (qt, *J*=7.4, 2H, CH_2CH_2C=), 2.10 (qt, *J*=7.3, 2H, $CH_2CH_2C_3$), 2.37 (td, *J*=7.3, 2.6, 2H, CH_2CO), 2.42 (t, *J*=7.4, 2H, CH_2CO), 2.50-2.53 (m, 2H, CH_2C_3), 2.58 (d, *J*=12.6, 1H, ½CH_2S), 2.67 (t, *J*=7.4, 2H, CH_2C=), 2.83 (dd, *J*=12.4, 5.1, 1H, ½CH_2S), 3.11-3.14 (m, 1H, CHS), 3.30 (d, *J*=6.7, 2H, $CH_2C_{3'}$), 3.95 (q, *J*=6.9, 2H, CH_2O), 4.13-4.15 (m, 1H, CHN), 4.30 (t, *J*=7.0, 2H, CH_2N), 4.29-4.31 (m, 1H, CHN), 4.99-5.01 (m, 1H, ½CH_2=), 5.06 (ddd, *J*=17.1, 3.2, 1.5, 1H, ½CH_2=), 5.97 (ddt, *J*=16.9, 10.1, 6.7, 1H, CH=), 6.37 (br s, 1H, NH), 6.45 (br s, 1H, NH), 6.80 (d, *J*=8.2, 1H, $H_{5'}$), 6.93 (d, *J*=8.1, 1H, H_5), 7.04-7.06 (m, 2H, H_2, H_4), 7.16 (dd, *J*=8.2, 2.0, 1H, $H_{6'}$), 7.24 (d, *J*=1.6, 1H, $H_{2'}$), 7.69 (dd, *J*=8.6, 1.7, 4H, 4$CH_{Ar}CCO$), 7.75 (dd, *J*=8.6, 3.0, 4H, 4$CH_{Ar}CN$), 7.92 (s, 1H, NCH=), 9.37 (br s, 1H, OH), 10.25 (br s, 1H, NHC_{Ar}), 10.26 (br s, 1H, NHC_{Ar}); ^{13}C-NMR (175 MHz, $(CD_3)_2SO$, δ): 14.7 (CH_3), 24.6 (CH_2C=), 24.8 (CH_2CH_2C=), 25.0 ($CH_2(CH_2)_2CHS$), 28.1, 28.2 ($(CH_2)_2CHS$), 31.1 (CH_2C_3), 31.6 ($CH_2CH_2C_3$), 33.8 ($CH_2C_{3'}$), 35.8, 36.4 (2CH_2CO), 39.9 (CH_2S), 48.7 (CH_2N), 55.4 (CHS), 59.2, 61.0 (2CHN), 63.5 (CH_2O), 112.9 (C_5), 114.4 ($C_{5'}$), 115.3 (CH_2=), 118.2 (4$CH_{Ar}CN$), 121.9 (NCH=), 125.2 ($C_{3'}$), 127.5 (C_4), 127.8 ($C_{6'}$), 128.8 ($C_{1'}$), 129.9 (C_1), 130.0 (C_2), 130.8 ($C_{2'}$), 130.9 (4$CH_{Ar}CCO$), 131.7 (2$C_{Ar}N$), 132.8 (C_3), 137.2 (CH=), 143.1 (2$C_{Ar}CO$), 146.4 (NC=), 153.6 (C_6), 153.9 ($C_{4'}$), 162.7 (NCON), 171.6, 171.8 (2CON), 193.4 (CO); HRMS (MALDI, *m/z*): calcd for $[M+H]^+$ $C_{49}H_{56}N_7O_6S$: 870.4013; found: 870.3966; HPLC-MS (ESI, *m/z*): 868.3 $[M-H]^-$; t_R (method B): 11.89 min.

***N*-[4-(4-{[4-(1-{3-[(3',5-Diallyl-4'-hydroxybiphenyl-2-yl)oxy]propyl}-1*H*-1,2,3-triazol-4-yl)butanoyl]amino}benzoyl)phenyl]biotinamide (58).** Obtained from alkyne **105** (20 mg, 37 μmol) and azide **62** (13 mg, 37 μmol) in 25% yield (8 mg). R_f: 0.13 (DCM/MeOH, 9:1).



IR (ATR, ν): 3333 (OH, NH), 1682 (CO), 1591, 1530, 1460 (Ar), 1265 (COC); ^{1}H-NMR (700 MHz, $(CD_3)_2SO$, δ): 1.35-1.42 (m, 2H, CH_2CH_2CHS), 1.49-1.53 (m, 1H, ½CH_2CHS), 1.60-1.67 (m, 3H, ½CH_2CHS, $CH_2(CH_2)_2CHS$), 1.91 (qt, *J*=7.5, 2H, CH_2CH_2C=), 2.16 (qt, *J*=6.3, 2H, CH_2CH_2O), 2.37 (td, *J*=7.3, 2.8, 2H, CH_2CO), 2.41 (t, *J*=7.6, 2H, CH_2CO), 2.58 (d, *J*=12.7, 1H, ½CH_2S), 2.65 (t, *J*=7.5, 2H, CH_2C=), 2.83 (dd, *J*=12.4, 5.1, 1H, ½CH_2S), 3.11-3.14 (m, 1H, CHS), 3.31-3.33 (m, 4H, CH_2C_5, $CH_2C_{3'}$), 3.87 (t, *J*=5.9, 2H, CH_2O), 4.13-4.15 (m, 1H, CHN), 4.31 (dd, *J*=7.5, 5.5, 1H, CHN) 4.38 (t, *J*=7.0, 2H, CH_2N), 4.94-4.95 (m, 1H, ½CH_2=$CHCH_2C_{3'}$), 5.00-5.04 (m, 2H, ½CH_2=$CHCH_2C_5$, ½CH_2=$CHCH_2C_{3'}$), 5.06 (ddd, *J*=16.9, 2.8, 1.5, 1H, ½CH_2=$CHCH_2C_5$), 5.92-5.98 (m, 2H, 2CH=), 6.37 (br s, 1H, NH), 6.45 (br s, 1H, NH), 6.84 (d, *J*=8.3, 1H, $H_{5'}$), 6.94 (d, *J*=8.2, 1H, H_3), 7.03 (dd, *J*=8.3, 2.2, 1H, H_4), 7.04 (d, *J*=2.1, 1H, H_6), 7.17 (dd, *J*=8.2, 2.1, 1H, $H_{6'}$), 7.25 (d, *J*=1.9, 1H, $H_{2'}$), 7.69 (d, *J*=8.6, 4H, $4CH_{Ar}CCO$), 7.76 (d, *J*=9.7, 4H, $4CH_{Ar}CN$), 7.77 (s, 1H, NCH=), 9.42 (br s, 1H, OH), 10.25 (br s, 1H, NHC_{Ar}), 10.26 (br s, 1H, NHC_{Ar}); ^{13}C-NMR (175 MHz, $(CD_3)_2SO$, δ): 24.6 (CH_2C=), 24.8 (CH_2CH_2C=), 25.0 ($CH_2(CH_2)_2CHS$), 28.1, 28.2 (($CH_2)_2CHS$), 29.6 (CH_2CH_2O), 33.8 ($CH_2C_{3'}$), 35.9, 36.4 ($2CH_2CO$), 38.7 (CH_2C_5), 39.9 (CH_2S), 46.3 (CH_2N), 55.4 (CHS), 59.2, 61.0 (2CHN), 64.7 (CH_2O), 112.9 (C_3), 114.5 ($C_{5'}$), 115.3, 115.5 ($2CH_2$=), 118.2 ($4CH_{Ar}CN$), 121.9 (NCH=), 125.4 ($C_{3'}$), 127.7 (C_4), 127.9 ($C_{6'}$), 128.8 ($C_{1'}$), 130.1 (C_1), 130.2 (C_6), 130.7 (C_2), 130.9 ($4CH_{Ar}CCO$), 131.7 ($2C_{Ar}N$, C_5), 137.2 ($CHCH_2C_{3'}$), 138.1 ($CHCH_2C_5$), 143.1 ($2C_{Ar}CO$), 146.4 (NC=), 153.4 (C_2), 154.0 ($C_{4'}$), 162.7 (NCON), 171.5, 171.8 (2CON), 193.4 (CO); HPLC-MS (ESI, *m/z*): 880.4 $[M-H]^-$; t_R (method B): 12.54 min.

(+)-6-(4-{[6-Ethoxy-4'-hydroxy-3'-(allyl)biphenyl-3-yl]methyl}-1*H*-1,2,3-triazol-1-yl)-*N*-biotinylamino)benzoyl]phenyl}hexanamide (59). Obtained from azide **106** (26 mg, 44 μmol) and alkyne **63** (13 mg, 44 μmol) in 49% yield (19 mg). Mp: 143-146 ºC. $[\alpha]_D^{20}$:+27.8 (c=0.23, DMSO). R_f: 0.12 (DCM/MeOH, 9:1)

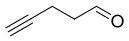


IR (ATR, ν): 3305 (OH, NH), 1688, 1593 (CO), 1525, 1459, 1405 (Ar), 1259 (COC); ^{1}H-NMR (700 MHz, $(CD_3)_2SO$, δ): 1.23 (t, *J*=6.9, 3H, CH_3), 1.25-1.28 (m, 2H, $CH_2(CH_2)_2N$), 1.35-1.46 (m, 2H, CH_2CH_2CHS), 1.48-1.53 (m, 1H, ½CH_2CHS), 1.59-1.68 (m, 5H, ½CH_2CHS, $CH_2(CH_2)_2CHS$, $CH_2(CH_2)_3N$), 1.81 (qt, *J*=7.3, 2H, CH_2CH_2N), 2.33 (t, *J*=7.4, 2H, CH_2CO), 2.35-2.38 (m, 2H, CH_2CO), 2.58 (d, *J*=12.5, 1H, ½CH_2S), 2.82 (dd, *J*=12.4, 5.1, 1H, ½CH_2S), 3.11-3.14 (m, 1H, CHS), 3.30 (d, *J*=6.6, 2H, $CH_2C_{3'}$), 3.91 (s, 2H, CH_2C_3) 3.94 (q, *J*=7.0, 2H, CH_2O), 4.13-4.15 (m, 1H, CHN), 4.28-4.32 (m, 3H, CHN, CH_2N), 5.00 (br d, *J*=9.9, 1H, ½CH_2=), 5.07 (dd, *J*=17.1, 1.3, 1H, ½CH_2=), 5.96 (ddt, *J*=17.0, 10.1, 6.8, 1H, CH=), 6.37 (br s, 1H, NH), 6.45 (br s, 1H, NH), 6.80 (d, *J*=8.2, 1H, $H_{5'}$), 6.93 (d, *J*=8.4, 1H, H_5), 7.06 (dd, *J*=8.40, 2.0, 1H, H_4), 7.10 (d, *J*=1.9, 1H, H_2), 7.12 (dd, *J*=8.3, 2.0, 1H, $H_{6'}$), 7.20 (d, *J*=1.6, 1H, $H_{2'}$), 7.69 (d, *J*=8.6, 4H, $4CH_{Ar}CCO$), 7.74 (d, *J*=8.0, 2H, $2CH_{Ar}CN$), 7.76 (d, *J*=8.4, 2H, $2CH_{Ar}CN$), 7.83 (m, 1H, NCH=), 9.39 (br s, 1H, OH), 10.23 (br s, 1H, NHC_{Ar}), 10.25 (br s, 1H, NHC_{Ar}); ^{13}C-NMR (175 MHz, $(CD_3)_2SO$, δ): 14.7 (CH_3), 24.4, 25.0, 25.5 ($CH_2(CH_2)_2CHS$, $(CH_2)_2CH_2CO$), 28.1, 28.2 ($(CH_2)_2CHS$), 29.6 (CH_2CH_2N), 30.5 (CH_2C_3), 33.8 ($CH_2C_{3'}$), 36.3, 36.4 ($2CH_2CO$), 40.0 (CH_2S), 49.0 (CH_2N), 55.4 (CHS), 59.2, 61.0 (2CHN), 63.5 (CH_2O), 112.9 (C_5), 114.4 ($C_{5'}$), 115.4 (CH_2=), 118.2 ($4CH_{Ar}CN$), 122.2 (NCH=), 125.2 ($C_{3'}$), 127.7 (C_4), 127.8 ($C_{6'}$), 128.8 ($C_{1'}$), 129.9 (C_1), 130.2 (C_2), 130.7 ($C_{2'}$), 130.9 ($4CH_{Ar}CCO$), 131.7 ($2C_{Ar}N$), 131.8 (C_3), 137.1 (CH=), 143.1 ($2C_{Ar}CO$), 146.4 (NC=), 153.7 (C_6), 153.9 ($C_{4'}$), 162.7 (NCON), 171.7, 171.8 (2CON), 193.4 (CO); HRMS (MALDI, *m/z*): calcd for $[M+H]^+$ $C_{49}H_{56}N_7O_6S$: 870.4013; found: 870.4027; HPLC-MS (ESI, *m/z*): 868.4 $[M-H]^-$; t_R (method B): 11.21 min.

6.1.6. Synthesis of clickable probes **64** and **65**

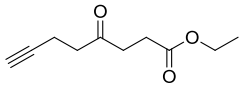
Pent-4-ynal (107). Derivative **107** was obtained following the experimental procedure previously described by Janza *et al.*,[158] and the spectroscopic data correspond with those reported.[159]

To a solution of oxalyl chloride (56 mL, 0.66 mol) in DCM (1 L) at -78 ºC, a solution of DMSO (93 mL, 1.3 mol) in DCM (80 mL) was added dropwise and the mixture was stirred at that temperature for 30 min, before a solution of 4-pentynol (50 g, 0.60 mol) in DCM (80 mL) was slowly added. The resulting mixture was stirred at -78 ºC for 1 h and then, a solution of triethylamine (330 mL, 2.4 mol) in DCM (80 mL) was slowly added. The mixture was stirred at -78 ºC for an additional 1 h and then allowed to warm to rt over 12 h. The mixture was diluted with DCM, washed with 2 M HCl (aq), saturated $NaHCO_3$ (aq) and brine, dried (Na_2SO_4), filtered, and evaporated under reduced pressure to obtain aldehyde **107** (42 g, 85%), which was used in the next step without further purification.



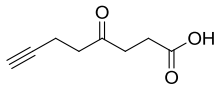
^{1}H-NMR (500 MHz, $CDCl_3$, δ): 1.99 (t, *J*=2.7, 1H, CH≡), 2.47 (td, *J*=7.1, 2.7, 2H, CH_2C≡), 2.67 (t, *J*=6.9, 2H, CH_2CHO), 9.75 (s, 1H, CHO).

Ethyl 4-oxooct-7-ynoate (108). A solution of aldehyde **107** (17.20 g, 210 mmol) and ethyl acrylate (46 mL, 420 mmol) in 1,4-dioxane (250 mL) was added dropwise over a period of 4 h to a suspension of 3-benzyl-5-(2-hydroxyethyl)-4-methyl-1,3-thiazolium chloride (7.88 g, 29 mmol) and triethylamine (20 mL, 147 mmol) in 1,4-dioxane (300 mL) at 80 ºC and under a nitrogen atmosphere. The mixture was stirred at this temperature for 54 h and then the solvents were removed under reduced pressure. The residue was resuspended in DCM (600 mL), washed with aqueous 10% H_2SO_4 (150 mL), saturated $NaHCO_3$ (aq) (250 mL) and brine (250 mL), dried (Na_2SO_4), filtered, and evaporated under reduced pressure. The crude was purified by chromatography (glass column, hexane to hexane/EtOAc, 8:2) to obtain **108** (10.70 g, 28%).



^{1}H-NMR ($CDCl_3$, δ): 1.33 (t, *J*=7.2, 3H, CH_3), 2.04 (t, *J*=2.7, 1H, CH≡), 2.54 (td, *J*=7.3, 2.6, 2H, CH_2C≡), 2.68 (t, *J*=6.5, 2H, CH_2), 2.76-2.86 (m, 4H, $2CH_2$), 4.20 (q, *J*=7.1, 2H, CH_2O); MS(ESI, *m/z*): 183.1 $[M+H]^+$.

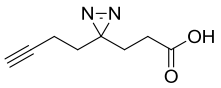
4-Oxooct-7-ynoic acid (109). To a solution of ester **108** (9.46 g, 52 mmol) in MeOH (400 mL) at rt and under a nitrogen atmosphere, LiOH (6.23 g, 260 mmol) and water (4.8 mL, 267 mmol) were added and the resulting solution was stirred at rt for 15 h. The solution was carefully acidified (pH=3) with 6 M HCl (aq). The resulting solution was extracted with DCM (2x) and the combined organic layers were dried (Na_2SO_4), filtered, and evaporated under reduced pressure to yield acid **109** (7.60 g, 95%), which was used in the next step without further purification.



^{1}H-NMR ($CDCl_3$, δ): 1.98 (t, *J*=2.5, 1H, CH≡), 2.48 (td, *J*=7.3, 2.5, 2H, CH_2C≡), 2.57-2.90 (m, 6H, $2CH_2CO$, CH_2COO); MS(ESI, *m/z*): 153.0 $[M+H]^+$.

3-[3-(But-3-yn-1-yl)-3*H*-diazirin-3-yl]propanoic acid (110). A dried round bottom flask containing **109** (3.10 g, 20 mmol) at 0 ºC and under a nitrogen atmosphere was charged with ammonia (195 mL, 7 M in MeOH) and the resulting solution was stirred at 0 ºC for 3 h. Then, a solution of hydroxylamine-*O*-sulfonic acid (3.20 g, 28 mmol) in anhydrous MeOH (25 mL) was added dropwise at 0 ºC and the mixture was stirred at this temperature for an additional 1 h and then allowed to warm to rt over 14 h. The suspension was evaporated to dryness and resuspended in MeOH (30 mL). The insoluble solid was filtered off and washed several times with MeOH. The solvent was evaporated under reduced pressure, and the residue was suspended in anhydrous MeOH (180 mL) and cooled to 0 ºC (protected from light). Diisopropylethylamine (DIPEA, 7.8 mL) was then added, followed by iodine (portion-wise), until a dark brown color persisted for more than 30 min, indicating total oxidation of the diaziridine group. The solution was then diluted with EtOAc, washed with 1 M HCl (aq), saturated $Na_2S_2O_3$ (aq, 3x) and brine. The combined aqueous phases were washed once with EtOAc and all organic layers were combined, dried (Na_2SO_4), filtered, and evaporated under reduced pressure. The residue was purified by chromatography (glass column, hexane to hexane/EtOAc, 8:2) to

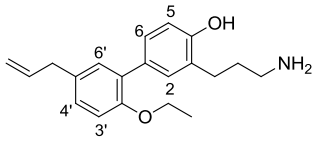
obtain diazirine **110** (889 mg, 28%). The spectroscopic data correspond with those previously reported.[160]



^{1}H-NMR ($CDCl_3$, δ): 1.66 (t, *J*=7.4, 2H, $CH_2CH_2C\equiv$), 1.81 (t, *J*=7.7, 2H, CH_2CH_2CO), 1.98-2.06 (m, 3H, CH≡, $CH_2C\equiv$), 2.18 (t, *J*=7.7, 2H, CH_2CO).

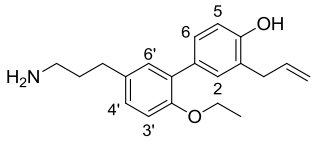
General procedure for the synthesis of amines 111 and 112. To a solution of the corresponding azide **60** or **61** (1 equiv) in anhydrous THF (7.8 mL/mmol) under a nitrogen atmosphere, PPh_3 (3 equiv) was added followed by water (10 equiv) and the resulting solution was heated under reflux for 3 h. The solvents were evaporated under reduced pressure and the residue was purified by chromatography (glass column, DCM to DCM/MeOH 8:2) to yield the corresponding amine.

5'-Allyl-3-(3-aminopropyl)-2'-ethoxybiphenyl-4-ol (111). Obtained from azide **60** (22 mg, 64 μmol) in 76% yield (15 mg). R_f: 0.16 (DCM/MeOH, 9:1).



IR (ATR, ν): 2976 (OH, NH), 1560, 1508 (Ar), 1270, 1238 (COC); ^{1}H-NMR (CD_3OD, δ): 1.31 (t, *J*=6.9, 3H, CH_3), 1.90 (br s, 2H, NH_2), 1.99 (qt, *J*=7.5, 2H, CH_2), 2.75 (t, *J*=7.2, 2H, CH_2C_3), 2.94 (app t, *J*=7.6, 2H, CH_2N), 3.34 (d, *J*=7.3, 2H, $CH_2C_{5'}$), 3.98 (q, *J*=7.0, 2H, CH_2O), 5.00-5.09 (m, 2H, CH_2=), 5.97 (ddt, *J*=16.9, 10.1, 6.7, 1H, CH=), 6.80 (d, *J*=8.3, 1H, H_5), 6.93 (d, *J*=8.9, 1H, $H_{3'}$), 7.03-7.06 (m, 2H, $H_{4'}$, $H_{6'}$), 7.21 (dd, *J*=8.3, 2.2, 1H, H_6), 7.26 (d, *J*=2.0, 1H, H_2); ^{13}C-NMR (CD_3OD, δ): 15.3 (CH_3), 28.0 (CH_2C_3), 29.3 (CH_2), 40.3, 40.4 (CH_2N, $CH_2C_{5'}$), 65.3 (CH_2O), 114.2 ($C_{3'}$), 115.4 (C_5), 115.6 (CH_2=), 127.2 (C_3), 128.9 ($C_{4'}$), 129.8 (C_6), 131.6 ($C_{6'}$, $C_{1'}$), 132.5 (C_2, C_1), 133.7 ($C_{5'}$), 139.4 (CH=), 155.4 (C_4), 155.5 ($C_{2'}$); HRMS (ESI, *m/z*): calcd for $[M+H]^+$ $C_{20}H_{26}NO_2$: 312.1964; found: 312.1958; HPLC-MS (ESI, *m/z*): 310.1 $[M-H]^-$; t_R (method A): 8.90 min.

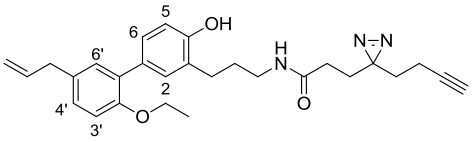
3-Allyl-5'-(3-aminopropyl)-2'-ethoxybiphenyl-4-ol (112). Obtained from azide **61** (40 mg, 0.12 mmol) in 49% yield (18 mg). R_f: 0.19 (DCM/MeOH/NH_3, 9:1:0.1).



IR (ATR, ν): 2977 (NH, OH), 1559, 1496 (Ar), 1271, 1238 (COC); ^{1}H-NMR (CD_3OD, δ): 1.30 (t, *J*=7.0, 3H, CH_3), 1.90 (br s, 2H, NH_2), 1.94 (qt, *J*=7.7, 2H, CH_2), 2.68 (t, *J*=7.6, 2H, $CH_2C_{5'}$), 2.91 (app t, *J*=7.8, 2H, CH_2N), 3.37 (d, *J*=6.5, 2H, CH_2C_3), 3.97 (q, *J*=7.0, 2H, CH_2O), 4.98-5.09 (m, 2H, CH_2=), 6.02 (ddt, *J*=16.9, 10.2, 6.6, 1H, CH=), 6.78 (d, *J*=8.2, 1H, H_5), 6.94 (d, *J*=8.2, 1H, $H_{3'}$), 7.08 (dd, *J*=8.3, 2.2, 1H, $H_{4'}$), 7.11 (d, *J*=2.1, 1H, $H_{6'}$), 7.18 (dd, *J*=8.3, 2.2, 1H, H_6), 7.24 (d, *J*=2.1, 1H, H_2); ^{13}C-NMR (CD_3OD, δ): 15.2 (CH_3), 30.9 (CH_2), 32.8 ($\underline{C}H_2C_{5'}$), 35.3 ($\underline{C}H_2C_3$), 40.3 (CH_2N), 65.4 (CH_2O), 114.4 ($C_{3'}$), 115.4 (C_5), 115.4 (CH_2=), 127.0 (C_3), 128.6 ($C_{4'}$), 129.2 (C_6), 131.2 (C_1/$C_{1'}$), 131.4 ($C_{6'}$), 132.3 (C_2), 132.6 (C_1/$C_{1'}$), 134.0 ($C_{5'}$), 138.6 (CH=), 155.2 (C_4), 155.8 ($C_{2'}$); HRMS (ESI, *m/z*): calcd for $[M+H]^+$ $C_{20}H_{26}NO_2$: 312.1964; found: 312.1958; HPLC-MS (ESI, *m/z*): 310.1 $[M-H]^-$; t_R (method A): 10.2 min.

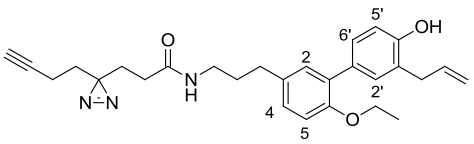
General procedure for the synthesis of final compounds 64 and 65. To a solution of acid **110** (1 equiv) in dry DCM (6.7 mL/mmol of acid), HOBt (1.2 equiv) was added under a nitrogen atmosphere and the mixture was stirred at rt for 10 min. Then, a solution of EDC (1.2 equiv) in dry DCM (6.7 mL/mmol of acid) was added and the reaction was stirred at rt for 30 min before a solution of the corresponding amine **111** or **112** (1 equiv) in dry DCM (6.7 mL/mmol of amine) was added, followed by DIPEA (2.2 equiv). The resulting mixture was stirred at rt for 16 h, diluted with EtOAc, washed with saturated $NaHCO_3$ (aq, 2x) and brine. The organic layer was dried (Na_2SO_4), filtered, and evaporated under reduced pressure. The residue was purified by chromatography using the appropriate eluent to afford the corresponding amide.

***N*-[3-(5'-Allyl-2'-ethoxy-4-hydroxybiphenyl-3-yl)propyl]-3-(3-but-3-yn-1-yl-3*H*-diaziren-3-yl)propanamide (64).** Obtained from from acid **110** (5 mg, 30 μmol) and amine **111** (9 mg, 30 μmol) in 45% yield (6 mg). Chromatography: glass column, DCM to DCM/EtOAc, 95:5. R_f: 0.07 (DCM/EtOAc, 95:5).



IR (ATR, ν): 3305, 3367 (NH, OH), 1644 (CO), 1550, 1509, 1491, 1469 (Ar), 1271, 1237 (COC); ^{1}H-NMR (700 MHz, CD_3OD, δ): 1.30 (t, *J*=7.0, 3H, CH_3), 1.60 (t, *J*=7.5, 2H, $CH_2CH_2C\equiv$), 1.73 (t, *J*=7.1, 1H, CH_2CH_2CO), 1.83 (qt, *J*=7.4, 2H, $CH_2CH_2C_3$), 1.998 (td, *J*=7.5, 2.7, 2H, $CH_2C\equiv$), 2.002 (t, *J*=7.7, 2H, CH_2CO), 2.21 (t, *J*=2.7, 1H, CH≡), 2.67 (t, *J*=7.5, 2H, CH_2C_3), 3.22 (t, *J*=7.1, 2H, CH_2N), 3.34 (d, *J*=6.7, 2H, $CH_2C_{5'}$), 3.97 (q, *J*=7.0, 2H, CH_2O), 5.01-5.03 (m, 1H, ½CH_2=), 5.06 (ddd, *J*=17.0, 3.6, 1.7, 1H, ½CH_2=), 5.97 (ddt, *J*=17.0, 10.1, 6.8, 1H, CH=), 6.76 (d, *J*=8.3, 1H, H_5), 6.91 (d, *J*=8.3, 1H, $H_{3'}$), 7.03 (dd, *J*=8.2, 2.2, 1H, $H_{4'}$), 7.06 (d, *J*=2.2, 1H, $H_{6'}$), 7.16 (dd, *J*=8.2, 2.3, 1H, H_6), 7.25 (d, *J*=2.2, 1H, H_2); ^{13}C-NMR (175 MHz, CD_3OD, δ): 13.8 ($CH_2C\equiv$), 15.3 (CH_3), 28.7 (CH_2CH_2CO), 28.8 (CN_2), 29.9 (CH_2CO), 30.6 ($CH_2CH_2C_3$), 31.1 ($CH_2CH_2C\equiv$), 33.3 (CH_2C_3), 40.3, 40.4 (CH_2N, $CH_2C_{5'}$), 65.4 (CH_2O), 70.2 (CH≡), 83.5 (C≡), 114.3 ($C_{3'}$), 115.3 (C_5), 115.6 (CH_2=), 128.4 (C_3), 128.7 ($C_{4'}$), 129.1 (C_6), 131.3 (C_1), 131.6 ($C_{6'}$), 132.3 ($C_{1'}$), 132.4 (C_2), 133.6 ($C_{5'}$), 139.3 (CH=), 155.2 (C_4), 155.4 ($C_{2'}$), 174.2 (CON); HRMS (ESI, *m/z*): calcd for $[M+Na]^+$ $C_{28}H_{33}N_3NaO_3$: 482.2420; found: 482.2414; HPLC-MS (ESI, *m/z*): 458.0 $[M-H]^-$; t_R (method A): 11.14 min.

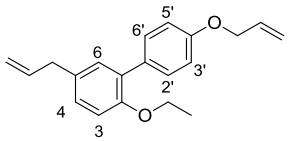
***N*-[3-(3'-Allyl-6-ethoxy-4'-hydroxybiphenyl-3-yl)propyl]-3-(3-but-3-yn-1-yl-3*H*-diaziren-3-yl)propanamide (65).** Obtained from acid **110** (5 mg, 30 μmol) and amine **112** (9 mg, 30 μmol) in 62% yield (9 mg). Chromatography: glass column, DCM to DCM/MeOH 95:5. R_f: 0.10 (DCM/EtOAc, 95:5).



IR (ATR, ν): 3293, 3078 (NH, OH), 1640 (CO), 1607, 1472, 1439 (Ar), 1269, 1235 (COC); ^{1}H-NMR (700 MHz, CD_3OD, δ): 1.29 (t, *J*=7.0, 3H, CH_3), 1.59 (t, *J*=7.5, 2H, $CH_2CH_2C{\equiv}$), 1.72 (t, *J*=7.2, 2H, CH_2CH_2CO), 1.81 (qt, *J*=7.3, 2H, $CH_2CH_2C_3$), 1.98-2.01 (m, 4H, $CH_2C{\equiv}$, CH_2CO), 2.22 (t, *J*=2.6, 1H, CH≡), 2.61 (t, *J*=7.6, 2H, CH_2C_3), 3.18 (t, *J*=7.0, 2H, CH_2N), 3.38 (d, *J*=6.6, 2H, $CH_2C_{3'}$), 3.95 (q, *J*=7.0, 2H, CH_2O), 4.99-5.01 (m, 1H, ½CH_2=), 5.06 (ddd, *J*=17.1, 3.5, 1.6, 1H, ½CH_2=), 6.02 (ddt, *J*=16.9, 10.2, 6.7, 1H, CH=), 6.78 (d, *J*=8.2, 1H, $H_{5'}$), 6.89 (d, *J*=8.3, 1H, H_5), 7.04 (dd, *J*=8.3, 2.2, 1H, H_4), 7.08 (d, *J*=2.2, 1H, H_2), 7.18 (dd, *J*=8.2, 2.2, 1H, $H_{6'}$), 7.25 (d, *J*=2.1, 1H, $H_{2'}$); ^{13}C-NMR (175 MHz, CD_3OD, δ): 13.8 ($CH_2C{\equiv}$), 15.3 (CH_3), 28.8 (CN_2), 29.8 (CH_2CH_2CO), 31.0 (CH_2CO), 32.3 ($CH_2CH_2C_3$), 33.3 ($CH_2CH_2C{\equiv}$), 33.4 (CH_2C_3), 35.2 ($CH_2C_{3'}$), 40.1 (CH_2N), 65.4 (CH_2O), 70.2 (CH≡), 83.5 (C≡), 114.3 (C_5), 115.3 ($C_{5'}$), 115.4 (CH_2=), 126.9 ($C_{3'}$), 128.5 (C_4), 129.2 ($C_{6'}$), 131.3 ($C_{1'}$), 131.4 (C_2), 132.3 ($C_{2'}$, C_1), 135.3 (C_3), 138.5 (CH=), 155.0 ($C_{4'}$), 155.3 (C_6), 174.2 (CON); HRMS (ESI, *m/z*): calcd for $[M+Na]^+$ $C_{28}H_{33}N_3NaO_3$: 482.2420; found: 482.2401; HPLC-MS (ESI, *m/z*): 458.1 $[M-H]^-$; t_R (method A): 10.71 min.

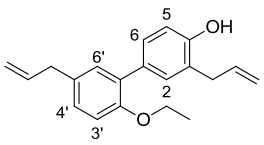
6.1.7. Synthesis of 2-*O*-ethylhonokiol

5-Allyl-4'-(allyloxy)-2-ethoxybiphenyl (113). Following the general procedure 6.1.1.2, allyl ether **113** was obtained from phenol **87** (30 mg, 0.12 mmol) and allyl bromide (13 µL, 0.14 mmol) in 92% yield (32 mg), and was used in the next step without further purification. R_f: 0.60 (hexane/EtOAc, 9:1).



^{1}H-NMR ($CDCl_3$, δ): 1.33 (t, *J*=6.9, 3H, CH_3), 3.36 (d, *J*=6.7, 2H, CH_2C_5), 4.00 (q, *J*=7.0, 2H, CH_2O), 4.58 (d, *J*=5.3, 2H, $CH_2OC_{4'}$), 5.03-5.12 (m, 2H, CH_2=$CHCH_2C_5$), 5.28-5.32 (m, 1H, ½CH_2=$CHCH_2O$), 5.41-5.47 (m, 1H, ½CH_2=$CHCH_2O$), 5.98 (ddt, *J*=16.9, 10.0, 6.7, 1H, $CHCH_2C_5$), 6.02 (ddt, *J*=17.1, 10.7, 5.4, 1H, $CHCH_2O$), 6.89 (d, *J*=8.3, 1H, H_3), 6.95 (d, *J*=9.0, 2H, $H_{3'}$, $H_{5'}$), 7.07 (dd, *J*=8.3, 2.2, 1H, H_4), 7.13 (d, *J*=2.2, 1H, H_6), 7.49 (d, *J*=8.9, 2H, $H_{2'}$, $H_{6'}$).

3,5'-Diallyl-2'-ethoxybiphenyl-4-ol (2-*O*-ethylhonokiol). Following the general procedure 6.1.1.9, 2-*O*-ethylhonokiol was obtained from allyl ether **113** (25 mg, 80 μmol) in 97% yield (24 mg). Chromatography: hexane to hexane/EtOAc, 9:1. R_f: 0.24 (hexane/EtOAc, 9:1). The spectroscopic data correspond with those reported.[126]



^{1}H-NMR ($CDCl_3$, δ): 1.33 (t, *J*=7.0, 3H, CH_3), 3.36 (d, *J*=6.8, 2H, $CH_2C_{5'}$), 3.45 (d, *J*=6.3, 2H, CH_2C_3), 4.00 (q, *J*=6.9, 2H, CH_2O), 5.09-5.12 (m, 2H, CH_2=), 5.15-5.24 (m, 2H, CH_2=), 5.98 (ddt, *J*=17.1, 10.2, 6.8, 1H, C<u>H</u>$CH_2C_{5'}$), 6.06 (ddt, *J*=17.0, 10.2, 6.6, 1H, C<u>H</u>CH_2C_3), 6.84 (d, *J*=8.8, 1H, H_5), 6.88 (d, *J*=8.2, 1H, $H_{3'}$), 7.06 (dd, *J*=8.4, 2.0, 1H, $H_{4'}$), 7.12 (d, *J*=2.1, 1H, $H_{6'}$), 7.32-7.35 (m, 2H, H_2, H_6); HRMS (ESI): calcd for $[M+Na]^+$ $C_{20}H_{22}NaO_2$: 317.1518; found: 317.1512; HPLC-MS (ESI, *m/z*): 293.1 $[M-H]^-$; t_R (method A): 11.38 min.

6.2. Biological assays

6.2.1. Binding assays

Membranes from HEK-293-EBNA cells expressing the hCB_1 or hCB_2 receptors were purchased from PerkinElmer and conserved at -80 ºC in packaging buffer for subsequent use. Competitive inhibition assays were performed according to standard procedures, briefly detailed below.

Cell membranes (8 mg/mL for CB_1 and 4 mg/mL for CB_2) were homogenized at 0 ºC using a glass dounce homogenizer in assay buffer (50 mM Tris·HCl, 2.5 mM ethylenediaminetetraacetic acid (EDTA), 5 mM $MgCl_2$ and 0.5 mg/mL bovine serum albumin (BSA), pH=7.4 for CB_1; or 50 mM Tris·HCl, 2.5 mM ethyleneglycoltetraacetic acid (EGTA), 5 mM $MgCl_2$ and 1 mg/mL fatty acid free BSA, pH=7.4 for CB_2) at 1:500 dilution. Fractions of 500 μL of the membrane suspension were incubated in 96-multiwell plates (Optiplate), which had been previously silanized with Sigmacote (Sigma) to prevent adsorption of the compounds, at 30 ºC for 90 min with [^{3}H]-CP55940 at a concentration of 0.4 nM for CB_1 and 0.53 nM for CB_2, respectively (144 Ci/mmol, PerkinElmer), in the absence or presence of different concentrations of the compound under study (ranging from 10^{-5} to 10^{-11} M), in a final volume of 550 μL. Nonspecific binding was determined by

radioligand binding in the presence of a saturating concentration of 10 μM (*R*)-(+)-WIN552122 (Tocris), and represented less than 15% of total binding.

For all binding assays, competing drug and nonspecific, total, and radioligand bindings were defined in triplicate. Incubation was terminated by rapid vacuum filtration through Wallac Filtermat A filters (PerkinElmer), presoaked in polyethylenimine (0.05%), using a FilterMate Unifilter 96-Harvester (PerkinElmer). The filters were then washed 9 times with 500 μL of ice-cold assay buffer and air-dried. Then, a MeltiLex solid scintillator sheet (PerkinElmer) was immediately melted onto the filter and the radioactivity bound to the filter was quantified by scintillation spectrometry, using a Microbeta TopCount instrument (PerkinElmer). The data were analyzed by an iterative curve-fitting procedure using GraphPad Prism program and K_i values were calculated from the IC_{50} values using the Cheng-Prusoff equation[87] and are expressed as the average and standard error obtained from two to four independent experiments carried out in triplicate.

6.2.2. Visualization of CB_1R in tonsil TMCs, pDCs and mDCs

TMCs and tonsil dendritic cell subsets (pDCs and mDCs) were purified following the protocol previously described by Palomares *et al*.[91]

For labelling experiments, TMCs or purified pDCs and mDCs from tonsils of atopic donors were suspended in phosphate buffered saline (PBS), centrifuged onto glass slides (cytospin, 200 rpm, 2 min), and fixed in 4% paraformaldehyde for 7 min. Then, cells were washed twice with PBS and treated with the endogenous biotin-blocking kit (Invitrogen) following the manufacturer's recommendations. After rinsing with PBS, cells were incubated with probe **1** at 0.5 μM and HU308 100 μM (for the visualization of CB_1 receptor) or HU210 0.5 μM (for the nonspecific fluorescence) in PBS for 60 min. Then, samples were rinsed twice with PBS and incubated with Streptavidin-Alexa Fluor 488 (1:1000, Invitrogen) in PBS for 1 h. After washing, cells were incubated with the corresponding primary anti-human antibody (CD3 [IgG_1 mouse, produced in-house], CD20 [IgG rabbit, Epitomics], CD123 [IgG_1 mouse, eBioscience], or HLA-DR-Alexa647 [IgG2b mouse, Biolegend]) or the proper isotype controls in PBS containing 4% normal goat serum for 45 min. Next, after washing, CD3-, CD20-, and CD123-binding antibodies were detected by using Alexa Fluor 546-conjugated goat anti-mouse, anti-rabbit, and

anti-mouse, respectively. Finally, cells were washed and treated with 1% paraformaldehyde and mounted with ProLong Gold antifade reagent with 4'-6-diamidino-2-phenylindole dihydrochloride (DAPI, Invitrogen) for nuclei staining. Images were acquired and analyzed by using the confocal microscope Leica DMI 4000B and the Leica TSC SPE system (Leica Microsystems GmBh). All assay and control samples were imaged under the same microscope conditions, and the images shown are representative of 2 independent experiments. There was no significant background in any of the assayed control conditions.

6.2.3. Flow cytometry analysis of PBMCs

PBMCs were isolated by Ficoll density gradient centrifugation from buffy coats of healthy donors as previously described.[91] Then, PBMCs (500000 cells/tube) were incubated with 1 μM of compound **22**, Alexa Fluor 488 alkyne (Life Technologies) or DMSO (vehicle) in PBS (200 μL) at rt for 30 min in the dark. Then, 1 mL of PBS was added and cells were isolated by centrifugation (3000 rpm, 3.5 min), resuspended in 0.01 mg/mL of propidium iodide in PBS (200 μL) and analyzed by flow cytometry. Flow cytometric analysis was performed on a Gallios flow cytometer (Beckman Coulter) and the data were analyzed by a Weasel v2.5 software.

6.2.4. Mass spectrometry profiling of ligand-binding proteins in cell membranes

- **Incubation and photocrosslinking of samples**

Membrane homogenates of HEK-293-EBNA (500 μL of 1.07 mg/mL for CB_2R) were incubated in the absence or presence of probe **39** (25 μM) in a final volume of 600 μL of incubation buffer in a 24-well polystyrene plate. After incubation at 37 ºC for 30 min, samples were irradiated at 360 nm using an UV lamp on ice for 1 h. Then, samples were transferred to microtubes (eppendorf) and 100 μL of a solution of 7% Triton X-100 in PBS were added to solubilize proteins and shaken at rt for 1 h, before 50 μL of a solution of 4% SDS in water and 50 μL of PBS were added. The samples were incubated with 100 μL of streptavidin-agarose beads (Pierce) for 12 h at 4 ºC, transferred to conical tubes and rinsed consecutively with 0.2% SDS in PBS (1x5 mL), PBS (3x5 mL), and water (3x5 mL). The beads were settled down by centrifugation (2500 rpm, 2 min) after each wash. The washed beads were suspended in 500 μL of 6 M urea in PBS and 25 μL of a 250 mM

solution of tris(2-carboxyethyl)phosphine (TCEP) in water were added and shaken at rt for 30 min. Then, 25 µL of a 400 mM iodoacetamide solution in water were added and allowed to react at rt for 30 min protected from light. Following reduction and alkylation, the beads were diluted in 800 µL of PBS, pelleted by centrifugation (4000 rpm, 3 min) and washed with PBS (3x1 mL).

- **Mass spectrometry analysis**

Proteins from pull-down samples were loaded onto SDS-PAGE and run in a unique band that concentrated all the proteins in the sample, stained with Coomassie Brilliant Blue G-250 and excised in 1 mm^3 small pieces, which were washed in ultrapure water. Samples were subjected to reduction and alkylation, and digested in-gel for 16 h at 37 ºC by adding modified porcine trypsin (Promega) at a final ratio of 1:20 (trypsin:protein). After digestion, peptides were vacuum-dried and finally dissolved in 0.1% formic acid for LC-MS/MS analysis. The tryptic peptide mixtures were subjected to nano-LC-MS for protein identification. Peptides were injected onto a C-18 reversed phase (RP) nano-column (100 mm I.D. and 12 cm, Mediterranea sea, Teknokroma) and analyzed in a continuous acetonitrile gradient consisting of 0-50% B in 90 min, 50-90% B in 1 min (B=95% acetonitrile, 0.5% acetic acid). A flow rate of 300 nL/min was used to elute peptides from the RP nano-column to an emitter nanospray needle for real time ionization and peptide fragmentation on an LTQ Orbitrap XL mass spectrometer (Thermo Fisher). An enhanced Fourier transform (FT)-resolution spectrum (resolution=30000) followed by the MS/MS spectra from most intense three parent ions (dissociated using CID activation) was analyzed along the chromatographic run (130 min). Dynamic exclusion was set at 1 min.

A second set of samples were processed as before, but the tryptic peptide mixtures were dissolved in 0.1% formic acid (gradient buffer A), injected onto a C-18 RP precolumn (Acclaim PepMap100 nanoViper Column, C18, 3 µm, 75 µm I.D. x 2 cm) and analyzed in a continuous acetonitrile gradient consisting of 8-31% B in 120 min, 31-90% B in 1 min (B=90% acetonitrile, 0.1% formic acid) using an Acclaim PepMap100, C18, 3 µm, 100Å, 75 µm i.d. x 25 cm nanocolumn. A flow rate of 200 nL/min was used to elute peptides from the RP nano-column to an emitter nanospray needle for real time ionization and peptide fragmentation on an Orbitrap Elite mass spectrometer (Thermo Fisher).

- **Database searching**

Tandem mass spectra were extracted by Proteome Discoverer v1.3 (for Orbi XL data) or Proteome Discoverer v1.4 (for Elite data) (Thermo Fisher). Charge state deconvolution and deisotoping were not performed. For protein identification, fragmentation spectra were searched against a curated subset of a human database (human_ref.fasta; 2003, April; 39414 entries) using Sequest (Thermo Fisher Scientific version 1.0.43.2) and X-Tandem S54 (The GPM, thegpm.org; version 2007.01.01.1) engines. Sequest and X-Tandem were searched allowing two missed trypsin cleavages, and a tolerance of 15 ppm or 0.8 Da was set for full MS or MS/MS spectra searches, respectively. Iodoacetamide alkylation of cysteine residues was selected as fixed modification and oxidation of methionine was allowed as variable modification. Finally, Scaffold v.3.00.02 software (Proteome Software Inc) was used to validate MS/MS based peptide and protein identifications.

6.2.5. Cell culture

MDA-MB-231 cells were grown in Dulbecco's modified Eagle medium (DMEM), while SKOV3 and OVCAR3 cells were grown in RPMI-1640 medium, both supplemented with 10% fetal bovine serum (FBS), 1% non-essential aminoacids, 1% sodium pyruvate, 100 U/mL penicillin, and 100 μg/mL streptomycin in a 5% CO_2 humidified atmosphere at 37 ^{o}C. For passage, cells were rinsed with PBS and incubated with 0.125% trypsin (0.02% EDTA solution) at 37 ^{o}C for 2 min. Detached cells were resuspended in growth medium, counted if necessary, and splitted onto fresh dishes.

6.2.6. Cytotoxicity assay

Cell viability was assessed by a colorimetric assay based on the metabolic reduction of 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) to formazan by the mitochondrial enzyme succinate dehydrogenase. MDA-MB-231, SKOV3, or OVCAR3 cells were plated in a 96-well plate (7500 cells/well) and treated with 50 μM of compounds **56-59**, **64**, and **65**, or with DMSO (vehicle-treated cells) in growth media (100 μL), in a 5% CO_2 humidified atmosphere at 37 ^{o}C for 48 h. Then, after removing the culture media, 100 μL of a premixed 2:3 solution of MTT/growth media (5 mg/mL of MTT in PBS) were added to each well, and cells were incubated at 37 ^{o}C for 4 h

protected from light. The medium was carefully removed and the precipitates of formazan were dissolved in 100 μL of DMSO and shaken mechanically for 5 min. The absorbance values were measured at a wavelength of 570 nm on a multi-well plate reader (Model Anthos Labtec 2010 1.7). Percentages of cell viability were relative to the DMSO controls.

6.2.7. In-gel analysis

For in situ labelling, cells were plated in 6 cm dishes and grown to 100% confluency, washed with PBS, and treated with the corresponding concentration of probe **64** or **65** (0.2-20 μM) in 1 mL of serum-free media. For the competition experiments, 10 μM of probe **64** or **65** and 100 μM of honokiol or 2-*O*-ethylhonokiol were used. After 30 min at 37 ºC, media was aspirated off and cells were irradiated (365 nm, 4 ºC, 10 min) using a Stratagene UV Stratalinker 1800 and scrapped in 1 mL of cold PBS (pH 7.4). Cell pellets were isolated by centrifugation (3000 rpm, 3 min), rinsed with PBS and centrifuged (3000 rpm, 3 min, 2x), lysed by probe sonication, and centrifuged (100000*g*, 45 min) to provide the soluble (supernatant) and membrane (pellet) fractions. Protein concentrations were determined using the bicinchoninic acid (BCA) protein assay (Bio-Rad) following the manufacturer's instructions, and adjusted to 1 mg/mL. Then, 50 μg of lysate were conjugated to rhodamine azide (Rh-N_3)[138] by treating with 6 μL of a pre-mixed solution containing 3 μL of 1.7 mM tris[(1-benzyl-1*H*-1,2,3-triazol-4-yl)methyl]amine (TBTA) in 4:1 DMSO:*t*-BuOH, 1 μL of 50 mM $CuSO_4$ in water, 1 μL of 50 mM TCEP in water (freshly prepared), and 1 μL of 1.25 mM Rh-N_3 in DMSO. After 1 h at rt in the dark, samples were mixed with Laemmli loading buffer and 30 μL of the click reaction mixture (20 μg of protein) were loaded into each gel lane and resolved using SDS-PAGE. Images were acquired using a Hitachi FMBIO-II flatbed fluorescence scanner. Fluorescent images are shown in gray scale.

6.2.8. Sample processing for SILAC quantitative mass spectrometry

MDA-MB-231 cells, cultured as described above, were plated in 10 cm dishes and grown to 100% confluency and washed with PBS. "Light" cells were treated with probe **65** (10 μM) and honokiol (100 μM), and "heavy" cells only with probe **65** (10 μM) in 2 mL of serum-free media. Cells were incubated with compound(s) at 37 ºC for 30 min, irradiated (365 nm, 4 ºC, 10 min), harvested, and lysed as described above. Heavy and

light proteomes were combined in equal amounts (0.75 mg of proteome each) and diluted with PBS to a final volume of 1.0 mL. The sample was treated with 110 µL of a pre-mixed solution containing 60 µL of 1.7 mM TBTA in 4:1 DMSO:*t*-BuOH, 20 µL of 50 mM $CuSO_4$ in water, 20 µL of 50 mM TCEP in water (freshly prepared) and 10 µL of 10 mM biotin-PEG_3-azide (ChemPepInc). After mixing for 1 h at rt, MeOH (2.0 mL), chloroform (0.5 mL), and PBS (1.0 mL) were added to the reaction mixture and the cloudy mixture was centrifuged at 5000 rpm for 15 min yielding a precipitated protein disc between the aqueous and organic layers. The top and bottom liquid phases were aspirated, and the protein disc was then washed with MeOH:chloroform (1:1 vol, 3x2 mL). MeOH (2 mL) was then added and the solution was sonicated to yield a cloudy mixture. Chloroform (0.5 mL) was added and the solution was centrifuged (5000 rpm, 10 min), aspirated, and the pellet was solubilized in urea (500 µL, 6 M in PBS). The solution was treated with 1:1 vol of TCEP:K_2CO_3 (50 µL, 200 mM:600 mM in PBS). The pellet was resuspended by sonication, and the resulting solution was incubated at 37 °C for 30 min. Then, iodoacetamide (70 µL, 400 mM in PBS) was added and the solution was incubated at rt for 30 min in the dark. 10% SDS in PBS was then added to each sample, followed by 5.5 mL of PBS to achieve a final concentration of 0.2% SDS. Streptavidin beads (Thermo Fisher) [100 µL slurry previously washed with PBS (3x)] were added and the mixture was rotated at rt for 2 h. Beads were washed with 0.2% SDS in PBS (5 mL), PBS (2x5 mL), and water (2x5 mL) before transferring to low-bind eppendorf tubes and centrifuged (1000 rpm, 2 min) into a pellet. Following aspiration of the supernatant, the beads were resuspended in 200 µL of urea (2 M in PBS) supplemented with $CaCl_2$ (1.0 mM final concentration) and sequencing grade porcine trypsin (Promega). Following overnight digestion at 37 °C, the tryptic digest solution (supernatant) was removed from the beads following centrifugation (1000 rpm, 2 min) and acidified with 5% formic acid. The tryptic digests were stored at -20 °C until analyzed by LC-MS/MS.

- **Mass spectrometry and data analysis**

Mass spectrometry was performed using a Thermo Orbitrap Velos mass spectrometer, as previously described.[141] Peptides were eluted using a five-step multidimensional LC-MS (MudPIT[161]) protocol (using 0%, 25%, 50%, 80% and 100% salt bumps of 500 mM aqueous NH_4OAc, followed by an increasing gradient of aqueous acetonitrile and 0.1% formic acid in each step), and data were collected in data-

dependent acquisition mode –two MS1 microscans (400–1800 mass to charge ratio [m/z]) and 30 data dependent fragmentation (MS2) scans– with dynamic exclusion enabled (repeat count of 1, exclusion duration of 20 s) with monoisotopic precursor selection enabled. All other parameters were left at default values. Prolucid searches allowed for variable oxidation of methionine (+15.9949 m/z), static modification of cysteine residues (+57.0215 m/z; iodoacetamide alkylation) and accepted only half or fully tryptic peptides. Each data set was independently searched with light and heavy parameter files; for the light search, all other amino acids were left at default masses; for the heavy search, static modifications on lysine (+8.0142 m/z) and arginine (+10.0082 m/z) were specified. The precursor-ion mass tolerance was set to 50 ppm and the fragment-ion mass tolerance was the default assignment of 0. The data were searched using the human reverse-concatenated nonredundant (gene-centric) FASTA database that assembled from the Uniprot database (www.uniprot.org). The resulting matched MS2 spectra were assembled into protein identifications, then filtered using DTASelect (version 2.0.47), and only half-tryptic or fully tryptic peptides were accepted for identification, and only fully-tryptic peptides were considered for quantification. Peptides were restricted to a specified false positive rate of 1%. Redundant peptide identifications common between multiple proteins were allowed, but the database was restricted to a single consensus splice variant. SILAC ratios were quantified using in-house software as described (CIMAGE[162]). Briefly, extracted MS1 ion chromatograms (±10 ppm) from both "light" and "heavy" targeted peptide masses (m/z) were generated using a retention time window (±10 min) centered on the time when the peptide ion was selected for MS/MS fragmentation, and subsequently identified. Next, the ratio of the peak areas under the light and heavy signals (signal-to-noise ratio>2.5) are calculated. Computational filters used to ensure that the correct peak-pair is used for quantification include a co-elution correlation score filter ($R^2 \geq 0.8$), removing target peptides with bad co-elution profile, and an "envelope correlation score" filter ($R^2 > 0.8$) that eliminates target peptides whose predicted pattern of the isotopic envelope distribution does not match the experimentally observed high-resolution MS1 spectrum. Also peptides detected as singletons, where only the heavy or light isotopically labelled peptide was detected and sequenced –but which passed all other filtering parameters– were given the maximum standard SILAC ratio of 20.

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7. REFERENCES

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SUPPLEMENTARY INFORMATION

8. SUPPLEMENTARY INFORMATION

8.1. Coomassie brilliant blue stained gels

In order to confirm that the amount of protein is the same in all comparable lanes, the gels shown in the results and discussion section of this work were stained with coomassie brilliant blue (0.1% in water/MeOH/AcOH, 4:5:1 vol) at rt for 16 h, and destained with the corresponding destaining solution (water/MeOH/AcOH, 5:4:1 vol) at rt for 2 h. Imaging was achieved using a B446-LI-COR Odyssey Infrared Imaging System. Staining is shown in red scale.

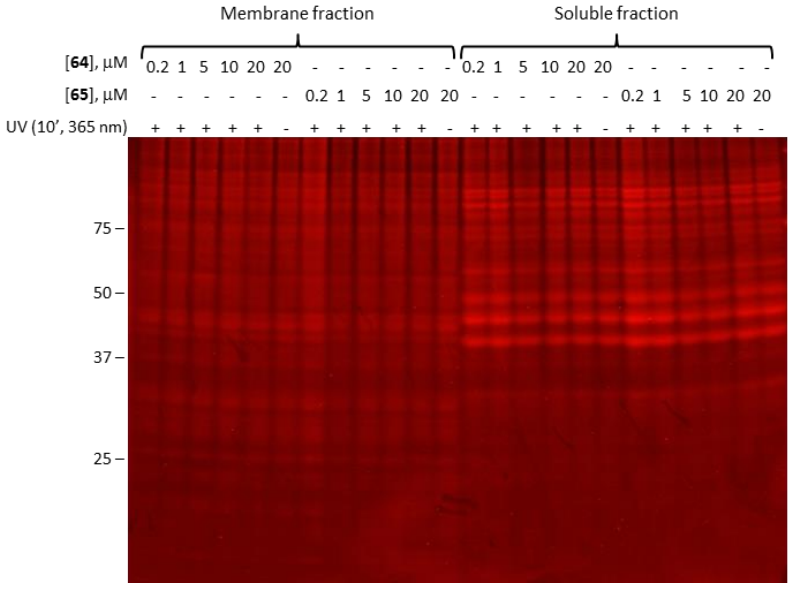

Figure S1. Coomassie staining of gel in Figure 26.

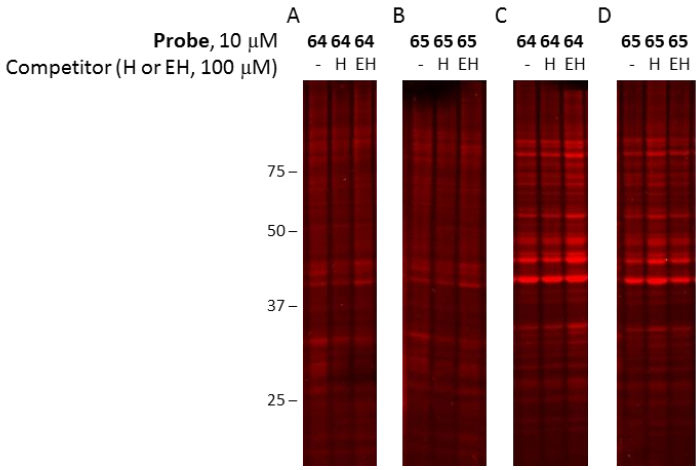

Figure S2. Coomassie staining of gel in Figure 27.

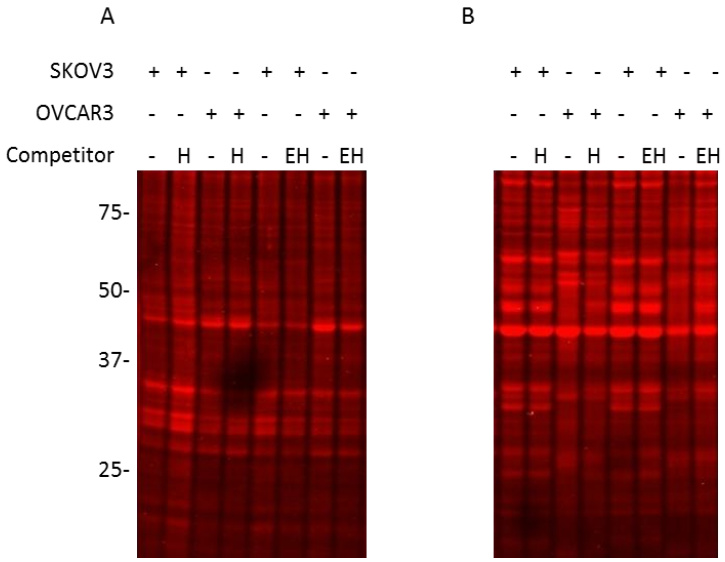

Figure S3. Coomassie staining of gel in Figure 28.