Doctoral Thesis

Chemoselective Amide Bond-Forming Reactions with Acylboron Compounds

Author(s):
Osuna Gaílvez, Alberto

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Chemoselective Amide Bond-Forming Reactions with Acylboron Compounds

A thesis submitted to attain the degree of
DOCTOR OF SCIENCES of ETH ZURICH
(Dr. sc. ETH Zurich)

presented by

Alberto Osuna Gálvez

Master of Science in Chemistry, Kyoto University
Born on 12.06.1987
Citizen of Spain

Accepted on the recommendation of
Prof. Dr. Jeffrey W. Bode, examiner
Prof. Dr. Helma Wennemers, co-examiner

2019
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Abstract

Amides constitute the core linkages of many industrially relevant polymers including Nylon®, and biomolecules such as proteins and peptides. The past 20 years have seen a considerable effort in the development of faster and more selective amide bond-forming synthetic processes. Among the many relevant breakthroughs in this field, the potential of acylboron compounds as acyl donors in amide bond-forming reactions has set this class of compounds in the spotlight of intensive research. Synthetic efforts from many different research groups have facilitated access to three main bench-stable acylboron categories applicable to the synthesis of amides: 1) potassium acyl trifluoroborates (KATs), 2) MIDA acylboronates, and 3) monofluoro acylboronates.

Acylborons can react as acyl donor compounds with suitable reactive amine surrogates, including hydroxylamines bearing an appropriate electron-withdrawing group on the O atom. Optimization efforts from our group have provided access to O-carbamoyl hydroxylamines as the reagents of choice, with an acceptable balance between reactivity and stability.

This dissertation begins with the examination of methods to enable the direct use of amines as reaction partners in the amide-forming reaction with KAT reagents. Our investigations identified in situ-formed N-chloramines from primary amines to react rapidly and selectively with KAT reagents to form the corresponding amides under acidic aqueous conditions. Among the functional groups screened, alcohols, aldehydes, carboxylic acids and even secondary amines were well tolerated. Primary N-chloroamides could be acylated with KATs to give imides in moderate to good yields. The reaction was applied to the late-stage labeling of antibiotic natural products and bovine serum albumin.
This protocol was further implemented in the preparation of hydrogels from the cross-linking of multi-arm PEG-KATs with multifunctional primary amines. The cross-linking event took place within minutes under mildly acidic media, and was completely suppressed at extremely acidic or near-neutral values of pH.

Having made a contribution to the amide bond-forming reactivity profile of KAT reagents, we turned our attention to further applications of monofluoro acylboronates previously developed in our group. Analogously to KATs, these acylborons undergo fast amide-forming ligation with O-carbamoylhydroxylamines without any extra reagent or catalyst. As the reaction occurs with concurrent C–B and N–O bond cleavage, we exploited this feature to develop a traceless template-assisted amide ligation platform by decorating the cleavable portions of the reactants with templating moieties to promote the reaction by proximity. As the templating system of choice, we used the streptavidin-desthiobiotin pair. We designed starting materials containing desthiobiotin ligands attached to their cleavable parts – the O-carbamoyl and the B-coordinating ligand – that undergo fast binding to externally added streptavidin. This binding event locates the reagents in spatial proximity to each other, which increases the local concentration and promotes the reaction. We could observe fast and traceless amide formation with conversion levels above 80% at 5 μM concentration of the hydroxylamine with just 2 equivalents of the acylboronate. The reaction kinetics were thoroughly studied for a variety of reaction conditions. We proposed a mathematical model to rationalize the conversion levels observed over time.
Zusammenfassung


Des Weiteren wurde die Reaktion für die Herstellung von Hydrogelen mittels Quervernetzung von mehrarmigen PEG-KATs und mehrfunktionalen primären Aminen angewandt. Während die Quervernetzung im schwach sauren Medium innerhalb weniger Minuten einsetzt, wird sie bei extrem saurem oder annähernd neutralem pH fast vollständig unterdrückt.

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## List of Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Name</th>
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<tbody>
<tr>
<td>$[\alpha]_D$</td>
<td>specific optical rotation at the wavelength of sodium D line</td>
</tr>
<tr>
<td>$\delta$</td>
<td>chemical shift</td>
</tr>
<tr>
<td>$\varepsilon$</td>
<td>extinction coefficient</td>
</tr>
<tr>
<td>$\mu$</td>
<td>micro</td>
</tr>
<tr>
<td>$\nu$</td>
<td>wavenumber</td>
</tr>
<tr>
<td>Ac</td>
<td>acetyl</td>
</tr>
<tr>
<td>aq</td>
<td>aqueous</td>
</tr>
<tr>
<td>Boc</td>
<td>tert-Butoxycarbonyl</td>
</tr>
<tr>
<td>br</td>
<td>broad</td>
</tr>
<tr>
<td>BSA</td>
<td>bovine serum albumin</td>
</tr>
<tr>
<td>Bu</td>
<td>butyl</td>
</tr>
<tr>
<td>Bz</td>
<td>benzoyl</td>
</tr>
<tr>
<td>Cbz</td>
<td>carbobenzoxy</td>
</tr>
<tr>
<td>d</td>
<td>doublet</td>
</tr>
<tr>
<td>DCH</td>
<td>1,3-dichloro-5,5-dimethylhydantoin</td>
</tr>
<tr>
<td>DIPEA</td>
<td>diisopropylethylamine</td>
</tr>
<tr>
<td>DMF</td>
<td>$N,N$-dimethylformamide</td>
</tr>
<tr>
<td>DMAP</td>
<td>$N,N$-dimethylaminopyridine</td>
</tr>
<tr>
<td>DMP</td>
<td>Dess-Martin periodinane</td>
</tr>
<tr>
<td>DMSO</td>
<td>dimethylsulfoxide</td>
</tr>
<tr>
<td>dr</td>
<td>diastereomeric ratio</td>
</tr>
<tr>
<td>$E$</td>
<td>entgegen (olefin geometry)</td>
</tr>
<tr>
<td>EDCI</td>
<td>1-ethyl-3-(3-dimethylaminopropyl) carbodiimide</td>
</tr>
<tr>
<td>ee</td>
<td>enantiomeric excess</td>
</tr>
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<td>------</td>
</tr>
<tr>
<td>equiv</td>
<td>equivalent</td>
</tr>
<tr>
<td>ESI</td>
<td>electrospray ionization</td>
</tr>
<tr>
<td>Et</td>
<td>ethyl</td>
</tr>
<tr>
<td>FPLC</td>
<td>fast protein liquid chromatography</td>
</tr>
<tr>
<td>g</td>
<td>gram</td>
</tr>
<tr>
<td>h</td>
<td>hour</td>
</tr>
<tr>
<td>HATU</td>
<td>1-[bis(dimethylamino)methylene-1H-1,2,3-triazolo[4,5-b]pyridinium-3-oxide hexafluorophosphate</td>
</tr>
<tr>
<td>HPLC</td>
<td>high performance liquid chromatography</td>
</tr>
<tr>
<td>Hz</td>
<td>hertz</td>
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<tr>
<td>i</td>
<td>iso</td>
</tr>
<tr>
<td>IR</td>
<td>infrared spectroscopy</td>
</tr>
<tr>
<td>J</td>
<td>coupling constant</td>
</tr>
<tr>
<td>KAHA</td>
<td>α-ketoacid-hydroxylamine</td>
</tr>
<tr>
<td>KAT</td>
<td>potassium acyltrifluoroborate</td>
</tr>
<tr>
<td>L</td>
<td>liter</td>
</tr>
<tr>
<td>LC</td>
<td>liquid chromatography</td>
</tr>
<tr>
<td>LED</td>
<td>light-emitting diode</td>
</tr>
<tr>
<td>M</td>
<td>molar</td>
</tr>
<tr>
<td>m</td>
<td>milli, multiplet</td>
</tr>
<tr>
<td>MALDI</td>
<td>matrix-assisted laser desorption/ionization</td>
</tr>
<tr>
<td>Me</td>
<td>methyl</td>
</tr>
<tr>
<td>MHz</td>
<td>megahertz</td>
</tr>
<tr>
<td>MIDA</td>
<td>methyliminodiacetic acid</td>
</tr>
<tr>
<td>min</td>
<td>minute</td>
</tr>
<tr>
<td>mmol</td>
<td>millimole</td>
</tr>
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<td>Abbreviation</td>
<td>Name</td>
</tr>
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<td>--------------</td>
<td>-------------------------------------------</td>
</tr>
<tr>
<td>mol%</td>
<td>mole percent</td>
</tr>
<tr>
<td>MOPS</td>
<td>3-(N-morpholino)propanesulfonic acid</td>
</tr>
<tr>
<td>m.p.</td>
<td>melting point</td>
</tr>
<tr>
<td>MS</td>
<td>mass spectrometry</td>
</tr>
<tr>
<td>Ms</td>
<td>methanesulfonyl</td>
</tr>
<tr>
<td>m/z</td>
<td>mass to charge ratio</td>
</tr>
<tr>
<td>nBuLi</td>
<td>n-butyllithium</td>
</tr>
<tr>
<td>NCL</td>
<td>native chemical ligation</td>
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<tr>
<td>NCS</td>
<td>N-chlorosuccinimide</td>
</tr>
<tr>
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<td>not detected</td>
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<tr>
<td>NMR</td>
<td>nuclear magnetic resonance</td>
</tr>
<tr>
<td>p</td>
<td>pentet, pico</td>
</tr>
<tr>
<td>PAGE</td>
<td>poly(acrylamide) gel electrophoresis</td>
</tr>
<tr>
<td>PBS</td>
<td>phosphate-buffered saline</td>
</tr>
<tr>
<td>PEG</td>
<td>poly(ethyleneglycol)</td>
</tr>
<tr>
<td>Ph</td>
<td>phenyl</td>
</tr>
<tr>
<td>pin</td>
<td>pinacol</td>
</tr>
<tr>
<td>Pr</td>
<td>propyl</td>
</tr>
<tr>
<td>R</td>
<td>general substituent</td>
</tr>
<tr>
<td>R (configuration)</td>
<td>rectus</td>
</tr>
<tr>
<td>rt</td>
<td>room temperature</td>
</tr>
<tr>
<td>S (configuration)</td>
<td>sinister</td>
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<tr>
<td>s</td>
<td>singlet</td>
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<tr>
<td>sat.</td>
<td>saturated</td>
</tr>
<tr>
<td>SDS</td>
<td>sodium dodecyl sulfate</td>
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<td>Abbreviation</td>
<td>Name</td>
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<td>--------------</td>
<td>----------------------------------------------------------------------</td>
</tr>
<tr>
<td>t</td>
<td>triplet, time</td>
</tr>
<tr>
<td>t, tert</td>
<td>tertiary</td>
</tr>
<tr>
<td>T</td>
<td>temperature</td>
</tr>
<tr>
<td>TBAF</td>
<td>tetrabutylammonium fluoride</td>
</tr>
<tr>
<td>TBTA</td>
<td>tris((1-benzyl-1H-1,2,3-triazol-4-yl)methyl)amine</td>
</tr>
<tr>
<td>TCCA</td>
<td>trichloroisocyanuric acid</td>
</tr>
<tr>
<td>TCEP</td>
<td>tris(2-carboxyethyl)phosphine</td>
</tr>
<tr>
<td>TFA</td>
<td>trifluoroacetic acid</td>
</tr>
<tr>
<td>THF</td>
<td>tetrahydrofuran</td>
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<tr>
<td>TLC</td>
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<tr>
<td>TMS</td>
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<td>TON</td>
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<tr>
<td>Tris</td>
<td>tris(hydroxymethyl)aminomethane</td>
</tr>
<tr>
<td>UV</td>
<td>ultraviolet</td>
</tr>
<tr>
<td>Z</td>
<td>zusammen (configuration)</td>
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Introduction to Acylborons for Amide Bond Formation
Chapter 1. Introduction to Acylborons for Amide Bond Formation

1.1 Opening remarks

Despite being an intriguing class of organic compounds, acylborons remained synthetically inaccessible for many years, presumably due to their high reactivity and tendency to rearrange.\(^1\) This instability arises from the empty p-orbital present on the tricoordinated boron atoms (Figure 1.1, left-side structure). Nozaki and co-workers pioneered the preparation of the first fully characterised acylboron example; its stability presumably arises from the high steric encumbrance of the coordinating ligand, which shields the empty p-orbital on boron (Figure 1.1, centre structure).\(^2\)

![Figure 1.1. Stability considerations of acylboron compounds having tri- and tetracoordinated boron atoms. Dipp = 2,6-iPr-C\(_6\)H\(_3\).](image)

From this landmark achievement, chemists sought to synthesize acylborons having a tetracoordinated, negatively charged boron to prevent instability issues derived from the empty p-orbital (Figure 1.1, right-side structure). The synthesis of these acylborons, however, stands out as a relatively complex task. From a retrosynthetic perspective, tetracoordinated acylboron compounds can be disconnected to a tricoordinated boron and an acyl anion synthon; nevertheless, borylation at the oxygen takes place preferentially over C-borylation due to the well-documented oxophilicity of boron.\(^3\) Over the past ten years, several strategies were adopted to overcome these synthetic challenges, enabling access to several bench-stable acylboron structures, including potassium acyltrifluoroborates (KATs) and MIDA acylboronates. Since then, acylborons have been demonstrated to be versatile building blocks to access previously inaccessible borylated structures including heterocycles. More importantly, their potential as acyl donors for chemoselective amide ligation has raised considerable interest from the scientific community.\(^4\)

This introduction focuses on acylboron species applicable as acyl donor reagents for amide synthesis. These can be summarized in three main categories according to the groups coordinating the boron atom: 1) potassium acyltrifluoroborates (KATs); 2) MIDA acylboronates; and 3) monofluoro acylboronates (Figure 1.2).
1.2 Potassium acyltrifluoroborates (KATs)

1.2.1 Synthesis

Potassium acyltrifluoroborates (KATs) are potassium salts of acyltrifluoroborate anions, where the boron atom is attached to an acyl residue and three fluorine atoms. KATs display remarkable bench stability, a feature that arises from the low lability of the coordinating fluoride anions that prevent the KAT from adopting a tricoordinated, less stable form. In 2010, Molander and co-workers disclosed the synthesis of the first KAT (compound 1-1), by generating the corresponding acyl anion equivalent followed by trapping with B(OiPr)₃ (Figure 1.3). Unfortunately, this method could not be extended to other substrates, so 1-1 remained the only accessible KAT.

Following an analogous strategy, the Bode group reported in 2012 the deprotonation and subsequent borylation of benzotriazole N,O-acetals (Figure 1.4). This route provided the first access to a variety of KATs bearing functional groups such as olefins, electron-poor aromatics and TMS-protected alkynes, albeit the reaction yields remained relatively low. In addition, very low temperatures (−110 °C) were necessary to avoid side reactions from the generated benzotriazole N,O-acetal anion. In subsequent optimization efforts, our group extended the scope to the synthesis of bifunctional KAT reagents having a fluorine, aldehyde, alcohol or alkyne group.
To avoid the synthetic inconveniences of the benzotriazole route, our group developed reagent 1-14, which enables the one-pot transfer of the acyltrifluoroborate functionality to aryl lithium species (Figure 1.5). The use of 1-14 considerably simplified the synthetic protocol and expanded the substrate scope to electron-rich aromatic and heteroaromatic substrates. Functional groups such as esters, nitro groups, nitriles or halides were tolerated and afforded the corresponding KAT in moderate to good yields.

KATs are chemically stable towards a number of reagents and reaction conditions. Our group has demonstrated their full tolerance to copper-catalyzed and strain-promoted 1,3-dipolar cycloadditions, nucleophilic substitutions, ester hydrolysis, TFA-mediated Rink Amide cleavage, Wittig reaction or thia-Michael reaction conditions.
The synthetic methods summarized above greatly expanded the substrate scope and granted access to previously inaccessible KAT reagents. A major drawback, however, arose when our group attempted to prepare aliphatic KATs from regent 1-14. In all cases, the alkyl-lithium reagent underwent double addition to 1-14 to afford the corresponding α-aminotrifluoroborates (double addition products) instead of KATs. To circumvent this, our group developed a protocol to generate KATs by the addition of aliphatic organocuprates to 1-14.\textsuperscript{11} By employing this procedure, the first tertiary KAT could be accessed in high yields (Figure 1.6).
Campos and Aldridge reported in 2015 the synthesis of tetrabutylammonium benzoyltrifluoroborate 1-31 from the hydrolysis of the corresponding acylborane 1-30 prepared by the reaction of benzyol chloride with borylzinc species 1-29 (Figure 1.7). However, a major drawback resides in accessing 1-29 from the corresponding bromoborane in a two-step procedure that requires inert atmosphere and very low temperatures. Despite the broad functional group tolerance observed in the formation of acylborons analogous to 1-30, the group only reported the transformation of this single example to the acyltrifluoroborate.

![Figure 1.7. Preparation of acyltrifluoroborate 1-31 from the reaction of nucleophilic boron species 1-29 and benzoyl chloride.](image)

Recently, Ito and co-workers disclosed the preparation of KATs by the chemoselective oxidation of $\alpha$-hydroxytrifluoroborates, compounds accessible through the Cu-catalyzed borylation of aldehydes (Figure 1.8). Their mild protocol enabled the synthesis of previously inaccessible scaffolds, including the first examples of enantiomerically enriched $\alpha$-amino acyltrifluoroborates. Epimerization of the $\alpha$-carbon was not observed under the reaction conditions.

![Figure 1.8. Chemoselective oxidation of $\alpha$-hydroxytrifluoroborates to KATs, including the first accessible $\alpha$-amino acyltrifluoroborates.](image)
1.2.2 Applications of KATs in amide synthesis

Along with their seminal preparation of the first KAT reagent, Molander et al. demonstrated the HBF₄⁻-promoted reaction with organic azides to form the corresponding amides (Figure 1.9).

Despite the relatively narrow substrate scope, this discovery constitutes the starting point for the synthetic applicability of KAT reagents for amide bond formation.

![Figure 1.9. HBF₄⁻-promoted amide-forming reaction of potassium acyltrifluoroborates with azides.]

The authors proposed a reaction mechanism involving initial Lewis acid-mediated defluorination of the KAT to generate acyldifluoroborinate I. Subsequent attack of the azide to I produces adduct II, which undergoes 1,2-acyl migration with elimination of N₂ gas to afford N-borylated intermediate III. Finally, aqueous workup liberates the amide product (Figure 1.10).

![Figure 1.10. Proposed mechanism for the reaction of azides with KAT reagents.]

The reaction of azides with KATs was implemented by the Bode group in the preparation of a [2]rotaxane that could be further elaborated into a series of lasso peptide structures. The strategy relied on the fast kinetics and chemoselectivity of the procedure to successfully cap the transient, reversible [2]rotaxane intermediate between azide 1-40 and crown ether 1-39 with KAT 1-10 (Figure 1.11).

![Figure 1.11. Amide-forming reaction of KAT 1-10 with transient [2]rotaxane from crown ether 1-39 and azide 1-40 for the preparation of lasso peptides.]
Our group disclosed in 2012 the fast amide-forming reaction of KATs with O-benzoylhydroxylamines as amine surrogates (Figure 1.12a). The choice of hydroxylamine was based on the need for a suitable leaving group on the N atom, as O-alkylhydroxylamines do not react with KATs under the same reaction conditions. The so-called KAT ligation happens in the absence of any other reagent or catalyst and with exquisite chemoselectivity. Competition experiments between KAT 1-49 and α-ketoacid 1-50 with hydroxylamine 1-51 showcased that KATs react at least 50 times faster than their α-ketoacid counterparts (Figure 1.12b).

Contrary to the reaction of azides and KATs shown above, the reaction on Figure 1.11 was hypothesized to follow a fundamentally different mechanism pathway. Instead of initial attack to the boron atom followed by 1,2-acyl migration, attack of the hydroxylamine to the carbonyl of the KAT was proposed as the key step of the mechanism (Figure 1.13, intermediate I). Subsequent extrusion of BF₃ with concurrent elimination of the benzoate group affords intermediate II that undergoes tautomerization to the amide product.
Figure 1.13. Mechanistic proposal for the reaction of KATs with \( \text{O-benzoylhydroxylamines} \).

Despite the fast and chemoselective ligation that \( \text{O-benzoylhydroxylamines} \) undergo with KATs, these hydroxylamines suffer from poor stability in the presence of amines. In search of reactants suitable to be used in peptide or protein bioconjugations with KAT reagents, our group screened a number of different hydroxylamines and selected \( \text{O-carbamoylhydroxylamines} \) as the most stable candidates while retaining optimal reactivity. The viability of these hydroxylamines was demonstrated by the preparation of unprotected GLP-1 peptide analogue 1-54 containing a diethylcarbamoylhydroxyamine moiety. Compound 1-54 reacted chemoselectively with KAT derivatives 1-55 to 1-57 bearing bioconjugation-relevant groups – such as PEG chains, biotin or dyes – even in the presence of unprotected arginine, histidine, aspartate or tryptophan chains, and the N-terminal amine (Figure 1.14). The ligations were completed within minutes at room temperature by just employing equimolar amounts of starting materials.\(^7\)

Figure 1.14. Chemoselective bioconjugation of carbamoylhydroxylamine-equipped GLP-1 peptide analogue 1-54 with KATs 1-55 to 1-57.

The ligation methodology was further applied to the bioconjugation of synthetic insulin by preparing photoprotected \( \text{O-carbamoylhydroxylamine-containing insulin derivative} \) 1-58. This enabled the mild deprotection of the reactive hydroxylamine by irradiation, leaving the protein intact. Hydroxylamine-equipped insulin could be subjected to late-stage PEGylation, lipidation or dye functionalization using the corresponding KAT reagents (Figure 1.15).\(^{17}\)
Figure 1.15. Chemoselective labelling of synthetic insulin equipped with a photoprotected O-carbamoylhydroxylamine. The red sphere accounts for rhodamine, myristyl or PEG groups.

In later studies, our group evaluated the influence that sterics surrounding the carbonyl atom have in KAT reactivity.\textsuperscript{11} Competition experiments between equimolar amounts of O-carbamoylhydroxylamine 1-59 with linear aliphatic KAT 1-60 and secondary KAT 1-61 resulted in a 2:1 mixture of linear and branched amides 1-62 and 1-63 (Figure 1.16a). Furthermore, an analogous experiment between 1-60 and tertiary KAT 1-25 produced the less hindered amide 1-62 in 99% yield with only traces of the sterically congested amide 1-64 (Figure 1.16b).

Intrigued by the remarkably high reaction rates displayed by the KAT ligation, the Bode group studied the kinetics of the most relevant bioconjugation reactions.\textsuperscript{18} The ligation of KATs with O-acylhydroxylamines was shown to proceed with a rate constant of 22 M\textsuperscript{-1} s\textsuperscript{-1} at pH 1-2 – far above
the rate of NCL, $0.26 \text{ M}^{-1} \text{s}^{-1}$. However, the reaction rate dramatically drops as the pH increases, becoming $0.39 \text{ M}^{-1} \text{s}^{-1}$ at pH 5.1 and lower than $0.01 \text{ M}^{-1} \text{s}^{-1}$ at pH 7.0.

During the course of investigations on faster KAT ligation under near-neutral pH, our group identified potassium 2-picolinoyltrifluoroborate to react 10-fold faster compared to the benzoyl trifluoroborate counterparts. This feature was exploited in the PEGylation of super-folded GFP (sfGFP) containing a O-carbamoylhydroxylamine (1-65) group with PEG derivatives 1-66 – 1-69 containing a 2-picolinoyltrifluoroborate moiety (Figure 1.17). Protein PEGylation happened with just a slight excess of the PEG-containing reagents in >90% conversion after 5 h of reaction.\textsuperscript{19}

![Figure 1.17. PEGylation of sfGFP 1-65 with PEG-labelled potassium 2-picolinyltrifluoroborates 1-66–1-69.](image)

In addition to applications in bioconjugation, the Bode group prepared an amide-crosslinked hydrogel from the reaction of four-arm-PEG materials 1-70 and 1-71, equipped with KAT and O-carbamoylhydroxylamines respectively (Figure 1.18).\textsuperscript{20} The gel formation rate was shown to exhibit pH dependence, being considerably slower at pH values close to neutrality. The biocompatibility of the KAT ligation was demonstrated by the encapsulation of bovine chondrocytes, which remained alive for 2 days inside the hydrogel.

![Figure 1.18. Preparation of amide-crosslinked hydrogels over a broad pH range from PEG-KAT 1-70 and PEG-hydroxylamine 1-71.](image)

Collaborative efforts from Johnson and Bode made use of the KAT ligation for the preparation of polymers with templated network topology in a traceless manner by installing templating moieties
onto the leaving groups on the hydroxylamine reactant. Topologically different polymers were prepared by crosslinking PEG-KAT 1-72 with either O-diethylcarbamoylhydroxylamine-PEG 1-73 – which does not undergo self-assembly – or O-dioctylcarbamoylhydroxylamine-PEG 1-74 – which self-assembles via hydrophobic interactions (Figure 1.19). By using either hydrogen- or deuterium-labelled starting materials, the specific topologies could be probed by selective network disassembly and mass analysis of the degradation products.21

![Figure 1.19. Preparation of topologically isomeric networks of PEG-KAT 1-72 with modular PEG-hydroxylamines 1-73 and 1-74 equipped with traceless templating leaving groups.](image)

1.3 MIDA boronates

1.3.1 Synthesis

The synthesis of the first N-methyliminodiacetyl (MIDA) acylboronates, pioneered by Yudin and co-workers, was achieved by the mild oxidation of MIDA α-hydroxyboronates (Figure 1.20).22 This protocol provided access to both aromatic and aliphatic acylboronates, and demonstrated their stability to a number of reaction conditions – including the α-bromination of aliphatic MIDA acylboronates – and their applicability in the synthesis of borylated heterocycles. However, the preparation of MIDA α-hydroxyboronates required a three-step synthesis from MIDA α-carboxyboronates – which need three additional steps to be synthesized from vinylboronates.23
Subsequent efforts from the Ito and Bode groups provided a more convenient route consisting of the ozonolysis of readily available MIDA alkenylboronates (Figure 1.21).\(^{24}\) This method greatly expanded the scope of accessible MIDA acylboronates and significantly reduced the number of steps required from commercial starting materials.

Perrin independently disclosed an analogous route to MIDA acylboronates by dihydroxylation and subsequent NaIO\(_4\)-mediated oxidation of MIDA alkenylboronates (Figure 1.22).\(^{25}\) The protocol
features remarkably mild aqueous conditions at room temperature. A variety of MIDA acylboronates having aldehyde, ester and carbamate among other functionalities could be synthesized in moderate to excellent yields.

Figure 1.22. Synthesis of MIDA acylboronates from sequential dihydroxylation / oxidation of MIDA alkenylboronates.

Our group has documented the transformation of potassium acyltrifluoroborates (KATs) into the corresponding MIDA acylboronates (Figure 1.23). The reaction was promoted by BF$_3$·OEt$_2$ to trigger the ligand exchange process.

Figure 1.23. Transformation of KATs into MIDA acylboronates.

Recently, Sharma and co-workers devised a strategic synthesis of differentially protected geminal diboronates from vinyl boronic esters or alkynes, and their subsequent oxidation to MIDA
Acylboronates (Figure 1.24). This approach reduced the number of steps required to access the target compounds from commercial starting materials.

Figure 1.24. Synthesis of MIDA acylboronates from the geminal diborylation and oxidation of vinyl boronate esters or alkynes. %Yield values correspond to isolated yields of the DMP-mediated oxidation of MIDA α-hydroxyboronates.

1.3.2 Applications in amide synthesis

In 2014, the Bode group disclosed the first amide-forming reaction of MIDA acylboronates (Figure 1.25). The reactivity of MIDA boronates was found to be superior to that of KAT reagents, as MIDA boronates could successfully react with less reactive O-alkylhydroxylamines, whereas KAT reagents failed to deliver the amide product under these conditions. The chemoselectivity of the amide formation event was confirmed by the ligation of unprotected peptide 1-100 having a β-alanine-derived O-methylhydroxylamine. Excess of MIDA acylboronate 1-77 was required to compensate the poor stability of these species in aqueous solutions.

Figure 1.25. Chemoselective ligation of O-methylhydroxylamine-containing peptide 1-100 with MIDA acylboronate 1-77.

Analogous to the reaction of KATs with O-acylhydroxylamines (Figure 1.13 above), MIDA boronates are proposed to react with O-methylhydroxylamines to give hemiaminal intermediate I (Figure 1.26). Extrusion of BF₃ and tautomerization of the iminol intermediate III affords the amide product.
product. The reactivity differences with KAT reagents are explained based on the equilibrium of hemiaminal \textbf{I} with oxime \textbf{II}. When KATs are used, intermediate \textbf{II} results in a neutral and more stable species, shifting the equilibrium to the oxime side – where extrusion of BF$_3$ is no longer possible. MIDA boronates, however, produce a cationic, less stable species \textbf{II}. The equilibrium is therefore shifted towards hemiaminal intermediate \textbf{I}.

![Figure 1.26. Mechanistic proposal for the amide bond-forming reaction of MIDA acylboronates with O-methylhydroxylamines.](image)

In addition, Yudin and co-workers demonstrated the iodine-promoted Beckmann rearrangement of in-situ-formed MIDA acylboronate oxime 1-103 to afford \textit{N}-borylated amide 1-104 (Figure 1.27). The reaction is proposed to occur from 1,2-boryl migration of the MIDA boronate group to the nitrogen atom, followed by hydrolysis. Byproduct 1-105 arising from the migration of the alkyl chain could be detected as well.

![Figure 1.27. Formation of oxime 1-103 from MIDA acylboronate 1-102 and subsequent Beckmann rearrangement to afford amide 1-104 and byproduct 1-105.](image)
1.4 Monofluoro acylboronates

1.4.1 Synthesis

In search for acylboronates with improved water stability and reactivity towards amide bond formation, the Bode group screened a number of ligands to replace the fluorine atoms in potassium acyltrifluoroborates (KATs). These investigations led to a new class of acylboronates having a single fluorine and a bidentate ligand coordinated to the boron atom – monofluoro acylboronates. These new acylboron compounds could be prepared in one step from the corresponding KATs and TMS-protected ligands mediated by BF$_3$·OEt$_2$ (Figure 1.28). Ligands having a 2-(2-hydroxyphenyl)pyridine-derived scaffold were found to deliver the most stable monofluoro acylboronates.

![Synthesis of monofluoro acylboronates from KATs and TMS-protected ligands.](image)

**Figure 1.28.** Synthesis of monofluoro acylboronates from KATs and TMS-protected ligands.

1.4.2 Applications in amide synthesis

In an analogous way to KAT regents, monofluoro acylboronates react with O-acylhydroxylamines to form amide-ligated products. The specific ligand structure coordinated to boron has considerable impact in the amide-forming reaction rate with hydroxylamine 1-115 (Figure 1.29).
Figure 1.29. Relative reactivities of several acylboronates in the amide-forming reaction with O-carbamoylhydroxylamine 1-115.

Derivative 1-107 having a methyl group close to the acylboron reacted with a 3.5-fold increased rate compared to 1-114, showing that the amide formation rate is strongly influenced by the sterics surrounding the acylboron moiety. On the other hand, derivative 1-109 failed in delivering any amide product. KAT 1-116 displayed only slightly decreased reaction rate over acylboronate 1-114, whereas the corresponding MIDA boronate 1-76 had a 2-fold increased reactivity.

1.5 Concluding remarks

Acylborons have recently emerged as compounds of special interest, partially due to their ability to participate in chemoselective amide bond formation events. Synthetic efforts from our group and others have enabled access to a broad variety of structures containing the acylboron functionality. These endeavours have expanded the scope of the fields towards the investigation of further applications in the bioconjugation of peptides and proteins, or in the preparation of amide-crosslinked polymeric networks.29,30

This thesis focuses on the development of new methodologies for the preparation of amides using acylboronates that circumvent problems associated with current procedures, with focus on chemoselectivity, water tolerance and fast reaction kinetics. In Chapter 2, the chemoselective amide formation of KATs with in situ-formed N-chloroamines under aqueous conditions that proceeds with excellent chemoselectivity is described and analyzed in detail. Chapter 3 discloses the preparation of amide-crosslinked hydrogels occurring among a dynamic range of pH values. Finally, Chapter 4
Chapter 1. Introduction to Acylborons for Amide Bond Formation

describes studies on the template-assisted ligation of monofluoro acylboronates with O-carbamoylhydroxylamines that operates under high dilution conditions.
Acylation of Amines and Amides with Potassium Acyltrifluoroborates

This work was done in collaboration with Dr. Cedric Schaack and Dr. Hidetoshi Noda, who contributed in conceiving the project and performed initial screening of conditions.
Chapter 2. Acylation of Amines and Amides with Potassium Acyltrifluoroborates

2.1 Introduction

Amides are key components of peptides and proteins, polymers, pharmaceuticals and agrochemicals among other commodities. Classical methods to produce amides mainly consist of the condensation of an activated carboxylic acid or the acid itself with an amine, which often requires expensive and wasteful coupling reagents as well as large excess or organic solvents (Figure 2.1). Amide bonds can be formed under aqueous conditions with preformed activated esters or by using peptide ligation methods, but these conditions and starting materials are appropriate only for intrinsically valuable intermediates such as large peptides or proteins. These factors contribute to the demand for new amide-forming processes that operate cleanly under more sustainable conditions.

![Diagram of amide bond formation](image.png)

**Figure 2.1. Classical approach to amide bond formation.**

In an effort to provide more environmentally-friendly and selective amide-forming reactions, chemists have reported considerable progress on catalytic processes for simple amide bond formation from carboxylic acids and amines. In an analogous way, catalytic amide formation methods from alternative starting materials such as aldehydes and alcohols, α-bromonitroalkanes or α,β-unsaturated aldehydes have proliferated. These methods are valuable in specific contexts but do not address the problems associated to condensation approaches, as they continue to require organic solvents or expensive stoichiometric additives.

The reaction of potassium acyltrifluoroborates (KATs) and O-acylhydroxylamines proceeds fast and chemoselectively under aqueous conditions. The main drawbacks, however, rely on the O-acylhydroxylamines. These compounds require several steps to be prepared, and they suffer occasionally from instability, especially in the case of α-amino acid derivatives. In light of this, we hypothesized that simple amines could be employed by in situ activation of the nitrogen atom (Figure 2.2). N-Chlorination of amines has been shown to proceed with fast rate in water. Such conditions, however, could also oxidize the acylboronate, as similar transformations of organoborons...
are well known.\textsuperscript{47-49} Furthermore, prior efforts on the conceptually related amide formation using \(\alpha\)-ketoacids and \(N\)-iodoamines gave mixed results.\textsuperscript{50}

![Figure 2.2. Conceptual approach for the reaction of KATs with in situ activated primary amines.](image)

### 2.2 Screening of reaction conditions

At the outset of our experiments, we chose potassium 4-fluorobenzyoltrifluoroborate (1a) and 2-phenethylamine (2a) in a mixture of aqueous citrate buffer and organic co-solvent as the model reaction for screening conditions for halogenating agents (Table 2.1). We identified \(N\)-chlorosuccinimide (NCS) and related reagents, particularly 1,3-dichloro-5,5-dimethylhydantoin (DCH), to efficiently promote the formation of amide 3a. These reagents were superior to their bromo or iodo analogues, a fact that we attributed to the poor stability of these reagents in water.\textsuperscript{51} As a control experiment, no amide formation was observed in the complete absence of halogenating source. 1a is stable towards DCH for several hours. However, after stirring a solution of 1a

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\textsuperscript{®}Performed on a 0.10 mmol scale with KAT 1a (1.1 equiv), amine 2a (1.0 equiv) and halogenating agent (1.1 equiv) in a mixture of THF and aqueous citrate buffer pH 3.0 (1:1, final concentration 0.10 M) at rt. (DCH), to efficiently promote the formation of amide 3a. These reagents were superior to their bromo or iodo analogues, a fact that we attributed to the poor stability of these reagents in water.\textsuperscript{51} As a control experiment, no amide formation was observed in the complete absence of halogenating source. 1a is stable towards DCH for several hours. However, after stirring a solution of 1a
(1.0 equiv) and DCH (1.1 equiv) in a mixture of THF and aqueous citrate buffer pH 3.0 (1:1, 2 mL) for 24 h, partial decomposition of 1a to 4-fluorobenzoic acid was observed by LC-MS analysis of the reaction mixture.

The reaction was tolerant towards a broad range of organic solvents in combination with aqueous buffers (Table 2.2). Selection of the reaction pH was an important consideration, as we expected that acidic conditions – which are typically favored for KAT ligation — would be detrimental to the amine chlorination due to the protonation of the amine and the subsequent decrease in nucleophilicity. Experimentally, the reaction performed well between pH 2 and 6, and we selected pH 3.0 as optimal. This optimal value may be due to the pKₐ of N-chloroamines – in the range of 0-2.

**Table 2.2. Solvent and pH screening.**

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*a Performed on a 0.10 mmol scale with KAT 1a (1.1 equiv), amine 2a (1.0 equiv) and DCH (1.1 equiv) in a mixture of organic solvent and aqueous buffer (1:1, final concentration 0.10 M) at rt.

– which would not be protonated under these conditions. After completion of the reaction, any excess chloramine or chlorinating agent was quenched by the addition of aqueous sodium bisulfite.
2.3 Substrate scope

2.3.1 Acylation of primary amines

The scope of the reaction was explored by using the conditions optimized above. A wide variety of primary amines underwent smooth amide formation using DCH as the chlorinating agent (Table 2.3). Either free amines or their salts could be used directly in the reaction (3b). Epimerization of the α-carbon was not observed when optically active L-phenylalanine methyl ester was used as substrate (product 3c, experimental details on Section 6.2), although this more sterically hindered amine required longer reaction times than amines on primary carbons. The reaction was found to

Table 2.3. Substrate scope for the amide-forming reaction.\(^a\)

\(^a\)Performed on a 0.10 mmol scale with KAT 1 (1.1 equiv), amine 2 (1.0 equiv) and DCH (1.1 equiv) in a mixture of THF and aqueous citrate buffer pH 3.0 (1:1, final concentration 0.10 M). Values in parentheses are isolated yields of the corresponding products 3a-3y. \(^b\) Amines used as HCl salts. \(^c\) For purification, the reaction mixture was treated with Ac₂O and pyridine in acetone. \(^d\) Isolated yield over two steps. \(^e\) NCS (2.0 equiv) was used instead of DCH. \(^f\) Amine was used as CF₃CO₂H salt. \(^g\) Performed with TCCA instead of DCH. \(^h\) Isolated as a CF₃CO₂H salt.
perform chemoselectively: it tolerated unprotected alcohols (3d, 3e), phenols (3f), ketones (3g) and carboxylic acids (3h). More importantly, acylation of primary amines occurs selectively in the presence of tertiary (3i) and unprotected secondary amine groups (3j). Substrates having double bonds – which may be sensitive to chlorinating agents – could be successfully acylated (3k), although small amounts of halogenated byproducts were observed. Electron-rich anilines often gave complex mixtures derived from ring chlorination or oxidation, but electron-poor aryl- (3l) and heteroarylamines (3m, 3n) could be smoothly acylated using 1,3,5-trichloroisocyanuric acid (TCCA). In contrast, classical amide formation using coupling agents often fails with electron-deficient amines.54 The reaction conditions also tolerated a variety of KATs having reactive groups such as an aldehyde (3s) or an activated ester (3t). Coumarin and sulforhodamine KATs afforded the corresponding amide (3u, 3v) in good yields. Azide and alkyne functionalities (3w – 3y) could be tolerated as well.

2.3.2 Acylation of primary amides

During the investigation of the primary amine substrate scope, we examined the acylation of NH₄Cl, expecting the formation of the primary amide. Surprisingly, we observed symmetrical imide 5a, suggesting that primary amides also undergo acylation under these conditions (Scheme 2.1).

![Scheme 2.1. Acylation of NH₄Cl.](image)

In comparison to primary amines, the corresponding primary amides displayed lower reactivity. After screening, we identified optimized conditions by employing the stronger chlorinating agent TCCA and mildly warming up to 40 °C. Aromatic (5b), aliphatic (5c) and α,β-unsaturated amides (5i) could be smoothly acylated to the corresponding imides (Table 2.4). Carbamates (5d), ureas (5e, 5f), sulfonamides (5g) and guanidines (5h) were also suitable partners for the reaction. Remarkably, primary amides could be acylated selectively in the presence of a secondary amine group (5j). Starting materials having triple bonds (5m), azides (5o) and activated ester functionalities (5n) afforded the corresponding imide in moderate to good yields. Coumarin-containing KAT 1u was tolerated as well (5p).
Furthermore, monoacylated guanidine 5h could be reacted with one more equivalent of KAT 1a to afford the corresponding diacylated derivative 6 in modest yield, along with recovery of starting material (Scheme 2.2). The reaction yield did not improve by prolonging the reaction time.
2.4 Influence of sterics

The substrate scope is limited to primary amines and amides. This appears to be a steric effect rather than a mechanistic limitation. Furthermore, the exceptional preference for the formation of the less hindered amide merits further studies. To explore this in more detail, we subjected lysine ethyl ester to the reaction conditions. Acylation took place cleanly at the \( \varepsilon \)-nitrogen in good yield (Scheme 2.3)

![Scheme 2.3](image)

Scheme 2.3. Selective acylation of L-lysine ethyl ester on the \( N^\varepsilon \) over the \( N^\alpha \) amine groups. Product 7 was isolated by preparative HPLC as a CF\(_3\)CO\(_2\)H salt.

As shown in Table 2.3 above, slight erosion of the yields was observed when primary amines on secondary carbons were employed. More hindered amines on tertiary carbons, such as tert-butylamine, did not react under the standard conditions (Scheme 2.4).

![Scheme 2.4](image)

Scheme 2.4. Attempted acylation of tert-butylamine with KAT 1a.

As observed during the substrate scope studies, secondary amines were found to be unreactive (Table 2.3, compound 3j). To clarify this reactivity difference, we conducted a number of control experiments. First, no reaction was observed between preformed \( N \)-chloromorpholine and 1a under the standard acylation conditions (Scheme 2.5.a). However, an excess of \( N \)-chloromorpholine underwent acylation with 1a under neat conditions (Scheme 2.5.b). More congested secondary \( N \)-chloramines, such as that derived from 2,2,6,6-tetramethylpiperidine, were unreactive even under neat conditions (Scheme 2.5.c).
Chapter 2. Acylation of Amines and Amides with Potassium Acyltrifluoroborates

2.5 Mechanistic insights

2.5.1 Elucidation experiments

To shed light into the reaction mechanism, a number of control experiments were conducted. In order to confirm the participation of $N$-chloroamines as the true reactive intermediates, we set up a control experiment by preparing $N$-chlorobutylamine ($2o$–$Cl$) from $n$-butylamine ($2o$) and NCS in CDCl$_3$; its purity and concentration were determined by NMR. This reacted within 30 min with KAT 1a, yielding amide 9 quantitatively (Scheme 2.6).

![Scheme 2.6. Preparation and reaction of $N$-chlorobutylamine $2o$–$Cl$ with 1a.](image)

In addition, by refluxing KAT 1a and 2-phenethylamine 2a in CH$_3$CN–$d_3$ in the presence of molecular sieves, we observed almost complete conversion to trifluoroborate imine 10, as elucidated by LC-MS and $^{19}$F-NMR studies of the mixture (Scheme 2.7). However, subsequent addition of NCS to the suspension yielded a complex mixture with only traces of the target amide 3a.
2.5.2 Proposed mechanism

The reaction is hypothesized to proceed via an ionic pathway, beginning with the chlorination of the amine to give a \( N \)-chloroamine I, which reacts with the KAT to give adduct II. Extrusion of BF\(_3\) and chloride yields iminol III that tautomerizes into the amide (Scheme 2.8, path A). As in the case of \( O \)-benzoylhydroxylamines, we favor direct interaction of the \( N \)-chloroamine and the KAT carbonyl over an interaction of the nucleophile with the boron and subsequent acyl migration.

We also considered an alternative pathway where the primary amine and the KAT would condense to an imine-like intermediate IV (path B). This intermediate could be chlorinated to afford intermediate V, which would lose BF\(_3\) to form nitrilium cation VI that undergoes hydrolysis to give the amide. However, in light of the control experiments conducted above, we considered path A a more plausible explanation mechanistically.

2.6 Kinetics experiments

We proceeded to study the kinetics profile and measure the rate constant involved in the reaction. As in the mechanistic proposal indicated above, the reaction is hypothesized to take place in two separate steps: 1) chlorination of the amine, and 2) reaction of the \( N \)-chloroamine with the KAT.
Chapter 2. Acylation of Amines and Amides with Potassium Acyltrifluoroborates

We first studied the rate constant for the formation of N-chloramines from NCS and primary amines under the reaction conditions. For this, the formation of N-chlorobutylamine from n-butylamine (1.0 equiv) and NCS (1.0 equiv) in a mixture of THF and aqueous citrate buffer pH 3.0 (1:1) was monitored by UV-Vis (λ = 270 nm) over time (Figure 2.3). Concentrations values were plotted over time and fitted using differential second-order Equation 1. From the fit equations, the rate constant for the chlorination step was estimated as 2.4 ± 0.4 M⁻¹ s⁻¹. NMR analysis of a 1000-fold concentrated mixture showed a clean chlorination step – no byproducts could be observed.

\[
\frac{d [2\omega-\text{Cl}]}{dt} = k \cdot ([2\omega-\text{Cl}]^2 + [2\omega]^2 - 2 \cdot [2\omega] \cdot [2\omega-\text{Cl}])
\]

\(k = \text{second-order rate constant} \quad [2\omega] = \text{initial concentration of } 2\omega\)

**Figure 2.3.** Kinetics studies of the chlorination of n-butylamine (2o, 1.0 equiv) with NCS (1.0 equiv) in a mixture of THF and aqueous buffer pH 3.0 (1:1). The plots correspond to three replicate experiments. Red dots correspond to single experimental values. Fitting curves are shown as thin black lines.

We also evaluated the reaction profile of the acylation of N-chloramines with KAT reagents (Figure 2.4). For this purpose, N-chlorobutylamine 2o–Cl was freshly synthesized and reacted with 1a (1.0 equiv) in a mixture of THF and aqueous citrate buffer pH 3.0 (1:1).

\[
\frac{d [2\omega-\text{Cl}]}{dt} = -k \cdot [2\omega-\text{Cl}]^2
\]

\(k = 8.3 \pm 1.5 \text{ M}^{-1} \text{ s}^{-1}\)

**Figure 2.4.** Kinetics studies of the acylation of N-chlorobutylamine 2o–Cl with 1a. Red dots correspond shows extrapolated average product concentration values from UV-Vis time points of two replicate experiments (error bars shown). The fitting curve to Equation (2) is shown as a thin black line.
Disappearance of the chloramine was monitored over time by UV-Vis ($\lambda = 245$ nm). Extrapolated concentration values were plotted against time and fitted to differential equation 2. An average rate constant of $8.3 \pm 1.5$ M$^{-1}$ s$^{-1}$ was obtained from the corresponding fitted curves.

These values demonstrate that this reaction takes place with very fast rate, several orders of magnitude faster than amide-forming processes using activated esters at room temperature. The rate constants for the reaction of KATs with O-acylhydroxylamines – previously measured in our lab – are within the same range than those observed above.

### 2.7 Application to natural product late-stage functionalization

Having established the chemoselectivity and fast kinetics of the KAT–chloramine reaction, we turned our attention to the application in the late-stage selective labeling of biologically relevant molecules. Among the possible targets, natural products typically display complex structures with many different reactive functionalities, making them an attractive platform to demonstrate the selectivity and the potential of this amidation method.

#### 2.7.1 Guanidine-selective acylation of streptomycin

Streptomycin hydrochloride is a broad-spectrum antibiotic that operates by binding to the 30S subunit of the bacterial ribosome, thereby inhibiting bacterial protein expression. Structurally, the molecule is a trisaccharide which contains two guanidine groups, one aldehyde and a secondary amine moiety. For the labelling experiments, we chose KAT $1l$ having an alkyne moiety that offers a handle amenable for further decoration with azide compounds via copper-catalyzed 1,3-dipolar cycloaddition. Acylation of streptomycin sesquisulfate with $1l$ under the standard conditions occurred selectively on the guanidino groups, yielding a mixture of isomers $11a$ and $11b$ (Scheme 2.9). Despite the low yields obtained after preparative HPLC, analysis of the reaction by HPLC and LC-MS showed a relatively clean trace with full consumption of $1l$ and two major peaks corresponding to $11a$ and $11b$ (details on the Experimental chapter, section 6.2). The products were isolated as internal hemiaminals and their structures fully elucidated by $^1$H- and $^{13}$C-NMR along with peak assignments. No acylation on the secondary amine could be detected.
2.7.2 Acylation of gentamicin C₁

Gentamicin C₁ is a broad-spectrum aminoglycoside. Analogous to streptomycin, it binds to the 30S ribosome subunit, which results in cell malfunction and death. The antibiotic is composed by three sugar rings functionalized with a number of primary and secondary amine groups. When subjected to the reaction conditions using KAT 1l as the limiting reagent, acylation took place exclusively on the primary amine groups (mixture of isomers, Scheme 2.10). The low isolated yields observed were attributed to incomplete conversion of 1l and to product loss during purification by preparative HPLC. The product mixture appeared as one single peak in the crude HPLC trace (details on the Experimental chapter, section 6.2).

2.7.3 PEGylation of polymyxin B

Polymyxin B is a mixture of cyclic peptide antibiotics extracted from Bacillus polymyxa. Polymyxin B is a potent bactericidal agent against Gram-negative bacteria. It inserts into the bacterial membrane, opening pores that allow water to freely flow inside the bacteria – which results in cell death. As Gram-positive bacteria possess a thicker cell wall, polymyxin B has little to no antibiotic effect against them.
PEGylation is an extended bioconjugation strategy to improve the solubility and plasmatic half-life of the conjugate compared to the non-conjugated molecule. We attempted the PEGylation of Polymyxin B – which contains five primary amines on its structure – by employing 5 kDa PEG-KAT 12 as the limiting reagent. We obtained a mixture of mono-PEGylated derivatives of polymyxin B in good yield (Scheme 2.11).

**Scheme 2.11. Mono-PEGylation of polymyxin B.**

### 2.8 Application to the labeling of bovine serum albumin

To further investigate the applicability of the method, we explored the potential of the amidation method in the conjugation of proteins. We chose bovine serum albumin (BSA) as its stability towards chlorinating agents has been documented in the literature. KAT 13 was used as the labeling reagent due to its alkyne unit that could be further reacted via copper-catalyzed 1,3-dipolar cyclization (CuAAC) with biotin-azide 14 to simplify the purification of labeled adducts. BSA was treated with HOCl for 15 min in aqueous citrate buffer pH 6.0, followed by addition of KAT 13. Subsequently, acylated protein was reacted with biotin-azide 14 via copper-catalyzed 1,3-dipolar cyclization (Figure 2.5).

**Figure 2.5.** Labeling studies of BSA with KAT 13.

Biotin-labeled conjugates were isolated by affinity chromatography using agarose-supported streptavidin. LC-MS/MS proteomics analysis of the eluted proteins showed that the biotin handle could be incorporated non-selectively to a number of lysine residues throughout the protein.
2.6. However, the process resulted in extensive side chlorination of tyrosines and oxidation of
cysteine and methionine residues.

2.9 Conclusions and outlook

We have developed a mild, water-tolerant, rapid and chemoselective acylation of in situ
generated \(N\)-chlorinated amines or amides using KAT reagents. This method complements classical
amide formations from amines by being completely tolerant to aqueous conditions, exceptionally fast
at acidic pH, and offering a unique chemoselectivity profile. Primary amines are selectively acylated
in the presence of nearly all other functional groups, including secondary amines. At the current
stage of development, the main disadvantage of this method in comparison to standard amide
formation is the use of KATs in place of the much more common carboxylic acids. KATs are,
however, easy to handle solids of considerable bench stability. Several synthetic routes have been
already disclosed by us and other groups, enabling access to a broad variety of KAT structures
including optically stable \(\alpha\)-aminoacyl trifluoroborates.\(^\text{60}\)

The acylation of primary amides, carbamates, and sulfonamides is especially remarkable.
There are relatively few methods known for preparation of their mixed acyclic imides and related
compounds, which are interesting components of bioactive natural products,\(^\text{61-63}\) pharmaceuticals,
and catalysts.\(^\text{64}\) Classical imide synthesis, such as acylation of amides with acyl chlorides or
anhydrides,\textsuperscript{65} or Mumm rearrangement of isoimides,\textsuperscript{66,67} are typically plagued by side reactions. Additional procedures include aminocarbonylation of aryl halides or olefins,\textsuperscript{68,69} oxidation of N-alkylamides\textsuperscript{70} and reaction of isonitriles with carboxylic acids\textsuperscript{71} among others.\textsuperscript{72-75} However, these approaches suffer from narrow substrate scope, harsh reaction conditions or side reactions. In this context, our method stands out as a mild and water-tolerant alternative with broad scope and functional group compatibility. Imide formation was demonstrated to occur selectively even in the presence of unprotected secondary amines, as showcased in the synthesis of 5j (Table 2.4) and in the selective acylation of streptomycin (Scheme 2.9).

Further improvements, such as electrocatalytic or enzyme-based chlorination, will contribute to improve the efficiency of this unique reaction. The labeling studies of BSA indicate that further implementations in protein bioconjugation might suffer from poor selectivity and excessive byproduct formation derived from the use of chlorinating agents. On the other hand, applications in the context of chemical biology that do not require the use of externally added chlorinating agents might be of special interest (see Chapter 5, Section 5.2 for details).
Amide-Crosslinked Hydrogel Preparation from Primary Amines and Multi-Arm PEG-KATs

This work was done in collaboration with Mr. Dominik Schauenburg, who expanded the scope and applications of the hydrogel formation protocol.
Chapter 3. Amide-Crosslinked Hydrogel Preparation from Primary Amines and Multi-Arm PEG-KATs

3.1 Introduction

Hydrogels are three-dimensional chemically cross-linked, hydrophilic polymeric networks capable of swelling when introduced into aqueous media. Hydrogels have relevant applications in tissue engineering, drug delivery, wound healing, and cell immobilization. Successful hydrogel formation requires water-tolerant reactions that proceed with fast kinetics and – ideally – in a traceless manner. The most extended methods for the preparation of chemically cross-linked hydrogels are thia-Michael addition, oxime ligation, thia-ene reaction, native chemical ligation and Huisgen azide–alkyne cycloaddition. Despite their success in the preparation of hydrogels, the need for elaborated, synthetically challenging starting materials often limits the preparation of more complex systems such as hydrogels bearing covalently immobilized functional molecules.

We have previously documented the biocompatible hydrogel formation from multifunctional polyethylene glycol (PEG)-KAT reagents. The method requires the use of O-acylhydroxylamines – reagents that require several synthetic steps and often suffer from stability problems. The use of in situ-formed N-chloramines enables the use of commercial primary amines, thus circumventing this problem. As described in Chapter 2, the reaction of KATs and N-chloroamines takes place under aqueous conditions with outstanding kinetics and exquisite chemoselectivity. These features make the reaction a suitable candidate for the development of a facile preparation of hydrogels. Furthermore, the reaction operates in a near-traceless manner – the only byproducts generated are BF$_3$ (subsequently hydrolyzed to borate salts), hydrogen chloride and chlorinating agent-derived byproducts. The reaction has been shown to be pH-sensitive, paving the way for the development of a pH-triggered gelation process.

3.2 Preliminary experiments

The gelation preliminary experiments were carried out using a four-armed 10 kDa PEG-KAT 15 (4 wt.%). As the cross-linking amine, we employed trifunctional amine 16. Gelation was initially tested by mixing PEG-KAT 15 with amine 16 (1 amine equivalent per KAT group) in aqueous citrate buffer pH 3.0, followed by addition of NCS from CH$_3$CN stock solution. Pleasingly, the mixture turned into a stiff gel within minutes (Figure 3.1).
Chapter 3. Amide-Crosslinked Hydrogel Preparation from Primary Amines and Multi-Arm PEG-KATs

3.3 Screening of gelation conditions

From this starting point, a number of reaction conditions were evaluated. The gel formation process was followed by real-time rheology measurements of the gelating mixture. We investigated the effect of NCS equivalencies in the gelation reaction. The corresponding storage moduli were plotted over time (Figure 3.2). The amount of added chlorinating agent played an important role on the gel formation rate; more homogeneous and faster gelation was observed when excess NCS was employed, as determined by rheological studies. No gel formation was observed in the absence of amine 16 or chlorinating agent.

Both the gelation rate and storage modulus plateau were dependent on the pH of the medium (Figure 3.3). The reaction showed an optimal value around pH 3. Gelation rate progressively slows down as pH increases; no gel formation could be observed at pH 7. The use of more acidic aqueous media also resulted in slower gelation rate (pH 2) or complete inhibition of the process (pH 1). This
observation is consistent with the protonation of the amine or chloroamine discussed in Section 2.2 above.

**Figure 3.3.** Effect of pH in hydrogel formation.

### 3.4 Conclusions and outlook

A mild and rapid method was developed for the formation of amide-cross-linked hydrogels in aqueous buffer at room temperature. Strong, transparent hydrogels could be readily prepared by reacting in situ chlorinated, commercial amine 16 with PEG-KAT 15. Hydrogel properties can be easily tailored by the equivalencies of amine and chlorinating agent added. The gel formation could be tuned over a dynamic pH range of 3–6. The complete suppression of the gelation at very acidic or neutral pH opens up interesting possibilities of developing systems where gel formation is triggered in response to pH changes.

The work described here was continued by Mr. Dominik Schauenburg, who expanded the primary amine scope and studied the decoration of hydrogels with small molecules such as dyes, fluorophores or bioconjugation tags.
Chapter 3. Amide-Crosslinked Hydrogel Preparation from Primary Amines and Multi-Arm PEG-KATs
Traceless Template-Assisted Ligation of Acylboronates and O-Acylhydroxylamines

During this project, Mr. Raphael Hofmann provided training and supportive discussions for the expression and purification of streptavidin mutants.
Chapter 4. Traceless Template-Assisted Ligation of Acylboronates and O-Acylhydroxylamines

4.1 Introduction and goal of this project

Biological processes have evolved over millions of years in order to enable organic reactions to occur in highly diluted systems. Among the different strategies adopted, Nature commonly makes use of biological templating scaffolds with affinity towards two given reagents so to bring them in close proximity to each other. This results in an increase of their local concentration which translates in an overall reaction acceleration. Well-known examples include ribosomal peptide synthesis or DNA ligation. Inspired by Nature, organic chemists have applied these principles in promoting organic reactions; the most notable contributions make use of biochemical templates such as DNA. Purely synthetic templates capable of promoting organic reactions have been developed as well with considerable success.90

Classical approaches to templated organic synthesis have made use of complementary scaffolds such as DNA strands to bring reaction substrates in close proximity to each other.91 Typically, reactants have been tethered covalently to the directing scaffolds, which therefore remain attached to the reaction product (Figure 4.1a).92-94 To address this issue, Diederichsen et al. disclosed a photocleavable PNA-templated native chemical ligation that allowed photocleavage of the directing PNA strands from the ligated peptide by irradiation95 – a strategy further used by Okamoto and co-workers in the photocleavable DNA-directed ligation of peptide fragments.96 Other implementations employed specific reaction platforms – such as the native chemical ligation – where the release of one directing strand was made possible (Figure 4.1b).97-99 Non-complementary systems where an external molecular template is employed have also been successfully disclosed (Figure 4.1c).100-102 Despite their successful application in areas such as proximity-driven target identification103,104 or combinatorial synthesis,105 the utility of non-cleavable templates or directing groups for broader synthetic applications is limited by the persistence of at least one of the templating groups.
To date, however, there are no successful implementations of a traceless templated ligation where the templating groups are cleaved concomitantly with bond formation. This may be due to the inherent requisites such a system must fulfill. First, the starting materials must be able to

**Figure 4.1.** Schematic representation of prior works on templated synthesis. a. Complementary template-assisted ligation without directing scaffold release. b. Complementary template-assisted ligation with partial directing scaffold release. c. External template-assisted ligation without directing scaffold release.

(a) Complementary template-assisted ligation without scaffold release (ref. XX–XX)

(b) Complementary template-assisted ligation with partial scaffold release (ref. XX–XX)

(c) Externally template-assisted reaction without scaffold release (ref. XX–XX)

**Figure 4.2.** Schematic representation of the goal of this project. a. Traceless templation approach relying on complementary directing scaffolds. b. Traceless templation approach relying on an external template.
react with each other without the intervention of any other external catalyst or reagent. Second, in order for the templating groups to be cleaved off concomitantly, the reactive groups must have cleavable portions amenable to functionalization that fragment during the reaction with each other (Figure 4.2 above).

In 2015, we disclosed a novel class of acylboron compounds – monofluoro acylboronates – capable of ligating with O-acylhydroxylamines to form amides (Figure 4.3). We recognized that the reaction fulfilled the rare criteria highlighted above – no extra reagent needed, and simultaneous cleavage of some portions of both molecules during the reaction—thus offering the possibility for true traceless templation. The present Chapter describes the development of a traceless templated reaction relying on the ligation of monofluoro acylboronates and O-carbamoylhydroxylamines.

To choose the ligand-template system, a number of factors had to be taken in consideration. First, the templating system should use ligands that are synthetically feasible to be incorporated into the starting materials structure. Second, the system should have a large association constant so that the binding event occurs even under diluted conditions. Additionally, the ligands should bind to the template with fast association rate. Based on these considerations, we chose to evaluate the following templating systems: the cyclodextrin-adamantane, and the streptavidin-biotin pairs.

4.2 Cyclodextrin-adamantane system

4.2.1 Overview

Cyclodextrins (CDs) are macrocyclic D-glucopyranose oligosaccharides consisting of three main types: α, β and γ-CD – containing six, seven or eight D-glucose units respectively. These glucose rings are arranged in a truncated cone structure with a hydrophobic cavity and a hydrophilic outer surface (Figure 4.4). CDs are capable of forming host-guest complexes with a large variety of organic molecules, both in aqueous solution and in the solid state. Guest molecules typically fit
into the hydrophobic cavity of CDs by non-covalent interactions such as van der Waals forces or hydrophobic interactions. CDs have been extensively studied not only in the context of molecular recognition,\textsuperscript{110} but also as chiral stationary phases\textsuperscript{111} and as carriers in drug delivery.\textsuperscript{112} The stability of the inclusion complex depends on the size, shape, polarity and steric hindrance of the guest molecule as well as the specific size of the CD.\textsuperscript{109,113,114}

\textbf{Figure 4.4.} Cyclodextrin structure and types.

Cyclodextrins have been demonstrated to be a good templating platform in organic chemistry.\textsuperscript{115} Among the different host-guest combinations, we chose the adamantane–\(\beta\)-CD pair (Scheme 4.1). Adamantane association to cyclodextrin takes place with acceptable thermodynamics (\(K_D \sim 10^{-3} - 10^{-4} \text{ M}\))\textsuperscript{116} and fast kinetics,\textsuperscript{117} which makes it a good platform candidate to develop a template-assisted acylboronate-hydroxylamine ligation.

\textbf{Scheme 4.1.} Host-guest association equilibrium of \(\beta\)-CD and adamantane.

\subsection*{4.2.2 Design and synthesis of reaction partners}

We required the installation of the reactive functional groups – acylboronate and hydroxylamine – on the host and guest scaffolds. Ideally, these starting materials would associate with each other and trigger the amide-forming reaction by spatial proximity of the reactive moieties (Scheme 4.2).
Chapter 4. Traceless Templated Ligation of Acylboronates and O-Acylhydroxylamines

Scheme 4.2. Conceptual scheme for the implementation of the β-CD–adamantane host-guest system in the template-assisted ligation of acylboronates and O-carbamoylhydroxylamines.

We chose to assign the adamantane unit to the acylboronate reactant and equip the hydroxylamine with a β-CD. For the late-stage reactive group decoration we employed the copper-catalysed 1,3-dipolar alkyne-azide cycloaddition (Cu-AAC) reaction. We first synthesized alkyne-containing hydroxylamine 21 starting from commercial N-Boc-hydroxylamine and N-methylpropargylamine 17 (Scheme 4.3). To enable the templating cyclodextrin to cleave off after the reaction, the alkyne was installed onto the cleavable O-acyl group. A coumarin fluorophore was also incorporated to enable reaction monitoring at low concentrations.


Azide-labelled β-CD was prepared according to a reported procedure\textsuperscript{118} (Scheme 4.4). Commercial β-CD was treated with tosyl chloride to produce monotosylated 22. Addition of sodium azide yielded mono-azole β-CD derivative 23.

Alkyne 21 was reacted with azide cyclodextrin 23 via copper-catalyzed 1,3-dipolar cycloaddition to give Boc-protected precursor 24 (Scheme 4.5a). Deprotection of the Boc group was conducted with HCl in dioxane (Scheme 4.5b). Deprotected hydroxylamine-β-CD 25 was stable in solution, but decomposed during concentration. To circumvent this, a stock of freshly deprotected material was prepared prior to each kinetics experiment.


4-Fluorobenzoyl trifluoroborate 1a was complexed with ligand 26 to form alkyne-equipped acylboronate 27. This was clicked to adamantane-azide derivatives 28 or 29 having spacers of different lengths to give adamantane-containing acylboronates 30 and 31 (Scheme 4.6).

Scheme 4.6. Synthesis of starting materials 30 and 31. TBTA = tris((1-benzyl-1H-1,2,3-triazol-4-yl)methyl)amine.
4.2.3 Kinetics experiments

With the materials in hand, we studied the conversion to product over time. Hydroxylamine 25 was mixed from freshly deprotected stock solution with acylboronates 30 or 31 in aqueous citrate buffer at pH 6.0. To follow the formation of product 32, aliquots (20 μL) were taken from the reaction at different time intervals, quenched with hydroxylamine 33 (> 100 equiv) and monitored by HPLC at 425 nm (Scheme 4.7).

Scheme 4.7. Reaction of CD-equipped hydroxylamine 25 with adamantane-acylboronates 30 or 31.

Integration values of the HPLC peak at 425 nm corresponding to product 32 were extrapolated to conversion values by the appropriate calibration curve and plotted over time (Figure 4.5). The kinetics profiles of the reactions using 30 or 31 – equipped with a shorter or longer linker respectively – were compared with a control experiment where 25 was reacted with KAT 1a (containing no adamantane unit). The reaction was found to be significantly faster when KAT 1a was used relative to the use of 30 or 31. This indicates that the reaction is not benefiting from, but rather being

Figure 4.5. Kinetics experiments for the β-CD-templated formation of amide 32. The plot corresponds to HPLC conversions over time (monitoring at 425 nm) using β-CD-equipped hydroxylamine 25 and adamantane-equipped acylboronates 30, 31 or KAT 1a.
hampered by the use of the CD–adamantane templating system. This may arise from the short link used to connect the hydroxylamine to β-CD. The lower reaction rate observed when using \textbf{30} compared to the use of \textbf{31} was attributed to the increased steric hindrance of the resulting \textbf{25–30} association complex due to the shorter spacer employed.

4.2.4 Conclusions and outlook

We examined the potential of the β-CD–adamantane host-guest complex to act as a templating system in the traceless ligation of O-acylhydroxylamines and acylboronates. Preliminary kinetics studies of the system were not supportive for further development, as little to no templating effect was observed in the preliminary reactions described above. This could possibly arise from the reactive hydroxylamine being too sterically hindered by cyclodextrin to be able to collide and react with a spatially close acylboronate-adamantane guest. Further experiments should include a system with a linker inserted between cyclodextrin and the reactive hydroxylamine.

4.3 Streptavidin-biotin system

4.3.1 Overview

Streptavidin is a homotetrameric protein produced by the bacteria \textit{Streptomyces avidinii}. Each of its monomers holds a binding pocket capable of strongly binding biotin with $K_D \approx 10^{14}$ M$^{-1}$ – one of the strongest non-covalent interactions ever measured$^{119}$ – and with very fast association kinetics ($k_{on} \approx 10^7$ M$^{-1}$ s$^{-1}$, $k_{off} \approx 10^{-7}$ s$^{-1}$)$^{120,121}$ Biotin–streptavidin complex displays considerable stability over high temperatures, extreme pH, denaturing agents and hydrolytic enzymes. $^{122}$ Geometrically, the binding pockets are arranged so that a pair of pockets are located spatially proximal to each other (18.8 Å, blue monomers) and distant (~31 Å, from blue to tanned monomers) from the other pair (Figure 4.6)$^{123}$

![Figure 4.6. Overview of the streptavidin-biotin system. Distance between proximal biotin binding pockets on blue monomers. Thermodynamic and kinetic parameters for the biotin-streptavidin association equilibrium.]

Because of these features, the streptavidin-biotin system poses a promising platform for the development of a templated ligation. The potential of the system has already been underlined by a
number of research groups. On one hand, Ward et al. have disclosed elegant implementations of streptavidin-biotin pairs as artificial metalloenzymatic systems,\textsuperscript{124} including methatesis, C–H activation and transfer hydrogenation to imines (Figure 4.7a).\textsuperscript{125} On the other hand, Winssinger and co-workers have successfully demonstrated the applicability of streptavidin in the template-assisted Ru-photocatalyzed azide reduction with release of a rhodamine reporter (Figure 4.7b).\textsuperscript{126}

![Figure 4.7](image)

**Figure 4.7.** Selected examples of applications of the streptavidin-biotin pair in organic synthesis. a. Enantioselective transfer hydrogenation of imines catalyzed by an iridium-containing biotin-streptavidin complex. b. Streptavidin-assisted Ru-photocatalyzed azide reduction (Winssinger, 2012)

Biotin dissociates remarkably slowly from streptavidin ($k_{\text{off}} \approx 10^{-7} \text{ s}^{-1}$), which is beneficial to guarantee that the reagents remain associated to the template. Given such a low dissociation constant, the reactive groups should be able to stay in close proximity enough time to react. However, this approach lacks complementarity – the addition of one equivalent of reagents and template will generate a distribution of association complexes. Among the possible combinations, some will display the regents properly located on the same face of the template (Figure 4.8, matching combinations). Other combinations, on the contrary, will have the wrong starting material arrangement (mismatching combination). A priori, template-assisted amide formation is expected to occur from matching combinations exclusively. In replacement of biotin, we chose to use desthiobiotin – a biotin analogue with lower affinity to streptavidin ($K_D \approx 10^{11} \text{ M}$, $k_{\text{off}} 10^{-4} – 10^{-5} \text{ s}^{-1}$) – to enhance ligand dissociation and reorganization from mismatched, non-productive states.\textsuperscript{127}
Figure 4.8. Conceptual overview of the streptavidin-assisted model reaction.

To avoid excessive formation of mismatching combinations, we designed our working model by careful experiment setup with sequential addition of reagents to better control association to streptavidin (Figure 4.9). The starting material equivalencies are expected to have an impact in the distribution of different combinations to streptavidin as well. To elucidate this, we examined the statistical distribution of matching and mismatching combinations generated by the sequential mixing of different starting material equivalencies with streptavidin. The use of one reagent in excess will positively increases the proportion of matching combinations by full saturation of any available streptavidin binding pocket. In contrast, the use of either 0.5 or 1.0 equivalents of streptavidin generates comparable proportions of matching combinations.

Figure 4.9. Combinations from the sequential addition of starting materials to streptavidin. The plot shows the relative proportion of combined matching and mismatching combinations for a number of streptavidin and acylboronate equivalencies.
4.3.2 Synthesis of reaction partners

We designed starting materials equipped with either biotin or desthiobiotin. The synthesis of hydroxylamine 21 and acylboronate 27 having an alkyne appended on their cleavable parts was carried out as described above (Schemes 4.3 and 4.6). The alkyne moiety was reacted with biotin- or desthiobiotin-azide (14 or 35 respectively) via copper-catalyzed 1,3-dipolar cycloaddition to readily access Boc-protected hydroxylamines 36 and 37, and acylboronates 38 and 39 (Scheme 4.8).

In an analogous procedure to cyclodextrin-equipped hydroxylamine 25, the deprotection of the Boc group on hydroxylamines 36 and 37 was carried out using HCl in dioxane to afford starting materials 40 and 41 (Scheme 4.9). As observed previously, free hydroxylamines suffered from poor stability over time; therefore, the deprotection of 36 and 37 was carried out prior to use for kinetics measurements. Following deprotection, the material was precipitated with Et₂O, excess of acid was washed away and the precipitate was dissolved in the reaction buffer.

4.3.3 Preliminary experiments
With the starting materials in hand, we proceeded to test the effect of the presence or absence of streptavidin in the reaction kinetics. Starting material purity was routinely checked by LC-MS prior to use in kinetics experiments. In a typical setup, commercial streptavidin (1.0 equiv) was mixed with a solution of 40 or 41 (5.0 µM) in aqueous citrate buffer pH 6.0, followed by addition of acylboronates 38 or 39 (2.0 equiv) (Figure 4.10).

**Figure 4.10.** Model streptavidin-assisted formation of amide 32 from biotin-equipped materials 38 and 40, or desthiobiotin-bearing 39 and 41.

To follow the formation of product 32, reaction aliquots were taken over time intervals, quenched with hydroxylamine 33 and monitored by HPLC (λ = 425 nm). Integration values of the peaks corresponding to 32 were extrapolated to conversions by the appropriate calibration curve, and plotted over time (Figure 4.11, green dots). Negative control experiments were carried out where biotin was added to prevent the binding of acylboronates 38 or 39 to occur (orange dots). The background reaction was tested by mixing the reaction partners in the absence of streptavidin (black dots). Despite the observation of promising conversion levels when biotin derivatives were used, the corresponding negative control experiment – where externally added biotin is used to poison the template – showed comparable conversions (Figure 4.11, centre plot). Desthiobiotin-bearing probes displayed similar conversion levels, but only marginal product formation was observed upon addition of biotin (right side plot).

**Figure 4.11.** Preliminary kinetics experiments for the templated formation of amide 32. The plots correspond to HPLC conversions over time using biotin-equipped (40 and 38, centre plot) or desthiobiotin-equipped starting materials (41 and 39, right-side plot) monitoring at 425 nm.

In every scenario, we suffered from considerable measurement error levels and very low reproducibility from run to run – drawbacks attributed to the need for aliquot quenching and the use
of HPLC as the measurement method. To be able to collect reliable data in a reproducible manner, we developed a workflow that avoids sample quenching and enables measurements in a real-time fashion.

### 4.3.4 Design and synthesis of a fluorescence-quenched hydroxylamine 42 for turn-on fluorescence studies

To solve the reproducibility issues encountered during the preliminary experiments, we turned to the use of a FRET-based strategy. FRET (Förster Resonance Energy Transfer) consists of the non-radiative transfer of energy from a donor (usually a fluorophore) to an acceptor through spatial dipole-dipole interactions. FRET is a very sensitive technique that allows for the real-time detection of fluorescence changes even at sub-nanomolar concentrations.

As the FRET pair, we chose to use a coumarin fluorescent donor and a non-radiative azo dye acceptor. To display a FRET effect, both donor and acceptor must be initially attached to be in close spatial proximity. In addition, the acceptor must be able to absorb energy at the emission wavelength of the donor. Finally, as the reaction takes place, the FRET components need to separate from each other so to show significant turn-on fluorescence effect. To implement these premises, we designed starting material 42 equipped with a coumarin donor ($\lambda_{em} = 485$ nm) on the N-side, and desthiobiotin along with 4-dimethylaminoazobenzene-4-sulfonyl (DABSYL)-derived azo quencher ($\lambda_{abs} = 500$ nm) on the O-side of the hydroxylamine. (Figure 4.12).

![Figure 4.12. Design of fluorescence-quenched hydroxylamine 42.](image)

To synthesize a suitable precursor, $N$-Boc-L-lysine methyl ester was allowed to react with DABSYL chloride followed by ester deprotection yielding carboxylic acid 43. This was coupled with azido amine 44 to afford compound 45. Following Boc deprotection, free amine was reacted with
desthiobiotin succinimidy ester 46 to give azide 47 (Scheme 4.11). Compound 47 underwent Cu-catalyzed 1,3-dipolar cyclization with alkyne 21 to produce Boc-hydroxylamine 48 with donor, acceptor and desthiobiotin ligand properly placed.


The deprotection of 48 was performed using HCl in dioxane to give free hydroxylamine 42 (Scheme 4.11). The product was precipitated and washed with Et₂O. The precipitated dry solid was stable for weeks in the fridge, but when making concentrated solutions, decomposition over time was observed. As a result, stock solutions of 42 were freshly prepared from the precipitated solid prior to its use in kinetics experiments.

Scheme 4.11. Deprotection of 48 to afford free hydroxylamine 42.

4.3.5 Expression of streptavidin mutants with modular binding pocket activity and orientation
Along with the preparation of adequately equipped starting materials, we adopted the elegant procedure disclosed by Howarth and co-workers to express and separate streptavidin mutants having different valencies and spatial arrangement of binding pockets (Figure 4.13). Among the expressed species, we expect that only “cis” (1,2-divalent) along with tetravalent and trivalent mutants would be efficient as templating scaffolds. Moreover, the use of 1,2-divalent streptavidin provides a simplified system as it possesses only two active binding pockets instead of four.

Figure 4.13. Expression and purification of streptavidin mutants with different valencies and orientation of binding pockets.

Given the excessive stability of the streptavidin-biotin association complex, we attempted to express mutants showing reduced affinity towards biotin and its analogues. There are four main amino acid residues involved in the interaction with biotin at the binding pocket: Asn23, Ser27, Ser45 and Asp128 (Figure 4.14).

Specifically, S45A and D128A mutations are reported to lower the association constant to biotin by approx. four orders of magnitude. More importantly, this decrease in affinity is not reflected in the association but rather in the dissociation rate (increasing from $\sim 10^{-6}$ to $\sim 10^{-2}$ s$^{-1}$).
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This opens the possibility of using such mutated streptavidins to enable template turnover while still retaining sufficient affinity toward starting materials to efficiently promote the reaction.

The desired streptavidin variants were prepared by following the same procedure as above. First of all, we introduced the desired mutations on the expression plasmid by PCR. Once having validated the mutated plasmid sequence, S45A and D128A tetravalent mutants were expressed and folded appropriately. The sequence of the expressed mutants was confirmed by MALDI-QTOF analysis of the samples.

4.3.6 Screening of reaction conditions

4.3.6.1 First experiments and negative controls

The reaction profile of the templated reaction using fluorescence-quenched hydroxylamine and acylboronate was evaluated. In a typical experiment, was dissolved in aqueous citrate buffer (pH 6.0) followed by the addition of tetravalent expressed streptavidin (0.50 equiv) and acylboronate (Scheme 4.12). The reaction was monitored by fluorescence (\(\lambda_{\text{ex}} = 430\) nm, \(\lambda_{\text{em}} = 485\) nm) over time. Conversion values were extrapolated and plotted over time.

Scheme 4.12. Amide-forming ligation of with occurring with turn-on fluorescence over time.

Significant acceleration was observed when expressed tetravalent streptavidin was used (Figure 4.15, red dots), as compared to the background reaction (orange dots) and to a negative control experiment where excess biotin was added to block streptavidin binding pockets (grey dots). The stability of reagent during the course of the reaction was confirmed by fluorescence measurement of a solution containing exclusively and streptavidin (green dots).
After reaching the plateau, experiments were analysed by LC-MS to confirm the final conversion values (Figure 4.16). Only remaining starting material 42, the desired amide product 32, and quencher-derived byproducts 49 and 50 could be observed in the LC-MS trace.

**Figure 4.16.** LC-MS analysis of the plateau section on the plot shown in Figure 4.15.

### 4.3.6.2 Mutant type

After having checked the preliminary experiments, we moved to evaluate the different expressed streptavidins with varied binding pocket dispositions and activities. The reaction exhibited acceleration only in the presence of tetravalent and 1,2-divalent ("cis") streptavidins; monovalent
and 1,3- or 1,4- (“trans”) streptavidins did not show any rate enhancement over the background reactivity (Figure 4.17).

![Figure 4.17. Performance of different streptavidin mutants in the reaction. Performed with 42 (5.0 μM), 39 (0-2.0 equiv) and S (0-0.8 equiv). Dots are mean conversion values of three replicates. Error bars are omitted for clarity.](image)

Next, we studied the performance of streptavidins mutated at the binding pocket. We observed less initial acceleration of the reaction when 0.5 equivalents of S45A streptavidin were employed, which was attributed to the lower affinity of the starting materials towards this mutant (Figure 4.18a). The use of the D128A mutant (0.5 equiv) gave, however, similar rate enhancement levels to wild type streptavidin were observed. Furthermore, conversion values reached the plateau after approximately 10-12 h when wild type streptavidin was used. On the contrary, the kinetic plot for the D128A mutant achieved slightly superior conversion levels without reaching the plateau after 19-20 h of reaction time. On the other hand, when substoichiometric amounts (0.1 equiv) of streptavidin were used, the conversion achieved after 16-17 h was significantly bigger when mutated streptavidins were used (1.5-fold for the S45A and 2.0-fold for the D128A mutants respectively), although the overall reaction rate was lower (Figure 4.18b). These results can be explained based on more efficient reactant and side product turnover from streptavidins having mutated binding pockets.

![Figure 4.18. Performance of streptavidins with mutated binding pocket aminoacids. a. Performed with 42 (5.0 μM), 39 (2.0 equiv) and S (0.5 equiv). b. Performed with 42 (5.0 μM), 39 (2.0 equiv) and S (0.1 equiv). Dots are conversion values from single runs.](image)
4.3.6.3 Streptavidin loading studies

We conducted studies on the effect of different streptavidin equivalencies in reaction conversion after 6 h (Figure 4.19). When tetravalent streptavidin was tested, optimal conversion was achieved at around 0.5 equiv.

![Figure 4.19. Streptavidin loading studies plot. Performed with 42 (5.0 μM), 39 (2.0 equiv) and S (0.1–4.0 equiv). Dots are conversion values from single runs after 6 h of reaction time.](image)

On the other hand, the use of 1,2-divalent streptavidin caused the conversion maximum to move to 1.0 equiv. These values are in agreement with the number of active binding pockets and, hence, the possible matching combinations of starting materials. As expected, conversion remained low over the entire equivalent range studied when 1,3- and 1,4-streptavidins were employed.

4.3.6.4 Acylboronate loading studies

After having established the optimal loading of streptavidin, we moved to evaluate the effect of different equivalencies of acylboronate 39 in the reaction (Figure 4.20). As expected, increasing amounts of 39 resulted in the progressive acceleration of the process. The conversion plateau doubled from 1.0 to 2.0 equivalents of 39, and achieved a saturation point at ≥ 4.0 equiv.
Figure 4.20. Acylboronate 39 loading studies. Performed with 42 (5.0 mM), 39 (1.00–10.0 equiv) and S (0.50 equiv). Dots are conversion values from single runs.

4.3.6.5 Linker length studies

Following the route established for the synthesis of 42 and 39, we prepared starting material analogues bearing different spacers between the desthiobiotin ligands and the reactive groups (Figure 4.21).

Figure 4.21. Starting materials equipped with linkers of different lengths between the desthiobiotin moieties and the reactive groups.

We examined the performance of these materials in the presence of tetravalent streptavidin (Figure 4.22). Optimal performance was found for the use of 42 and 39 equipped with short polyethylene glycol (PEG) linker. The use of longer PEG chains (51, 54 and 52, 55) resulted in slightly diminished performance. Starting materials bearing a very short butyl spacer (53 and 56) showed no acceleration over the background rate.
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4.3.6.6 Influence of pH

To evaluate the influence of pH, we carried out the reaction of 42 (5.0 μM) and 39 (2.0 equiv) templated by tetravalent streptavidin (0.50 equiv) in aqueous buffers at different pH values (Figure 4.23). Consistent with our prior works on the ligation of O-acylhydroxylamines and potassium acyltrifluoroborates, we observed a significant decrease in reaction rate when increasing the pH from 6.0 to 7.0 or 7.4.

4.3.6.7 Influence of concentration

Experiments were conducted at different concentrations of starting material 42 but with exactly the same equivalencies of 39 (10.0 equiv) and tetravalent streptavidin (0.50 equiv) in aqueous citrate buffer pH 6.0 (Figure 4.24). We observed comparable initial reaction rates when the
reaction was carried out at 5.0 $\mu$M, 1.0 $\mu$M or 0.50 $\mu$M. In contrast, the reactions reached a higher conversion in shorter times when conducted at higher concentration.

These observations are in agreement with conversion to product initially dominated by first-order templation followed by non-templated background where the influence of concentration would have higher impact.

4.3.7 Isolation and evaluation of streptavidin association complexes with compound 42.

In an effort to confirm that the starting materials do bind to streptavidin, we proceeded to prepare and isolate the association complexes from hydroxylamine 42 with divalent streptavidin mutants. The association complexes were formed by adding 42 to a solution of “cis” (1,2-divalent) or “trans” (1,3- and 1,4-divalent) streptavidins in aqueous citrate buffer at pH 6.0. Following incubation for 5 min at rt, the complexes were purified by anion-exchange FPLC. The chromatogram peaks show UV-Vis peaks at 280 nm – corresponding to the protein backbone – and at 425 and 500 nm – arising from the coumarin and DABSYL moieties on compound 42 (Figure 4.25). These results show that 42 binds and co-elutes with either mutant regardless of the binding pocket disposition. To rule out non-specific binding, biotin was added prior to compound 42 to the protein solutions to block the binding pockets on streptavidin. This resulted in chromatogram peaks showing no absorption at 425 or 500 nm (details on Section 6.4).
Complex 57 contains an active binding pocket next to bound 42, so we expected it to form quantitative matching combinations. Following addition of 39 (2.0 equiv) to a solution of 56 (5.0 μM) in aqueous citrate buffer at pH 6.0, the formation of amide 32 was monitored by fluorescent over time (Figure 4.26). The corresponding conversion plot showed rapid formation of the amide product and good overall conversion. Addition of biotin to block the binding pockets again resulted in the decrease of initial rate and conversion. The reaction of KAT 1a containing no desthiobiotin with complex 57 gave lower conversion than the negative control experiment using biotin. These results provide evidence that productive binding of the acylboronate and hydroxylamine in adjacent streptavidin binding sites is responsible for the increase in the observed rate of amide formation.

Figure 4.26. Reaction of association complex 57 (5.0 μM) with 39 (2.0 equiv) or 1a (2.0 equiv) in the presence or absence of biotin (5.0 equiv) in aqueous citrate buffer pH 6.0. The conversion plots over time are shown on the right side.

4.3.8 Kinetics modelling

To rationalize the experimental kinetic plots obtained during the course of our investigations, we built a simplified model starting from the following premises. On one hand, the association of
reagents to the template is assumed to occur instantly and irreversibly relative to product formation. The implication is that, given a mixture of 42 and streptavidin, all 42 will be bound to the protein. As discussed in section 4.4.1, the association of 42 and 39 to streptavidin produces an array of combinations (Figure 4.27). These combinations can display the correct alignment of reactants (M, matching combinations) or display the reactants so that they are not spatially close to each other (X, mismatching combinations).

On the other hand, conversion to product is assumed to follow two main processes (Equation 4.1): (1) template-assisted formation form matching combinations (M) of 42 and 39 on streptavidin (the templation term) and (2) non-templated reaction of 42 and 39 (the background term). \( k_T \) and \( k_B \) are the rate constants corresponding to the templation and the background term respectively.

\[
- \frac{d ([M] + [X])}{dt} = \frac{d [32]}{dt} = k_T \cdot [M] + k_B \cdot [39] \cdot [X]
\]

\( k_T \) = first order rate constant of templated amide formation  
\( k_B \) = second order background rate constant

To simplify the subsequent integration of Equation 4.1, pseudo-first order conditions were assumed ([39] >> [42]). Integrated Equation 4.2 adopts a bi-exponential form where each exponential phase corresponds to either the templation or the background terms. The amplitude factor governing the templation term \( (F) \) is the fraction of initial matching complex M.
Equation 4.2.

\[
\% \text{conv} = \frac{100 \cdot [32]}{[42]_0} = F \cdot \left| 1 - e^{(-k_T \cdot t)} \right| + (100 - F) \cdot \left| 1 - e^{(-k_B \cdot [39] \cdot t)} \right|
\]

\(F\) = amplitude factor of template amide formation

To evaluate the validity of our model, the curves on Figure 4.24 – which were recorded under pseudo-first order conditions – along with a background reaction curve, were fitted using Equation 4.2. Among the multiple possible solutions, we picked the parameters which best explained the experimental results (Figure 4.28).

**Figure 4.28.** Conversion over time plots from Figure 4.21 showing the corresponding fitting curves (black thin lines) to Equation 4.2. The fitting parameters for each curve are shown below the graph.
4.3.9 Conclusions and outlook

The development of a traceless template-assisted amide ligation of monofluoro acylboronates and O-acylhydroxylamines was achieved by using the streptavidin-biotin system. The ligation takes place with concomitant release of the directing desthiobiotin-linker units and achieves high levels of conversion even at concentrations as low as 0.50 \( \mu \text{M} \). By expressing streptavidin mutants with different valencies, we demonstrated that the reaction is accelerated exclusively in the presence of tetravalent or 1,2-divalent streptavidins – the only mutants which possess the right orientation of active binding pockets. The use of starting materials having different spacers between desthiobiotin and the reactive group highlighted the requirement of a linker of optimal length to achieve maximum performance. The isolation of association complexes 57 and 58 was performed to showcase the ability of starting material 42 to bind to streptavidin regardless of the arrangement of binding pockets. Kinetics analysis and modelling was provided to explain experimental conversions.

The present system was designed to prove that concurrently traceless templation can provide substantial conversion enhancement in the ligation of two different fragments. This paves the way for a generic approach to overcome the inherent limitations in the rate of non-enzymatic conjugation reactions. For future implementations, this approach can be further applied in fields such as receptor-templated cell signaling, or chemoselective conjugations under dilute aqueous conditions. In this regard, the stability of monofluoro acylboronates could be a limiting factor for the late-stage decoration with more biochemically relevant structures. Analogous to the preparation of acylboronate 27, we synthesized acylboronate 59 having an aldehyde handle appended on the benzyol part along with the alkyne moiety present on the ligand scaffold (Scheme 4.12). This structure should offer a platform for the elaboration of more elaborated materials containing PEG chains, peptides or proteins to use on bioconjugation applications.


The system could be further applicable to other chemical reactions that fulfill the necessary requisites to be used as model processes. For example, the Wittig reaction between
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N-sulfonylimines and stabilized phosphonium ylides to yield alkenes occurs with no extra reagent needed. Both starting materials have structural sections – the N-sulfonyl and the triphenylphosphine groups – that are cleaved along with alkene formation, offering the possibility to decorate them desthiobiotin ligands (Figure 4.29).

Figure 4.29. Conceptual scheme of a streptavidin-assisted Wittig reaction of desthiobiotin-equipped N-sulfonylimines with phosphonium ylides.
Summary and Outlook
5.1 Summary

This dissertation began with the exploration of the amide-forming reaction of potassium acyltrifluoroborates (KATs) with in situ-formed N-chloroamines and N-chloroamides. The reaction operates with fast kinetics under acidic aqueous conditions. The studies demonstrated the broad substrate scope of this methodology, including tolerance to unprotected secondary amines. The acylation of primary amides in the presence of secondary amines is a remarkable example of chemoselectivity that would not be achieved by any other existing method in literature. This dissertation also included applications of the procedure to the labelling of natural products and BSA.

More elaborated studies on the crosslinking of four-arm PEGs equipped with KAT moieties to form hydrogels were conducted in Chapter 3. Rheology measurements showed that hydrogels could be prepared by employing appropriate amounts of a small molecule polyamine and the chlorinating agent. Furthermore, the gel formation event was shown to occur in the acidic pH range.

In Chapter 4, the special features of the reaction of monofluoro acylboronates with O-acylhydroxylamines was exploited to develop a template-assisted ligation method that could deliver the amide-ligated product in a traceless manner. Two templating systems were examined in detail: the cyclodextrin-adamantane host-guest pair and the streptavidin-biotin system. The former one did not provide promising preliminary results to support further insight, although additional experiments would be necessary to fully rule out the system as an alternative. The latter pair, streptavidin and biotin, feature fast and irreversible binding properties which proved to be an ideal choice for this project. Upon preliminary experiments using simplified reagents having desthiobiotin as the ligand and streptavidin as the molecular template, a more elaborated set of starting materials was designed and synthesized based on internal non-radiative fluorescence quenching to enable a real-time readout as the reaction proceeds. The ligation was remarkably accelerated in the presence of streptavidin over the background reaction, as supported by negative control experiments where biotin was added to block the template. The influence of a large variety of reaction conditions and streptavidin mutants was methodically studied. Finally, a mathematical model was proposed to explain the conversion over time achieved at different micromolar to sub-micromolar concentrations.
5.2 Outlook

5.2.1 Application of the KAT–chloramine ligation in the detection and analysis of naturally N-chlorinated proteins in cellulo

As a method of exquisite chemoselectivity and fast kinetics, the reaction of KAT reagents with in situ-formed $N$-chloramines stands out as a strong candidate to be employed for bioconjugation purposes. However, the use of chlorinating conditions precludes this protocol as an alternative to current methodologies, as byproducts are expected to occur from the side reaction of biomolecules with chlorinating agents.

On the other hand, the procedure could be suitable for use in a chemical biology context. Specifically, macrophages and white cells are known to generate HOCl among other reactive oxygen species (ROS) during endocytic processes as part of the innate immune system response to destroy invasive microorganisms or toxins. The process, known as respiratory burst, is responsible for extensive oxidation and modification of cell biomolecules, like amino acid residues in proteins. Among these modifications, lysine residues are thought to be N-chlorinated by HOCl; the corresponding $N$-chlorolysines could directly serve as oxidants or act as a $\text{Cl}^+$–transfer agents for tyrosine chlorination, activation of enzymes or induction of apoptosis, among other possibilities. Despite that there is a large body of knowledge about intracellular HOCl detection, detection and identification of N-chlorinated proteins has not been achieved.

Figure 5.1. Proposed workflow and rationale for the targeting and identification of putative N-chlorinated proteins involved in phagosome respiratory burst with KAT 13. LPS = lipopolysaccharide. POI = protein of interest.
In light of the remarkable selectivity towards N-chloramines displayed by KAT reagents, it would be conceivable to develop a KAT probe suitable for its use in the activity-based protein profiling (ABPP) probe for the downstream targeting and proteomics identification of the putative naturally occurring N-chlorinated proteins involved in these biochemical processes (Figure 5.1).

### 5.2.2 Traceless templated amide-forming ligation of proteins

The covalent, non-enzymatic ligation of large molecules such as proteins (> 10 kDa) remains a challenging task. Ligation reactions are required to be tolerant to potentially reactive residues including lysines and cysteines. In addition, the large molecular weight and often limited solubility precludes the preparation of solutions above micromolar concentrations. A near stoichiometric bimolecular reaction at 10 μM would require a rate constant \( k \approx 20 \text{ M}^{-1} \text{ s}^{-1} \) to achieve 90% conversion after 12 h. There are very few organic reactions that fulfill such chemoselectivity and fast kinetics requirements.

The use of template assistance palliates the need for kinetically fast reactions by increasing the effective local concentration of reaction partners. In Chapter 4, we demonstrated the efficiency of the traceless streptavidin-templated amide-forming ligation of acylboronates with O-carbamoylhydroxylamines. Upon careful design of reaction partners amenable to late-stage decoration with protein molecules, we anticipate that the system could be implemented in the ligation of large peptide fragments or whole proteins of biological or pharmacological interest with full control over the ligation sites (Figure 5.2).

**Figure 5.2.** Proposed approach to the synthesis of protein-protein conjugates using the traceless streptavidin-templated amide-forming reaction of acylboronates and O-carbamoylhydroxylamines.
5.2.3 Targeted drug delivery based on the in-situ assembly of active drugs assisted by target cell membrane receptors

The treatment of cancer often relies on the use of potent but toxic medicines that typically induce devastating side effects in patients.\textsuperscript{140} One strategy to address this issue is the targeted delivery of drugs into cancer tissues, which reduces the required dose and lowers the overall toxicity.\textsuperscript{141} Ligand-targeted drug delivery makes use of drug-ligand conjugates with affinity towards cancer cell receptors. By employing the appropriate cleavable linker, the drug can be released upon binding of the conjugate, delivering a higher local drug concentration around the tumour area. However, a problem arises from the use of already active drugs that can detach prematurely from the conjugate and cause harmful side effects.

One possible solution to this issue would be the in situ assembly of an active drug from inactive fragments in the locality of malign tumours. In this context, the traceless template-assisted ligation system described in Chapter 4 could pose a promising platform to develop prodrug-equipped reaction partners having affinity tags for cancer cell membrane receptors. The binding to the membrane receptor subsequently triggers the corresponding template-assisted amide-forming reaction that reconstitutes and releases a fully active drug directly on the tumour area (Figure 5.3).

\textbf{Figure 5.3.} Proximity-driven in-situ drug assembly and release triggered binding to cancer cell membrane receptors.
References
References


(55) These trifluoroborate iminium species were further isolated and characterized by our group, and disclosed in the literature. Shiro, T.; Schuhmacher, A.; Jackl, M. K.; Bode, J. W. Facile synthesis of $\alpha$-aminoboronic acids from amines and potassium acyltrifluoroborates (KATs) via trifluoroborate-iminiums (TIMs). Chem. Sci. 2018, 9, 5191–5196.


(60) See Chapter 1, Section 1.2.1 for details.


Experimental Section
7.1 General methods

All reactions were carried out in over-dried glassware and were stirred with Teflon-coated magnetic stir bars. Air- or moisture-sensitive reactions were conducted in dry glassware sealed with a rubber septum under N₂ atmosphere. Thin layer chromatography (TLC) was performed on glass backed plates pre-coated with silica gel (Merck, Silica Gel 60 F254) which were visualized by fluorescence quenching under UV light or by staining (using ninhydrin or KMnO₄ solutions). Flash column chromatography was performed on Silicycle Silica Flash F60 (230–400 Mesh) using a forced flow of air at 0.5–1.0 bar. HPLC (high performance liquid chromatography) was performed on JASCO analytical and preparative instruments. Analytical and preparative reverse-phase HPLC were performed using Shiseido C18 UG120 5 μm (4.6 mm I.D. × 250 mm) and YMC C18 (20 mm I.D. × 250 mm) columns. Unless otherwise noted, elution was carried out with flow rates 1.0 mL/min and 10 mL/min respectively using MilliQ-H₂O with 0.1% TFA (eluent A) and HPLC grade CH₃CN with 0.1% TFA (eluent B). LC-MS was performed either on a Dionex UltiMate 3000 RSLC connected to a Surveyor MSQ Plus mass spectrometer, or on a Waters H-class Acuity HPLC connected to a SQ detector.

NMR spectra were measured on VARIAN Mercury 300 MHz, 75 MHz, Bruker Avance 400 MHz, 100 MHz or Bruker AV-II 600 MHz, 150 MHz. Chemical shifts are expressed in parts per million (ppm) and are referenced to CDCl₃ 7.26 ppm, 77.0 ppm; acetone-d₆ 2.05 ppm, 29.8 and 206.3 ppm; DMSO-d₆ 2.50 ppm, 39.5 ppm; CD₃OD 3.31 ppm, 49.0 ppm. Coupling constants are reported as Hertz (Hz). Splitting patterns are indicated as follows: br, broad; br s, broad singlet; s, singlet; d, doublet; t, triplet; q, quartet; p, pentet; sx, sextet; sp, septet; dd, doublet of doublets; dt, doublet of triplets; td, triplet of doublets; qd, quartet of doublets; ddd, doublet of doublet of doublets; tdd, triplet of doublet of doublets; m, multiplet. Infrared (IR) spectra were recorded on a JASCO FT/IR-4100 spectrophotometer and are reported as wavenumber (cm⁻¹). Optical rotations were measured with a Jasco P-2000 polarimeter with a 100-mm path length cell operating at the sodium D line (589 nm), at 23-24 °C and reported as [α]D (concentration g/100 mL, solvent). High-resolution mass spectra were measured on a Bruker Daltonics maXis ESI– QTOF or Bruker solariX ESI/MALDI–FTICR by the mass spectrometry service of the Laboratorium für Organische Chemie at the ETH Zürich. UV-Vis spectra and DNA and protein concentration measurements were performed on a Thermo NanoDrop 2000c spectrophotometer. Rheology measurements were recorded on an Anton Paar MCR 301 instrument (5% amplitude, f = 1 Hz).
For protein analysis, sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was carried out in a Mini-PROTEAN Tetra Cell system (Bio-Rad) connected to a PowerPac Basic (BioRad) programmable power supply. Protein electrophoresis samples (2 – 5 µg protein) were treated with an equal volume of a sample buffer, Laemmli 2x Concentrate (Sigma) and heated to 95 °C for 5 minutes to denature the protein sample when necessary.1 Samples were loaded (typically a 10 µL loading volume) onto commercially available Mini-PROTEAN TGX Precast Protein Gels (Bio-Rad) with a gradient gel percentage of either 8 – 16% or 7.5%. A commercially available 10 – 180 kDa pre-stained protein ladder (Thermo Fisher) was typically applied to one well (5 µL) of each gel for the assignment of apparent molecular masses. Gels were run for 30 minutes at 200 V in a running buffer (25 mM Tris, 192 mM glycine, 0.1% (w/v) SDS, pH 8.3). Protein bands were subsequently visualized by incubating the gels in a staining solution (0.1% Coommasie Brilliant Blue R 250, 40% MeOH, 10% acetic acid) with gentle agitation for 1 hour. This was followed by a destaining protocol in which the gels were gently agitated in a destaining solution (40% MeOH, 10% acetic acid) for several hours, replenishing the solution every hour until the background of the gel became fully destained. All FPLC (Fast Protein Liquid Chromatography) protein purifications were performed on an ÄKTA pure chromatography system (GE Healthcare) using the UNICORN 6.3 Workstation system control software. All purifications were carried out at 4 °C. All buffers were freshly prepared, filtered and degassed immediately prior to use. Anion exchange chromatography purifications were performed using the strong ion exchange Mono Q 5/50 GL column (purchased from GE Healthcare). All protein purifications were monitored at 280 nm. Fluorescence was measured using a Tecan Infinite® 200 PRO plate reader. 96-Well plates were purchased from TPP. Measurements were typically performed with the plate cover on. Fluorescence was measured at $\lambda_{ex} = 430$ nm, $\lambda_{em} = 485$ nm.

In general, organic solvents were used as supplied (ACS or HPLC grade). CH$_2$Cl$_2$, EtOAc and hexanes were distilled prior to use. Reagents were purchased from Acros, Sigma-Aldrich, TCI, Fluka, Fluorochem or ABCR and used without further purification, with the following exceptions. NCS was recrystallized following the literature procedure. DCH was recrystallized from EtOAc/hexanes. TMSCl and N,O-bis(trimethylsilyl)trifluoroacetamide (68) were distilled over CaH$_2$. Amines 2k$^2$, 2n$^3$ and 2o$^2$, potassium acyltrifluoroborates 1a$^1$, 1b-e$^5$, 1f-h$^6$, 1j$^6$, 1k$^7$ and 12$^4$, 4-armed PEG-KAT 15$^8$ and compounds 23, 28$^{10}$, 34$^{11}$ and 67$^{12}$ were synthesized according to the corresponding literature procedures. Gentamicin C$_1$ was isolated from the commercial complex (purchased from Apollo, contains gentamicins C$_1$, C$_{1a}$ and C$_{2a/b}$) by following the reported procedure.$^{13}$ N-Chloromorpholine$^{14}$ and 1-chloro-2,2,6,6-tetramethylpiperidine$^{15}$ were prepared as previously reported.
7.2 Experimental procedures and characterization data for Chapter 2

Preparation of starting materials.

Preparation of 2-(6-(Diethylamino)-3-(diethyliminio)-3H-xanthen-9-yl)-5-(N-(2-(2-(2-(4-N-(2-phenylethyl)carbamoyl)benzamido)ethoxy)ethoxy)ethyl)sulfamoyl)benzenesulfonate (3v).

A round-bottom flask was charged with KAT 1g (130 mg, 0.30 mmol, 1.1 equiv) and 2-(6-(diethylamino)-3-(diethyliminio)-3H-xanthen-9-yl)-5-(N-(2-(2-aminoethoxy)ethoxy)ethyl)sulfamoyl)benzenesulfonate (60, 38.4 µL, 0.60 mmol, 1.2 equiv). DMF (1 mL) and aqueous buffer pH 9.2 (1.5 mL, 0.1 M, prepared by dissolving anhydrous K$_2$CO$_3$ (1.01 g, 7.31 mmol) and anhydrous KHCO$_3$ (9.27 g, 92.7 mmol) in distilled water (1 L), final pH was adjusted using 1 M NaOH and HCl solutions) were added and the solution stirred at rt for 6h. The solution was purified by preparative reverse phase HPLC (Shiseido C18 column 50 x 250 mm, flow = 40 mL/min, 20% A in 10 min, then 20-95% A/B in 40 min, monitoring at $\lambda = 301$ nm, $t_R = 31.6$ min). The fractions were pooled, neutralized with anhydrous KF (pH 5–6) and lyophilized. The residue was dissolved in MeOH, filtered and evaporated to give a magenta solid (161 mg, 62%) in > 90% purity (by NMR). The structure was confirmed after conversion to amide 3v, as described in general procedure A.

Preparation of potassium 4-(N-propargylcarbamoyl)benzoyl trifluoroborate 1l.
A round-bottom flask was charged with KAT 1g (213.0 mg, 0.50 mmol, 1.2 equiv) and propargylamine (38.4 μL, 0.60 mmol, 1.2 equiv). DMF (1 mL) and aqueous buffer pH 9.2 (2 mL, 0.1 M, prepared as described above for the synthesis of 1i) were added and the solution stirred at rt for 4h. The solution was evaporated until a yellowish oil remained in the flask. Addition of CH₂Cl₂ caused the product to precipitate. The suspension was filtered and the filter cake was redissolved in anhydrous acetone. The solution was filtered and evaporated to give 1l as a white solid (95.2 mg, 65%). m.p. >180 °C (decomp); ¹H-NMR (600 MHz, acetone-d₆): δ 8.37 (t, J = 5.3 Hz, 1H), 8.10 (d, J = 8.0 Hz, 2H), 7.94 (d, J = 8.4 Hz, 2H), 4.21 (dd, J = 5.6, 2.5 Hz, 2H), 2.67 (t, J = 2.5 Hz, 1H); ¹³C-NMR (151 MHz, acetone-d₆): δ 235.0 (br), 167.1, 143.9, 137.2, 129.1, 127.8, 81.5, 71.9, 29.5; ¹⁹F-NMR (470 MHz, acetone-d₆): δ −144.8; ¹¹B-NMR (160 MHz, acetone-d₆): δ −0.74; IR (thin film): ν 3296, 1677, 1634, 1536, 1206, 1133 cm⁻¹; ESI-HRMS calcd for C₁₁H₇BF₃NO₂ [M –K]⁻ 254.0606, found 254.0612.

Preparation of Fmoc-L-glutamine methyl ester 4b.

L-Glutamine methyl ester derivative 62 was prepared according to a reported procedure.¹⁶ To a round-bottom flask charged with Nα-Fmoc-Nα-Trt-L-glutamine (61, 3.05 g, 5.0 mmol, 1.0 equiv), TMSCl (3.2 mL, 25 mmol, 5.0 equiv) and MeOH (10 mL) were added sequentially and the mixture was stirred at rt for 3 h. The suspended solid formed during the reaction was filtered off, dissolved in EtOAc and washed with saturated aqueous NaHCO₃ to afford 62 (2.98 g, 95%), which was pure enough to be used in the next step.

Amide 4b was prepared by subjecting 62 to standard trityl deprotection conditions.¹⁷ To a round-bottom flask containing 62 (2.98 g, 4.77 mmol, 1.0 equiv) were added CH₂Cl₂ (30 mL), TIPS (2 mL, 10 mmol, 2.1 equiv) and TFA (10 mL). The solution was stirred at rt for 30 min and evaporated in vacuo. The crude material was purified by column chromatography on silica gel (CH₂Cl₂/MeOH = 100:5, 1% AcOH) to give 4b as a white solid (1.2 g, 66%). The spectral data was in accordance with the literature.¹⁸
Table 1. Screening of halogenating agents

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In a round bottom flask, 1a (23.0 mg, 0.10 mmol, 1.0 equiv) was dissolved in 1 mL of a 1:1 mixture of THF and aqueous buffer pH 3.0, 25 mM (prepared by dissolving citric acid monohydrate (4.48 g, 21.35 mmol) and trisodium citrate dihydrate (1.07 g, 3.64 mmol) in distilled water (1 L), final pH was adjusted using 0.1 M NaOH and HCl solutions). 2-Phenethylamine (2a, 13.6 μL, 0.11 mmol, 1.1 equiv) and halogenating agent (1.1 equiv) were added sequentially and the mixture stirred at rt for 30 to 90 min. The solution was quenched with saturated aqueous NaHSO₃ (0.3 mL), poured into H₂O/brine and extracted with EtOAc (3 x 3 mL). The organic extracts were collected, dried over Na₂SO₄, filtered and evaporated in vacuo. The reaction yield was measured by NMR analysis of the crude, using nitromethane (5.4 μL, 0.10 mmol) as internal standard.
Table 2. Screening of solvents and pH.

<table>
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</table>

In a round bottom flask, 1a (23.0 mg, 0.10 mmol, 1.0 equiv) was dissolved in 1 mL of a 1:1 mixture of organic solvent and aqueous buffer (pH values from 1.4 to 9). 2-Phenethylamine (2a, 13.6 μL, 0.11 mmol, 1.1 equiv) and DCH (21.7 mg, 0.11 mmol, 1.1 equiv) were added sequentially and the mixture allowed to stir at rt for 30 min. The solution was quenched with saturated aqueous NaHSO₃ (0.3 mL), poured into H₂O/brine and extracted with EtOAc (3 x 3 mL). The organic extracts were collected, dried over Na₂SO₄, filtered and evaporated in vacuo. The reaction yield was measured by NMR analysis of the crude, using nitromethane (5.4 μL, 0.10 mmol) as internal standard.
General procedure A: Synthesis of amides from aliphatic or aromatic amines

In a round-bottom flask, the corresponding potassium acyltrifluoroborate 1 (0.11 mmol, 1.1 equiv) was dissolved in 1 mL of a 1:1 mixture of THF and aqueous buffer pH 3.0, 25 mM (prepared by dissolving citric acid monohydrate (4.48 g, 21.35 mmol) and trisodium citrate dihydrate (1.07 g, 3.64 mmol) in distilled water (1 L), final pH was adjusted using 0.1 M NaOH and HCl solutions). Amine 2 (0.10 mmol, 1.0 equiv) and 1,3-dichloro-5,5-dimethylhydantoin (DCH, 21.7 mg, 0.11 mmol, 1.1 equiv) were added and the mixture stirred at rt. The solution was quenched with saturated aqueous NaHSO₃ (0.3 mL), poured into H₂O/brine and extracted with EtOAc (3 x 3 mL). The organic extracts were collected, dried over Na₂SO₄, filtered and evaporated in vacuo. Unless otherwise noted, the residue was purified by column chromatography on silica gel to afford the corresponding amide.

N-(2-Phenylethyl)-4-fluorobenzamide (3a).

Prepared by following general procedure A for 30 min with potassium 4-fluorobenzoyltrifluoroborate (1a, 25.6 mg, 0.11 mmol, 1.1 equiv) and 2-phenethylamine (2a, 11.6 μL, 0.10 mmol, 1.0 equiv). The crude material was purified by column chromatography on silica gel (eluting with EtOAc/hexanes = 1:3) to give 3a as a white solid (22.3 mg, 92%); m.p. 117-119 °C; ¹H-NMR (400 MHz, CDCl₃): δ 7.72 (dd, J = 8.8, 5.3 Hz, 2H), 7.38-7.34 (m, 2H), 7.30-7.25 (m, 3H), 7.1 (t, J = 8.6 Hz, 2H), 6.11 (br s, 1H), 3.74 (dt, J = 7.3, 6.3 Hz, 2H), 2.96 (t, J = 6.9 Hz, 2H); ¹³C-NMR (100 MHz, CDCl₃): δ 166.4, 164.7 (d, J = 251.6 Hz), 138.8, 130.8 (d, J = 3.1 Hz), 129.1 (d, J = 8.9 Hz), 128.8, 128.7, 126.7, 115.6 (d, J = 21.8 Hz), 41.2, 35.7; ¹⁹F-NMR (375 MHz, CDCl₃): δ −108.4; IR (thin film): ν 3348, 3229, 1770, 1735, 1643, 1355, 1216 cm⁻¹; ESI-HRMS calcd for C₁₅H₁₅FNO [M + H]⁺ 244.1132, found 244.1126.

N-(2,2,2-Trifluoroethyl)-4-fluorobenzamide (3b).

Prepared by following general procedure A for 30 min with potassium 4-fluorobenzoyltrifluoroborate (1a, 28.7 mg, 0.13 mmol, 1.3 equiv) and 2,2,2-trifluoroethamine hydrochloride (2b, 13.5 μL, 0.10 mmol, 1.0 equiv). The crude material was purified by column chromatography on silica gel (eluting with EtOAc/hexanes = 1:3) to give 3b as a white solid (16.3 mg, 74%); m.p. 98-100 °C; ¹H-
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**NMR** (400 MHz, CDCl$_3$): $\delta$ 7.84 (dd, $J = 8.8$, 5.2 Hz, 2H), 7.15 (td, $J = 8.5$, 1.3 Hz, 2H), 6.57 (br, 1H), 4.18-4.09 (m, 2H); $^{13}$C-NMR (100 MHz, CDCl$_3$): $\delta$ 166.6, 165.1 (d, $J = 253.3$ Hz), 129.6 (d, $J = 9.1$ Hz), 129.3 (d, $J = 3.2$ Hz), 124.1 (q, $J = 278.4$ Hz), 116.9 (d, $J = 22.0$ Hz), 41.1 (q, $J = 34.8$ Hz); $^{19}$F-NMR (375 MHz, CDCl$_3$): $\delta$ -72.3, -106.7; IR (thin film): $\nu$ 3317, 2923, 1653, 1543, 1505, 1267, 1159 cm$^{-1}$; ESI-HRMS calcd for C$_9$H$_8$F$_4$NO $[M + H]^+$ 222.0537, found 222.0531.

**N-(4-Fluorobenzoyl)-phenylalanine methyl ester (3c).**

Prepared by following general procedure A for 1 h with potassium 4-fluorobenzoyl trifluoroborate (1a, 25.6 mg, 0.11 mmol, 1.1 equiv) and L-phenylalanine methyl ester hydrochloride (2c, 21.6 mg, 0.10 mmol, 1.0 equiv). The crude material was purified by column chromatography on silica gel (eluting with EtOAc/hexanes = 1:2) to give 3c as a white solid (24.3 mg, 81%); m.p. 61-64 °C; $^{1}$H-NMR (400 MHz, CDCl$_3$): $\delta$ 7.75 (dd, $J = 8.8$, 5.2 Hz, 2H), 7.35-7.27 (m, 3H), 7.16-7.11 (m, 4H), 6.57 (br, 1H), 5.10 (dt, $J = 7.6$, 5.6 Hz, 1H), 3.78 (s, 3H), 3.32 (dd, $J = 13.9$, 5.8 Hz, 1H), 3.25 (dd, $J = 13.9$, 5.4 Hz, 1H); $^{13}$C NMR (100 MHz, CDCl$_3$): $\delta$ 172.0, 166.1, 165.0 (d, $J = 252.6$ Hz), 135.7, 129.9 (d, $J = 3.1$ Hz), 129.4 (d, $J = 9.0$ Hz), 129.3, 128.7, 127.3, 115.8 (d, $J = 22.0$ Hz), 53.6, 52.5, 37.8; $^{19}$F-NMR (375 MHz, CDCl$_3$): $\delta$ -107.2; IR (thin film): $\nu$ 3309, 3030, 2953, 1745, 1644, 1503, 1229, 1160 cm$^{-1}$; ESI-HRMS calcd for C$_{17}$H$_{17}$FNO$_3$ [M + H]$^+$ 302.1187, found 302.1187; $\left[\alpha\right]_D^{25}$: +89.4 ($c = 0.22$, CHCl$_3$).

**N-(4-Fluorobenzoyl)-serine methyl ester (3d).**

Prepared by following general procedure A for 1 h with potassium 4-fluorobenzoyl trifluoroborate (1a, 25.6 mg, 0.11 mmol, 1.1 equiv) and L-serine methyl ester hydrochloride (2d, 15.6 mg, 0.10 mmol, 1.0 equiv). The crude material was purified by column chromatography on silica gel (eluting with CH$_2$Cl$_2$/MeOH = 100:5) to give 3d as a white solid (19.3 mg, 80%); m.p. 97-99 °C; $^{1}$H-NMR (400 MHz, CDCl$_3$): $\delta$ 7.84 (dd, $J = 8.8$, 5.5 Hz, 2H), 7.12 (t, $J = 8.7$ Hz, 3H), 4.85 (dt, $J = 7.2$, 3.5 Hz, 1H), 4.09 (dd, $J = 11.2$, 3.8 Hz, 1H), 4.03 (ddd, $J = 11.2$, 3.4, 0.8 Hz, 1H), 3.82 (s, 3H), 2.82 (br, 1H); $^{13}$C-NMR (100 MHz, CDCl$_3$): $\delta$ 171.0, 166.6, 165.0 (d, $J = 252.8$ Hz), 129.7, 129.6 (d, $J = 9.0$ Hz), 115.7 (d, $J = 21.9$ Hz), 63.4, 55.2, 52.9; $^{19}$F-NMR (375 MHz, CDCl$_3$): $\delta$ -107.2; IR (thin film): $\nu$ 3363, 2924, 1741, 1645, 1504, 1230, 1162 cm$^{-1}$; ESI-HRMS calcd for C$_{11}$H$_{12}$FNNaO$_4$ [M + Na]$^+$ 264.0643, found 264.0644; $\left[\alpha\right]_D^{25}$: +39.6 ($c = 0.32$, CHCl$_3$).
2-(4-Fluorobenzamido)-2-deoxy-1,3,4,6-tetra-O-acetyl-α/β-D-glucopyranose (3e).

Prepared with potassium 4-fluorobenzoyltrifluoroborate (1a, 25.6 mg, 0.11 mmol, 1.1 equiv) and D-glucosamine hydrochloride (2e, 21.5 mg, 0.10 mmol, 1.0 equiv) by a modification of general procedure A. After the reaction was stirred for 1 h at rt and quenched, the aqueous phase containing the product was washed twice with EtOAc and lyophilized. The crude material was resuspended in acetone (5 mL) and Ac₂O (188 μL, 2.0 mmol, 20 equiv) and pyridine (162 μL, 2.0 mmol, 20 equiv) were added at rt. The mixture was stirred for 15 h, quenched with saturated aqueous NaHCO₃ and extracted with EtOAc (3 x 3 mL). The organic extracts were collected, dried over Na₂SO₄, filtered and evaporated in vacuo. The residue was purified by column chromatography on silica gel (eluting with EtOAc/hexanes = 1:1) to give 3e as a white solid (31.1 mg, 66% (over 2 steps), mixture of α and β isomers (α/β ~2:1 ratio, determined by NMR analysis of the reaction crude)); α-3e: m.p. 107-110 °C; ¹H-NMR (400 MHz, CDCl₃): δ 7.73 (dd, J = 8.8, 5.2 Hz, 2H), 7.14 (t, J = 8.5 Hz, 2H), 6.35 (d, J = 3.7 Hz, 1H), 6.33 (d, J = 8.6 Hz, 1H), 5.42-5.29 (m, 2H), 4.63 (dd, J = 10.7, 8.5, 3.7 Hz, 1H), 4.31 (dd, J = 12.6, 4.2 Hz, 1H), 4.12 (dd, J = 12.5, 2.2 Hz, 1H), 4.06 (dd, J = 9.9, 4.1, 2.4 Hz, 1H), 2.19 (s, 3H), 2.13 (s, 3H), 2.10 (s, 3H), 2.07 (s, 3H); ¹³C-NMR (100 MHz, CDCl₃): δ 172.4, 170.8, 169.1, 168.6, 166.1, 165.1 (d, J = 253.1 Hz), 129.5, 129.3 (d, J = 9.1 Hz), 115.9 (d, J = 22.0 Hz), 90.5, 70.8, 69.8, 67.3, 61.6, 52.1, 20.9, 20.8, 20.7, 20.6; ¹⁹F-NMR (375 MHz, CDCl₃): δ -106.7; IR (thin film): ν 3342, 2962, 1748, 1653, 1503 cm⁻¹; ESI-HRMS calcd for C₂₁H₂₄FNNaO₁₀ [M + Na]⁺ 492.1267, found 492.1274; [α]D²⁵: +87.6 (c = 0.69, CHCl₃).

β-3e: m.p. 194-196 °C; ¹H-NMR (400 MHz, CDCl₃): δ 7.73 (dd, J = 8.9, 5.2 Hz, 2H), 7.09 (t, J = 8.6 Hz, 2H), 6.29 (d, J = 9.2 Hz, 1H), 5.80 (d, J = 8.7 Hz, 1H), 5.32-5.22 (m, 2H), 4.54 (dt, J = 10.1, 9.0 Hz, 1H), 4.30 (dd, J = 12.4, 4.7 Hz, 1H), 4.16 (dd, J = 12.4, 2.3 Hz, 1H), 3.88 (ddd, J = 9.6, 4.6, 2.2 Hz, 1H), 2.11 (s, 3H), 2.07 (s, 3H), 2.06 (s, 3H), 1.99 (s, 3H); ¹³C-NMR (100 MHz, CDCl₃): δ 171.5, 170.7, 169.6, 169.2, 166.2, 165.0 (d, J = 253.0 Hz), 129.7, 129.3 (d, J = 3.2 Hz), 129.3 (d, J = 9.1 Hz), 115.9 (d, J = 22.0 Hz), 92.9, 73.2, 72.7, 67.6, 61.7, 53.6, 20.9, 20.7, 20.6, 20.6; ¹⁹F-NMR (375 MHz, CDCl₃): δ -107.1; IR (thin film): ν 3341, 2958, 1748, 1659, 1503, 1225 cm⁻¹; ESI-HRMS calcd for C₂₁H₂₄FNNaO₁₀ [M + Na]⁺ 492.1267, found 492.1273; [α]D²⁵: +50.9 (c = 0.29, CHCl₃).
**N-(2-(4-Hydroxyphenyl)ethyl)-4-fluorobenzamide (3f).**

Prepared by following general procedure A for 30 min using potassium 4-fluorobenzoyltrifluoroborate (1a, 25.6 mg, 0.11 mmol, 1.1 equiv) and tyramine (2f, 13.7 mg, 0.10 mmol, 1.0 equiv). The crude material was purified by column chromatography on silica gel (eluting with CH$_2$Cl$_2$/acetone = 10:1, 0.5% AcOH) to give 3f as a white solid (18.1 mg, 70%); m.p. 136-140 °C; $^1$H-NMR (400 MHz, DMSO-d$_6$): $\delta$ 9.18 (s, 1H), 8.55 (t, $J$ = 5.6 Hz, 1H), 7.89 (dd, $J$ = 8.7, 5.6 Hz, 2H), 7.29 (t, $J$ = 8.9 Hz, 2H), 7.03 (d, $J$ = 8.4 Hz, 2H), 6.69 (d, $J$ = 8.4 Hz, 2H), 3.44-3.39 (m, 2H), 2.73 (dd, $J$ = 8.4, 6.6 Hz, 2H); $^{13}$C-NMR (100 MHz, DMSO-d$_6$): $\delta$ 165.0, 164.2 (d, $J$ = 248.2 Hz), 155.6, 131.6 (d, $J$ = 2.9 Hz), 130.2 (d, $J$ = 9.0 Hz), 129.5, 129.5, 115.6 (d, $J$ = 21.6 Hz), 115.1, 41.3, 34.3; $^{19}$F-NMR (375 MHz, DMSO-d$_6$): $\delta$ −109.8; IR (thin film): $\nu$ 3285, 2927, 1639, 1503, 1235, 1159 cm$^{-1}$; ESI-HRMS calcd for C$_{15}$H$_{14}$FNNaO$_2$ [M + Na]$^+$ 282.0901, found 282.0903.

**N-(2-Oxo-2-phenylethyl)-4-fluorobenzamide (3g).**

Prepared by following general procedure A for 30 min with potassium 4-fluorobenzoyltrifluoroborate (1a, 25.6 mg, 0.11 mmol, 1.1 equiv) and 2-aminoacetophenone hydrochloride (2g, 17.2 mg, 0.10 mmol, 1.0 equiv). The crude material was purified by column chromatography on silica gel (eluting with EtOAc/hexanes = 1:2) to give 3g as a white solid (24.5 mg, 95%); m.p. 122-126 °C; $^1$H-NMR (400 MHz, CDCl$_3$): $\delta$ 8.05 (dt, $J$ = 8.3, 1.1 Hz, 2H), 7.92 (dd, $J$ = 8.8, 5.2 Hz, 2H), 7.67 (t, $J$ = 7.5 Hz, 1H), 7.55 (t, $J$ = 7.4 Hz, 2H), 7.32 (br s, 1H), 7.16 (t, $J$ = 8.6 Hz, 2H), 4.97 (dd, $J$ = 4.2, 0.7 Hz, 2H); $^{13}$C-NMR (100 MHz, CDCl$_3$): $\delta$ 196.3, 166.4, 164.9 (d, $J$ = 252.2 Hz), 134.3, 134.3, 130.0 (d, $J$ = 3.2 Hz), 129.5 (d, $J$ = 9.0 Hz), 129.0, 128.0, 115.7 (d, $J$ = 21.9 Hz), 46.9; $^{19}$F-NMR (375 MHz, CDCl$_3$): $\delta$ −107.7; IR (thin film): $\nu$ 3363, 1690, 1635, 1498, 1364, 1224 cm$^{-1}$; ESI-HRMS calcd for C$_{15}$H$_{13}$FNO$_2$ [M + H]$^+$ 258.0925, found 258.0740.

**4-(4-Fluorobenzamido)methylbenzoic acid (3h).**

Prepared by following general procedure A for 1 h using potassium 4-fluorobenzoyltrifluoroborate (1a, 25.6 mg, 0.11 mmol, 1.1 equiv) and 4-(aminomethyl)benzoic acid (2h, 15.1 mg, 0.10 mmol, 1.0 equiv). The crude material was purified by column chromatography on silica gel (eluting with CH$_2$Cl$_2$/MeOH = 100:5, 1% AcOH) to give 3h as a white solid (21.3 mg, 78%) in >90% purity by NMR. For characterization purposes, the product was further purified by preparative reverse-phase HPLC (YMC C18 column, A/B eluent gradient: 20-95% for 28 min, $t_R$ = 15.7 min); m.p. 220-224 °C; $^1$H-NMR (400 MHz, DMSO-d$_6$): $\delta$ 12.86 (br s, 1H), 9.15 (t, $J$ = 6.0 Hz, 1H), 7.99 (dd, $J$ = 8.9, 5.5 Hz, 2H), 7.92 (d, $J$ = 8.3 Hz, 2H), 7.43 (d, $J$ = 8.3 Hz, 2H), 7.33
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(t, J = 8.9 Hz, 2H), 4.55 (d, J = 5.9 Hz, 2H); $^{13}$C-NMR (100 MHz, DMSO-d$_6$): $\delta$ 167.6, 165.7, 164.4 
(d, J = 248.6 Hz), 145.2, 131.1 (d, J = 2.9 Hz), 130.4 (d, J = 9.0 Hz), 129.9, 129.8, 127.6, 115.8 (d, 
J = 21.8 Hz), 43.0; $^{19}$F-NMR (375 MHz, DMSO-d$_6$): $\delta$ -109.3; IR (thin film): $\nu$ 3419, 3324, 1682, 1636, 
1540, 1504, 1288 cm$^{-1}$; ESI-HRMS calcd for C$_{15}$H$_{13}$FNO$_3$ [M + H]$^+$ 274.0874, found 274.0877.

$\text{N-(2-Dimethylaminoethyl)-4-fluorobenzamide (3i).}$

Prepared with potassium 4-fluorobenzoyltrifluoroborate (1a, 23 mg, 0.10 mmol, 1.0 equiv), 
$N,N$-dimethylethylenediamine (2i, 13.1 $\mu$L, 0.12 mmol, 1.2 equiv). Following addition of NaHSO$_3$, 
the reaction crude was poured into 1 M NaOH, extracted with CH$_2$Cl$_2$, washed with H$_2$O and brine, 
dried over Na$_2$SO$_4$, filtered and evaporated in vacuo. The residue was purified by column chromatography on silica gel (eluting with CH$_2$Cl$_2$/MeOH = 100:5, +1% NEt$_3$) to give 3i as a yellow oil (14.8 mg, 70%); $^1$H-NMR 
(400 MHz, acetone-d$_6$): $\delta$ 7.95 (dd, J = 8.7, 5.6 Hz, 2H), 7.70 (br s, 1H), 7.20 (t, J = 8.7 Hz, 2H), 3.48 
(q, J = 6.3 Hz, 2H), 2.50 (t, J = 6.5 Hz, 2H), 2.24 (s, 6H); $^{13}$C-NMR (100 MHz, acetone-d$_6$): $\delta$ 165.3, 164.4 
(d, J = 251.7 Hz), 131.5 (d, J = 3.0 Hz), 129.6 (d, J = 8.9 Hz), 115.0 (d, J = 21.9 Hz), 58.2, 44.7, 37.4; $^{19}$F-NMR 
(282 MHz, acetone-d$_6$): $\delta$ -111.4; IR (thin film): $\nu$ 3314, 2945, 2773, 1639, 1547, 1502, 1231, 1159 cm$^{-1}$; ESI-HRMS calcd for C$_{11}$H$_{16}$FN$_2$O [M + H]$^+$ 211.1241, found 211.1243.

$\text{N-(Piperidin-4-ylmethyl)-4-fluorobenzamide (3j).}$

Prepared with potassium 4-fluorobenzoyltrifluoroborate (1a, 29.7 mg, 0.129 mmol, 1.1 equiv), 4-(aminomethyl)piperidine (2j 13.4 mg, 0.117 mmol, 1.0 equiv) and NCS (31.3 mg, 0.234 mmol, 2.0 equiv) by a slight modification of general procedure A. After 30 min stirring at rt, the reaction was quenched with saturated aqueous NaHSO$_3$ (0.5 mL), poured into 1 M NaOH and extracted with EtOAc (3 x 3 mL). The aqueous extracts were collected, basified with 5 M NaOH until pH >12, extracted again with CH$_2$Cl$_2$, washed with H$_2$O and brine, dried over Na$_2$SO$_4$, filtered and evaporated in vacuo to give 3j as a yellowish solid (16.8 mg, 61%); m.p. 107-109 °C; $^1$H-NMR (400 MHz, CDCl$_3$): $\delta$ 7.76 (dd, J = 8.8, 5.3 Hz, 2H), 7.08 (t, J = 8.6 Hz, 2H), 6.33 (s, 1H), 3.31 (t, J = 6.2 Hz, 2H), 3.08 (d, J = 12.6 Hz, 2H), 2.58 (td, J = 12.2, 2.4 Hz, 2H), 1.93 (br, 1H), 1.77-1.67 (m, 3H), 1.27-1.14 (m, 2H); $^{13}$C-NMR (100 MHz, CDCl$_3$): $\delta$ 166.7, 164.7 (d, J = 251.7 Hz), 131.0 (d, J = 3.1 Hz), 129.3 (d, J = 8.9 Hz), 115.7 (d, J = 21.9 Hz), 46.4, 46.1, 36.7, 31.2; $^{19}$F-NMR (375 MHz, CDCl$_3$): $\delta$ -108.4; IR (thin film): $\nu$ 3298, 2924, 1640, 1551, 1504, 1319, 1233, 1159 cm$^{-1}$; ESI-HRMS calcd for C$_{13}$H$_{18}$FN$_2$O [M + H]$^+$ 237.1398, found 237.1402.
**N-(2-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)ethyl)-4-fluorobenzamide (3k).**

Prepared by following general procedure A for 45 min with potassium 4-fluorobenzoyle trifluoroborate (1a, 23.0 mg, 0.10 mmol, 1.0 equiv) and 1-(2-aminoethyl)maleimide trifluoroacetic acid salt (2k, 30.5 mg, 0.12 mmol, 1.2 equiv). The crude material was purified by column chromatography on silica gel (eluting with CH$_2$Cl$_2$/MeOH = 100:5 + 0.5% AcOH), and recrystallized from EtOAc/hexanes (1:2), filtered and dried in vacuo to give 3k as a white solid (13.1 mg, 51%); m.p. 170-172 °C; $^1$H-NMR (400 MHz, CDCl$_3$): $\delta$ 7.77 (dd, $J$ = 8.8, 5.3 Hz, 2H), 7.10 (t, $J$ = 8.6 Hz, 2H), 6.74 (s, 2H), 6.66 (br s, 1H), 3.85-3.82 (m, 2H), 3.67-3.63 (m, 2H); $^{13}$C-NMR (100 MHz, CDCl$_3$): $\delta$ 171.1, 166.6, 164.8 (d, $J$ = 251.8 Hz), 134.3, 130.2 (d, $J$ = 3.0 Hz), 129.3 (d, $J$ = 8.9 Hz), 115.6 (d, $J$ = 21.9 Hz), 40.1, 37.5; $^{19}$F-NMR (375 MHz, CDCl$_3$): $\delta$ -108.1; IR (thin film): $\nu$ 3335, 3109, 2941, 1707, 1635, 1504, 1405, 1234 cm$^{-1}$; ESI-HRMS calcd for C$_{13}$H$_{12}$FN$_2$O$_3$ [M + H]$^+$ 263.0826, found 263.0829.

**N-(4-Nitrophenyl)-4-fluorobenzamide (3l).**

Prepared by following general procedure A for 1 h with potassium 4-fluorobenzoyle trifluoroborate (1a, 23.0 mg, 0.10 mmol, 1.0 equiv), 4-nitroaniline (2l, 15.2 mg, 0.11 mmol, 1.1 equiv), and 1,3,5-trichloroisocyanuric acid (TCCA, 8.4 mg, 0.036 mmol, 0.36 equiv) as a replacement for DCH. The crude material was purified by column chromatography on silica gel (eluting with EtOAc/CH$_2$Cl$_2$/hexanes = 1:1:3) to give 3l as a pale-yellow solid in >90% purity by NMR (20.8 mg, 80%). For characterization purposes, 3l was further purified by preparative reverse-phase HPLC (YMC C18 column, gradient of 40-95% MeCN/H$_2$O + 0.1% TFA for 28 min, $t_R$ = 15.4 min). The spectral data was in accordance with the literature; $^1$H-NMR (400 MHz, CDCl$_3$): $\delta$ 8.26 (d, $J$ = 9.2 Hz, 2H), 8.07 (br s, 1H), 7.91 (dd, $J$ = 8.8, 5.1 Hz, 2H), 7.83 (d, $J$ = 9.2 Hz, 2H), 7.20 (t, $J$ = 8.6 Hz, 2H); $^{13}$C-NMR (100 MHz, CDCl$_3$): $\delta$ 165.3 (d, $J$ = 254.4 Hz), 164.7, 143.8, 143.6, 130.1 (d, $J$ = 3.2 Hz), 129.6 (d, $J$ = 9.1 Hz), 125.2, 119.5, 116.2 (d, $J$ = 22.0 Hz); $^{19}$F-NMR (375 MHz, CDCl$_3$): $\delta$ -105.7.
N-(2-Pyridyl)-4-fluorobenzamide (3m).

Prepared by following general procedure A for 1 h with potassium 4-fluorobenzoyltrifluoroborate (1a, 23.0 mg, 0.10 mmol, 1.0 equiv) and 2-aminopyridine (2m, 25.2 mg, 0.11 mmol, 1.1 equiv). The crude material was purified by column chromatography on silica gel (eluting with EtOAc/hexanes = 2:5) to give 3m as a pale yellow solid (15.1 mg, 70%); m.p. 115-117 °C; $^1$H-NMR (400 MHz, CDCl$_3$): δ 8.76 (br, 1H), 8.36 (d, $J$ = 8.4 Hz, 1H), 8.24 (br s, 1H), 7.97 (dd, $J$ = 8.8, 5.2 Hz, 2H), 7.76 (t, $J$ = 8.3 Hz, 1H), 7.17 (t, $J$ = 8.7 Hz, 2H), 7.09-7.05 (m, 1H); $^{13}$C-NMR (100 MHz, CDCl$_3$): δ 165.0 (d, $J$ = 253.4 Hz), 164.7, 151.5, 147.9, 138.5, 130.5 (br), 129.7 (d, $J$ = 9.1 Hz), 120.1, 116.0 (d, $J$ = 22.0 Hz), 114.2; $^{19}$F-NMR (375 MHz, CDCl$_3$): δ −106.7; IR (thin film): ν 3243, 3070, 2924, 1676, 1504, 1433, 1310, 1231 cm$^{-1}$; ESI-HRMS calcd for C$_{12}$H$_{10}$FN$_2$O [M + H]$^+$ 217.0772, found 217.0775.

1-(6-(4-Fluorobenzamido)-9H-purin-9-yl)-1-deoxy-2,3,5-tri-O-acetyladenosine trifluoroacetate salt (3n).

Prepared with potassium 4-fluorobenzoyltrifluoroborate (1a, 25.6 mg, 0.11 mmol, 1.1 equiv), adenosine (2n, 26.7 mg, 0.10 mmol, 1.0 equiv) and 1,3,5-trichloroisocyanuric acid (TCCA, 9.4 mg, 0.04 mmol, 0.4 equiv) as a replacement for DCH by a modification of general procedure A. After the reaction was stirred for 2 h at rt and quenched, THF was evaporated in vacuo and the aqueous phase containing the product was lyophilized. The crude material was resuspended in acetone (5 mL), filtered and the solvent evaporated to reduce the volume to ca. 1 mL. Ac$_2$O (500 μL, 5.3 mmol, 53 equiv) and pyridine (250 μL, 1.6 mmol, 16 equiv) were added at rt. The mixture was stirred for 15 h, quenched with saturated aqueous NaHCO$_3$, extracted with EtOAc, dried over Na$_2$SO$_4$ and evaporated in vacuo. The residue was purified by preparative reverse-phase HPLC (YMC C18 column, gradient of 20-95% MeCN/H$_2$O + 0.1% TFA for 28 min, t$_{R}$ = 16.5 min) to give 3n as a viscous, sticky oil [28.3 mg, 45% (over 2 steps)]; $^1$H-NMR (400 MHz, CDCl$_3$): δ 9.26 (br, 2H), 8.76 (s, 1H), 8.36 (s, 1H), 8.09 (dd, $J$ = 8.8, 5.2 Hz, 2H), 7.20 (t, $J$ = 8.6 Hz, 2H), 6.28 (d, $J$ = 5.4 Hz, 1H), 5.92 (t, $J$ = 5.4 Hz, 1H), 5.62 (t, $J$ = 5.0 Hz, 1H), 4.49 (q, $J$ = 10.7, 8.5, 3.7 Hz, 1H), 4.46-4.38 (m, 2H), 2.16 (s, 3H), 2.13 (s, 3H), 2.08 (s, 3H); $^{13}$C-NMR (100 MHz, CDCl$_3$): δ 170.3, 169.6, 169.4, 165.8 (d, $J$ = 255.1 Hz), 164.2, 161.6 (q, $J$ = 40.1 Hz), 152.0, 151.3, 149.4, 142.0, 131.0 (d, $J$ = 9.4 Hz), 128.8 (d, $J$ = 3.0 Hz), 123.3, 116.0 (d, $J$ = 22.2 Hz), 115.2 (q, $J$ = 287.6 Hz), 86.6, 80.7, 73.2, 70.6, 62.9, 20.7, 20.5, 20.3; $^{19}$F-NMR (375 MHz, CDCl$_3$): δ −76.0, −104.8; IR (thin film): ν 3113, 1748, 1603, 1504, 1373, 1223 cm$^{-1}$; ESI-HRMS calcd for C$_{23}$H$_{23}$FN$_5$O$_8$ [M + H]$^+$ 516.1525, found 516.1520; $[\alpha]_D$: −22.8 (c = 1.25, CHCl$_3$).
**N-(2-Phenylethyl)-4-methoxybenzamide (3o)**\(^{20}\).

![Chemical Structure of 3o](image)

Prepared by following general procedure A for 30 min with potassium 4-methoxybenzoyltrifluoroborate (1b, 24.2 mg, 0.10 mmol, 1.0 equiv) and 2-phenethylamine (2a, 13.8 µL, 0.11 mmol, 1.1 equiv). The crude material was purified by column chromatography on silica gel (eluting with EtOAc/hexanes = 1:2) to give 3o as a white solid (21.4 mg, 84%). The spectral data was in accordance with the literature; \(^1\)H-NMR (400 MHz, CDCl\(_3\)): \(\delta\) 7.69 (d, \(J = 8.8\) Hz, 2H), 7.35 (t, \(J = 7.3\) Hz, 3H), 7.27 (t, \(J = 7.3\) Hz, 3H), 6.92 (d, \(J = 8.8\) Hz, 2H), 6.13 (br, 1H), 3.86 (s, 3H), 3.73 (q, \(J = 6.6\) Hz, 2H), 2.95 (t, \(J = 6.9\) Hz, 2H); \(^{13}\)C-NMR (100 MHz, CDCl\(_3\)): \(\delta\) 167.0, 162.1, 139.0, 128.7, 128.6, 126.9, 126.6, 113.7, 55.4, 41.1, 35.8.

**N-(2-Phenylethyl)-2-picolinamide (3p)**\(^{21}\).

![Chemical Structure of 3p](image)

Prepared by following general procedure A for 1 h with potassium 2-picolinoyltrifluoroborate (1c, 21.3 mg, 0.10 mmol, 1.0 equiv) and 2-phenethylamine (2a, 13.8 µL, 0.11 mmol, 1.1 equiv). The crude material was purified by column chromatography on silica gel (eluting with EtOAc/hexanes = 2:3) to give 3p as an oily solid (14.6 mg, 64%). The spectral data was in accordance with the literature; \(^1\)H-NMR (400 MHz, CDCl\(_3\)): \(\delta\) 8.52 (ddd, \(J = 4.7, 1.7, 0.9\) Hz, 1H), 8.20 (dt, \(J = 7.9, 1.1\) Hz, 1H), 8.16 (br s, 1H), 7.84 (td, \(J = 7.7, 1.7\) Hz, 1H), 7.41 (ddd, \(J = 7.6, 4.8, 1.2\) Hz, 1H), 7.34-7.29 (m, 1H), 7.28-7.21 (m, 4H), 3.74 (q, \(J = 7.2\) Hz, 2H), 2.95 (t, \(J = 7.3\) Hz, 2H); \(^{13}\)C-NMR (100 MHz, CDCl\(_3\)): \(\delta\) 164.3, 149.9, 148.0, 139.0, 137.3, 128.8, 128.6, 126.5, 126.2, 122.2, 40.8, 36.0.

**N-(2-Phenylethyl)-3-furamide (3q).**

![Chemical Structure of 3q](image)

Prepared by following general procedure A for 1 h with potassium 3-furoyltrifluoroborate (1d, 20.2 mg, 0.10 mmol, 1.0 equiv) and 2-phenethylamine (2a, 13.8 µL, 0.11 mmol, 1.1 equiv). The crude material was purified by column chromatography on silica gel (eluting with EtOAc/hexanes = 1:2) to give 3q as an oily solid (14.6 mg, 64%); m.p. 87-89 °C; \(^1\)H-NMR (400 MHz, CDCl\(_3\)): \(\delta\) 7.86 (dd, \(J = 0.9, 0.6\) Hz, 1H), 7.40 (t, \(J = 1.8\) Hz, 1H), 7.32 (t, \(J = 7.3\) Hz, 2H), 7.27-7.21 (m, 3H), 6.53 (m, 1H), 5.87 (br s, 1H), 3.65 (q, \(J = 6.3\) Hz, 2H), 2.90 (t, \(J = 6.9\) Hz, 2H); \(^{13}\)C-NMR (100 MHz, CDCl\(_3\)): \(\delta\) 162.6, 144.5, 143.7, 138.8, 128.8, 128.7, 126.6, 122.6, 108.2, 40.6, 35.7; IR (thin film): \(\nu\) 3317, 2926, 1633, 1537, 1327, 1196, 1160 cm\(^{-1}\); ESI-HRMS calcd for C\(_{13}\)H\(_{14}\)NO\(_2\) [M + H]\(^+\) 216.1019, found 216.1020.
N-(2-Phenylethyl)-4-phenylbutanamide (3r)\textsuperscript{22}.

Prepared by following general procedure A for 30 min with potassium 4-phenylbutanoyltrifluoroborate (1e, 25.4 mg, 0.10 mmol, 1.0 equiv) and 2-phenethylamine (2a, 13.8 µL, 0.11 mmol, 1.1 equiv). The crude material was purified by column chromatography on silica gel (eluting with EtOAc/hexanes = 1:3) to give 3r as a white solid (23.5 mg, 88%). The spectral data was in accordance with the literature; \textit{\textsuperscript{1}H-NMR} (400 MHz, CDCl\textsubscript{3}): \(\delta\) 7.36-7.16 (m, 10H), 5.43 (br s, 1H), 3.55 (q, \(J = 6.9\) Hz, 2H), 2.84 (t, \(J = 6.9\) Hz, 2H), 2.65 (t, \(J = 7.5\) Hz, 2H), 2.15 (t, \(J = 7.2\) Hz, 2H), 1.97 (p, \(J = 7.5\) Hz, 2H); \textit{\textsuperscript{13}C-NMR} (100 MHz, CDCl\textsubscript{3}): \(\delta\) 172.6, 141.5, 138.9, 128.8, 128.7, 128.5, 128.4, 126.5, 125.9, 40.5, 35.9, 35.7, 35.1, 27.1.

N-(2-Phenylethyl)-4-formylbenzamide (3s).

Prepared by following general procedure A for 50 min with potassium 4-formylbenzoyltrifluoroborate (1f, 24.0 mg, 0.10 mmol, 1.0 equiv) and 2-phenethylamine (2a, 13.8 µL, 0.11 mmol, 1.1 equiv). The crude material was purified by column chromatography on silica gel (eluting with EtOAc/hexanes = 2:3) to give 3s as a white solid (13.6 mg, 54%); m.p. 102-104 °C; \textit{\textsuperscript{1}H-NMR} (400 MHz, CDCl\textsubscript{3}): \(\delta\) 10.07 (s, 1H), 7.93 (d, \(J = 8.2\) Hz, 2H), 7.85 (d, \(J = 8.2\) Hz, 2H), 7.33 (t, \(J = 7.1\) Hz, 2H), 7.28-7.22 (m, 3H), 6.29 (br s, 1H), 3.76 (q, \(J = 6.7\) Hz, 2H), 2.98 (t, \(J = 6.9\) Hz, 2H); \textit{\textsuperscript{13}C-NMR} (100 MHz, CDCl\textsubscript{3}): \(\delta\) 191.5, 166.4, 139.8, 138.6, 138.2, 129.8, 128.8, 128.8, 127.5, 126.7, 41.3, 35.6; IR (thin film): \(\nu\) 3316, 2925, 1705, 1638, 1543, 1317, 1205 cm\(^{-1}\); ESI-HRMS calcd for C\textsubscript{16}H\textsubscript{15}FNNaO\textsubscript{2} [M + H]\(^+\) 276.0995, found 276.1000.

Pentafluorophenyl 4-(N-(2-phenylethyl)carbamoyl)benzoate (3t).

Prepared by following general procedure A for 50 min with potassium 4-(pentafluorophenoxycarbonyl)benzoyltrifluoroborate (1g, 42.2 mg, 0.10 mmol, 1.0 equiv) and 2-phenethylamine (2a, 13.8 µL, 0.11 mmol, 1.1 equiv). The crude material was purified by column chromatography on silica gel (eluting with EtOAc/hexanes = 1:3) to give 3t as a crystalline solid (15.6 mg, 36%); m.p. 150-152 °C; \textit{\textsuperscript{1}H-NMR} (600 MHz, CDCl\textsubscript{3}): \(\delta\) 7.94 (dd, \(J = 8.9, 5.1\) Hz, 2H), 7.35 (br s, 1H), 7.20 (t, \(J = 8.5\) Hz, 2H); \textit{\textsuperscript{13}C-NMR} (151 MHz, CDCl\textsubscript{3}): \(\delta\) 166.1, 161.8, 141.3 (\(\text{\textsuperscript{19}F}\)), 140.2, 139.7 (\(\text{\textsuperscript{19}F}\)), 138.6, 137.9 (\(\text{\textsuperscript{19}F}\)), 131.0, 129.4, 128.8, 128.8, 127.3, 126.8, 125.1 (\(\text{\textsuperscript{19}F}\)), 41.3, 35.6; \textit{\textsuperscript{19}F-NMR} (375 MHz, CDCl\textsubscript{3}): \(\delta\) -152.3, -157.4, -162.0; IR (thin film): \(\nu\) 3317, 2924, 1763, 1636, 1520 cm\(^{-1}\); ESI-HRMS calcd for C\textsubscript{22}H\textsubscript{13}F\textsubscript{5}NO\textsubscript{3} [M − H]\(^−\) 434.0821, found 434.0810.
3-((7-(Diethylamino)-2-oxo-2H-chromen-4-yl)methoxy)-N-(2-phenylethyl)benzamide (3u).

Prepared by a slight modification of general procedure A. 2-Phenethylamine (2a, 15.6 µL, 0.12 mmol, 1.2 equiv) and DCH (23.6 mg, 0.12 mmol, 1.2 equiv) were dissolved in a mixture of THF and aqueous buffer pH 3.0 (1:1, 2 mL), and stirred for 5 min. Potassium 3-((7-(diethylamino)-2H-chromen-2-one)methoxy) benzoyltrifluoroborate (1h, 45.6 mg, 0.10 mmol, 1.0 equiv) was added and the mixture stirred for 1 h. The crude material was purified by column chromatography on silica gel (eluting with CH₂Cl₂/MeOH = 100:2) to give 3u as an orange solid (30.6 mg, 65%); m.p. 48-52 °C; ¹H-NMR (400 MHz, CDCl₃): δ 7.41 (dd, J = 2.6, 1.5 Hz, 1H), 7.37-7.30 (m, 4H), 7.27-7.19 (m, 4H), 7.10 (ddd, J = 8.2, 2.7, 1.0 Hz, 1H), 6.59 (d, J = 1.3 Hz, 1H), 6.53 (d, J = 2.5 Hz, 1H), 6.27 (t, J = 1.1 Hz, 1H), 6.17 (t, J = 5.6 Hz, 1H), 5.18 (d, J = 1.3 Hz, 2H), 3.72 (q, J = 6.7 Hz, 2H), 3.42 (q, J = 7.1 Hz, 4H), 2.94 (t, J = 6.9 Hz, 2H), 1.22 (t, J = 7.1 Hz, 6H); ¹³C-NMR (100 MHz, CDCl₃): δ 167.2, 162.1, 158.4, 156.5, 150.8, 150.1, 139.0, 136.5, 130.0, 129.0, 128.9, 126.8, 124.6, 119.5, 118.8, 113.1, 108.8, 107.0, 106.2, 98.0, 66.0, 44.9, 41.4, 35.8, 12.6; IR (thin film): ν 3584, 2971, 2927, 1712, 1604, 1529, 1352 cm⁻¹; ESI-HRMS calcd for C₂₉H₃₁N₂O₄ [M + H]⁺ 471.2278, found 471.2276.

2-(6-(Diethylamino)-3-(diethyliminio)-3H-xanthen-9-yl)-5-(N-(2-(2-(2-(4-(N-(2-phenylethyl)carbamoyl)benzamido)ethoxy)ethoxy)ethyl)sulfamoyl)benzenesulfonate (3v).

Prepared by a slight modification of general procedure A. 2-Phenethylamine (2a, 7.8 µL, 0.06 mmol, 1.5 equiv) and DCH (11.8 mg, 0.06 mmol, 1.5 equiv) were dissolved in a mixture of THF and aqueous buffer pH 3.0 (1:1, 2 mL), and stirred for 5 min. Potassium 4-(N-(2-(2-(2-(4-(6-(diethylamino)-3-(diethyliminio)-3H-xanthen-9-yl)-3-sulfonatobenzenesulfonamido)ethoxy)ethoxy)ethyl) carbamoyl)benzoyltrifluoroborate (1i, 37.0 mg, 0.04 mmol, 1.0 equiv) was added and the mixture stirred for 1 h. The crude material was purified by column chromatography on silica gel (eluting with CH₂Cl₂/MeOH = 100:8) to give 3v as a magenta solid (23.3 mg, 62%); m.p. 154-157 °C; ¹H-NMR (400 MHz, CDCl₃): δ 8.92 (d, J = 1.9 Hz, 1H), 8.00 (dd, J = 7.8, 1.9 Hz, 1H), 7.81 (s, 3H), 7.72 (t, J = 5.5 Hz, 1H), 7.66 (t, J = 5.7 Hz, 1H), 7.22-7.15 (m, 6H), 7.12 (t, J = 8.6 Hz, 1H), 6.74 (t, J = 6.0 Hz, 1H), 6.69 (dd, J = 9.6, 2.5 Hz, 2H), 6.46 (d, J = 2.5 Hz, 1H), 3.63 (t, J = 5.2 Hz, 2H), 3.61-3.55 (m, 6H), 3.52 (q, J = 5.3 Hz, 2H), 3.48-3.39 (m, 9H), 3.20 (q, J = 5.8 Hz, 2H), 2.79-2.75 (m, 2H), 1.20 (t, J = 7.2 Hz, 12H); ¹³C-NMR (100 MHz, CDCl₃): δ 166.8, 166.6, 158.1, 157.7, 155.5, 147.5, 142.0, 139.7, 136.7, 136.3, 133.9, 133.3, 130.0, 129.0, 128.4,
127.6, 127.5, 127.4, 126.1, 114.3, 113.8, 95.7, 70.7, 70.5, 69.8, 69.5, 46.0, 43.2, 41.7, 40.0, 35.8, 29.8, 12.7; IR (thin film): ν 3440, 3345, 2928, 1648, 1591, 1416, 1337, 1180, 1076 cm⁻¹; ESI-HRMS calcd for C₄₉H₅₈N₅O₁₀S₂ [M + H]⁺ 940.3620, found 940.3608.

4-(3-Azidopropoxy)-N-(2-phenylethyl)benzamide (3w).

Prepared by following general procedure A for 50 min with potassium 4-(3-azidopropoxy)benzoic trifluoroborate (1j, 31.1 mg, 0.10 mmol, 1.0 equiv) and 2-phenethylamine (2a, 13.8 μL, 0.11 mmol, 1.1 equiv). The crude material was purified by column chromatography on silica gel (eluting with EtOAc/hexanes = 2:3) to give 3w as a white solid (24.0 mg, 74%); m.p. 80-83 °C; ¹H-NMR (400 MHz, CDCl₃): δ 7.68 (d, J = 8.8 Hz, 2H), 7.35 (t, J = 7.2 Hz, 1H), 7.29-7.23 (m, 2H), 6.91 (d, J = 8.8 Hz, 2H), 6.15 (t, J = 5.9 Hz, 1H), 4.10 (t, J = 6.0 Hz, 2H), 3.72 (td, J = 6.9, 5.8 Hz, 2H), 3.54 (t, J = 6.6 Hz, 2H), 2.94 (t, J = 6.9 Hz, 2H), 2.08 (p, J = 6.3 Hz, 2H); ¹³C-NMR (100 MHz, CDCl₃): δ 167.0, 161.3, 139.1, 128.9, 128.8, 128.8, 127.3, 126.7, 114.3, 64.8, 48.2, 41.2, 35.9, 28.8; IR (thin film): ν 3295, 2942, 2920, 2021, 1607, 1540, 1504, 1300, 1261 cm⁻¹; ESI-HRMS calcd for C₁₈H₂₁N₄O₂ [M + H]⁺ 325.1659, found 325.1658.

N-(2-phenylethyl)-4-(2-trimethylsilylethynyl)benzamide (3x).

Prepared by following general procedure A for 1 h with potassium 4-(2-trimethylsilylethynyl)benzoic trifluoroborate (1k, 30.8 mg, 0.10 mmol, 1.0 equiv) and 2-phenethylamine (2a, 15.5 μL, 0.12 mmol, 1.2 equiv). The crude material was purified by preparative TLC (eluting with EtOAc/hexanes = 1:4) to give 3x as a white solid (22.1 mg, 69%). The spectral data was in accordance with the literature; ¹H-NMR (400 MHz, CDCl₃): δ 7.62 (d, J = 8.5 Hz, 2H), 7.48 (d, J = 8.5 Hz, 2H), 7.33 (d, J = 7.2 Hz, 2H), 7.30-7.24 (m, 2H), 6.14 (br s, 1H), 3.71 (q, J = 6.9 Hz, 2H), 2.93 (t, J = 6.9 Hz, 2H), 0.25 (s, 9H); ¹³C-NMR (100 MHz, CDCl₃): δ 166.8, 138.9, 134.3, 132.2, 128.9, 128.9, 126.8, 126.8, 126.5, 104.1, 97.1, 41.3, 35.8, 0.0.
N-(2-Phenylethyl)-N'-propargylterephthalimide (3y).

Prepared by following general procedure A for 1 h with potassium 4-(N-propargylcarbamoyl)benzoyltrifluoroborate (1l, 29.2 mg, 0.10 mmol, 1.0 equiv) and 2-phenethylamine (2a, 15.5 μL, 0.12 mmol, 1.2 equiv). While stirring the reaction mixture, the product precipitated as a white, insoluble solid. This solid was filtered, washed with H₂O and CH₂Cl₂ and dried in vacuo to give 3y (24.2 mg, 79%); m.p. >210 °C (decomp.); ¹H-NMR (400 MHz, DMSO-d₆): δ 9.04 (t, J = 5.5 Hz, 1H), 8.69 (t, J = 5.6 Hz, 1H), 7.92 (d, J = 8.7 Hz, 2H), 7.88 (d, J = 8.7 Hz, 2H), 7.33-7.17 (m, 5H), 4.07 (m, 2H), 3.53-3.46 (m, 2H), 3.13 (t, J = 2.5 Hz, 1H), 2.85 (t, J = 7.5 Hz, 2H); ¹³C-NMR (100 MHz, DMSO-d₆): δ 165.4, 165.3, 139.5, 137.0, 135.9, 128.6, 128.3, 127.2, 127.1, 126.1, 81.1, 73.0, 40.9, 35.0, 28.6; IR (thin film): ν 3284, 2918, 1624, 1546, 1265 cm⁻¹; ESI-HRMS calcd for C₁₉H₁₉N₂O₂ [M + H]⁺ 307.1441, found 307.1442.

Preparation of N-(4-fluorobenzoyl)-4-fluorobenzamide (5a).

In a round-bottom flask, potassium 4-fluorobenzoyltrifluoroborate (1a, 69 mg, 0.30 mmol, 3.0 equiv) and ammonium chloride (5.4 mg, 0.10 mmol, 1.0 equiv) were dissolved in 1 mL of a 1:1 mixture of THF and aqueous buffer pH 3.0, 25 mM (prepared as described in general procedure A). To this solution, 1,3-dichloro-5,5-dimethylhydantoin (DCH, 43.3 mg, 0.22 mmol, 2.2 equiv) was added, and the mixture stirred at 40 ºC for 3 h. The reaction was quenched with saturated aqueous NaHSO₃ (0.5 mL), poured into H₂O/brine and extracted with EtOAc (3 x 3 mL). The organic extracts were collected, dried over Na₂SO₄, filtered and evaporated in vacuo. The residue was purified by preparative TLC (eluting with EtOAc/hexanes 1:2) to afford 5a as a white solid (16.2 mg, 62%); m.p. 153-157 ºC; ¹H-NMR (400 MHz, CDCl₃): δ 8.97 (br s, 1H), 7.89 (dd, J = 8.8, 5.1 Hz, 4H), 7.17 (t, J = 8.6 Hz, 4H); ¹³C-NMR (100 MHz, CDCl₃): δ 166.0, 165.7 (d, J = 255.3 Hz), 130.8 (d, J = 9.3 Hz), 129.3 (d, J = 3.1 Hz), 116.0 (d, J = 22.2 Hz); ¹⁹F-NMR (375 MHz, CDCl₃): δ −104.6; IR (thin film): ν 3273, 3076, 2923, 1681, 1602, 1471, 1224, 1159 cm⁻¹; ESI-HRMS calcd for C₁₄H₁₀F₂NO₂ [M + H]⁺ 262.0674, found 262.0678.
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General procedure B: Synthesis of imides from primary amides and sulfonamides

In a round-bottom flask, potassium acyltrifluoroborate (1, 0.11 mmol, 1.1 equiv) was dissolved in 1 mL of a 1:1 mixture of THF and aqueous buffer pH 3.0, 25 mM (prepared as described for general procedure A). The amide or sulfonamide (4, 0.10 mmol, 1.0 equiv) and 1,3,5-trichloroisocyanuric acid (TCCA, 8.5 mg, 0.036 mmol, 0.36 equiv) were added sequentially. The mixture was stirred at 40 °C, quenched with saturated aqueous NaHSO₃ (0.3 mL), poured into H₂O/brine and extracted with EtOAc (3 x 3 mL). The organic extracts were collected, dried over Na₂SO₄, filtered and evaporated in vacuo. The residue was purified by column chromatography on silica gel to afford the imide.

N-Benzoyl-4-fluorobenzamide (5b). Prepared by following general procedure B for 4 h with potassium 4-fluorobenzoyltrifluoroborate (1a, 25.6 mg, 0.11 mmol, 1.1 equiv) and benzamide (4b, 12.1 mg, 0.10 mmol, 1.0 equiv). The crude material was purified by column chromatography on silica gel (eluting with CH₂Cl₂/MeOH = 100:4) to give 5b as a white solid (15.8 mg, 65%). The spectral data was in accordance with the literature; ¹H-NMR (400 MHz, CDCl₃): δ 9.01 (br s, 1H), 7.94-7.88 (m, 4H), 7.64 (t, J = 7.5 Hz, 1H), 7.53 (t, J = 7.5 Hz, 2H), 7.20 (t, J = 8.6 Hz, 2H); ¹³C-NMR (100 MHz, CDCl₃): δ 166.5, 165.9, 165.6 (d, J = 255.0 Hz), 133.2, 133.1, 130.8 (d, J = 9.3 Hz), 129.5 (d, J = 3.0 Hz), 128.9, 128.0, 116.0 (d, J = 22.1 Hz); ¹⁹F-NMR (375 MHz, CDCl₃): δ -104.9.

(S)-Methyl 2-(9H-fluoren-9-ylmethoxycarbonylamino)-5-(4-fluorobenzamido)-5-oxopentanoate (5c). Prepared by following general procedure B for 3 h with potassium 4-fluorobenzoyltrifluoroborate (1a, 25.6 mg, 0.11 mmol, 1.1 equiv) and Fmoc-L-glutamine methyl ester (4c, 38.3 mg, 0.10 mmol, 1.0 equiv). The crude material was purified by column chromatography on silica gel (eluting with EtOAc/hexanes = 1:2) to give 5c as a white solid in >90% purity (by NMR) [33.3 mg, 66%]. For characterization purposes, 5c was further purified by preparative reverse-phase HPLC (YMC C18 column, A/B eluent gradient: 40-95% for 28 min,
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$t_\text{R} = 18.6$ min; **m.p.** 162-164 °C; $^1$H-NMR (400 MHz, CDCl$_3$): $\delta$ 9.09 (br, 1H), 7.87 (dd, $J = 8.9$, 5.1 Hz, 2H), 7.75 (d, $J = 7.5$ Hz, 2H), 7.58 (d, $J = 7.5$ Hz, 2H), 7.38 (t, $J = 7.4$ Hz, 2H), 7.29 (td, $J = 7.5$, 1.2 Hz, 2H), 7.14 (t, $J = 8.6$ Hz, 2H), 5.63 (d, $J = 8.2$ Hz, 1H), 4.50 (td, $J = 8.4$, 4.7 Hz, 1H), 4.36 (dd, $J = 7.3$, 2.0 Hz, 2H), 4.21 (t, $J = 7.1$ Hz, 1H), 3.77 (s, 3H), 3.15-3.01 (m, 2H), 2.34 (dq, $J = 13.1$, 6.9 Hz, 1H), 2.08 (dq, $J = 14.5$, 7.1 Hz, 1H), 1.84 (br, 1H); $^{13}$C-NMR (100 MHz, CDCl$_3$): $\delta$ 175.2, 172.5, 165.7 (d, $J = 255.3$ Hz), 164.6, 156.1, 143.8, 143.7, 141.3, 130.4 (d, $J = 9.3$ Hz), 128.7 (d, $J = 3.2$ Hz), 127.7, 127.1, 125.1, 120.0, 116.1 (d, $J = 22.2$ Hz), 67.2, 53.2, 52.6, 47.1, 33.5, 27.1; $^{19}$F-NMR (375 MHz, CDCl$_3$): $\delta$ −104.6; IR (thin film): $\nu$ 3318, 3019, 2952, 1714, 1683, 1477, 1236, 1161 cm$^{-1}$; ESI-HRMS calcd for C$_{28}$H$_{25}$FN$_2$NaO$_6$ [M + Na]$^+ 527.1589$, found 527.1589; $[\alpha]_D$: +13.4 (c = 1.15, CHCl$_3$).

**tert-Butyl N-4-fluorobenzoylcarbamate (5d).**

Prepared by following general procedure B for 4 h with potassium 4-fluorobenzoyl trifluoroborate (1a, 25.6 mg, 0.11 mmol, 1.1 equiv) and tert-butyl carbamate (4d, 11.7 mg, 0.10 mmol, 1.0 equiv). The crude material was purified by column chromatography on silica gel (eluting with EtOAc/hexanes = 1:3) to give 5d as a white solid (10.3 mg, 43%); **m.p.** 124-127 °C; $^1$H-NMR (400 MHz, CDCl$_3$): $\delta$ 7.83 (dd, $J = 8.7$, 5.2 Hz, 3H), 7.16 (t, $J = 8.6$ Hz, 2H), 1.56 (s, 9H); $^{13}$C-NMR (100 MHz, CDCl$_3$): $\delta$ 165.4 (d, $J = 254.3$ Hz), 164.2, 149.5, 130.1 (d, $J = 9.2$ Hz), 129.6 (d, $J = 3.2$ Hz), 116.0 (d, $J = 22.2$ Hz), 83.0, 28.0; $^{19}$F-NMR (282 MHz, CDCl$_3$): $\delta$ −105.6; IR (thin film): $\nu$ 3270, 2980, 2926, 1749, 1603, 1500, 1220, 1147 cm$^{-1}$; ESI-HRMS calcd for C$_{12}$H$_{14}$FNNaO$_3$ [M + Na]$^+ 262.0850$, found 262.0850.

**N-(4-Fluorobenzoyl)-N'-methylurea (5e).**

Prepared by following general procedure B for 4 h with potassium 4-fluorobenzoyl trifluoroborate (1a, 25.6 mg, 0.11 mmol, 1.1 equiv) and methylurea (4e, 7.4 mg, 0.10 mmol, 1.0 equiv). The crude material was purified by column chromatography on silica gel (eluting with EtOAc/hexanes = 1:1) to give 5e as a white solid (11.7 mg, 60%); **m.p.** 222-226 °C; $^1$H-NMR (400 MHz, DMSO-d$_6$): $\delta$ 10.70 (br s, 1H), 8.49 (q, $J = 4.9$ Hz, 1H), 8.04 (dd, $J = 9.0$, 5.4 Hz, 2H), 7.33 (t, $J = 8.7$ Hz, 2H), 2.78 (s, $J = 4.7$ Hz, 3H); $^{13}$C-NMR (100 MHz, DMSO-d$_6$): $\delta$ 167.4, 165.2 (d, $J = 250.8$ Hz), 154.5, 131.5 (d, $J = 9.5$ Hz), 129.3 (d, $J = 2.9$ Hz), 116.0 (d, $J = 22.0$ Hz), 26.6; $^{19}$F-NMR (375 MHz, CDCl$_3$): $\delta$ −105.2; IR (thin film): $\nu$ 3332, 1698, 1567, 1280, 1225 cm$^{-1}$; ESI-HRMS calcd for C$_9$H$_9$FN$_2$NaO$_2$ [M + Na]$^+ 219.0540$, found 219.0543.

**N-((2,5-Dioxoimidazolidin-4-yl)carbamoyl)-4-fluorobenzamide (5f).**
Prepared by a modification of general procedure B for 4 h with potassium 4-fluorobenzoyltrifluoroborate (1a, 25.6 mg, 0.11 mmol, 1.1 equiv), DL-allantoin (4f, 31.6 mg, 0.10 mmol, 1.0 equiv) and 1,3-dichloro-5,5-dimethylhydantoin (DCH, 21.7 mg, 0.11 mmol, 1.1 equiv) as a replacement for TCCA. While stirring the reaction mixture, the product precipitated as a white, insoluble solid. This solid was filtered, washed with H2O and CH2Cl2 and dried in vacuo to give 5f (22.4 mg, 80%); m.p. >250 °C (no melting); 1H-NMR (400 MHz, DMSO-d6): δ 10.95 (s, 1H), 10.73 (s, 1H), 9.23 (d, J = 7.1 Hz, 1H), 8.21 (s, 1H), 8.05 (dd, J = 8.7, 5.4 Hz, 2H), 7.36 (t, J = 8.6 Hz, 2H), 5.46 (d, J = 7.0 Hz, 1H); 13C-NMR (100 MHz, DMSO-d6): δ 172.9, 167.5, 165.3 (d, J = 251.3 Hz), 157.4, 153.3, 131.7 (d, J = 9.5 Hz), 129.3 (d, J = 2.9 Hz), 116.1 (d, J = 22.0 Hz), 62.6; 19F-NMR (282 MHz, DMSO-d6): δ −106.5; IR (thin film): ν 3345, 1777, 1698, 1526, 1434, 1228, 1167 cm−1; ESI-HRMS calcd for C11H9FN4NaO4 [M + Na]+ 303.0500, found 303.0506.

N-Methanesulfonyl-4-fluorobenzamide (5g).

Prepared by following general procedure B for 2.5 h at 60 °C with potassium 4-fluorobenzoyltrifluoroborate (1a, 25.6 mg, 0.11 mmol, 1.1 equiv) and methanesulfonamide (4g, 9.5 mg, 0.10 mmol, 1.0 equiv). The crude material was purified by column chromatography on silica gel (eluting with hexanes/acetone = 3:2 + 0.5% AcOH) to give 5g as a white solid (14.7 mg, 68%); m.p. 178-182 °C; 1H-NMR (400 MHz, acetone-d6): δ 10.66 (br, 1H), 8.10 (dd, J = 8.9, 5.3 Hz, 2H), 7.31 (t, J = 8.8 Hz, 2H), 3.39 (s, 3H); 13C-NMR (100 MHz, acetone-d6): δ 165.6 (d, J = 252.2 Hz), 164.9, 131.1 (d, J = 9.5 Hz), 128.6 (d, J = 2.9 Hz), 115.6 (d, J = 22.3 Hz), 40.8; 19F-NMR (375 MHz, acetone-d6): δ −107.4; IR (thin film): ν 3252, 1677, 1602, 1443, 1339, 1233, 1157 cm−1; ESI-HRMS calcd for C8H7FNO3S [M − H]− 216.0136, found 216.0139.

Nα-benzoyl-Nω-(4-fluorobenzoyl)-L-arginine ethyl ester (5h).

Prepared by following general procedure B for 3 h with potassium 4-fluorobenzoyltrifluoroborate (1a, 25.6 mg, 0.11 mmol, 1.1 equiv) and Nα-benzoyl-L-arginine ethyl ester hydrochloride (4h, 102.8 mg, 0.30 mmol, 1.5 equiv). The crude material was purified by column chromatography on silica gel (eluting with CH2Cl2/MeOH 100:5, +1% NEt3) to give 5h as a white solid in >90% purity (by NMR) [64.1 mg, 75%]. For characterization purposes, 5h was further purified by preparative reverse-phase HPLC (YMC C18 column, A/B eluent gradient: 20-95% for 28 min, tR = 15.8 min). The collected fraction was washed with saturated aqueous NaHCO3 (to remove CF3CO2H), extracted with CH2Cl2, dried over
Na₂SO₄, filtered and evaporated in vacuo to give 5n as a crystalline solid; m.p. 52-56 °C; ¹H-NMR (400 MHz, CDCl₃): δ 8.15 (dd, J = 8.5, 5.7 Hz, 2H), 7.78 (d, J = 7.6 Hz, 2H), 7.56 (d, J = 7.4 Hz, 1H), 7.44 (t, J = 7.6 Hz, 2H), 7.02 (t, J = 8.7 Hz, 2H), 6.98-4.79 (m, 1H), 4.25 (q, J = 7.2 Hz, 2H), 3.64-3.52 (m, 1H), 3.21 (br, 1H), 2.07-1.95 (m, 1H), 1.85-1.62 (m, 3H), 1.30 (t, J = 7.2 Hz, 2H); ¹³C-NMR (100 MHz, CDCl₃): δ 176.0, 172.2, 168.0, 164.8 (d, J = 250.5 Hz), 161.3, 134.3, 133.2, 132.3, 131.1 (d, J = 8.9 Hz), 128.8, 127.1, 114.7 (d, J = 21.5 Hz), 62.1, 51.3, 40.5, 31.1, 25.0, 14.2; ¹⁹F-NMR (375 MHz, CDCl₃): δ −109.7; IR (thin film): ν 3329, 2982, 2936, 1734, 1600, 1556, 1353, 1219, 1144 cm⁻¹; ESI-HRMS calcd for C₂₂H₂₆F₄N₄O₄ [M + H]⁺ 429.1933, found 429.1932; [α]D: +4.9 (c = 1.06, CHCl₃).

N-(E)-Cinnamoyl-4-fluorobenzamide (5i).

Prepared by following general procedure B for 3 h with potassium 4-fluorobenzoyltrifluoroborate (1a, 23.0 mg, 0.10 mmol, 1.0 equiv) and trans-cinnamamide (4i, 16.1 mg, 0.125 mmol, 1.25 equiv). The crude material was purified by column chromatography on silica gel (eluting with EtOAc/hexanes = 1:5 + 0.5% AcOH) to give 5i as a white solid (15.3 mg, 57%); m.p. 179-182 °C; ¹H-NMR (400 MHz, CDCl₃): δ 8.84 (br s, 1H), 7.98-7.90 (m, 3H), 7.81 (d, J = 15.7 Hz, 1H), 7.68-7.62 (m, 2H), 7.44-7.39 (m, 3H), 7.21 (t, J = 8.6 Hz, 2H); ¹³C-NMR (100 MHz, CDCl₃): δ 167.7, 165.8 (d, J = 255.3 Hz), 164.8, 147.0, 134.5, 130.8, 130.4 (d, J = 9.3 Hz), 129.2 (d, J = 3.2 Hz), 128.9, 128.7, 119.1, 116.2 (d, J = 22.2 Hz); ¹⁹F-NMR (375 MHz, CDCl₃): δ −104.6; IR (thin film): ν 3279, 1661, 1469, 1355, 1248 cm⁻¹; ESI-HRMS calcd for C₁₆H₁₂FN₂O₂Na [M + Na]⁺ 292.0744, found 292.0745.

tert-Butyl 4-((4-fluorobenzoyl)carbamoyl)piperidine-1-carboxylate (5j).

Prepared with potassium 4-fluorobenzoyltrifluoroborate (1a, 25.6 mg, 0.11 mmol, 1.1 equiv), piperidine-4-carboxamide (4j, 12.8 mg, 0.10 mmol, 1.0 equiv) and 1,3,5-trichloroisocyanuric acid (TCCA, 15.3 mg, 0.066 mmol, 0.66 equiv) by a modification of general procedure B. The reaction was stirred for 3 h at 40 °C and quenched with saturated aqueous NaHSO₃ (0.6 mL). Saturated aqueous NaHCO₃ was added until the solution turned slightly basic (pH between 7-8). Boc₂O (340 µL, 1.5 mmol) was added, and the mixture stirred for 1 h at rt, poured into H₂O/brine and extracted with EtOAc (3 x 3 mL). The organic extracts were collected, dried over Na₂SO₄, filtered and evaporated in vacuo. The residue was purified by column chromatography on silica gel (eluting with EtOAc/hexanes = 1:2) to give 5j as a white solid [15.6 mg, 44% (over 2 steps)]; m.p. 173-175 °C; ¹H-NMR (400 MHz, CDCl₃): δ 8.78 (br s, 1H), 7.93 (dd, J = 8.9, 5.1 Hz, 2H), 7.21 (t, J = 8.5 Hz, 2H), 4.28-4.10 (m, 2H), 3.61 (tt, J = 11.2,
3.6 Hz, 1H), 2.89 (t, J = 12.7 Hz, 2H), 1.97 (d, J = 13.0 Hz, 2H), 1.70 (ddd, J = 24.5, 12.5, 4.3 Hz, 2H), 1.46 (s, 9H); 13C-NMR (100 MHz, CDCl₃): δ 177.7, 165.8 (d, J = 255.6 Hz), 164.3, 154.7, 130.4 (d, J = 9.3 Hz), 128.8 (d, J = 3.1 Hz), 116.3 (d, J = 22.2 Hz), 79.6, 43.1, 42.7, 28.5, 27.9; 19F-NMR (375 MHz, CDCl₃): δ −104.5; IR (thin film): ν 3280, 2975, 2929, 1731, 1692, 1603, 1493, 1240, 1159 cm⁻¹; ESI-HRMS calcd for C₁₈H₂₃FN₂NaO₄ [M + Na]+ 373.1534, found 373.1540.

_N-Benzoyl-4-phenylbutanamide (5k).⁴_

![Chemical Structure](image)

Prepared by following general procedure B for 3 h with potassium 4-phenylbutanoyl trifluoroborate (1e, 25.4 mg, 0.10 mmol, 1.0 equiv) and benzamide (4a, 13.3 mg, 0.11 mmol, 1.1 equiv). The crude material was purified by preparative reverse-phase HPLC (YMC C18 column, gradient of 20-95% MeCN/H₂O + 0.1% TFA for 28 min, tᵣ = 22.2 min) to give 5k as a white solid (12.0 mg, 45%). The spectral data was in accordance with the literature; ¹H-NMR (400 MHz, CDCl₃): δ 8.57 (s, 1H), 7.83 (d, J = 7.8 Hz, 2H), 7.61 (tt, J = 7.8, 1.2 Hz, 1H), 7.50 (t, J = 7.8 Hz, 2H), 7.35-7.19 (m, 5H), 3.04 (t, J = 7.4 Hz, 2H), 2.74 (t, J = 7.2 Hz, 2H), 2.06 (p, J = 7.5 Hz, 2H); ¹³C-NMR (100 MHz, CDCl₃): δ 175.7, 165.4, 141.6, 133.2, 132.8, 129.0, 128.5, 128.4, 127.6, 126.0, 37.0, 35.2, 25.7.

_Ethyl 4-(benzoylcarbamoyl)benzoate (5l).⁴_

![Chemical Structure](image)

Prepared by following general procedure B for 4 h with potassium 4-(ethoxycarbonyl)benzoyl trifluoroborate (1m, 28.4 mg, 0.10 mmol, 1.0 equiv) and benzamide (4a, 13.3 mg, 0.11 mmol, 1.1 equiv). The crude material was purified by column chromatography on silica gel (eluting with EtOAc/hexanes = 2:3) to give 5l as a white solid (12.5 mg, 42%). The spectral data was in accordance with the literature; ¹H-NMR (400 MHz, CDCl₃): δ 9.17 (s, 1H), 8.11 (d, J = 8.0 Hz, 2H), 7.92-7.85 (m, 3H), 7.61 (t, J = 7.4 Hz, 1H), 7.49 (t, J = 7.6 Hz, 2H), 4.39 (q, J = 7.1 Hz, 2H), 1.40 (t, J = 7.1 Hz, 3H); ¹³C-NMR (100 MHz, CDCl₃): δ 166.6, 166.3, 165.5, 137.2, 134.2, 133.3, 133.0, 129.9, 128.9, 128.0, 128.0, 61.6, 14.3.
Chapter 7. Experimental Section

**N-Benzoyl-4-(2-trimethylsilylethynyl)benzamide (5m).**

Prepared by following general procedure B for 5 h with potassium 4-(2-trimethylsilylethynyl)benzoyltrifluoroborate (1k, 30.8 mg, 0.10 mmol, 1.0 equiv), benzamide (4a, 15.4 mg, 0.12 mmol, 1.2 equiv) and TCCA (9.3 mg, 0.04 mmol, 0.4 equiv). The crude material was purified by preparative TLC (eluting with EtOAc/hexanes = 1:3) to give 5m as a viscous oil (18.1 mg, 57%); \(^1\)H-NMR (400 MHz, CDCl\(_3\)): \(\delta\) 8.99 (s, 1H), 7.86 (d, \(J = 7.6\) Hz, 2H), 7.80 (d, \(J = 8.3\) Hz, 2H), 7.61 (t, \(J = 7.5\) Hz, 1H), 7.56 (d, \(J = 8.4\) Hz, 2H), 7.50 (d, \(J = 7.6\) Hz, 2H), 0.27 (s, 9H); \(^{13}\)C-NMR (100 MHz, CDCl\(_3\)): \(\delta\) 166.6, 166.1, 133.4, 133.3, 132.8, 132.4, 129.0, 128.3, 128.1, 128.0, 103.8, 98.6, 0.0; IR (thin film): \(\nu\) 3275, 2959, 2159, 1723, 1604, 1480, 1225 cm\(^{-1}\); ESI-HRMS calcd for C\(_{19}\)H\(_{20}\)NO\(_2\)Si [M + H]\(^+\) 322.1258, found 322.1264.

**Pentafluorophenyl 4-(N-benzoylcarbamoyl)benzoate (5n).**

Prepared by following general procedure B for 12 h with potassium 4-(pentafluorophenoxycarbonyl)benzoyltrifluoroborate (1g, 42.6 mg, 0.10 mmol, 1.0 equiv), benzamide (4a, 15.5 mg, 0.12 mmol, 1.2 equiv) and TCCA (11.6 mg, 0.05 mmol, 0.5 equiv). The crude material was purified by column chromatography on silica gel (eluting with EtOAc/hexanes = 1:3 to 2:3) to give 5n as a viscous oil (22.6 mg, 52%); \(^1\)H-NMR (600 MHz, CDCl\(_3\)): \(\delta\) 8.52 (br s, 1H, NH), 8.32 (d, \(J = 8.7\) Hz, 2H, ArH), 7.97 (d, \(J = 8.3\) Hz, 2H, ArH), 7.90 (dd, \(J = 8.4, 1.2\) Hz, 2H, ArH), 7.66 (t, \(J = 7.5\) Hz, 1H, ArH), 7.56-7.53 (m, 2H, ArH); \(^{13}\)C-NMR (151 MHz, CDCl\(_3\)): \(\delta\) 167.1, 166.0, 161.8, 141.4 (\(^{19}\)F), 139.9 (\(^{19}\)F), 139.2, 138.1 (\(^{19}\)F), 133.8, 132.7, 131.1, 130.6, 129.2, 128.6, 128.1 125.2 (\(^{19}\)F); \(^{19}\)F-NMR (375 MHz, CDCl\(_3\)): \(\delta\) −152.3, −157.2, −161.9; IR (thin film): \(\nu\) 3274, 1764, 1724, 1521, 1481, 1481, 1228, 1055 cm\(^{-1}\); ESI-HRMS calcd for C\(_{21}\)H\(_9\)F\(_5\)NO\(_4\) [M − H]\(^−\) 434.0457, found 434.0446.

**4-(3-Azidopropoxy)-N-benzoylbenzamide (5o).**

Prepared by following general procedure 2 for 6 h with potassium 4-(3-azidopropoxy)benzoyltrifluoroborate (1j, 31.1 mg, 0.10 mmol, 1.0 equiv) and benzamide (4a, 15.5 mg, 0.12 mmol, 1.2 equiv). The crude material was purified by preparative TLC (eluting with EtOAc/hexanes = 1:1) to give 5o as a colourless oil (13.0 mg, 40%); \(^1\)H-NMR (400 MHz, CDCl\(_3\)): \(\delta\) 8.98 (br s, 1H), 7.89-7.82 (m, 4H), 7.60 (t, \(J = 7.4\) Hz, 1H), 7.50 (t, \(J = 7.5\) Hz, 2H), 6.97 (d, \(J = 8.7\) Hz, 2H), 4.12 (t, \(J = 5.9\) Hz, 2H), 3.54 (t, \(J = 6.5\) Hz, 2H), 2.09 (p, \(J = 6.2\) Hz, 2H); \(^{13}\)C-
NMR (100 MHz, CDCl₃): δ 167.0, 166.1, 162.9, 133.6, 133.2, 130.5, 129.0, 128.1, 125.6, 114.7, 65.0, 48.2, 28.8; IR (thin film): ν 3274, 2939, 2099, 1722, 1605, 1469, 1225, 1174 cm⁻¹; ESI-HRMS calcd for C₁₇H₁₇N₄O₃ [M + H]^⁺ 325.1295, found 325.1289.

3-((7-(Diethylamino)-2-oxo-2H-chromen-4-yl)methoxy)-N-benzyolbenzamide (5p).

Prepared by a slight modification of general procedure 1. Benzamide (4a, 15.5 mg, 0.12 mmol, 1.2 equiv) and TCCA (14 mg, 0.06 mmol, 0.6 equiv) were dissolved in a mixture of THF and aqueous buffer pH 3.0 (1:1, 2 mL), and stirred for 10 min at rt. Potassium 3-((7-(diethylamino)-2H-chromen-2-one)methoxy) benzoyletrifluoroborate (1h, 45.6 mg, 0.10 mmol, 1.0 equiv) was added and the mixture stirred for 12 h at 40 °C. The crude material was purified by preparative TLC (eluting with CH₂Cl₂/MeOH = 100:2) to give 5p as an orange solid (11.7 mg, 25%); m.p. 132-136 °C; ¹H-NMR (400 MHz, CDCl₃): δ 9.03 (br s, 1H), 7.03 (d, J = 8.2 Hz, 2H), 7.59 (t, J = 7.4 Hz, 1H), 7.51.37-7.40 (m, 5H), 7.38 (d, J = 9.0 Hz, 1H), 7.23-7.18 (m, 1H), 6.65 (dd, J = 9.0, 2.5 Hz, 1H), 6.57 (d, J = 2.5 Hz, 1H), 6.28 (br, 1H), 5.20 (d, J = 1.3 Hz, 2H), 3.41 (q, J = 7.1 Hz, 4H), 1.18 (t, J = 7.1 Hz, 6H); ¹³C-NMR (100 MHz, CDCl₃): δ 166.6, 166.1, 162.3, 158.5, 156.3, 150.2, 150.1, 135.0, 133.5, 133.2, 130.4, 129.1, 128.1, 124.8, 120.6, 120.6, 114.1, 109.9, 107.5, 107.1, 99.1, 66.0, 45.6, 12.4; IR (thin film): ν 3263, 2974, 1722, 1603, 1472, 1241, 1199 cm⁻¹; ESI-HRMS calcd for C₂₈H₂₇N₂O₅ [M + H]^⁺ 471.1914, found 471.1917.

Preparation of Nα-benzoyl-Nα,ω'-bis(4-fluorobenzoyl)-L-arginine ethyl ester (6).

In a round-bottom flask, potassium 4-fluorobenzoyltrifluoroborate (1a, 25.6 mg, 0.11 mmol, 1.2 equiv) and Nα-benzoyl-Nα-(4-fluorobenzoyl)-L-arginine ethyl ester (5h, 38.5 mg, 0.09 mmol, 1.0 equiv) were dissolved in 2 mL of a 1:1 mixture of THF and aqueous buffer pH 3.0 (prepared as described in general procedure A). To this solution, 1,3,5-trichloroisocyanuric acid (TCCA, 11.2 mg, 0.048 mmol, 0.53 equiv) was added and the mixture stirred at 40 °C for 4 h. The mixture was quenched with saturated aqueous NaHSO₃ (0.6 mL), poured into H₂O/brine and extracted with
EtOAc (3 x 3 mL). The organic extracts were collected, washed with saturated aqueous NaHCO₃, dried over Na₂SO₄, filtered and evaporated in vacuo. The residue was purified by column chromatography on silica gel (eluting with EtOAc/hexanes 2:5) to afford 6 as a white solid [19.6 mg, 38%] along with recovered 5h (18.5 mg, 48%). m. p. 170-173 °C; ¹H-NMR (400 MHz, CDCl₃): δ 14.49 (s, 1H), 9.48 (t, J = 5.8 Hz, 1H), 8.25 (dd, J = 8.8, 5.6 Hz, 2H), 8.05 (dd, J = 8.9, 5.2 Hz, 2H), 7.80-7.77 (m, 2H), 7.50 (t, J = 7.6 Hz, 1H), 7.41 (t, J = 7.5 Hz, 2H), 7.22 (t, J = 8.6 Hz, 2H), 7.06 (t, J = 8.6 Hz, 2H), 6.78 (d, J = 6.8 Hz, 1H), 4.92 (q, J = 6.4 Hz, 1H), 4.23 (q, J = 7.1 Hz, 2H), 3.73 (ddd, J = 17.0, 13.3, 6.1 Hz, 2H), 2.22-2.10 (m, 1H), 2.00-1.77 (m, 2H), 1.27 (t, J = 7.1 Hz, 3H); ¹³C-NMR (100 MHz, CDCl₃): δ 177.6, 172.3, 167.1, 167.1, 166.0 (d, J = 255.8 Hz), 165.4 (d, J = 252.6 Hz), 157.1, 133.8, 133.7 (d, J = 2.7 Hz), 131.9, 131.9 (d, J = 9.1 Hz), 130.6 (d, J = 9.5 Hz), 128.6, 128.2 (d, J = 3.0 Hz), 127.0, 116.4 (d, J = 22.2 Hz), 115.0 (d, J = 21.6 Hz), 61.9, 52.2, 40.8, 30.2, 25.1, 14.2; ¹⁹F-NMR (375 MHz, CDCl₃): δ −104.1, −107.8; IR (thin film): ν 3398, 2928, 1734, 1598, 1576, 1340, 1219, 1142 cm⁻¹; ESI-HRMS calcd for C₂₉H₂₉F₂N₄O₅ [M + H]^⁺ 551.2101, found 551.2100; [α]D: +22.7 (c = 0.70, CHCl₃).

Preparation of N’-(4-fluorobenzoyl)-L-lysine ethyl ester trifluoroacetate salt (7).

In a round-bottom flask, L-lysine ethyl ester dihydrochloride (124 mg, 0.50 mmol, 2.5 equiv) was dissolved in 5 mL of a 1:1 mixture of THF and aqueous buffer pH 3.0 (prepared as described in general procedure A). NCS (133.5 mg, 1.0 mmol, 5.0 equiv) was introduced and the solution stirred for 5 min. Potassium 4-fluorobenzoyltrifluoroborate (1a, 46 mg, 0.20 mmol, 1.0 equiv) was added, and the mixture stirred for 1 h at rt. The mixture was quenched with saturated aqueous NaHSO₃ (1 mL), poured into 1 M NaOH and extracted with EtOAc (3 x 3 mL). The organic extracts were collected, dried over Na₂SO₄, filtered and evaporated in vacuo. The residue was purified by preparative reverse-phase HPLC (YMC C18 column, A/B eluent gradient: 5-95% for 32 min, tR = 15.4 min). The fraction collected was lyophilized to give 7 as a hygroscopic white solid (50.5 mg, 61%). ¹H-NMR (400 MHz, DMSO-d₆): δ 8.58-8.40 (br, 3H), 7.91 (dd, J = 8.6, 5.7 Hz, 2H), 7.28 (t, J = 8.6 Hz, 2H), 4.19 (qd, J = 7.1, 2.6 Hz, 2H), 4.06-3.94 (m, 1H), 3.25 (q, J = 6.4 Hz, 2H), 1.81 (q, J = 7.9 Hz, 2H), 1.60-1.26 (m 4H), 1.20 (t, J = 7.1 Hz, 1H); ¹³C-NMR (100 MHz, DMSO-d₆): δ 170.0, 165.5, 164.2 (d, J = 248.2 Hz), 159.0 (q, J = 33.7 Hz), 131.5 (d, J = 2.8 Hz), 130.2 (d, J = 9.0 Hz), 117.1 (q, J = 295.5 Hz), 115.5 (d, J = 21.7 Hz), 62.2, 52.3, 39.2, 30.3, 29.0, 22.1, 14.3; ¹⁹F-NMR
(375 MHz, DMSO-<sup>d6</sup>): δ −74.1, −109.9; IR (thin film): ν 3310, 2940, 1745, 1677, 1638, 1201, 1134 cm<sup>−1</sup>; ESI-HRMS calcd for C<sub>15</sub>H<sub>22</sub>FN<sub>2</sub>O<sub>3</sub> [M + H]<sup>+</sup> 297.1609, found 297.1610; [α]<sub>D</sub>: +8.3 (c = 0.81, MeOH).

**Preparation of N-(4-fluorobenzoyl)morpholine (8).**<sup>25</sup>

![Chemical structure of 1a and 8](image)

In a round-bottom flask, potassium 4-fluorobenzoyltrifluoroborate (1a, 23 mg, 0.10 mmol, 1.0 equiv) and N-chloromorpholine (150.0 μL, 1.95 mmol, 19.5 equiv) were stirred neat for 30 min at rt. The reaction was quenched with saturated aqueous NaHSO<sub>3</sub> (0.5 mL), poured in H<sub>2</sub>O/brine and extracted with EtOAc (3 x 3 mL). The organic extracts were collected, dried over Na<sub>2</sub>SO<sub>4</sub>, filtered and evaporated in vacuo. The residue was purified by column chromatography on silica gel (eluting with CH<sub>2</sub>Cl<sub>2</sub>/MeOH 100:2.5) to afford 8 as a colorless oil (14.7 mg, 70%). The spectral data was in accordance with the literature.

**Preparation and acylation of N-chlorobutylamine.**

![Chemical structure of 2o and 9](image)

In a Schlenk flask under N<sub>2</sub> atmosphere, CDCl<sub>3</sub> (2 mL), n-butylamine (2o, 198 μL, 2.0 mmol) and NCS (267 mg, 2.0 mmol, 1.0 equiv) were introduced, stirred at rt for 10 min, and filtered. The concentration of N-chlorobutylamine (2o–Cl<sub>1</sub>) on the filtrate was calculated by NMR analysis (using nitromethane (5.4 μL, 0.10 mmol) as internal standard) and found to be ca. 0.58 M. The solution was used for the next step without further manipulation. The spectral data of N-chlorobutylamine was in accordance with the literature.<sup>26</sup>

A round bottom flask was charged with potassium 4-fluorobenzoyltrifluoroborate 1a (23.0 mg, 0.10 mmol, 1.0 equiv) and THF (0.5 mL). To this suspension, the freshly prepared solution of N-chlorobutylamine (2o–Cl<sub>1</sub>, 206 μL, 0.12 mmol, 1.2 equiv, ca. 0.58 M in CDCl<sub>3</sub>) and aqueous buffer pH3.0 (0.5 mL, citric acid and trisodium citrate, 25mM) were sequentially added. The mixture was
stirred at rt for 45 min, quenched with saturated aqueous NaHSO$_3$ (0.3 mL), poured in H$_2$O/brine and extracted with EtoAc (3 x 3 mL). The organic extracts were collected, dried over Na$_2$SO$_4$, filtered and evaporated in vacuo. The residue was purified by column chromatography on silica gel (eluting with EtoAc/hexanes 1:2) to afford N-butyl-4-fluorobenzamide 9 as a white solid (17.4 mg, 89%). The spectral data was in accordance with the literature.$^{27}$

$^1$H-NMR (400 MHz, CDCl$_3$): $\delta$ 7.76 (dd, $J = 8.8, 5.3$ Hz, 2H), 7.09 (t, $J = 8.6$ Hz, 2H), 3.44 (td, $J = 7.2, 5.7$ Hz, 2H), 1.59 (p, $J = 7.8$ Hz, 2H), 1.40 (sx, $J = 7.5$ Hz, 2H), 0.95 (t, $J = 7.3$ Hz, 2H); $^{13}$C-NMR (100 MHz, CDCl$_3$): $\delta$ 166.5, 164.6 (d, $J = 251.5$ Hz), 131.0 (d, $J = 3.2$ Hz), 129.1 (d, $J = 8.8$ Hz), 115.5 (d, $J = 21.9$ Hz), 39.9, 31.7, 20.2, 13.8; $^{19}$F-NMR (375 MHz, CDCl$_3$): $\delta$ $-$108.6.

**Attempted oxidation of imine 10.**

![Diagram](image)

MS 4Å (200 mg) were introduced in a round-bottom flask and flame-dried in vacuo. 1a (23.0 mg, 0.10 mmol, 1.0 equiv), CD$_3$CN (1 mL) and 2-phenethylamine 2a (12.4 µL, 0.10 mmol, 1.0 equiv) were added sequentially and the mixture refluxed for 1 h and cooled to rt. The formation of imine 10 was confirmed by LC-MS analysis of the mixture. $^{19}$F-NMR experiments revealed the disappearance of the fluorine peak from 1a, and the formation of two more BF$_3$ peaks that might correspond to the two possible stereoisomers of 10. NCS (13.4 mg, 0.10 mmol, 1 equiv) was added and the suspension stirred at rt for 1 h. The reaction was quenched with saturated aqueous NaHSO$_3$ (0.3 mL), filtered through celite, poured in H$_2$O/brine and extracted with EtoAc (3 x 3 mL). The organic extracts were collected, dried over Na$_2$SO$_4$, filtered and evaporated in vacuo. Analysis of the NMR of the mixture revealed only traces of amide 3a.
Stability of KAT 1a towards chlorinating agents.

1a is stable towards DCH for several hours. However, after stirring a solution of 1a (23.0 mg, 0.10 mmol, 1.0 equiv) and DCH (21.7 mg, 0.11 mmol, 1.1 equiv) in a mixture of THF and aqueous citrate buffer pH 3.0 (1:1, 2 mL), partial decomposition of 1a to 4-fluorobenzoic acid was observed by LC-MS analysis of the reaction mixture. The solution was quenched with saturated aqueous NaHSO₃ (0.5 mL), poured into 1 M aqueous HCl and brine and extracted with EtOAc (3 x 3 mL). The organic extracts were collected, dried over Na₂SO₄, filtered and evaporated in vacuo.
4-Fluorobenzoic acid was formed in 51% yield as determined by NMR analysis of the residue using CH$_3$NO$_2$ (5.41 µL, 0.10 mmol) as internal standard.

**Determination of molar absorptivity and rate constant for the formation of N-chlorobutylamine.**

\[
\text{N}-\text{Chlorobutylamine (2o–Cl)} \text{ was prepared and its concentration determined in an analogous manner as described above, but using CD$_3$CN in replacement of CDCl$_3$. To determine the molar absorptivity of N-chlorobutylamine, seriated solutions (final concentrations 0.50-1.25 mM) were prepared in a 1:1 mixture of THF and aqueous buffer pH 3.0 (prepared by dissolving citric acid monohydrate (4.48 g, 21.35 mmol) and trisodium citrate dihydrate (1.07 g, 3.64 mmol) in distilled water (1 L), final pH was adjusted using 0.10 M NaOH and HCl solutions). Absorbances at 270 nm are measured and plotted over molar concentration. The extinction coefficient of 1a was found to be 296.1 ± 6.0 M$^{-1}$ cm$^{-1}$.}
\]

![](image)

\[
y = 296.1x + 0.007
\]
\[
R^2 = 0.9947
\]

A solution of N-butylamine (2o) in a 1:1 mixture of THF and aqueous buffer pH 3.0 was prepared (2 mL, 2.5 mM). NCS (50 µL from 100 mM solution, final concentration 2.5 mM) was added. The absorbance at 270 nm was measured while mildly stirring the solution. Concentrations of N–chlorobutylamine produced were calculated and plotted over time.

To properly fit the product concentration ([P]) over time (t) plot, the starting material concentration at a time t ([A]) needs to be properly converted to product concentration, as follows:

\[
[A] = [A_0] - [P]
\]

Where \(A_0\) is the initial starting material concentration. This gives the following differential equation:

\[
v = \frac{d[P]}{dt} = k[P]^2 + k[A_0]^2 - 2k[A_0][P]
\]
By fitting the data with the differential equation above, the corresponding best-fit rate constants were obtained with excellent $R^2$ values. The average second order rate constant was found to be $k = 2.4 \pm 0.4 \text{ M}^{-1} \text{s}^{-1}$.

**UV-Vis spectrum and determination of molar absorptivity of 1a**

To determine the molar absorptivity of 4-fluorobenzoyltrifluoroborate 1a, seriated solutions of 1a (final concentrations 3-15 mM) were prepared in a 1:1 mixture of THF and aqueous buffer pH 3.0 (prepared by dissolving citric acid monohydrate (4.48 g, 21.35 mmol) and trisodium citrate dihydrate (1.07 g, 3.64 mmol) in distilled water (1 L), final pH was adjusted using 0.1 M NaOH and HCl solutions). Absorbances at 245 nm are measured and plotted over molar concentration. The extinction coefficient ($\varepsilon$) of 1a was found to be $6.97 \cdot 10^3 \text{ M}^{-1} \text{ cm}^{-1}$. 

$y = 6969x - 0.005523$

$r^2 = 0.9996$
Kinetic studies of the acylation of \( N \)-chlorobutylamine monitored by UV.

\[
\begin{align*}
\text{1a} & \quad \text{BF}_3K & \quad \text{Cl} - \text{N}^+ \text{nBu} \\
\text{THF / aqueous buffer pH 3.0 (1:1)} & \quad \text{245 nm} \\
\lambda_{\text{max}} & = 245 \text{ nm} \\
\varepsilon & = 6.97 \times 10^3 \text{ M}^{-1} \text{ cm}^{-1} \\
\varepsilon & = 3.74 \times 10^3 \text{ M}^{-1} \text{ cm}^{-1}
\end{align*}
\]

A solution of around 0.10 mM concentration of \( 1a \) in a 1:1 mixture of THF and aqueous buffer pH 3.0 was prepared. The concentration was measured by UV-Vis (measuring the absorbance at 245 nm, \( \varepsilon = 6.97 \times 10^3 \text{ M}^{-1} \text{ cm}^{-1} \)) and found to be 0.12 mM. 2 mL (0.24 \( \mu \)mol, 1 equiv) were transferred to a quartz cuvette equipped with a stir bar. Freshly prepared \( N \)-chlorobutylamine (prepared as described above, 50.3 mM, 4.89 \( \mu \)L, 0.24 \( \mu \)mol, 1.0 equiv) was added and the absorbance at 245 nm was monitored at rt while stirring slowly. Concentrations of starting material were calculated from the molar absorptivities of both \( 1a \) and \( 9 \), by the following equation:

\[
[C_{1a}] = \frac{A_t - (\varepsilon_9 C_0)}{\varepsilon_{1a} - \varepsilon_9}
\]

Where \( A_t \) is total absorbance value measured, \( C_0 \) is the initial concentration value and \( \varepsilon_{1a} \) and \( \varepsilon_9 \) are the extinction coefficients of \( 1a \) and \( 9 \) respectively.

The reaction between \( 1a \) and \( N \)-chlorobutylamine is a bimolecular process that follows a second-order kinetics. Reaction rates can be written as follows:

\[
\nu = -\frac{d[A]}{dt} = k[A][B]
\]

Since the reaction was carried out with stoichiometric amounts of both reagents, the equation above can be converted to the following equation:

\[
\nu = -\frac{d[A]}{dt} = k[A]^2
\]

The differential equation above shows that second-order rate constants can be determined by plotting \([A]\) against \(t\). Non-linear fit of the data by using this equation gave the corresponding best-fit second-order rate constants with reasonable \( r^2 \) values.

From the plots below, a second-order kinetic constant of 8.3 \( \pm \) 1.5 M\(^{-1}\) s\(^{-1}\) was obtained.
Epimerization Studies of Compound 3c.

**HPLC:** Daicel Chiralcel (4.6 × 250 mm); iPrOH/hexanes (1:4), flow 0.5 mL/min. Retention times: 13.2 min (minor), 18.9 min (major). ee = 99.5%.

**General procedure C: Late-stage labeling of natural products**

In a round-bottom flask, the corresponding natural product (5.0-10.0 equiv) was dissolved in a mixture of THF and aqueous buffer pH 3.0 (prepared as described in general procedure A). DCH or NCS (2.0-10.0 equiv) were added and the mixture stirred at rt for 10 min. KAT 11 or 12 (1.0 equiv) were subsequently added and the solution stirred at rt. The reaction was monitored by analytical
HPLC and quenched with saturated aqueous NaHSO₃. The crude solution was purified by preparative reverse phase HPLC

\( N^6(C^1/C^3)-(4-(N\text{-propargylcarbamoyl})\text{benzoyl})\text{streptomycin trifluoroacetate 11a/11b.} \)

Prepared by following general procedure C with streptomycin sesquisulfate (391 mg, 0.50 mmol, 5.0 equiv), KAT 11 (29.3 mg, 0.10 mmol, 1.0 equiv) and DCH (39.4 mg, 0.20 mmol, 2.0 equiv) in 4 mL of a 1:3 mixture of THF and aqueous buffer pH 3.0, 25 mM (prepared as described in general procedure A) for 8 h. The solution was purified by preparative reverse phase HPLC (YMC C18 column, A/B eluent gradient: 5-95% for 25 min) to give the title compound as a mixture of four isomers in two main peaks. Based on the chemical shift of guanidine carbons, we have tentatively assigned the main structure of the isolated peaks to 11a (t<sub>r</sub> = 10.5 min, 15.8 mg, ~80% purity, 0.016 mmol, 15%) and 11b (t<sub>r</sub> = 11.5 min, 14.9 mg, > 95% purity, 0.016 mmol, 17%), each of them containing two inseparable hemiaminal tautomers. From the crude analytical HPLC and MALDI traces (see below), the reaction appeared to be complete, so we attribute the low yields obtained to technical problems during the purification step.

11a: m.p. >120 °C (decomp.); \(^1\text{H-NMR}\) (600 MHz, CD<sub>3</sub>OD): δ 8.17 (m, 4H, Ar<sup>c</sup>H), 8.04 (m, 4H, Ar<sup>d</sup>H), 5.38-5.31 (m, 4H, C<sup>1'</sup>H, C<sup>1''</sup>H), 4.48 (br s, 1H, C<sup>3'</sup>–C<sup>H</sup>O), 4.45 (br s, 1H, C<sup>3'</sup>–C<sup>H</sup>O), 4.42-4.36 (m, 4H, C<sup>2'</sup>H, C<sup>4'</sup>H), 4.17 (d, J = 2.6 Hz, 4H, C<sup>2</sup>H<sup>2</sup>C≡C), 3.96-3.88 (m, 4H, C<sup>3</sup>H, C<sup>6''</sup>H), 3.80-3.68 (m, 6H, C<sup>3''</sup>H, C<sup>5''</sup>H, C<sup>4</sup>H), 3.66-3.59 (m, 4H, C<sup>3</sup>H, C<sup>6''</sup>H), 3.56-3.50 (m, 2H, C<sup>5</sup>H), 3.44-3.38 (m, 4H, C<sup>1</sup>H, C<sup>6</sup>H), 2.99-2.93 (m, 2H, C<sup>4</sup>H<sup>2</sup>C≡C), 2.86-2.70 (m, 8H, C<sup>2</sup>–NC<sub>H</sub>₃, C<sup>2</sup>–C≡C), 2.63 (t, J = 2.5 Hz, 2H, C≡CH), 1.25-1.22 (m, 6H, C<sup>5</sup>H<sub>3</sub>); \(^13\text{C-NMR}\) (151 MHz, CD<sub>3</sub>OD): δ 169.3 (C<sup>a</sup>), 168.2 (C<sup>f</sup>), 163.3 (q, J = 34.7 Hz, CF<sub>3</sub>CO<sub>2</sub>H), 160.5 (C<sup>1</sup>–NH–C(NH)NHR), 157.0 (C<sup>3</sup>–NH–C(NH)NH₂), 140.0 (C<sup>o</sup>), 135.7 (C<sup>c</sup>), 129.8 (C<sup>c</sup>), 129.1 (C<sup>c</sup>), 118.2 (q, J = 292.9 Hz, CF<sub>3</sub>CO<sub>2</sub>H), 107.9 (C<sup>c</sup>), 107.8 (C<sup>c</sup>), 98.5 (C<sup>3</sup>–CHO), 98.4 (C<sup>3</sup>–CHO), 97.0 (C<sup>c</sup>'), 96.8 (C<sup>c</sup>'), 88.4 (C<sup>c</sup>'), 87.4 (C<sup>c</sup>), 83.2 (C<sup>c</sup>), 82.6 (C<sup>c</sup>), 81.1 (C<sup>c</sup>), 80.9 (C<sup>c</sup>), 80.4 (CH₂C≡C), 78.6 (C<sup>c</sup>), 78.4 (C<sup>c</sup>), 75.7 (C<sup>c</sup>), 75.5 (C<sup>c</sup>), 75.3 (C<sup>c</sup>), 73.3 (C<sup>c</sup>), 72.3 (C≡CH), 72.2 (C<sup>c</sup>), 72.1 (C<sup>c</sup>), 72.0 (C<sup>c</sup>), 70.6 (C<sup>c</sup>), 70.6 (C<sup>c</sup>), 63.5 (C<sup>c</sup>), 63.3 (C<sup>c</sup>), 62.5 (C<sup>c</sup>), 60.7 (C<sup>c</sup>), 59.9 (C<sup>c</sup>), 33.0 (C<sup>2</sup>–NCH₂), 32.6 (C<sup>2</sup>–NCH₂), 30.1 (CH₂C≡C), 24.9,
13.2 \((C^5)\), 12.9 \((C^5)\); IR (thin film): \(\nu\) 3322, 2983, 1668, 1206, 1143 \(cm^{-1}\); \textbf{ESI-HRMS} calcd for \(C_{33}H_{51}N_8O_{15} [M + H + MeOH]^+\) 799.3468, found 799.3455; \([\alpha]_D: -27.8 \(c = 0.35,\ MeOH\)\).

\textbf{11b: m.p.} >140 °C (decomp.); \(\textbf{\(^1H-NMR}\) (600 MHz, CD\textsubscript{3}OD): \(\delta\) 8.10 (d, \(J = 8.5\ Hz, 4H, Ar^I H\), 7.98 (d, \(J = 8.4\ Hz, 4H, Ar^II H\), 5.47 (d, \(J = 3.6\ Hz, 1H, C^I H\), 5.45 (d, \(J = 3.6\ Hz, 1H, C^{I '} H\), 5.28 (d, \(J = 1.9\ Hz, 1H, C^I ' H\), 5.27 (d, \(J = 2.1\ Hz, 1H, C^I H\), 4.52 (s, 1H, C\(^{3−}−\text{CHO}\)), 4.50 (s, 1H, C\(^{3−}−\text{CHO}\)), 4.48-4.36 (m, 4H, C\(^{2−} H\), C\(^4− H\), 4.17 (d, \(J = 2.5\ Hz, 4H, CH_2C≡C\), 4.08-4.04 (m, 2H), C\(^{6−}−\text{NCH}_3\), 2.62 (t, \(J = 2.5\ Hz, 2H, C≡CH\), 1.26 (d, \(J = 6.3\ Hz, 3H\), 1.25 (d, \(J = 6.3\ Hz, 3H\); \(\textbf{\(^{13C-NMR}\)} (151 MHz, CD\textsubscript{3}OD): \(\delta\) 169.5 \((C^a)\), 168.3 \((C^i)\), 163.3 \((q, J = 33.7\ Hz, CF_3CO_2H\), 159.8 \((C^−\text{NHC(NH)NHR}\), 157.5 \((C^−\text{NHC(NH)NH}_2\), 140.0 \((C^o)\), 135.6 \((C^o)\), 129.7 \((C^o)\), 129.0 \((C^i)\), 118.2 \((q, J = 292.2\ Hz, CF_3CO_2H\), 108.3 \((C^I)\), 108.2 \((C^I ')\), 98.6 \((C^{3−}−\text{CHO}\)), 98.4 \((C^{3−}−\text{CHO}\)), 97.1 \((C^o)\), 96.9 \((C^o)\), 88.4 \((C^2)\), 87.5 \((C^2)\), 83.2 \((C^3)\), 82.7 \((C^3)\), 81.2 \((C^i)\), 80.4 \((CH_2C≡C\), 78.5 \((C^4)\), 78.3 \((C^4)\), 75.6 \((C^5)\), 75.6 \((C^5)\), 75.4 \((C^5)\), 72.9 \((C^6)\), 72.9 \((C^6)\), 72.3 \((C≡CH\), 72.1 \((C^I)\), 70.6 \((C^3)\), 63.3 \((C^2)\), 63.1 \((C^2)\), 62.8 \((C^6)\), 62.7 \((C^6)\), 60.7 \((C^i)\), 59.9 \((C^o)\), 59.8 \((C^o)\), 33.1 \((C^{2−−}\text{NCH}_3\), 32.7 \((C^{2−−}\text{NCH}_3\), 30.1 \((CH_2C≡C\), 13.2 \((C^5)\), 12.9 \((C^5)\); IR (thin film): \(\nu\) 3308, 1673, 1203, 1138 \(cm^{-1}\); \textbf{ESI-HRMS} calcd for \(C_{33}H_{51}N_8O_{15} [M + H + MeOH]^+\) 799.3468, found 799.3458; \([\alpha]_D: -37.2 \(c = 0.48,\ MeOH\)\).

**Crude analytical HPLC**

(5-95% A/B, monitoring at \(\lambda = 254\ nm\) for 17 min):

![Crude analytical HPLC graph](image_url)
A negative control experiment was performed where we mixed streptomycin sesquisulfate (72.8 mg, 0.10 mmol, 1.0 equiv) and DCH (19.7 mg, 0.10 mmol, 1.0 equiv) in 2 mL of a 1:3 mixture of THF and aqueous buffer pH 3.0, 25 mM (prepared as described in general procedure A). The solution was stirred at rt for 8 h, quenched with aqueous NaHSO₃ and lyophilized. The residue was dissolved in D₂O and the ¹H-NMR spectrum was recorded and compared with the ¹H-NMR of the starting material. We observed that several proton and carbon signals split to two after treatment with chlorinating agent (see Figure 6.1 below for a comparison between the spectra, zoomed at the aliphatic methyl peaks). This effect is more pronounced in the signals assigned to and close to the streptose ring, so we attribute this to a possible tautomerization of the aldehyde group at the streptose ring (middle, five-membered ring), to give the corresponding internal hemiaminal products.

![Crude MALDI-TOF spectrum](image)

**Figure 6.1.** Comparison between ¹H-NMR (4.5-6.0 ppm-zoomed region) of streptomycin before and after treatment with DCH.
**N-(4-(N-Propargylcarbamoyl)benzoyl)gentamicin C<sub>1</sub>.**

Prepared by following general procedure C using gentamicin C<sub>1</sub> free base (139 mg, 0.29 mmol, 3.0 equiv), NCS (40 mg, 0.29 mmol, 3.0 equiv) and KAT 1I (28.4 mg, 0.10 mmol, 1.0 equiv) in 4 mL of a 1:3 mixture of THF and aqueous buffer pH 3.0, 25 mM (prepared as described in general procedure A) for 3 h. The solution was lyophilized and the residue purified by preparative reverse phase HPLC (YMC C18 column, A/B eluent gradient: 5-50% for 25 min, t<sub>R</sub> = 18.2 min) to give the title compound as a white powder (20.2 mg, 25%). m.p. >140 °C (decomp); <sup>1</sup>H-NMR (600 MHz, CD<sub>3</sub>OD): δ 7.94-7.89 (m, 4H), 6.00 (br s, 1H), 5.10 (d, J = 7.4 Hz, 1H), 4.31-4.22 (m, 2H), 4.16 (d, J = 2.6 Hz, 2H), 4.11-4.07 (m, 1H), 4.05 (d, J = 12.5 Hz, 1H), 4.00 (dd, J = 10.7, 3.4 Hz, 1H), 3.83-3.75 (m, 2H), 3.47-3.36 (m, 3H), 3.34-3.30 (m, 1H), 3.16 (d, J = 10.7 Hz, 1H), 2.90-2.85 (m, 1H), 2.75 (br s, 3H), 2.66 (br s, 3H), 2.61 (t, J = 2.5 Hz, 1H), 2.40-2.33 (m, 1H), 2.19-2.09 (m, 2H), 2.07-1.93 (m, 2H), 1.87-1.81 (m, 1H), 1.68-1.56 (m, 2H), 1.31 (d, J = 6.6 Hz, 3H), 1.24 (s, 3H); <sup>13</sup>C-NMR (150 MHz, CD<sub>3</sub>OD): δ 169.6, 168.9, 138.6, 138.2, 129.0, 128.7, 100.6, 96.6, 83.7, 80.7, 78.8, 76.9, 72.3, 70.8, 70.0, 69.1, 68.3, 66.1, 59.3, 50.9, 50.5, 50.2, 36.1, 32.3, 32.1, 30.2, 25.1, 22.3, 22.2, 10.6; IR (thin film): ν 3293, 3041, 1675, 1201, 1134 cm<sup>-1</sup>; ESI-HRMS calcd for C<sub>32</sub>H<sub>51</sub>N<sub>6</sub>O<sub>9</sub> [M + H]<sup>+</sup> 663.3712, found 663.3706; [α]<sub>D</sub>: +57.9 (c = 0.14, MeOH). Note: from the corresponding control experiments, no substantial change was observed in the NMR spectra of gentamicin C<sub>1</sub> after treatment with NCS.

**Crude analytical HPLC**

(5-95% A/B, monitoring at λ = 254 nm for 17 min):

![Crude analytical HPLC graph](image)

**N-MonoPEGylated polymyxin B**
Prepared by following general procedure C using polymyxin sulfate (130.3 mg, 0.10 mmol, 10.0 equiv), NCS (13.3 mg, 0.10 mmol, 10.0 equiv) and PEG-KAT 12 (MW ~5,000 Da, 50.0 mg, 0.010 mmol, 1.0 equiv) in 1 mL of a 1:3 mixture of THF and aqueous buffer pH 3.0, 25 mM (prepared as described in general procedure 1) for 15 h. The solution was purified by spin filtration (MWCO = 3,000 Da, washing with CH$_3$CN / 0.1 M HCl (1:3, 4 x 10 mL)) and lyophilized to give the title compound as a white powder (46.5 mg, 74%).

**Analytical HPLC (20-95% A/B, monitoring at $\lambda = 354$ nm, 17 min):**

![HPLC graph](image)

**MALDI-TOF:**

![MALDI-TOF graphs](image)

**Labelling studies of BSA with KAT 13**
To an Eppendorf tube containing a solution of commercial bovine serum albumin (BSA, 50 μL, 75 μM, 3.75 nmol) in PBS buffer pH 6.0 was added NaOCl (2.5 μL from 20 mM stock solution, 50.0 nmol, 13.3 equiv). The solution was shaken gently at rt for 20 min. KAT 13 (2 μL from 50 mM stock solution, 100.0 nmol, 26.6 equiv) was added and the mixture shaken gently at rt for 5 h. Biotin-azide 14 (44.4 μg, 100.0 nmol, 26.6 equiv), copper sulfate pentahydrate (25.0 μg, 100.0 nmol, 26.6 equiv) and tris((1-benzyl-1H-1,2,3-triazol-4-yl)methyl)amine (TBTA, 5.6 μg, 10.0 mmol, 2.6 equiv) were added sequentially followed by sodium ascorbate (100.0 μg, 1.0 μmol, 133.0 equiv). The mixture was shaken gently at rt overnight, spin-filtered (MWCO 10 kDa) ans washed with PBS pH 7.4. The solution was purified by affinity chromatography using agarose-supported monomeric avidin supported in agarose. Eluted fractions were pooled, buffer replaced with 0.01% HCO₂H by spin filtration (MWCO 10 kDa), collected in a LoBind® Eppendorf tube and analyzed by LC-MS/MS proteomics (MW increase = 538 Da / modified residue).

LC-MS/MS analysis showed that BSA sequence could not be successfully mapped by direct search of the expected mass increase at the modified residues. However, by introducing side reactions such as tyrosine chlorination and cysteine/methionine oxidation, the protein sequence could be covered in > 95% by LC-MS/MS. The following lysine residues showed the expected mass increase corresponding to the biotin label:
MKWTVFISLL LLFSSAYSRG VFRRDTHKSE IAHRFKDLGE EHKFLVLIA
FSQYLQQCFP DEHVKLVNEL TEFAKTCVAD ESHAGCEKSL HTLFQGDELCK
VSLRETYGD MADCEKQEP ERNECFLSHK DDSPDLFPKLK PDPNTLCDEF
KADKKFPGK YLYEIAARRHP YFYAPELLEYY ANKYNGVFQBE CCQAEDKGAC
LPKIERMRE KVLSARQKR LQCASIQKFG ERALKAWSVA RLSQKFPKAE
FVEVTKLVTLD TKVHKECHGD LLLCADDRE DLAKYICDN QDTISSKLKE
CCDKFLLEKS HCIAEVEKDA IPENLPPLTA DFAEDKVCK NYQEAADFL
GSFLYEYSRR HPEYAVSVLL RLAKYEATL EECCAKDDPH ACYSTVFDKL
KHLVDEPQNL IKQNCQFKEK LGEYGQNAL IVRYTRKVQ PVSTPLVEVS
RSLEGVQTRGLCTKHELMR CTEYDSLIL NRCVLHEKT PVSEKVTKCC
TESLVRNRFPC FSALTPETY VPKAFDEKLF TFHADICTLP DTEKVTKKQT
ALVELLKHKP KATEEQLKTV MENFVAFVDK CCAADDKKEAC FAVEGPKLVV
STQTALA K = Lysines modified with the biotin tag
7.3 Experimental procedures and characterization data for Chapter 3

Gel formation rheometry measurements.

4-Armed PEG 15 (2.0 mg, 0.2 μmol, 1.0 equiv) and amine 16 (38.9 μg, 0.27 μmol, 1.33 equiv) were dissolved in aqueous buffer (50 μL, pH 1-7, 4 wt.% PEG). The solution was placed on the plate of the rheometer, NCS was added (from 1 M solution in CH$_3$CN, 0–20 equiv) and rheology measurement started. The corresponding plots of storage and loss moduli over time for all conditions tested are shown below as the mean of 3 runs. Error bars are omitted for clarity.
7.4 Experimental procedures and characterization data for Chapter 4

7.4.1 Synthesis of common precursors and products

Synthesis of 7-diethylamino-2-oxo-2H-chromene-3-carboxamidoethylammonium trifluoroacetate (20)

A round-bottom flask was charged with 7-diethylaminocoumarin-3-carboxylic acid (63, 960 mg, 3.7 mmol, 1.00 equiv) and HATU (1.55 g, 4.1 mmol, 1.10 equiv). The solids were suspended in CH$_2$Cl$_2$ (30 mL) and DIPEA (1.66 mL, 9.2 mmol, 2.50 equiv) was added. The suspension was stirred at rt until all the suspended solids completely dissolved. tert-Butyl-2-aminoethyl carbamate (760 µL, 4.8 mmol, 1.30 equiv) was added and the solution stirred at rt overnight. The solution was poured into 0.5 M aqueous HCl and extracted with CH$_2$Cl$_2$. The organic phase was collected, neutralized with saturated aqueous NaHCO$_3$ and extracted again with CH$_2$Cl$_2$. The organic extracts were collected, dried with Na$_2$SO$_4$, filtered and evaporated in vacuo. The residue was purified by column chromatography on silica gel (eluting with CH$_2$Cl$_2$/MeOH = 100:3) to give 64 as a bright yellow solid (1.20 g, 80%);$^{28}$ $^1$H-NMR (500 MHz, CDCl$_3$): $\delta$ 8.95 (t, $J = 6.2$ Hz, 1H), 8.68 (s, 1H), 7.42 (dd, 3H, ArH), 7.1 (t, $J = 8.9$, 1.1 Hz, 1H), 6.66 (ddd, $J = 8.9$, 2.5, 1.0 Hz, 1H), 6.51 (br s, 1H), 5.10 (br s, 1H), 3.55 (q, $J = 6.0$ Hz, 2H), 3.45 (q, $J = 6.8$ Hz, 4H), 3.35 (t, $J = 5.9$ Hz, 2H), 1.43 (s, 9H), 1.23 (t, $J = 6.9$ Hz, 6H); $^{13}$C-NMR (126 MHz, CDCl$_3$): $\delta$ 164.0, 162.7, 157.7, 156.1, 152.5, 148.2, 131.3, 110.3, 108.7, 97.0, 79.4, 45.4, 41.1, 39.8, 28.5, 12.5.

In a round-bottom flask, carbamate 64 (810 mg, 2.0 mmol, 1.0 equiv) was dissolved in CH$_2$Cl$_2$ (6 mL). Trifluoroacetic acid (2 mL) was added and the mixture stirred at rt for 1 h. The solution was evaporated by rotavap and Et$_2$O was added to the crude. The solids were collected by vacuum filtration, washed with Et$_2$O and dried in vacuo to give ammonium salt 20 as a bright yellow solid (786 mg, 94%). m.p. 198-202 °C; $^1$H-NMR (500 MHz, CD$_3$OD): $\delta$ 8.63 (m, 1H), 7.56-7.52 (m, 1H), 6.85-6.80 (m, 1H), 6.56 (s, 1H), 3.69 (t, $J = 5.8$ Hz, 2H), 3.53 (q, $J = 7.2$ Hz, 4H), 3.17 (t, $J = 5.6$ Hz, 2H), 1.24 (t, $J = 7.1$ Hz, 6H); $^{13}$C-NMR (126 MHz, CD$_3$OD): $\delta$ 166.8, 163.9, 162.9 (q, $J = 35.6$ Hz), 159.3, 154.8, 149.6, 132.7, 119.3 (q, $J = 290.0$ Hz), 111.7, 109.7, 109.4, 97.3, 46.0, 41.1, 38.5, 12.7; IR (thin film): ν 3318, 2976, 1691, 1616, 1578, 1509, 1418, 1352, 1187, 1131 cm$^{-1}$; ESI-HRMS calcd for C$_{16}$H$_{22}$N$_3$O$_3$ [M + H]$^+$ 304.1656, found 304.1652.
Synthesis of 4-(N-tert-butoxycarbonyl-N-(N'-methyl-N'-propargylcarbamoyloxy) amino)butanoic acid (19)

Following a reported procedure, a 100 mL Schlenk flask was charged with triphosgene (2.96 g, 10.0 mmol, 0.33 equiv) and purged with N₂ gas. CH₂Cl₂ (40 mL) was added under N₂ and the solution cooled to –78 ºC. Pyridine (242 µL, 3.0 mmol, 0.10 equiv) and a solution of N-methylpropargylamine (17, 2.66 mL, 30.0 mmol, 1.00 equiv) in CH₂Cl₂ (5 mL) were added sequentially under N₂. The reaction was removed from the cooling bath and stirred at rt for 72 h. The solvents were removed by evaporation and the residue purified by Kugelrohr distillation (100 ºC, high vacuum) to give the corresponding carbamoyl chloride as a colorless oil (2.22 g, 56%), which was taken directly to the next step.

In a round-bottom flask, N-tert-butoxycarbonylhydroxylamine (2.93 g, 22.0 mmol, 1.30 equiv) and DMAP (210 mg, 1.70 mmol, 0.10 equiv) were added. The solids were dissolved in CH₂Cl₂ (35 mL) under N₂. Triethylamine (3.13 mL, 22.0 mmol, 1.30 equiv) followed by a solution of the distilled carbamoyl chloride above (2.22 g, 16.8 mmol, 1.00 equiv) in CH₂Cl₂ (3 mL) were added to the mixture. The reaction was refluxed and stirred overnight. After cooling to rt, the reaction was quenched with 0.5 M aqueous HCl. The phases were separated and the aqueous phase extracted with CH₂Cl₂. The organic extracts were collected, dried with Na₂SO₄, filtered and evaporated in vacuo. The residue was purified by column chromatography on silica gel (eluting with EtOAc/hexanes = 1:4) to give hydroxylamine 18 as a viscous oil (16.5 mmol, quant.); ¹H-NMR (500 MHz, CDCl₃): δ 7.85 (br s, 1H), 4.17-4.10 (m, 2H), 3.09-2.99 (m, 3H), 2.28 (br s, 1H), 1.48 (s, 9H); ¹³C-NMR (126 MHz, CDCl₃): δ 156.3, 156.1 (CO rotamers), 155.5 (CO rotamers), 83.1, 77.7, 73.1, 39.2 (N-CH₃ rotamers), 38.1 (N-CH₃ rotamers), 34.7 (N-CH₂ rotamers), 33.2 (N-CH₂ rotamers), 28.1; IR (thin film): ν 3261, 2979, 1725, 1369, 1251, 1120 cm⁻¹; ESI-HRMS calcd for C₁₀H₁₆N₂NaO₄ [M + Na]⁺ 251.1002, found 251.1011.

A round-bottom flask was charged with hydroxylamine 18 (2.0 g, 8.8 mmol, 1.00 equiv) and K₂CO₃ (3 g, 22 mmol, 2.50 equiv). The solids were suspended in DMF (40 mL) and ethyl 3-bromopropanoate (1.85 mL, 95% purity, 12.3 mmol, 1.40 equiv) was added. The suspension was stirred at 60 ºC overnight and filtered through celite. The filtrate was diluted with EtOAc/hexanes (4:1) and washed with H₂O/brine. The organic extracts were collected, dried with Na₂SO₄, filtered
Chapter 7. Experimental Section

and evaporated in vacuo. The residue was purified by column chromatography on silica gel (eluting with EtOAc/hexanes = 1:5 to 1:3) to give hydroxylamine 65 as a viscous oil (2.23 g, 74%); \textsuperscript{1}H-NMR (500 MHz, CDCl\textsubscript{3}): \(\delta\) 4.14-4.10 (m, 4H), 3.66 (t, \(J = 7.2\) Hz, 2H), 3.03 (br s, 3H), 2.40 (t, \(J = 7.5\) Hz, 2H), 2.27 (t, \(J = 2.4\) Hz, 1H), 1.91 (p, \(J = 7.1\) Hz, 2H), 1.46 (s, 9H), 1.24 (t, \(J = 7.1\) Hz, 3H); \textsuperscript{13}C-NMR (126 MHz, CDCl\textsubscript{3}): \(\delta\) 173.2, 154.9, 154.7 (C\textsubscript{O} rotamers), 82.3, 78.0, 72.9, 60.5, 49.9, 39.1 (N-C\textsubscript{H}\textsubscript{3} rotamers), 38.4 (N-C\textsubscript{H}\textsubscript{2} rotamers), 34.9 (N-C\textsubscript{H}\textsubscript{2} rotamers), 31.4, 28.3, 22.8, 14.4; \textsuperscript{IR} (thin film): \(\nu\) 3261, 2979, 1728, 1368, 1159, 1105 cm\textsuperscript{-1}; \textsuperscript{ESI-HRMS} calcd for C\textsubscript{16}H\textsubscript{27}N\textsubscript{2}O\textsubscript{6} \([M + H]\)\textsuperscript{+} 343.1864, found 343.1862.

In a round-bottom flask ethyl ester 65 (1.14 g, 3.33 mmol, 1.00 equiv) and lithium hydroxide monohydrate (420 mg, 10.0 mmol, 3.00 equiv) were introduced and dissolved in THF/MeOH/H\textsubscript{2}O (5:2:1, 10 mL). The solution was stirred for 3 h at rt and acidified with 1 M HCl to pH 1-2. The suspension was extracted with CH\textsubscript{2}Cl\textsubscript{2}. The organic extracts were collected, dried with Na\textsubscript{2}SO\textsubscript{4}, filtered and evaporated in vacuo. The residue was purified by column chromatography on silica gel (eluting with CH\textsubscript{2}Cl\textsubscript{2}/MeOH/AcOH = 100:2:0.5) to give carboxylic acid 19 as a white solid (609 mg, 58%). m.p. 63-68 °C; \textsuperscript{1}H-NMR (500 MHz, CDCl\textsubscript{3}): \(\delta\) 4.14 (br s, 2H), 3.70 (t, \(J = 6.7\) Hz, 2H), 3.05 (br s, 3H), 2.50 (t, \(J = 7.3\) Hz, 2H), 2.29 (t, \(J = 2.4\) Hz, 1H), 1.94 (p, \(J = 7.0\) Hz, 2H), 1.48 (s, 9H); \textsuperscript{13}C-NMR (126 MHz, CDCl\textsubscript{3}): \(\delta\) 173.2, 154.9, 154.7 (C\textsubscript{O} rotamers), 154.2 (C\textsubscript{O} rotamers), 82.3, 78.0, 72.9, 60.5, 49.9, 39.1 (N-C\textsubscript{H}\textsubscript{3} rotamers), 38.4 (N-C\textsubscript{H}\textsubscript{2} rotamers), 34.9 (N-C\textsubscript{H}\textsubscript{2} rotamers), 33.4 (N-C\textsubscript{H}\textsubscript{2} rotamers), 31.4, 28.3, 22.8, 14.4; \textsuperscript{IR} (thin film): \(\nu\) 3261, 2979, 1728, 1368, 1159, 1105 cm\textsuperscript{-1}; \textsuperscript{ESI-HRMS} calcd for C\textsubscript{16}H\textsubscript{27}N\textsubscript{2}O\textsubscript{6} \([M + H]\)\textsuperscript{+} 343.1864, found 343.1862.

Synthesis of N-(7-diethylamino-2-oxo-2H-chromene-3-carboxamidoethylcarbamoyl)propyl)-N-\textit{tert}-butoxycarbonyl-\textit{O-}(N'-methyl-N'-propargylcarbamoyl)hydroxylamine (21)

A round-bottom flask was charged with carboxylic acid 19 (491 mg, 1.56 mmol, 1.00 equiv) and HATU (654 mg, 1.72 mmol, 1.10 equiv). The solids were dissolved in CH\textsubscript{2}Cl\textsubscript{2} (10 mL) and DIPEA (840 \(\mu\)L, 4.7 mmol, 3.00 equiv) was added. After stirring for 5 min at rt, the mixture became a clear yellowish solution. Ammonium salt 20 (780 mg, 1.87 mmol, 1.20 equiv) was added and the reaction stirred at rt for 5 h. The reaction was quenched with 0.5 M HCl, extracted with CH\textsubscript{2}Cl\textsubscript{2} and the organic phase washed with saturated aqueous NaHCO\textsubscript{3} and H\textsubscript{2}O/brine. The organic extracts were collected, dried with Na\textsubscript{2}SO\textsubscript{4}, filtered and evaporated in vacuo. The residue was purified by column
chromatography on silica gel (eluting with CH₂Cl₂/MeOH/NEt₃ = 100:1:1) followed by another column chromatography on silica gel (eluting with EtOAc/NEt₃ = 100:1) to give 21 as a yellow solid (572 mg, 61%). m.p. 109-114 °C; ¹H-NMR (500 MHz, CDCl₃): δ 9.01 (t, J = 6.0 Hz, 1H), 8.66 (s, 1H), 7.43 (d, J = 9.0 Hz, 1H), 6.69 (br s, 1H), 6.66 (dd, J = 9.0, 2.4 Hz, 1H), 6.50 (dd, J = 2.4, 0.7 Hz, 1H), 4.10 (br s, 2H), 3.64 (t, J = 6.6 Hz, 2H), 3.57 (q, J = 6.1 Hz, 2H), 3.47-3.41 (m, 6H), 3.01 (br s, 3H), 2.33-2.24 (m, 3H), 1.93 (p, J = 7.0 Hz, 2H), 1.44 (s, 9H), 1.23 (t, J = 7.1 Hz, 6H); ¹³C-NMR (126 MHz, CDCl₃): δ 172.9, 164.6, 162.7, 157.8, 155.1, 154.9 (CO rotamers), 154.2 (CO rotamers), 152.6, 148.3, 131.4, 110.3, 110.1, 108.6, 96.9, 82.3, 78.0, 72.9, 50.0, 45.4, 40.9, 39.3, 39.1 (N-CH₂ rotamers), 38.4 (N-CH₂ rotamers), 34.9 (N-CH₃ rotamers), 33.6, 33.5 (N-CH₃ rotamers), 28.3, 23.6, 12.5; IR (thin film): ν 3309, 2975, 1698, 1616, 1509, 1417, 1351, 1228, 1134 cm⁻¹; ESI-HRMS calcd for C₃₀H₄₂N₅O₈ [M + H]+ 600.3028, found 600.3020.

Synthesis of 10-(4-fluorobenzoyl)-3-ethynyl-10-fluoro-9-oxa-10a-aza-10-boraphenanthrene (27)

A Schlenk flask was charged with 2-bromo-4-formylpyridine (1.86 g, 10.0 mmol, 1.00 equiv), 2-hydroxyphenylboronic acid (1.66 g, 12.0 mmol, 1.20 equiv), Pd(PPh₃)₄ (115 mg, 0.10 mmol, 0.01 equiv) and potassium carbonate (2.80 g, 20.0 mmol, 2.00 equiv) and the atmosphere replaced with N₂ gas. A mixture of toluene/EtOH/H₂O (10:5:1, 45 mL, degassed) was introduced and the suspension vigorously stirred at 80 ºC overnight. The reaction was cooled to rt, diluted with EtOAc and filtered through Celite. The solvents were removed by rotary evaporation and the residue purified by column chromatography on silica gel (eluting with toluene) to give 66 as an orange solid (1.31 g, 66%). m.p. 136-138 °C; ¹H-NMR (500 MHz, CDCl₃): δ 10.15-10.12 (m, 1H), 8.77-8.70 (m, 1H), 8.33-8.25 (m, 1H), 7.92-7.83 (m, 1H), 7.67-7.60 (m, 1H), 7.39-7.33 (m, 1H), 7.08-7.02 (m, 1H), 6.98-6.92 (m, 1H); ¹³C-NMR (126 MHz, CD₂OD): δ 191.1, 160.0, 159.7, 147.6, 142.9, 132.5, 126.5, 119.6, 119.3, 119.0, 118.6, 118.3; IR (thin film): ν 2837, 2719, 1697, 1552, 1485, 1423, 1312, 1230 cm⁻¹; ESI-HRMS calcd for C₁₂H₁₀NO₂ [M + H]+ 200.0706, found 200.0705.

Aldehyde 66 (890 mg, 4.5 mmol, 1.00 equiv) and potassium carbonate (1.25 g, 9.0 mmol, 2.00 equiv) were introduced into a round-bottom flask. The solids were suspended in MeOH (15 mL) and Ohira-Bestmann reagent (67, 950 mg, 5.0 mmol, 1.10 equiv) was added. The reaction was
stirred at rt for 2 h; strong gas evolution was observed during the course of the reaction. The reaction was filtered through Celite and silica gel was added to the filtrate (to support the crude material in silica). The suspension was evaporated and dried in vacuo. The silica-supported crude was purified by column chromatography on silica gel (eluting with toluene/hexanes (1:1)) to give 26 as a yellow crystalline solid (452 mg, 51%).

Acylboronate 27 was synthesized according to a modification of our reported procedure.30 A Schlenk flask was charged with 26 (309 mg, 1.58 mmol, 1.00 equiv) and the atmosphere replaced with N \textsubscript{2} gas. CH\textsubscript{3}CN (1.5 mL) and reagent 68 (525 \mu L, 2.0 mmol, 1.25 equiv) were added sequentially and the mixture stirred at 60 °C for 1 h. The reaction was allowed to cool to rt and the solvent was evaporated to dryness. The residue was redissolved in CH\textsubscript{3}CN (2 mL). The solution was transferred to a round-bottom flask containing a suspension of KAT \textsubscript{1a} (368 mg, 1.60 mmol, 1.00 equiv) in CH\textsubscript{3}CN (8 mL), followed by the addition of TMSCl (203 \mu L, 1.60 mmol, 1.00 equiv). The suspension was stirred at rt for 5 h, neutralized with saturated aqueous NaHCO\textsubscript{3} and extracted with CH\textsubscript{2}Cl\textsubscript{2}. The organic extracts were collected, dried with Na\textsubscript{2}SO\textsubscript{4}, filtered and evaporated in vacuo (bath temperature < 30 °C). The residue was purified by column chromatography on silica gel (eluting with acetone/hexanes = 1:5, crude material was dry-loaded) to give 27 as a crystalline yellow solid (416 mg, 76%).

**Synthesis of N-(2-(4-(4-fluorobenzamido)butanamido)ethyl)-7-(diethylamino)-2-oxo-2H-chromene-3-carboxamide (32)**
Boc-protected hydroxylamine 21 (100 mg, 0.16 mmol, 1.00 equiv) was introduced in a round-bottom flask. HCl (4 M in 1,4-dioxane, 0.50 mL, 2.0 mmol, 12.5 equiv) was added and the mixture stirred at rt for 30 min. Et₂O (20 mL) was added and the resulting precipitate collected by vacuum filtration and dried in vacuo. The solid was transferred to a round-bottom flask charged with KAT 1a (73.6 mg, 0.32 mmol, 2.00 equiv). The solids were dissolved in THF / aqueous buffer pH 6.0 (1:1, 4 mL) and the solution stirred at rt for 3 h. The reaction was diluted with CH₂Cl₂, washed with H₂O/brine and extracted with CH₂Cl₂. The organic extracts were collected, dried with Na₂SO₄, filtered and evaporated in vacuo. The residue was purified by column chromatography on silica gel (eluting with CH₂Cl₂/MeOH = 100:3) to give amide 32 as a yellow solid (66.4 mg, 81%). m.p. 171-174 °C; ¹H-NMR (500 MHz, CDCl₃): δ 9.08 (t, J = 6.1 Hz, 1H), 8.58 (s, 1H), 7.86 (dd, J = 8.8, 5.3 Hz, 2H), 7.66 (t, J = 5.2 Hz, 1H), 7.34 (d, J = 9.0 Hz, 1H), 7.07-6.99 (m, 3H), 6.62 (dd, J = 9.0, 2.5 Hz, 1H), 6.44 (d, J = 2.3 Hz, 1H), 3.60-3.55 (m, 2H), 3.49-3.40 (m, 8H), 2.38-2.33 (m, 2H), 1.95 (p, J = 6.3 Hz, 2H), 1.23 (t, J = 7.1 Hz, 6H); ¹³C-NMR (126 MHz, CDCl₃): δ 173.7, 166.5, 165.0, 164.7 (d, J = 251.0 Hz), 162.8, 157.8, 152.9, 148.3, 131.3, 130.8 (d, J = 3.1 Hz), 129.5 (d, J = 8.8 Hz), 115.4 (d, J = 15.8 Hz), 110.2, 109.6, 108.4, 96.6, 45.2, 41.3, 40.2, 39.3, 34.4, 24.5, 12.5; ¹⁹F-NMR (471 MHz, CDCl₃): δ −109.1; IR (thin film): ν 3309, 2927, 1698, 1615, 1579, 1529, 1506, 1417, 1351, 1227, 1188, 1134 cm⁻¹; ESI-HRMS calcd for C₂₇H₂₂F₅N₄O₅ [M + H]⁺ 511.2351, found 511.2345.
7.4.2 Experimental procedures and characterization data for Section 4.2

**Synthesis of Boc-hydroxylamine–β-CD 24**

A round-bottom flask was charged with azide-containing β-CD 23 (143.0 mg, 0.12 mmol, 1.00 equiv), 21 (74.0 mg, 0.12 mmol, 1.00 equiv), copper sulfate pentahydrate (30.0 mg, 0.12 mmol, 1.00 equiv) and tris((1-benzyl-1H-1,2,3-triazol-4-yl)methyl)amine (TBTA, 13.0 mg, 0.025 mmol, 0.20 equiv). The solids were suspended in CH$_3$CN/H$_2$O (1:1, 5 mL) and sodium ascorbate (100. mg, 0.50 mmol, 4.00 equiv) was added. The reaction was stirred at rt for 15 h and filtered. The filtrate was purified by preparative reverse-phase HPLC (YMC C18 column, gradient of 20-95% MeOH/H$_2$O + 0.1% TFA for 28 min, $t_R$ = 21.1 min). The corresponding peak was collected and lyophilized to give 24 as a sticky yellow solid (32.7 mg, 16%). ESI-HRMS calcd for C$_{72}$H$_{110}$N$_{8}$NaO$_{42}$ [M + Na]$^+$ 1781.6610, found 1781.6613.
Deprotection of Boc-hydroxylamine 24

Precursor 24 (5.6 mg, 3.2 μmol, 1.00 equiv) was introduced in a vial and dissolved in the minimal amount of MeOH (~10 μL). HCl (4 M in dioxane, 100 μL, > 100 equiv) was added and the reaction stirred at rt for 30 min. Acetone (2 mL) was added to precipitate the product. The mixture was centrifuged, the supernatant discarded. The precipitate was washed with acetone, centrifuged again and dried in vacuo after discarding the supernatant to give a yellow solid (4.4 mg, 83%). The solid was unstable as a concentrated solution at rt, so the spectral data could not be collected. Stock solutions were prepared prior to use for kinetics experiments. A typical LC-MS trace of a freshly prepared solution of 25 in CH$_3$CN/H$_2$O is shown below.

Synthesis of $N$-(11-(4-(10-(4-fluorobenzoyl)-10-fluoro-9-oxa-10a-aza-10-boraphenanthren-3-yl)-1H-1,2,3-triazol-1-yl)-3,6,9-trioxaundecyl)adamantane-1-carboxamide (30)
A round-bottom flask was charged with 27 (17.0 mg, 0.05 mmol, 1.00 equiv), 28 (22.1 mg, 0.06 mmol, 1.20 equiv), copper sulfate pentahydrate (12.5 mg, 0.05 mmol, 1.00 equiv) and tris((1-benzyl-1H-1,2,3-triazol-4-yl)methyl)amine (TBTA, 10.0 mg, 0.02 mmol, 0.40 equiv). The solids were suspended in CH$_3$CN/H$_2$O (1:1, 3 mL) and sodium ascorbate (20 mg, 0.10 mmol, 2.00 equiv) was added. The reaction was stirred at rt for 2 h and filtered. The filtrate was washed with H$_2$O/brine and extracted with CH$_2$Cl$_2$. The organic extracts were collected, dried with Na$_2$SO$_4$, filtered and evaporated in vacuo. The residue was purified by column chromatography on silica gel (eluting with CH$_2$Cl$_2$/MeOH = 100:2.5) to give 30 as a sticky, hygroscopic yellow solid (30.9 mg, 85%).

\[ \text{1H-NMR (600 MHz, CDCl}_3\text{)}: \delta 8.63 (d, J = 1.6 Hz, 1H), 8.49 (s, 1H), 8.46 (d, J = 6.3 Hz, 1H), 8.25 (dd, J = 8.6, 5.8 Hz, 2H), 7.93-7.87 (m, 2H), 7.36 (ddd, J = 8.5, 7.2, 1.6 Hz, 1H), 7.13 (t, J = 8.8 Hz, 2H), 7.02 (dd, J = 8.3, 1.1 Hz, 1H), 6.96 (ddd, J = 8.3, 7.2, 1.2 Hz, 1H), 6.04 (t, J = 5.2 Hz, 1H), 4.65 (td, J = 4.5, 1.2 Hz, 2H), 3.95 (t, J = 5.0 Hz, 2H), 3.69-3.60 (m, 8H), 3.53-3.49 (m, 2H), 3.41-3.37 (m, 2H), 2.02-1.97 (m, 3H), 1.83-1.78 (m, 6H), 1.74-1.69 (m, 3H), 1.67-1.62 (m, 3H); \text{13C-NMR (151 MHz, CDCl}_3\text{)}: \delta 227.8 (br), 178.2, 165.4 (d, J = 252.7 Hz), 155.1 (d, J = 4.9 Hz), 150.2, 144.4, 143.3, 142.8 (d, J = 5.8 Hz), 136.5, 134.7, 131.4 (d, J = 9.1 Hz), 125.8, 125.0, 120.8, 120.4, 118.9, 116.7, 115.7, 115.3 (d, J = 21.5 Hz), 70.7, 70.7, 70.5, 70.2, 70.0, 69.2, 50.8, 39.3, 39.0, 36.6, 28.2; \text{19F-NMR (471 MHz, CDCl}_3\text{)}: \delta -107.1, -158.4; \text{11B-NMR (160 MHz, CDCl}_3\text{)}: \delta 2.00; \text{IR (thin film): } \nu 2903, 2850, 1632, 1587, 1499, 1228, 1119 \text{ cm}^{-1}; \text{ESI-HRMS calcd for C}_{39}H_{45}BF_{2}N_{5}O_{6} [\text{M + H}]^+ 728.3432, \text{found 728.3427.}

Synthesis of N-(20-azido-3,6,9,12,15,18-hexaoxaecosyl)adamantane-1-carboxamide (29)

Amine XX (100.0 mg, 90% purity, 0.26 mmol, 1.00 equiv) was introduced in a round-bottom flask and dissolved in CH$_2$Cl$_2$ (3 mL). NEt$_3$ (71.0 µL, 0.50 mmol, 2.00 equiv) was added followed by adamantane-1-carbony chloride (65.0 mg, 0.33 mmol, 1.25 equiv). The solution was stirred at rt overnight, quenched with 1 M aqueous HCl, washed with H$_2$O/brine and extracted with CH$_2$Cl$_2$. The organic extracts were collected, dried with Na$_2$SO$_4$, filtered and evaporated in vacuo. The residue was purified by column chromatography on silica gel (eluting with acetone/hexanes = 1:2) to give 29 as a pale yellow oil (126.0 mg, 95%).

\[ \text{1H-NMR (600 MHz, CDCl}_3\text{)}: \delta 6.10 (br s, 1H), 3.74-3.60 (m, 22 H), 3.55 (t, J = 5.2 Hz, 2H), 3.47-3.42 (m, 2H), 3.41-3.38 (m, 2H), 2.07-2.01 (m, 3H), 1.88-1.83 (m, 6H), 1.77-1.66 (m, 6H); \text{13C-NMR (151 MHz, CDCl}_3\text{)}: \delta 178.1, 70.9, 70.8, 70.8, 70.8, 70.7, 70.7, 70.7, 70.4, 70.2, 70.1, 50.8, 40.7, 39.3, 39.1, 36.7, 28.3; \text{IR (thin film): } \nu 3368, 2901, 2850,
1639, 1520, 1451, 1284, 1096 cm⁻¹; **ESI-HRMS** calcd for C_{25}H_{44}KN_{4}O_{7} [M + K]^+ 551.2842, found 551.2834.

**Synthesis of N-(20-(4-(10-(4-fluorobenzoyl)-10-fluoro-9-oxa-10a-aza-10-boraphenanthren-3-yl)-1H-1,2,3-triazol-1-yl)-3,6,9,12,15,18-hexaoxaeicosyl)adamantane-1-carboxamide (31)**

![Synthesis diagram]

A round-bottom flask was charged with 27 (45.0 mg, 0.12 mmol, 1.10 equiv), 29 (58.6 mg, 0.11 mmol, 1.00 equiv), copper sulfate pentahydrate (28.5 mg, 0.11 mmol, 1.00 equiv) and tris((1-benzyl-1H-1,2,3-triazol-4-yl)methyl)amine (TBTA, 12.0 mg, 0.03 mmol, 0.20 equiv). The solids were suspended in CH₃CN/H₂O (1:1, 2 mL) and sodium ascorbate (100 mg, 0.50 mmol, 4.00 equiv) was added. The reaction was stirred at rt for 2 h and filtered. The filtrate was washed with H₂O/brine and extracted with CH₂Cl₂. The organic extracts were collected, dried with Na₂SO₄, filtered and evaporated in vacuo. The residue was purified by column chromatography on silica gel (eluting with CH₂Cl₂/MeOH = 100:8) to give 31 as a sticky, hygroscopic yellow solid (52.8 mg, 54%).

**¹H-NMR** (600 MHz, CDCl₃): δ 8.67 (d, J = 1.7 Hz, 1H), 8.59 (s, 1H), 8.46 (d, J = 6.3 Hz, 1H), 8.24 (dd, J = 8.6, 5.8 Hz, 2H), 7.97 (dd, J = 6.3, 1.7 Hz, 1H), 7.93 (dd, J = 8.1, 1.6 Hz, 1H), 7.36 (ddd, J = 8.5, 7.2, 1.5 Hz, 1H), 7.12 (t, J = 8.8 Hz, 2H), 7.02 (dd, J = 8.3, 1.1 Hz, 1H), 6.96 (ddd, J = 8.2, 7.2, 1.2 Hz, 1H), 6.10 (t, J = 5.2 Hz, 1H), 4.69-4.62 (m, 2H), 3.96 (t, J = 4.9 Hz, 2H), 3.69-3.53 (m, 20H), 3.52-3.47 (m, 2H), 3.43-3.38 (m, 2H), 2.05-1.97 (m, 3H), 1.86-1.79 (m, 6H), 1.76-1.64 (m, 6H); **¹³C-NMR** (151 MHz, CDCl₃): δ 227.5 (br), 178.1, 165.4 (d, J = 252.5 Hz), 155.1 (d, J = 4.9 Hz), 150.1, 144.5, 143.3, 142.8 (d, J = 5.8 Hz), 136.5, 134.6, 131.3 (d, J = 9.1 Hz), 125.8, 125.4, 120.7, 120.3, 118.9, 116.7, 115.7, 115.3 (d, J = 21.4 Hz), 70.7, 70.7, 70.6, 70.6, 70.6, 70.3, 70.0, 69.2, 50.9, 40.7, 39.3, 39.1, 36.7, 28.3; **¹⁹F-NMR** (471 MHz, CDCl₃): δ −107.3, −158.3; **¹¹B-NMR** (160 MHz, CDCl₃): δ 2.05; IR (thin film): ν 2902, 2850, 1632, 1587, 1498, 1452, 1227, 1103 cm⁻¹; **ESI-HRMS** calcd for C_{45}H_{56}BF₂N₅NaO₉ [M + Na]^+ 882.4039, found 882.4031.
General procedure for the kinetics experiments using β-CD–hydroxylamine 25

To an Eppendorf tube were added 25 (17.5 μL from 0.57 mM stock solution, 10.0 nmol, 1.0 equiv, final concentration 50 μM) and aqueous citrate buffer pH 6.0 (180 μL) followed by acylboronate 30-31 or KAT 1a (added from stock solution, 10.0 nmol, 1.0 equiv). The reaction was gently shaken at rt. Aliquots (20 μL) were taken at time intervals, quenched with hydroxylamine 33 (4 μL from 0.10 M stock solution, 40 equiv) and injected into analytical HPLC (YMC C18 column, gradient of 20-95% MeOH/H₂O + 0.1% TFA for 17 min). The peaks corresponding to product 32 were integrated and the integral values extrapolated to concentrations with the appropriate calibration curve. %Conversion values were obtained by dividing extrapolated concentrations over initial concentration of 25 and plotted over time.
7.4.3 Experimental procedures and characterization data for Section 4.3

Synthesis of \( N\)-(11-azido-2,6,9-trioxaundecyl)-6-((4\text{R},5\text{S})-5\text{methyl}-2\text{oxoimidazolidin-4-yl})\)hexanamide (35)

In a round-bottom flask were introduced d-desthiobiotin succinimidyl ester (46, 211 mg, 0.68 mmol, 1.00 equiv) and 11-azido-3,6,9-trioxaundecanamine (44, 160 \( \mu \)L, 0.75, 1.10 equiv). The solids were dissolved in DMF (1 mL) and 1 M aqueous buffer pH 9.0 (1 mL, prepared by dissolving NaHCO\(_3\) (0.42 g, 5.0 mmol) and Na\(_2\)CO\(_3\) (10.0 g, 95.0 mmol) in distilled water (final volume 100 mL), final pH was adjusted using 1 M NaOH and HCl solutions). The reaction was stirred at rt overnight, diluted with CH\(_2\)Cl\(_2\), washed with H\(_2\)O/brine and extracted with CH\(_2\)Cl\(_2\). The organic extracts were collected, dried with Na\(_2\)SO\(_4\), filtered and evaporated in vacuo. The residue was purified by column chromatography on silica gel (eluting with EtOAc/MeOH = 100:20) to give 35 as a pale-yellow oil (271 mg, quant.). The spectral data was in accordance with the literature.\(^{31}\) \(^{1}\text{H}\)-NMR (500 MHz, CDCl\(_3\)): \( \delta \) 6.27 (br s, 1H), 5.11 (br s, 1H), 4.55 (br s, 1H), 3.87-3.80 (m, 1H), 3.73-3.61 (m, 11H), 3.56 (dd, \( J = 5.7 \), 4.6 Hz, 2H), 3.47-3.42 (m, 2H), 3.39 (dd, \( J = 5.5 \), 4.6 Hz, 2H), 2.18 (t, \( J = 7.4 \) Hz, 2H), 1.66 (p, \( J = 7.2 \) Hz, 2H), 1.55-1.22 (m, 6H), 1.13 (d, \( J = 6.5 \) Hz, 3H); \(^{13}\text{C}\)-NMR (126 MHz, CDCl\(_3\)): \( \delta \) 173.1, 163.4, 70.9, 70.7, 70.7, 70.4, 70.2, 70.1, 56.2, 51.5, 50.8, 39.3, 36.3, 29.6, 28.9, 26.2, 25.4, 15.9.

Preparation of \( N\)-(7-diethylamino-2-oxo-2\text{H}-chromene-3-carboxamidoethylcarbamoyl)propyl)-\( N\)-tert-butoxycarbonyl-(\( N\')-(11-(5-[(3\text{a},4\text{S},6\text{a}R)-2\text{-oxohexahydro-1\text{H}-thieno[3,4-d]imidazol-4-yl}]pentanamido)-3,6,9-trioxaundecyl)-1\text{H}-1,2,3-triazol-4-yl)methyl)-\( N\')-methylcarbamoyl)hydroxylamine (36)

To a round-bottom flask were added alkyne 21 (60.0 mg, 0.10 mmol, 1.00 equiv), azide 14 (49.0 mg, 0.11 mmol, 1.10 equiv), copper sulfate pentahydrate (25.0 mg, 0.10 mmol, 1.00 equiv) and tris((1-benzyl-1\text{H}-1,2,3-triazol-4-yl)methyl)amine (TBTA, 11.0 mg, 0.02 mmol, 0.20 equiv). The solids were suspended in CH\(_3\)CN/H\(_2\)O (1:1, 3 mL), and sodium ascorbate (50.0 mg, 0.25 mmol,
2.50 equiv) was added. The reaction was stirred at rt for 3 h and filtered. The filtrate was purified by preparative reverse-phase HPLC (YMC C18 column, gradient of 20-95% CH$_3$CN/H$_2$O + 0.1% TFA for 28 min, $t_R = 20.7$ min). The corresponding peak was collected and lyophilized to give 36 as a sticky yellow solid (38.3 mg, 37%). $^1$H-NMR (500 MHz, CDCl$_3$): $\delta$ 9.07 (t, $J = 6.0$ Hz, 1H), 8.65 (s, 1H), 8.06-7.74 (br, 1H), 7.43 (d, $J = 8.9$ Hz, 1H), 7.20-7.05 (br, 1H), 6.85-6.73 (br, 2H), 6.65 (dd, $J = 9.0$, 2.4 Hz, 1H), 6.48 (d, $J = 2.3$ Hz, 1H), 4.64-4.49 (m, 5H), 4.41-4.35 (m, 1H), 3.95-3.84 (m, 2H), 3.68-3.51 (m, 14H), 3.49-3.38 (m, 8H), 3.18 (td, $J = 7.4$, 4.5 Hz, 1H), 3.05-2.96 (m, 3H), 2.92 (dd, $J = 13.0$, 4.9 Hz, 1H), 2.77 (d, $J = 12.9$ Hz, 1H), 2.32 (t, $J = 7.5$ Hz, 2H), 2.23 (t, $J = 7.3$ Hz, 2H), 1.91 (p, $J = 7.0$ Hz, 2H), 1.79-1.57 (m, 4H), 1.51-1.36 (m, 10H), 1.24 (t, $J = 7.1$ Hz, 6H); $^{13}$C-NMR (126 MHz, CDCl$_3$): $\delta$ 173.9, 173.5, 164.8, 164.7, 162.7, 160.5 (q, $J = 39.7$ Hz) 157.8, 155.3 (CO rotamers), 155.2, 154.4 (CO rotamers), 152.9, 151.4, 143.5, 131.4, 124.2, 115.3 (q, $J = 287.2$ Hz) 110.3, 109.5, 108.4, 96.6, 82.4, 70.6, 70.5, 70.2, 69.8, 69.4, 62.3, 60.8, 55.4, 50.4, 50.2, 45.2, 45.0 (N-CH$_2$ rotamers), 44.1 (N-CH$_2$ rotamers), 40.8, 40.5, 39.4, 39.3, 35.6, 35.4 (N-CH$_3$ rotamers), 34.4 (N-CH$_3$ rotamers), 33.4, 28.2, 28.0, 27.9, 25.5, 23.6, 12.5; $^{19}$F-NMR (471 MHz, CDCl$_3$): $\delta$ –76.0; IR (thin film): $\nu$ 3308, 2932, 1699, 1617, 1580, 1511, 1419, 1352, 1135 cm$^{-1}$; ESI-HRMS calc'd for C$_{48}$H$_{74}$N$_{11}$O$_{13}$S [M + H]$^+$ 1044.5183, found 1044.5175; $[^\alpha]_D^{25}$: +15.3 (c = 1.35, CHCl$_3$).

Preparation of N-(7-diethylamino-2-oxo-2H-chromene-3-carboxamidoethylcarbamoyl)propyl)-N-tert-butoxycarbonyl-O-(N'-(1-(11-(6-((4R,5S)-5-methyl-2-oximidazolidin-4-yl)hexanamido)-3,6,9-trioxaundecyl)-1H-1,2,3-triazol-4-yl)methyl)-N'-methylcarbamoyl)hydroxylamine (37)

To a round-bottom flask were added alkyne 21 (30.0 mg, 0.05 mmol, 1.00 equiv), azide 35 (20.7 mg, 0.05 mmol, 1.00 equiv), copper sulfate pentahydrate (12.5 mg, 0.05 mmol, 1.00 equiv) and tris((1-benzyl-1H-1,2,3-triazol-4-yl)methyl)amine (TBTA, 5.3 mg, 0.01 mmol, 0.20 equiv). The solids were suspended in CH$_3$CN/H$_2$O (1:1, 2 mL), and sodium ascorbate (40.0 mg, 0.20 mmol, 4.00 equiv) was added. The reaction was stirred at rt for 3 h and filtered. The filtrate was purified by preparative reverse-phase HPLC (YMC C18 column, gradient of 20-95% CH$_3$CN/H$_2$O + 0.1% TFA for 28 min, $t_R = 18.2$ min). The corresponding peak was collected, neutralized with saturated aqueous NaHCO$_3$, diluted with brine, extracted with CH$_2$Cl$_2$, dried with Na$_2$SO$_4$, filtered and evaporated in vacuo to give 37 as a sticky yellow solid (28.2 mg, 56%). $^1$H-NMR (500 MHz, CDCl$_3$): $\delta$ 9.01 (t, $J = 6.0$ Hz, 1H), 8.66 (s, 1H), 8.02-7.72 (br, 1H), 7.42 (d, $J = 9.0$ Hz, 1H), 7.04-6.86 (br,
1H), 6.64 (dd, J = 9.0, 2.3 Hz, 1H), 6.57 (br, 1H), 6.47 (s, 1H), 5.44 (br, 1H), 4.84 (br, 1H), 4.60-4.48 (m, 4H), 3.93-3.85 (m, 2H), 3.85-3.78 (m, 1H), 3.71-3.51 (m, 15H), 3.49-3.37 (m, 8H), 3.04-2.95 (m, 3H), 2.28 (t, J = 7.5 Hz, 2H), 2.17 (t, J = 7.4 Hz, 2H), 1.91 (p, J = 7.1 Hz, 2H), 1.64 (p, J = 7.2 Hz, 2H), 1.52-1.17 (m, 23H), 1.09 (d, J = 6.5 Hz, 3H); \textsuperscript{13}C-NMR (126 MHz, CDCl\textsubscript{3}): \( \delta \) 173.2, 172.8, 164.5, 163.6, 162.7, 157.8, 155.2 (CO rotamers), 155.1, 154.3 (CO rotamers), 152.8, 148.3, 143.6, 131.3, 124.0, 110.2, 109.8, 108.4, 96.6, 82.1, 70.7, 70.6, 70.5, 70.2, 70.0, 69.5, 56.1, 51.5, 50.3, 50.2, 45.2, 45.1 (N-CH\textsubscript{2} rotamers), 44.2 (N-CH\textsubscript{2} rotamers), 40.7, 39.3, 39.2, 36.1, 35.4 (N-CH\textsubscript{3} rotamers), 34.4 (N-CH\textsubscript{3} rotamers), 33.5, 29.8, 29.6, 28.9, 28.3, 26.1, 25.4, 23.5, 15.9, 12.5; IR (thin film): \( \nu \) 3313, 2930, 1616, 1580, 1510, 1417, 1351, 1228, 1134, 1106 cm\textsuperscript{-1}; ESI-HRMS calcd for C\textsubscript{48}H\textsubscript{75}N\textsubscript{11}NaO\textsubscript{13} [M + Na]\textsuperscript{+} 1036.5438, found 1036.5439; \( [\alpha]_D^{25} \): +1.9 (c = 1.36, CHCl\textsubscript{3}).

**Deprotection of Boc-hydroxylamine precursor 36**

Precursor 36 (6.4 mg, 6.1 \( \mu \)mol, 1.00 equiv) was introduced in a vial and dissolved in the minimal amount of MeOH (~10 \( \mu \)L). HCl (4 M in dioxane, 150 \( \mu \)L, > 100 equiv) was added and the reaction stirred at rt for 30 min. Et\textsubscript{2}O (2 mL) was added to precipitate the product. The mixture was centrifuged, the supernatant discarded. The precipitate was washed with Et\textsubscript{2}O, centrifuged again and dried in vacuo after discarding the supernatant to give a yellow solid (4.9 mg, 85%). The solid was unstable as a concentrated solution at rt, so the spectral data could not be collected. Stock solutions were prepared prior to use for kinetics experiments. A typical LC-MS trace of a freshly prepared solution of 40 in CH\textsubscript{3}CN/H\textsubscript{2}O is shown below.

**Deprotection of Boc-hydroxylamine precursor 37**
Precursor 36 (5.6 mg, 5.5 µmol, 1.00 equiv) was introduced in a vial and dissolved in the minimal amount of MeOH (~10 µL). HCl (4 M in dioxide, 100 µL, > 100 equiv) was added and the reaction stirred at rt for 30 min. Et₂O (2 mL) was added to precipitate the product. The mixture was centrifuged, the supernatant discarded. The precipitate was washed with Et₂O, centrifuged again and dried in vacuo after discarding the supernatant to give a yellow solid (4.1 mg, 81%). The solid was unstable as a concentrated solution at rt, so the spectral data could not be collected. Stock solutions were prepared prior to use for kinetics experiments. A typical LC-MS trace of a freshly prepared solution of 41 in CH₃CN/H₂O is shown below.

**Synthesis of N-(11-(4-(10-(4-fluorobenzoyl)-10-fluoro-9-oxa-10a-aza-10-boraphenanthren-3-yl)-1H-1,2,3-triazol-1-yl)-3,6,9-trioxaundecyl)-5-[(3aS,4S,6aR)-2-oxohexahydro-1H-thieno[3,4-d]imidazol-4-yl]pentanamide (38)**

A round-bottom flask was charged with 27 (20.0 mg, 0.05 mmol, 1.00 equiv), 14 (35.6 mg, 0.08 mmol, 1.50 equiv), copper sulfate pentahydrate (9.6 mg, 0.06 mmol, 1.10 equiv) and tris((1-benzyl-1H-1,2,3-triazol-4-yl)methyl)amine (TBTA, 16.0 mg, 0.03 mmol, 0.60 equiv). The solids were suspended in CH₃CN/H₂O (1:1, 5 mL) and sodium ascorbate (40 mg, 0.20 mmol, 4.0 equiv) was added. The reaction was stirred at rt overnight and filtered. The filtrate was purified by preparative
reverse-phase HPLC (YMC C18 column, gradient of 20-95% CH$_3$CN/H$_2$O + 0.1% TFA for 28 min, $t_R = 21.4$ min). The corresponding peak was collected, neutralized with saturated aqueous NaHCO$_3$, diluted with brine, extracted with CH$_2$Cl$_2$, dried with Na$_2$SO$_4$, filtered and evaporated in vacuo to give 38 as a yellow solid (12.2 mg, 31%). $^1$H-NMR (600 MHz, CDCl$_3$): $\delta$ 8.64 (br s, 1H), 8.62 (d, $J = 2.0$ Hz, 1H), 8.44 (d, $J = 6.2$ Hz, 1H), 8.22 (dd, $J = 8.6, 5.8$ Hz, 2H), 7.96 (d, $J = 6.3$ Hz, 1H), 7.91 (d, $J = 8.2$ Hz, 1H), 7.35 (ddd, $J = 8.5, 1.6$ Hz, 1H), 7.12 (t, $J = 8.7$ Hz, 2H), 7.01 (dd, $J = 8.3, 1.2$ Hz, 1H), 6.95 (t, $J = 7.6$ Hz, 1H), 6.69 (br, 1H), 6.25 (d, $J = 9.3$ Hz, 1H), 5.53 (br, 1H), 4.68 (dd, $J = 4.9$ Hz, 2H), 4.46-4.40 (m, 1H), 4.24 (dd, $J = 7.9, 4.6$ Hz, 1H), 3.96 (t, $J = 5.0$ Hz, 2H), 3.68-3.57 (m, 8H), 3.55-3.48 (m, 2H), 3.45-3.33 (m, 2H), 3.11-3.05 (m, 1H), 2.85 (dd, $J = 12.8, 4.8$ Hz, 1H), 2.18-2.11 (m, 2H), 1.69-1.52 (m, 4H), 1.40-1.32 (m, 2H); $^{13}$C-NMR (151 MHz, CDCl$_3$): $\delta$ 228.2 (br), 173.7, 165.9 (d, $J = 252.7$ Hz), 164.2, 155.1 (d, $J = 4.9$ Hz), 150.1, 144.5, 143.3, 142.8 (d, $J = 5.8$ Hz), 136.5, 134.7, 131.3 (d, $J = 9.1$ Hz), 125.9, 125.6, 120.8, 120.3, 119.0, 116.7, 115.8, 115.4 (d, $J = 21.5$ Hz), 70.6, 70.3, 70.2, 70.2, 70.0, 69.3, 61.9, 60.4, 55.6, 50.7, 40.6, 39.1, 35.7, 28.1, 28.0, 25.5; $^{19}$F-NMR (471 MHz, CDCl$_3$): $\delta$ –106.9, –158.2; $^{11}$B-NMR (160 MHz, CDCl$_3$): $\delta$ 2.07; IR (thin film): $\nu$ 3301, 2924, 2870, 1695, 1633, 1587, 1546, 1230, 1120 cm$^{-1}$; ESI-HRMS calcd for C$_{38}$H$_{44}$BF$_2$N$_7$NaO$_7$S [M + Na]$^+$ 814.2983, found 814.2990; [$\alpha$]$_D^{25}$: +16.4 (c = 0.21, CHCl$_3$).

**Synthesis of N-(11-(4-(10-(4-fluorobenzoyl)-10-fluoro-9-oxa-10a-aza-10-boraphenanthren-3-yl)-1H-1,2,3-triazol-1-yl)-3,6,9-trioxaundecyl)-6-((4$^R$,5$^S$)-5-methyl-2-oxoimidazolidin-4-yl)hexanamide (39)**

A round-bottom flask was charged with 27 (48.9 mg, 0.14 mmol, 1.00 equiv), 35 (57.2 mg, 0.14 mmol, 1.00 equiv), copper sulfate pentahydrate (34.0 mg, 0.14 mmol, 1.00 equiv) and tris((1-benzyl-1H-1,2,3-triazol-4-yl)methyl)amine (TBTA, 17.0 mg, 0.03 mmol, 0.20 equiv). The solids were suspended in CH$_3$CN/H$_2$O (1:1, 2 mL) and sodium ascorbate (80 mg, 0.40 mmol, 2.80 equiv) was added. The reaction was stirred at rt overnight and filtered. The filtrate was purified by preparative reverse-phase HPLC (YMC C18 column, gradient of 20-95% CH$_3$CN/H$_2$O + 0.1% TFA for 28 min, $t_R = 21.5$ min). The corresponding peak was collected, neutralized with saturated aqueous NaHCO$_3$, diluted with brine, extracted with CH$_2$Cl$_2$, dried with Na$_2$SO$_4$, filtered and evaporated in vacuo to give 39 as a yellow solid (23 mg, 22%). m.p. 69-75 °C; $^1$H-NMR (600 MHz, CDCl$_3$): $\delta$ 8.65 (br s, 1H), 8.56 (s, 1H), 8.45 (d, $J = 6.2$ Hz, 1H), 8.23 (dd, $J = 8.4, 5.8$ Hz, 2H), 7.94 (ddd, $J = 6.3, 1.7, 0.9$ Hz, 1H), 7.81 (d, $J = 7.9$ Hz, 1H), 7.58 (d, $J = 8.7$ Hz, 1H), 7.43 (d, $J = 6.3$ Hz, 1H), 7.30 (ddd, $J = 8.5, 7.2, 1.6$ Hz, 1H), 7.21 (t, $J = 8.7$ Hz, 2H), 7.04 (dd, $J = 8.3, 1.2$ Hz, 1H), 6.97 (t, $J = 7.6$ Hz, 1H), 6.64 (br, 1H), 6.28 (d, $J = 9.3$ Hz, 1H), 5.55 (br, 1H), 4.63 (dd, $J = 4.9$ Hz, 2H), 4.47-4.40 (m, 1H), 4.23 (dd, $J = 7.9, 4.6$ Hz, 1H), 3.95 (t, $J = 5.0$ Hz, 2H), 3.67-3.57 (m, 8H), 3.54-3.47 (m, 2H), 3.45-3.33 (m, 2H), 3.10-3.04 (m, 1H), 2.86 (dd, $J = 12.8, 4.8$ Hz, 1H), 2.19-2.12 (m, 2H), 1.69-1.52 (m, 4H), 1.41-1.32 (m, 2H); $^{13}$C-NMR (151 MHz, CDCl$_3$): $\delta$ 228.2 (br), 173.7, 165.9 (d, $J = 252.7$ Hz), 164.2, 155.1 (d, $J = 4.9$ Hz), 150.1, 144.5, 143.3, 142.8 (d, $J = 5.8$ Hz), 136.5, 134.7, 131.3 (d, $J = 9.1$ Hz), 125.9, 125.6, 120.8, 120.3, 119.0, 116.7, 115.8, 115.4 (d, $J = 21.5$ Hz), 70.6, 70.3, 70.2, 70.2, 70.0, 69.3, 61.9, 60.4, 55.6, 50.7, 40.6, 39.1, 35.7, 28.1, 28.0, 25.5; $^{19}$F-NMR (471 MHz, CDCl$_3$): $\delta$ –106.9, –158.2; $^{11}$B-NMR (160 MHz, CDCl$_3$): $\delta$ 2.07; IR (thin film): $\nu$ 3301, 2924, 2870, 1695, 1633, 1587, 1546, 1455, 1230, 1120 cm$^{-1}$; ESI-HRMS calcd for C$_{38}$H$_{44}$BF$_2$N$_7$NaO$_7$S [M + Na]$^+$ 814.2983, found 814.2990; [$\alpha$]$_D^{25}$: +16.4 (c = 0.21, CHCl$_3$).
7.92 (dd, $J = 8.1$, 1.6 Hz, 1H), 7.36 (ddd, $J = 8.5$, 7.2, 1.5 Hz, 1H), 7.12 (t, $J = 8.8$ Hz, 2H), 7.02 (dd, $J = 8.3$, 1.1 Hz, 1H), 6.96 (ddd, $J = 8.2$, 7.2, 1.2 Hz, 1H), 6.37 (s, 1H), 5.29 (br, 1H), 4.67 (dd, $J = 5.8$, 4.1 Hz, 2H), 3.95 (t, $J = 5.4$ Hz, 2H), 3.80 (p, $J = 7.1$ Hz, 1H), 3.69-3.58 (m, 8H), 3.53-3.47 (m, 2H), 3.38 (q, $J = 5.4$ Hz, 2H), 2.10 (t, $J = 7.4$ Hz, 2H), 1.59 (p, $J = 7.3$ Hz, 2H), 1.49-1.17 (m, 6H), 1.09 (d, $J = 6.5$ Hz, 3H); $^{13}$C-NMR (151 MHz, CDCl$_3$): $\delta$ 227.9 (br), 173.2, 165.5 ($J = 252.7$ Hz), 163.5, 155.1 ($d$, $J = 4.9$ Hz), 150.2, 144.3, 143.3, 142.9 ($d$, $J = 5.8$ Hz), 136.5, 134.7, 131.3 ($d$, $J = 9.1$ Hz), 125.8, 125.3, 120.8, 120.4, 119.0, 116.7, 115.8, 115.4 ($d$, $J = 21.5$ Hz), 70.6, 70.6, 70.5, 70.1, 70.0, 69.2, 56.1, 51.5, 50.8, 39.2, 36.1, 29.6, 28.8, 26.0, 25.4, 15.9; $^{19}$F-NMR (471 MHz, CDCl$_3$): $\delta$ –107.0, –158.2; $^{11}$B-NMR (160 MHz, CDCl$_3$): $\delta$ 2.09; IR (thin film): $\nu$ 3298, 2932, 2867, 1696, 1634, 1588, 1546, 1499, 1454, 1230 cm$^{-1}$; ESI-HRMS calcd for C$_{38}$H$_{47}$BF$_2$N$_7$O$_7$ [M + H]$^+$ 762.3599, found 762.3593; $[\alpha]_D^{25}$: +9.9 ($c = 0.34$, CHCl$_3$).

**Representative procedure for the kinetics experiments of acylboronates 38, 39 and hydroxylamines 40, 41 monitored by HPLC**

To an Eppendorf tube were added hydroxylamine 40 (4.4 μL from 0.45 mM stock solution, 2.0 nmol, 1.0 equiv, final concentration 5.0 μM), aqueous citrate buffer pH 6.0 (270 μL) and in the presence or absence of commercial streptavidin (117.6 μL from 17.0 μM stock solution, 2.0 nmol, 1.0 equiv) and externally added biotin (11.1 μL from 0.90 mM stock solution, 10.0 nmol, 5.0 equiv). The mixture was incubated at rt for 5 min and acylboronate 38 (4.0 μL from 1.0 mM stock solution, 4.0 nmol, 2.0 equiv) was added. The reaction was gently shaken at rt. Aliquots (20 μL) were taken at time intervals, quenched with hydroxylamine 33 (4 μL from 0.10 M stock solution, 400 equiv) and injected into analytical HPLC (YMC C18 column, gradient of 20-95% CH$_3$CN/H$_2$O + 0.1% TFA for 17 min). The peaks corresponding to product 32 were integrated and the integral values extrapolated to concentrations with the appropriate calibration curve. %Conversion values were obtained by dividing extrapolated concentrations over initial concentration of 40 and plotted over time.
Chapter 7. Experimental Section

Synthesis of \( N^\alpha-(4\text{-dimethylaminophenylazo})\text{benzenesulfonyl})-N^\varepsilon\text{-tert-butoxycarbonyl-L-lysine (43)} \)

To a round-bottom flask \( N^\alpha\text{-tert-butoxycarbonyl-L-lysine methyl ester hydrochloride (594 mg, 2.00 mmol, 1.10 equiv)} \) was added and dissolved in \( CH_2Cl_2 \). Triethylamine (711 \( \mu \)L, 5.0 mmol, 2.50 equiv) and \( 4\text{-dimethylaminophenylazo})\text{benzenesulfonyl chloride (576 mg, 1.78 mmol, 1.00 equiv)} \) were added sequentially. The reaction was stirred at rt for 2 h, poured into a separation funnel, washed with \( H_2O/\text{brine} \) and extracted with \( CH_2Cl_2 \). The organic extracts were collected, dried with \( Na_2SO_4 \), filtered and evaporated in vacuo. The residue was purified by column chromatography on silica gel (eluting with \( CH_2Cl_2/MeOH = 100:2.5 \)) to give 69 as a bright red solid (633 mg, 65%).

**m.p.** 192-195 °C; **\( ^1H\text{-NMR (500 MHz, CDCl}_3 \): }\delta 7.96-7.89 (m, 6H), 6.78 (d, \( J = 9.4 \text{ Hz, 2H} \)), 5.27 (d, \( J = 9.1 \text{ Hz, 1H} \)), 4.56 (br s, 1H), 3.94 (ddd, \( J = 9.1, 7.8, 5.0 \text{ Hz, 1H} \)), 3.49 (s, 3H), 3.14 (s, 6H), 3.11-
3.03 (m, 2H), 1.80-1.72 (m, 1H), 1.70-1.60 (m, 1H), 1.47-1.33 (m, 12H); $^{13}$C-NMR (126 MHz, CDCl$_3$): \(\delta\) 172.1, 156.2, 155.5, 153.4, 143.6, 139.0, 128.4, 126.2, 122.6, 111.8, 79.3, 55.7, 52.7, 40.5, 40.2, 33.0, 29.5, 28.6, 22.2; IR (thin film): \(\nu\) 3311, 2932, 1742, 1681, 1606, 1520, 1366, 1161 cm$^{-1}$; ESI-HRMS calcd for C$_{26}$H$_{38}$N$_5$O$_6$S [M + H]$^+$ 548.2537, found 548.2533; \([\alpha]_D^{25}\): +117.3 (c = 0.48, CHCl$_3$).

A round-bottom flask was charged with 69 (273 mg, 0.50 mmol, 1.00 equiv) and lithium hydroxide monohydrate (84 mg, 2.0 mmol, 2.00 equiv). The solids were dissolved in THF/MeOH/H$_2$O (4:1:1, 5 mL) and the mixture stirred at rt overnight. The reaction was quenched with 1 M aqueous oxalic acid till the solution displayed a pH 1-2. The suspension was extracted with CH$_2$Cl$_2$, the organic extracts collected, dried with Na$_2$SO$_4$, filtered and evaporated in vacuo to give 43 as a red solid (340 mg, quant). m.p. 149-153 °C; $^1$H-NMR (500 MHz, CD$_3$OD): \(\delta\) 7.95 (d, \(J = 8.9\) Hz, 2H), 7.89 (d, \(J = 8.9\) Hz, 2H), 7.86 (d, \(J = 9.2\) Hz, 2H), 6.84 (d, \(J = 9.3\) Hz, 2H), 3.85 (dd, \(J = 8.5, 5.3\) Hz, 1H), 3.12 (s, 6H), 3.02-2.94 (m, 2H), 1.79-1.71 (m, 1H), 1.67-1.59 (m, 1H), 1.47-1.31 (m, 13H); $^{13}$C-NMR (126 MHz, CD$_3$OD): \(\delta\) 174.8, 158.5, 156.8, 154.9, 144.8, 142.0, 129.3, 126.7, 123.3, 112.6, 79.9, 57.1, 41.0, 40.4, 33.7, 30.3, 28.8, 23.8; IR (thin film): \(\nu\) 3273, 2931, 1699, 1603, 1520, 1364, 1135 cm$^{-1}$; ESI-HRMS calcd for C$_{25}$H$_{35}$N$_5$NaO$_6$S [M + Na]$^+$ 556.2200, found 556.2194; \([\alpha]_D^{25}\): +55.2 (c = 0.28, CH$_3$OH).

**General procedure D: Preparation of precursors 45, 72 and 73.**

To a round-bottom flask containing carboxylic acid 43 and HATU was added CH$_2$Cl$_2$ (final concentration 0.1 M). DIPEA was added and the mixture stirred for 5 min at rt, turning from a suspension to a clear solution. O-(2-Aminooethyl)-O’-(2-azidoethyl)polyethylene glycol (44, 70 or 71) was added and the mixture stirred at rt overnight. The reaction was washed with saturated aqueous NaHCO$_3$ and extracted with CH$_2$Cl$_2$. The organic extracts were collected, dried with Na$_2$SO$_4$, filtered and evaporated in vacuo. The residue was purified by column chromatography on silica gel.

\(\text{(S)-N-(11-Azido-3,6,9-trioxaundecyl)-2-(4-dimethylaminophenylazo)benzenesulfonamido)-6-}
\(\text{(tert-butoxycarbonylamino) hexanamide (45)}\)
Prepared following General procedure D with 43 (300 mg, 0.56 mmol, 1.00 equiv), 11-azido-3,6,9-trioxaundecan-1-amine (44, 139 μL, 95% purity, 0.67 mmol, 1.20 equiv), HATU (240 mg, 0.63 mmol, 1.10 equiv) and DIPEA (293 μL, 1.70 mmol, 3.00 equiv). Isolated by column chromatography on silica gel (eluting with CH$_2$Cl$_2$/MeOH = 100:4) to give 45 as a red solid (310 mg, 85%). m.p. 135-139 °C; $^1$H-NMR (500 MHz, CDCl$_3$): δ 7.99-7.87 (m, 6H), 6.78 (d, $J = 9.0$ Hz, 2H), 6.75 (br s, 1H), 5.93-5.81 (m, 1H), 4.69 (br s, 1H), 3.72-3.60 (m, 10H), 3.59-3.52 (m, 2H), 3.44-3.35 (m, 4H), 3.33-3.27 (m, 2H), 3.12 (s, 6H), 3.10-3.03 (m, 1H), 3.02-2.94 (m, 1H), 1.74-1.59 (m, 2H), 1.48-1.22 (m, 13H); $^{13}$C-NMR (126 MHz, CDCl$_3$): δ 170.9, 156.6, 155.3, 153.4, 143.5, 139.1, 128.5, 126.3, 122.6, 111.8, 79.5, 70.8, 70.7, 70.4, 70.1, 69.5, 56.8, 50.8, 40.5, 39.5, 39.4, 32.4, 29.6, 28.6, 21.6; IR (thin film): ν 3347, 3259, 2930, 2864, 2103, 1682, 1646, 1534, 1393 cm$^{-1}$; ESI-HRMS calcd for C$_{33}$H$_{52}$N$_9$O$_8$S $[M + H]^+$ 734.3654, found 734.3648; $[\alpha]_D^{25}$: +47.4 (c = 0.62, CHCl$_3$).

(S)-N-(20-Azido-3,6,9,12,15,18-hexaoxaecosyl)-2-(4-dimethylaminophenylazo)benzenesulfonamido)-6-(tert-butoxycarbonylamino)hexanamide (72)

Prepared following General procedure D with 43 (71.6 mg, 0.13 mmol, 1.00 equiv), 20-azido-3,6,9,12,15,18-hexaoxaecosan-1-amine (70, 50 μL, 90% purity, 0.13 mmol, 1.0 equiv), HATU (53.5 mg, 0.14 mmol, 1.10 equiv) and DIPEA (72 μL, 0.4 mmol, 3.00 equiv). Isolated by column chromatography on silica gel (eluting with CH$_2$Cl$_2$/MeOH = 100:4) to give 72 as a red solid (76.3 mg, 69%). m.p. 87-90 °C; $^1$H-NMR (500 MHz, CDCl$_3$): δ 7.94 (d, $J = 8.7$ Hz, 2H), 7.91-7.86 (m, 4H), 6.80 (br, 1H), 6.75 (d, $J = 9.2$ Hz, 2H), 5.91 (br, 1H), 4.73 (br, 1H), 3.72-3.49 (m, 23H), 3.45-3.35 (m, 4H), 3.32-3.27 (m, 2H), 3.12 (s, 6H), 3.10-3.02 (m, 1H), 3.01-2.93 (m, 1H), 1.75-1.58 (m, 2H), 1.45 (s, 9H), 1.43-1.18 (m, 4H); $^{13}$C-NMR (126 MHz, CDCl$_3$): δ 170.9, 156.6, 155.7, 153.4, 143.7, 139.2, 128.5, 125.9, 122.6, 111.6, 79.4, 70.8, 70.7, 70.4, 70.1, 69.5, 56.8, 50.8, 40.4, 39.5, 39.4, 32.4, 29.6, 28.6, 21.6; IR (thin film): ν 3335, 3259, 2930, 2864, 2103, 1682, 1646, 1523, 1369, 1141 cm$^{-1}$; ESI-HRMS calcd for C$_{39}$H$_{63}$N$_9$O$_{11}$S $[M + Na]^+$ 888.4260, found 888.4252; $[\alpha]_D^{25}$: +30.9 (c = 0.78, CHCl$_3$).

(S)-N-(32-Azido-3,6,9,12,15,18,21,24,27,30-decaoxadotriacontyl)-2-(4-dimethylaminophenylazo)benzenesulfonamido)-6-(tert-butoxycarbonylamino)hexanamide (73)
Prepared following General procedure D with 43 (40.6 mg, 76 \( \mu \)mol, 1.00 equiv), 32-azido-3,6,9,12,15,18,21,24,27,30-decaoxadotriacontan-1-amine (50 \( \mu \)L, 90% purity, 80 \( \mu \)mol, 1.05 equiv), HATU (32 mg, 85 \( \mu \)mol, 1.10 equiv) and DIPEA (42 \( \mu \)L, 250 \( \mu \)mol, 3.00 equiv). Isolated by column chromatography on silica gel (eluting with \( \text{CH}_2\text{Cl}_2/\text{MeOH} = 100:5 \)) to give 73 as a red solid (46.8 mg, 59%).

\[ \text{\textit{H}-NMR (500 MHz, CDCl}_3): \delta 7.94 (d, J = 8.7 Hz, 2H), 7.92-7.87 (m, 4H), 6.83 (br, 1H), 6.76 (d, J = 9.3 Hz, 2H), 5.91 (d, J = 7.3 Hz, 1H), 4.74 (br, 1H), 3.68-3.62 (m, 31H), 3.61-3.58 (m, 2H), 3.57-3.51 (m, 2H), 3.45-3.35 (m, 4H), 3.34-3.24 (m, 2H), 3.12 (s, 6H), 3.01-2.93 (m, 1H), 1.74-1.56 (m, 2H), 1.45 (s, 9H), 1.42-1.34 (m, 2H), 1.33-1.20 (m, 2H); \textit{C}-NMR (126 MHz, CDCl}_3): \delta 170.9, 156.6, 155.6, 153.3, 143.6, 139.2, 128.5, 126.0, 122.6, 111.7, 79.4, 70.8, 70.8, 70.7, 70.7, 70.6, 70.6, 70.6, 70.5, 70.4, 70.1, 69.6, 56.8, 50.8, 40.5, 39.6, 39.4, 32.5, 29.6, 28.6, 21.6; \textit{IR (thin film):} \nu 3334, 3258, 2867, 2104, 1682, 1646, 1607, 1522, 1369, 1140 cm\(^{-1}\); \textit{ESI-HRMS} calcd for C\(_{47}\)H\(_{83}\)N\(_{10}\)O\(_{15}\)S \([\text{M + NH}_4]^+ \) 1059.5755, found 1059.5736; [\( \alpha \)]\(_D\)\(^{25} \): +35.3 (c = 0.65, CHCl\(_3\)).

**Synthesis of (S)-N-(4-Azidobutyl)-2-(4-dimethylaminophenylazo)benzenesulfonamido)-6-(tert-butoxycarbonylamino) hexanamide (75)**

A round-bottom flask was charged with \( N \)-(4-bromobutyl)phthalimide\(^{32} \) (1.41 g, 5.0 mmol, 1.00 equiv) and sodium azide (1.0 g, 15.0 mmol, 3.00 equiv). The solids were suspended in DMF (10 mL) and the mixture stirred for 6 h at 60 °C. The reaction was cooled to rt, poured into H\(_2\)O/brine and extracted with EtOAc/hexanes (2:1). The organic extracts were collected, dried with Na\(_2\)SO\(_4\), filtered and evaporated in vacuo to give 74 as a white solid (1.08 g, 89%) without further purification.

\[ \text{\textit{H}-NMR (500 MHz, CDCl}_3): \delta 7.87 (m, 2H), 7.75-7.70 (m, 2H), 3.72 (t, J = 7.0 Hz, 2H), 3.33 (t, J = 6.8 Hz, 2H), 1.81-1.74 (m, 2H), 1.68-1.60 (m, 2H); \textit{C}-NMR (126 MHz, CDCl}_3): \delta 168.5, 134.1, 132.2, 123.4, 51.0, 37.4, 26.4, 26.0; \textit{IR (thin film):} \nu 2942, 2094, 1708, 1396, 1043 cm\(^{-1}\); \textit{ESI-HRMS} calcd for C\(_{47}\)H\(_{83}\)N\(_{10}\)O\(_{15}\)S \([\text{M + Na}^+ \) 267.0852, found 267.0855.

To a round-bottom flask containing a solution of 74 (37.0 mg, 0.15 mmol, 1.10 equiv) in THF (2 mL) was added hydrazine monohydrate (50 \( \mu \)L, 1.0 mmol, 7.00 equiv). The solution was stirred
at 60 ºC for 4 h, cooled to rt, diluted with CH₂Cl₂ and extracted with 0.2 M HCl. The acidic aqueous extracts were basified with 1M aq. NaOH until pH >14, extracted with CH₂Cl₂, dried with Na₂SO₄, filtered and concentrated in rotavap (complete drying of the sample was avoided).

A separate round-bottom flask was charged with carboxylic acid 43 (74.6 mg, 0.14 mmol, 1.00 equiv) and HATU (57 mg, 0.15 mmol, 1.10 equiv). The solid were suspended in CH₂Cl₂ (1 mL) and DIPEA (100 µL, 0.56 mmol, 4.00 equiv) was added. The mixture was stirred for 5 min at rt, turning from a suspension to a clear solution. To this solution were added the concentrated extracts from above, and the mixture was stirred at rt overnight. The mixture was poured into a separation funnel, washed with H₂O/brine and extracted with CH₂Cl₂. The organic extracts were collected, dried with Na₂SO₄, filtered and evaporated in vacuo. The residue was purified by column chromatography on silica gel (eluting with CH₂Cl₂/MeOH = 100:4) to give 75 as a red solid (42.4 mg, 48% over two steps). m.p. 166-169 ºC; ¹H-NMR (500 MHz, CDCl₃): δ 7.96 (d, J = 8.7 Hz, 2H), 7.92-7.88 (m, 4H), 6.76 (d, J = 9.2 Hz, 2H), 6.57 (t, J = 5.4 Hz, 1H), 5.88 (d, J = 6.7 Hz, 1H), 4.68 (t, J = 6.0 Hz, 1H), 3.63 (td, J = 6.9, 4.4 Hz, 1H), 3.24-3.20 (m, 2H), 3.20-3.16 (m, 2H), 3.12 (s, 6H), 2.98-2.91 (m, 1H), 1.73-1.64 (m, 2H), 1.54-1.17 (m, 18H); ¹³C-NMR (126 MHz, CDCl₃): δ 171.1, 157.1, 155.9, 153.3, 143.7, 138.6, 128.6, 125.9, 122.7, 111.6, 79.9, 56.6, 51.1, 40.4, 39.1, 38.7, 31.4, 29.4, 28.6, 26.8, 26.2, 21.0; IR (thin film): ν 3356, 3262, 2928, 1683, 1650, 1541, 1367, 1162, 1142 cm⁻¹; ESI-HRMS calcd for C₂₉H₄₃N₇NaO₅S [M + Na]+ 652.3000, found 652.2995; [α]ᵢ₀²⁵: +13.1 (c = 0.25, CHCl₃).
General procedure E: Preparation of precursors 47, 76-78

Boc-carbamate (45, 72, 73 or 75) was dissolved in CH₂Cl₂ / CF₃CO₂H (1:1, 0.05 M final concentration) and the mixture stirred for 1 h at rt. The reaction was basified with 1 M NaOH to pH >12, extracted with CH₂Cl₂, dried with Na₂SO₄, filtered and evaporated in vacuo.

To the crude obtained above was added d-desthiobiotin succinimidyl ester (46). The solids were dissolved in DMF and 1 M aqueous buffer pH 9.0 [prepared by dissolving NaHCO₃ (0.42 g, 5.0 mmol) and Na₂CO₃ (10.0 g, 95.0 mmol) in distilled water (final volume 100 mL), final pH was adjusted using 1 M NaOH and HCl solutions]. The reaction was stirred at rt overnight, diluted with CH₂Cl₂, washed with H₂O/brine and extracted with CH₂Cl₂. The organic extracts were collected, dried with Na₂SO₄, filtered and evaporated in vacuo. The residue was purified by column chromatography on silica gel.

(S)-N-(11-Azido-3,6,9-trioxaundecyl)-2-(4-dimethylaminophenylazo)benzenesulfonamido)-6-(6-((4R,5S)-5-methyl-2-oxoimidazolidin-4-yl)hexanamido)hexanamide (47)

Prepared following General procedure E with 45 (150 mg, 0.20 mmol, 1.00 equiv) and 46 (90 mg, 0.27 mmol, 1.30 equiv). Isolated by column chromatography on silica gel (eluting with CH₂Cl₂/MeOH = 100:7) to give 47 as a red solid (134 mg, 81%). m.p. 135-139 °C; ¹H-NMR (500 MHz, CD₃OD): δ 7.95 (d, J = 8.9 Hz, 2H), 7.91 (d, J = 8.8 Hz, 2H), 7.88 (d, J = 9.2 Hz, 2H), 6.85 (d, J = 9.2 Hz, 2H), 3.84-3.73 (m, 2H), 3.70-3.67 (m, 1H), 3.66-3.61 (m, 6H), 3.60-3.57 (m, 2H), 3.52-3.49 (m, 2H), 3.37-3.34 (m, 2H), 3.33-3.25 (m, 2H), 3.15-3.06 (m, 9H), 2.15 (t, J = 7.6 Hz, 2H), 1.69-1.53 (m, 4H), 1.51-1.24 (m, 11H), 1.10 (d, J = 6.5 Hz, 3H); ¹³C-NMR (126 MHz, CD₃OD): δ 176.1, 173.6, 166.2, 166.2, 156.8, 154.9, 144.8, 141.5, 129.4, 126.8, 123.4, 112.6, 71.6, 71.5, 71.3, 71.1, 70.2, 57.9, 57.4, 52.7, 51.8, 40.4, 40.2, 40.0, 37.0,
34.0, 30.7, 29.7, 27.1, 26.9, 23.9, 15.6; IR (thin film): $\nu$ 3250, 2931, 2860, 2100, 1709, 1645, 1607, 1369, 1141 cm$^{-1}$; ESI-HRMS calcd for C$_{38}$H$_{59}$N$_{11}$NaO$_{8}$S [M + Na]$^+$ 852.4161, found 852.4144; $[\alpha]_D^{25}$: +48.9 (c = 0.33, CH$_3$OH).

(S)-N-(20-Azido-3,6,9,12,15,18-hexaoxaeicosyl)-2-(4-dimethylaminophenylazo)benzenesulfonamido)-6-(6-((4$R$,5$S$)-5-methyl-2-oxoimidazolidin-4-yl)hexanamido)hexanamide (76)

Prepared following General procedure E with 72 (60 mg, 0.07 mmol, 1.00 equiv) and 46 (25 mg, 0.07 mmol, 1.00 equiv). Isolated by column chromatography on silica gel (eluting with CH$_2$Cl$_2$/MeOH = 100:8 to 100:10) to give 76 as a red solid (45 mg, 67%). m.p. 105-109 °C; $^1$H-NMR (500 MHz, CDCl$_3$): $\delta$ 7.94 (d, $J$ = 8.6 Hz, 2H), 7.89-7.84 (m, 4H), 7.18 (d, $J$ = 8.2 Hz, 2H), 6.74 (d, $J$ = 9.2 Hz, 2H), 6.33 (br, 1H), 6.01 (br, 1H), 5.10 (br, 1H), 3.90-3.82 (m, 1H), 3.82-3.75 (m, 1H), 3.74-3.68 (m, 1H), 3.67-3.59 (m, 18H), 3.59-3.54 (m, 2H), 3.52-3.47 (m, 2H), 3.39-3.29 (m, 4H), 3.24-3.15 (m, 4H), 3.10 (s, 6H), 2.23-2.13 (m, 2H), 1.75-1.57 (m, 4H), 1.55-1.22 (m, 10 H), 1.12 (d, $J$ = 6.7 Hz, 3H); $^{13}$C-NMR (126 MHz, CDCl$_3$): $\delta$ 173.6, 171.5, 164.2, 155.5, 153.2, 143.6, 139.7, 128.2, 125.8, 122.6, 111.6, 70.8, 70.7, 70.6, 70.6, 70.5, 70.5, 70.1, 69.5, 56.7, 56.1, 51.6, 50.8, 40.4, 39.3, 38.7, 35.8, 32.4, 29.6, 28.4, 28.2, 25.6, 25.3, 22.0, 15.8; IR (thin film): $\nu$ 3245, 2927, 2860, 2102, 1709, 1617, 1551, 1370, 1139, 1087 cm$^{-1}$; ESI-HRMS calcd for C$_{44}$H$_{75}$N$_{12}$O$_{11}$S [M + NH$_4$]$^+$ 979.5393, found 979.5381; $[\alpha]_D^{25}$: +44.4 (c = 1.37, CHCl$_3$).

(S)-N-(32-Azido-3,6,9,12,15,18,21,24,27,30-decaoxadotriacontyl)-2-(4-dimethylaminophenylazo)benzenesulfonamido)-6-(6-((4$R$,5$S$)-5-methyl-2-oxoimidazolidin-4-yl)hexanamido)hexanamide (77)

Prepared following General procedure E with 73 (33 mg, 32 $\mu$mol, 1.00 equiv) and 46 (15 mg, 50 $\mu$mol, 1.50 equiv). Isolated by column chromatography on silica gel (eluting with CH$_2$Cl$_2$ /MeOH = 100:10) to give 77 as a red gum (30 mg, 84%). $^1$H-NMR (500 MHz, CDCl$_3$): $\delta$ 7.95 (d, $J$ = 8.9 Hz, 2H), 7.92-7.87 (m, 4H), 7.16 (d, $J$ = 8.4 Hz, 1H), 7.07 (t, $J$ = 5.6 Hz, 1H), 6.76 (d, $J$ = 9.3 Hz, 2H), 6.21 (t, $J$ = 4.8 Hz, 1H), 5.72 (br, 1H), 4.82 (br, 1H), 3.92-3.85 (m, 1H), 3.84-3.78 (m, 1H), 3.74-3.69 (m, 1H), 3.68-3.60 (m, 38H), 3.58-3.54 (m, 2H), 3.52-3.47 (m, 2H), 3.39-3.29 (m, 4H), 3.24-3.15 (m, 4H), 3.10 (s, 6H), 2.23-2.13 (m, 2H), 1.75-1.57 (m, 4H), 1.55-1.22 (m, 10 H), 1.12 (d, $J$ = 6.7 Hz, 3H); $^{13}$C-NMR (126 MHz, CDCl$_3$): $\delta$ 173.6, 171.5, 164.2, 155.5, 153.2, 143.6, 139.7, 128.2, 125.8, 122.6, 111.6, 70.8, 70.7, 70.6, 70.6, 70.5, 70.5, 70.1, 69.5, 56.7, 56.1, 51.6, 50.8, 40.4, 39.3, 38.7, 35.8, 32.4, 29.6, 28.4, 28.2, 25.6, 25.3, 22.0, 15.8; IR (thin film): $\nu$ 3245, 2927, 2860, 2102, 1709, 1617, 1551, 1370, 1139, 1087 cm$^{-1}$; ESI-HRMS calcd for C$_{44}$H$_{75}$N$_{12}$O$_{11}$S [M + NH$_4$]$^+$ 979.5393, found 979.5381; $[\alpha]_D^{25}$: +44.4 (c = 1.37, CHCl$_3$).
(S)-N-(4-Azidobutyl)-2-(4-dimethylaminophenylazo)benzenesulfonamido)-6-(6-((4R,5S)-5-methyl-2-oxoimidazolidin-4-yl)hexanamido)hexanamide (78)

Prepared following General procedure B with 75 (37.0 mg, 0.06 mmol, 1.00 equiv) and 46 (31.0 mg, 0.10 mmol, 1.70 equiv). Isolated by column chromatography on silica gel (eluting with CH₂Cl₂/MeOH = 100:7) to give 78 as a red solid (32.1 mg, 74%).

**Physical Data:**
- **m.p.:** 140-144 °C
- **1H-NMR** (500 MHz, CD₃OD): δ 7.94 (d, J = 8.7 Hz, 2H), 7.92-7.84 (m, 4H), 6.85 (d, J = 9.2 Hz, 2H), 3.85-3.77 (m, 1H), 3.73-3.63 (m, 2H), 3.20-3.15 (m, 2H), 3.14-3.04 (m, 7H), 3.01-2.89 (m, 2H), 2.14 (t, J = 7.5 Hz, 2H), 1.67-1.52 (m, 4H), 1.51-1.23 (m, 15H), 1.09 (d, J = 6.5 Hz, 3H); **13C-NMR** (126 MHz, CD₃OD): δ 176.1, 173.6, 166.2, 166.2, 156.9, 155.0, 144.8, 141.5, 129.4, 126.8, 123.4, 112.6, 57.9, 57.4, 52.7, 52.1, 40.4, 40.0, 39.8, 37.0, 34.0, 30.7, 30.2, 29.7, 27.5, 27.2, 27.1, 26.9, 23.9, 15.6; **IR** (thin film): ν 3252, 2928, 2098, 1706, 1645, 1608, 1557, 1371, 1162 cm⁻¹; **ESI-HRMS** calcd for C₃₄H₅₂N₁₁O₅S [M + H]^+ 726.3868, found 726.3859; [α]D²⁵: +15.2 (c = 0.65, CH₃OH).

To a round-bottom flask were added alkyne 21, azide reagent (47, 76, 77 or 78), copper sulfate pentahydrate and tris((1-benzyl-1H-1,2,3-triazol-4-yl)methyl)amine (TBTA). The solids were suspended in CH$_3$CN/H$_2$O (1:1, 2 mL), and sodium ascorbate was added. The reaction was stirred at rt for 3 h and filtered. The filtrate was purified by preparative reverse-phase HPLC (YMC C18 column, gradient of 20-95% CH$_3$CN/H$_2$O + 0.1% TFA for 28 min).

Boc-hydroxylamine 48

Prepared following General procedure F with 21 (22.5 mg, 38 µmol, 1.00 equiv), 47 (31.2 mg, 38 µmol, 1.00 equiv), copper sulfate pentahydrate (9.8 mg, 38 µmol, 1.00 equiv), tris((1-benzyl-1H-1,2,3-triazol-4-yl)methyl)amine (4.0 mg, 8 µmol, 0.20 equiv) and sodium ascorbate (30 mg, 150 µmol, 4.00 equiv). Purified by preparative reverse-phase HPLC ($t_R = 23.0$ min). The corresponding peak was collected, neutralized with saturated aqueous NaHCO$_3$, diluted with brine, extracted with CH$_2$Cl$_2$/MeOH, dried with Na$_2$SO$_4$, filtered and evaporated in vacuo to give 48 as an orange solid (39.1 mg, 73%). m.p. 169-174 °C; $^1$H-NMR (500 MHz, CDCl$_3$): $\delta$ 9.01 (t, $J = 6.0$ Hz, 1H), 8.65 (s, 1H), 7.92 (d, $J = 8.6$ Hz, 2H), 7.87-7.82 (m, 4H), 7.41 (d, $J = 8.9$ Hz, 1H), 7.26 (br, 1H), 7.16 (d, $J = 7.0$ Hz, 1H), 6.72 (d, $J = 9.2$ Hz, 2H), 6.62 (dd, $J = 9.0, 2.5$ Hz, 1H), 6.45 (d, $J = 2.4$ Hz, 1H), 6.35 (br, 1H), 5.82 (br s, 1H), 5.05 (br s, 1H), 4.52 (br, 4H), 3.90-3.77 (m, 4H), 3.72-3.38 (m, 19H), 3.34-3.27 (m, 2H), 3.24-3.18 (m, 2H), 3.17-3.11 (m, 2H), 3.09 (s, 6H), 3.02-2.93 (m, 3H), 2.28 (t, $J = 7.5$ Hz, 2H), 2.15 (t, $J = 7.2$ Hz, 2H), 1.90 (p, $J = 6.8$ Hz, 2H), 1.74-1.55 (m, 4H), 1.50-1.17 (m, 28H), 1.10 (d, $J = 6.5$ Hz, 3H); $^{13}$C-NMR (126 MHz, CDCl$_3$): $\delta$ 173.4, 172.8, 171.4, 164.4, 163.9, 162.6, 157.7, 155.4, 155.1 (N-CO rotamers), 155.0, 154.3 (N-CO rotamers), 153.1, 152.7, 148.2, 148.2, 143.5, 139.6, 131.3, 128.2, 125.7, 124.0, 122.5, 111.4, 110.1, 109.6, 108.3, 96.5, 82.2, 82.1, 70.5, 70.4, 70.2, 69.4, 69.3, 56.7, 56.0, 51.5, 50.2, 50.1.
Chapter 7. Experimental Section

45.1, 45.0 (N-CH₂ rotamers), 44.1 (N-CH₂ rotamers), 40.5, 40.4, 40.3, 39.2, 39.2, 38.6, 35.8, 35.3 (N-CH₃ rotamers), 34.3 (N-CH₃ rotamers), 33.4, 32.4, 29.7, 29.5, 28.4, 28.2, 25.6, 25.3, 23.5, 22.0, 15.7, 12.4; IR (thin film): ν 3302, 2927, 1697, 1603, 1511, 1419, 1362, 1228, 1133 cm⁻¹; ESI-HRMS calcd for C₆₈H₁₀₁N₁₆O₁₆S [M + H]⁺ 1429.7297, found 1429.7279; [α]D₂₅: +22.8 (c = 1.65, CHCl₃).

Boc-hydroxylamine 79

Prepared following General procedure F with 21 (21.8 mg, 36 µmol, 1.00 equiv), 76 (35.0 mg, 36 µmol, 1.00 equiv), copper sulfate pentahydrate (9.1 mg, 36 µmol, 1.00 equiv), tris((1-benzyl-1H-1,2,3-triazol-4-yl)methyl)amine (4.0 mg, 8 µmol, 0.20 equiv) and sodium ascorbate (30 mg, 150 µmol, 4.00 equiv). Purified by preparative reverse-phase HPLC (tᵣ = 23.2 min). The corresponding peak was collected, neutralized with saturated aqueous NaHCO₃, diluted with brine, extracted with CH₂Cl₂/MeOH, dried with Na₂SO₄, filtered and evaporated in vacuo to give 79 as a sticky orange solid (28.3 mg, 50%).

¹H-NMR (500 MHz, CDCl₃): δ 9.01 (m, 1H), 8.65 (s, 1H), 7.99-7.72 (m, 7H), 7.42 (d, J = 8.9 Hz, 1H), 7.24 (br, 1H), 7.15 (d, J = 8.2 Hz, 1H), 6.99-6.82 (br, 1H), 6.73 (d, J = 8.7 Hz, 2H), 6.63 (dd, J = 9.0, 2.3 Hz, 1H), 6.46 (s, 1H), 6.36 (br, 1H), 5.78 (br, 1H), 4.98 (br, 1H), 4.60-4.47 (m, 4H), 3.91-3.77 (m, 4H), 3.73-3.67 (m, 1H), 3.65-3.53 (m, 19H), 3.51-3.40 (m, 8H), 3.37-3.29 (m, 2H), 3.25-3.15 (m, 3H), 3.10 (s, 6H), 3.03-2.94 (m, 3H), 2.28 (t, J = 7.5 Hz, 2H), 2.22-2.14 (m, 2H), 1.91 (p, J = 7.1 Hz, 2H), 1.76-1.56 (m, 4H), 1.52-1.26 (m, 19H), 1.24-1.16 (m, 8H), 1.12 (d, J = 6.5 Hz, 3H); ¹³C-NMR (126 MHz, CDCl₃): δ 173.5, 172.9, 171.5, 164.5, 164.0, 162.7, 157.8, 156.6, 155.6, 155.2 (N-CO rotamers), 155.1, 154.4 (N-CO rotamers), 153.2, 152.8, 148.3, 143.6, 143.5, 139.7, 131.4, 128.3, 125.8, 124.0, 122.6, 111.6, 110.2, 109.8, 108.4, 96.6, 82.3 (N-(CH₃)₃ rotamers), 82.1 (N-(CH₃)₃ rotamers), 70.6, 70.5, 70.5, 70.2, 69.6, 69.5, 56.7, 56.1, 51.6, 50.3, 50.1, 45.2, 45.1 (N-CH₂ rotamers), 44.2 (N-CH₂ rotamers), 40.7, 40.4, 39.3, 39.3, 38.7, 35.8, 33.5, 33.5 (N-CH₃ rotamers), 34.4 (N-CH₃ rotamers), 33.6, 32.4, 29.6, 28.5, 28.3, 28.1, 25.7, 25.3, 23.6, 22.0, 15.8, 12.5; IR (thin film): ν 3308, 2931, 2867, 1697, 1603, 1511, 1353, 1133 cm⁻¹; ESI-HRMS calcd for C₇₄H₁₁₂N₁₆NaO₁₉S [M + Na]⁺ 1583.7903, found 1583.7896; [α]D₂₅: +15.7 (c = 1.4, CHCl₃).
Boc-hydroxylamine 80

Prepared following General procedure F with 21 (20.0 mg, 18 \( \mu \text{mol} \), 1.00 equiv), 77 (12.0 mg, 18 \( \mu \text{mol} \), 1.00 equiv), copper sulfate pentahydrate (4.4 mg, 18 \( \mu \text{mol} \), 1.00 equiv), tris((1-benzyl-1H-1,2,3-triazol-4-yl)methyl)amine (2.0 mg, 4 \( \mu \text{mol} \), 0.20 equiv) and sodium ascorbate (16 mg, 80 \( \mu \text{mol} \), 5.00 equiv). Purified by preparative reverse-phase HPLC \((t_R = 23.1 \text{ min})\). The corresponding peak was collected, neutralized with saturated aqueous NaHCO\(_3\), diluted with brine, extracted with CH\(_2\)Cl\(_2\)/MeOH, dried with Na\(_2\)SO\(_4\), filtered and evaporated in vacuo to give 80 as a sticky orange solid (19.5 mg, 63%).

\(^1\text{H-NMR}\) (500 MHz, CDCl\(_3\)): \(\delta\) 9.01 (d, \(J = 6.0 \text{ Hz} \), 1H), 8.66 (s, 1H), 7.95 (d, \(J = 8.6 \text{ Hz} \), 2H), 7.90-7.85 (m, 4H), 7.42 (d, \(J = 8.9 \text{ Hz} \), 1H), 7.26 (br, 1H), 7.17 (d, \(J = 8.2 \text{ Hz} \), 1H), 6.94-6.78 (br, 1H), 6.74 (d, \(J = 9.2 \text{ Hz} \), 2H), 6.64 (dd, \(J = 9.0, 2.4 \text{ Hz} \), 1H), 6.47 (d, \(J = 9.0, 2.4 \text{ Hz} \), 1H), 6.39 (br, 1H), 5.77 (br, 1H), 4.93 (br, 1H), 4.60-4.49 (m, 4H), 3.90-3.83 (m, 3H), 3.83-3.77 (m, 1H), 3.73-3.68 (m, 1H), 3.66-3.54 (m, 35H), 3.52-3.49 (m, 2H), 3.47-3.41 (m, 6H), 3.38-3.31 (m, 2H), 3.26-3.16 (m, 3H), 3.11 (s, 6H), 3.02-2.95 (m, 3H), 2.29 (t, \(J = 7.6 \text{ Hz} \), 2H), 2.24-2.16 (m, 2H), 1.91 (p, \(J = 7.1 \text{ Hz} \), 2H), 1.76-1.58 (m, 4H), 1.53-1.33 (m, 19H), 1.31-1.19 (m, 8H + H grease), 1.13 (d, \(J = 6.5 \text{ Hz} \), 3H); \(^{13}\text{C-NMR}\) (126 MHz, CDCl\(_3\)): \(\delta\) 173.6, 172.9, 171.6, 164.6, 164.0, 162.7, 157.8, 155.6, 155.2 (N-CO rotamers), 155.1, 154.4 (N-CO rotamers), 153.2, 152.8, 148.3, 143.6, 143.5, 139.7, 131.4, 128.3, 125.8, 124.0, 122.6, 111.6, 110.2, 109.9, 108.4, 96.7, 82.3 (N-C(CH\(_3\))\(_3\) rotamers), 82.1 (N-C(CH\(_3\))\(_3\) rotamers), 70.7, 70.6, 70.6, 70.6, 70.5, 70.5, 70.4, 70.4, 70.4, 70.1, 69.7, 69.5, 56.7, 56.2, 51.7, 50.3, 50.1, 45.2, 45.1 (N-CH\(_2\) rotamers), 44.2 (N-CH\(_2\) rotamers), 40.7, 40.4, 39.3, 39.3, 38.6, 35.8, 35.3 (N-CH\(_3\) rotamers), 34.4 (N-CH\(_3\) rotamers), 33.6, 32.3, 29.8, 28.4, 28.3, 28.0, 25.7, 25.3, 23.6, 21.9, 15.8, 12.5; \(\text{IR} (\text{thin film})\): \(\nu\) 3310, 2924, 1699, 1603, 1513, 1364, 1135 cm\(^{-1}\); \(\text{ESI-HRMS}\) calcd for C\(_{82}\)H\(_{130}\)N\(_{16}\)O\(_{23}\)S [M + 2H]\(^{2+}\) 869.4602, found 869.4619; \([\alpha]\)\(_D\)\(^{25}\) : +17.9 (c = 0.95, CHCl\(_3\)).
Boc-hydroxylamine 81

Prepared following General procedure F with 21 (16.5 mg, 28 \( \mu \)mol, 1.00 equiv), 78 (20.0 mg, 28 \( \mu \)mol, 1.0 equiv), copper sulfate pentahydrate (7.1 mg, 28 \( \mu \)mol, 1.00 equiv), tris((1-benzyl-1H-1,2,3-triazol-4-yl)methyl)amine (3.0 mg, 6 \( \mu \)mol, 0.20 equiv) and sodium ascorbate (25 mg, 125 \( \mu \)mol, 4.50 equiv). Purified by preparative reverse-phase HPLC (\( t_R = 23.4 \) min). The corresponding peak was collected, neutralized with saturated aqueous NaHCO\(_3\), diluted with brine, extracted with CH\(_2\)Cl\(_2\)/MeOH, dried with Na\(_2\)SO\(_4\), filtered and evaporated in vacuo to give 81 as an orange solid (16.6 mg, 45%). m.p. 97-102 °C; \(^1\)H-NMR (500 MHz, CDCl\(_3\)): \( \delta \) 9.04 (t, \( J = 6.0 \) Hz, 1H), 8.66 (s, 1H), 7.94 (d, \( J = 8.7 \) Hz, 2H), 7.91-7.81 (m, 4H), 7.43 (d, \( J = 8.9 \) Hz, 1H), 7.28-7.22 (br, 1H), 7.22-7.07 (br, 1H), 6.98-6.84 (br, 1H), 6.73 (d, \( J = 8.8 \) Hz, 2H), 6.64 (dd, \( J = 9.0, 2.4 \) Hz, 1H), 6.47 (d, \( J = 2.4 \) Hz, 1H), 6.29 (br, 1H), 5.82 (br s, 1H), 5.12 (br s, 1H), 4.58-4.44 (m, 2H), 4.30-4.15 (m, 2H), 3.91 (m, 1H), 3.84 (td, \( J = 9.2, 4.0 \) Hz, 1H), 3.74-3.68 (m, 1H), 3.67-3.60 (m, 1H), 3.59-3.53 (m, 1H), 3.49-3.38 (m, 6H), 3.24 (br, 1H), 3.16 (br, 1H), 3.10 (s, 6H), 3.07-2.93 (m, 5H), 2.32-2.25 (m, 2H), 2.23-2.15 (m, 2H), 1.89 (p, \( J = 7.0 \) Hz, 2H), 1.83-1.55 (m, 6H), 1.53-1.36 (m, 16H), 1.35-1.26 (m, 4H), 1.26-1.19 (m, 7H), 1.15 (d, \( J = 6.5 \) Hz, 3H); \(^{13}\)C-NMR (126 MHz, CDCl\(_3\)): \( \delta \) 173.6, 173.0, 171.6, 164.7, 164.0, 162.8, 157.8, 155.5, 155.3 (N-CO rotamers), 155.2, 154.3 (N-CO rotamers), 153.3, 152.9, 148.4, 143.7, 143.5, 139.6, 131.4, 128.2, 126.0, 123.0, 122.6, 111.7, 110.3, 109.7, 108.4, 96.6, 82.4 (N-C(CH\(_3\))\(_3\) rotamers), 82.3 (N-C(CH\(_3\))\(_3\) rotamers), 56.7, 56.2, 51.8, 50.1, 50.0, 45.3 (N-CH\(_2\) rotamers), 45.2, 44.3 (N-CH\(_2\) rotamers), 40.8, 40.5, 39.3, 38.8, 38.6, 35.7, 35.5 (N-CH\(_3\) rotamers), 34.6 (N-CH\(_3\) rotamers), 33.5, 32.4, 29.8, 29.6, 28.3, 27.8, 27.5, 26.3, 25.7, 25.3, 23.5, 22.0, 15.8, 12.6; IR (thin film): \( \nu \) 3306, 2931, 1697, 1603, 1511, 1364 cm\(^{-1}\); ESI-HRMS calcd for C\(_{64}\)H\(_{92}\)N\(_{16}\)NaO\(_{13}\)S [M + Na]\(^+\) 1347.6643, found 1347.6630; \([\alpha]_D^{25}\): +30.4 (c = 0.83, CHCl\(_3\)).

Representative procedure for the synthesis of starting materials 42, 51-53

Precursor 48 (25.0 mg, 17.5 \( \mu \)mol, 1.00 equiv) was introduced in a vial and dissolved in the minimal amount of MeOH (~50 \( \mu \)L). HCl (4 M in dioxane, 0.5 mL, > 100 equiv) was added and the
reaction stirred at rt for 30 min. Et₂O (5 mL) was added to precipitate the product. The mixture was centrifuged, the supernatant discarded. The precipitate was washed with Et₂O, centrifuged again and dried in vacuo after discarding the supernatant to give a red solid (19.1 mg, 83%). The solid was unstable as a concentrated solution at rt, so the spectral data could not be collected. Instead, the solid was stored in the fridge (stable for several months) and stock solutions were prepared prior to use for kinetics experiments. A typical LC-MS trace of a freshly prepared solution of 42 in CH₃CN/H₂O is shown below.

**Synthesis of N-(20-(4-(10-(4-fluorobenzoyl)-10-fluoro-9-oxa-10a-aza-10-boraphenanthren-3-yl)-1H-1,2,3-triazol-1-yl)-3,6,9,12,15,18-hexaoxaicosyl)-6-((4R,5S)-5-methyl-2-oxoimidazolidin-4-yl)hexanamide (54)**

To a round-bottom flask were added 46 (50.0 mg, 0.16 mmol, 1.30 equiv) and 20-azido-3,6,9,12,15,18-hexaoxaicosan-1-amine (50 μL, 90% purity, 0.13 mmol, 1.00 equiv). The solids were suspended in CH₃CN (1.5 mL) and aqueous buffer pH 9.0 (0.5 mL, prepared as describe above). The mixture was stirred at rt for 2 h and 27 (52 mg, 0.15 mmol, 1.20 equiv) was added, followed by copper sulfate pentahydrate (32.0 mg, 0.13 mmol, 1.00 equiv), tris((1-benzyl-1H-1,2,3-
triazol-4-yl)methyl)amine (TBTA, 15.0 mg, 0.03 mmol, 0.20 equiv) and sodium ascorbate (120 mg, 0.60 mmol, 4.6 equiv). The solution was stirred at rt for 2 h and filtered. The filtrate was purified by preparative reverse-phase HPLC (YMC C18 column, gradient of 20-95% CH$_3$CN/H$_2$O + 0.1% TFA for 28 min, $t_R = 22.2$ min). The corresponding peak was collected, diluted with brine, extracted with CH$_2$Cl$_2$, dried with Na$_2$SO$_4$, filtered and evaporated in vacuo to give 54 as a yellow solid (24.1 mg, 21% over two steps).

$^1$H-NMR (600 MHz, CDCl$_3$): $\delta$ 8.66 (d, $J = 1.6$ Hz, 1H), 8.62 (s, 1H), 8.45 (d, $J = 6.3$ Hz, 1H), 7.97 (dd, $J = 6.3$, 1.7 Hz, 1H), 7.92 (dd, $J = 8.2$, 1.6 Hz, 1H), 7.35 (ddd, $J = 8.5$, 7.2, 1.6 Hz, 1H), 7.10 (t, $J = 8.7$ Hz, 2H), 7.00 (dd, $J = 8.3$, 1.1 Hz, 1H), 6.95 (ddd, $J = 8.2$, 1.2 Hz, 1H), 6.54 (t, $J = 5.6$ Hz, 1H), 5.45 (br s, 1H), 4.78 (br s, 1H), 4.67-4.63 (m, 2H), 3.96-3.92 (m, 2H), 3.79 (p, $J = 7.0$ Hz, 1H), 3.63-3.60 (m, 7H), 3.57-3.52 (m, 8H), 3.52-3.49 (m, 2H), 3.41-3.36 (m, 2H), 2.15 (t, $J = 7.4$ Hz, 2H), 1.62 (p, $J = 7.2$ Hz, 2H), 1.50-1.18 (m, 6H), 1.08 (d, $J = 6.5$ Hz, 3H); $^{13}$C-NMR (151 MHz, CDCl$_3$): $\delta$ 227.6, 173.2, 165.4 (d, $J = 252.5$ Hz), 163.7, 155.1 (d, $J = 4.8$ Hz), 150.1, 144.5, 143.2, 142.8 (d, $J = 5.7$ Hz), 136.5, 134.6, 131.3 (d, $J = 7.1$ Hz), 125.9, 125.5, 120.7, 120.3, 119.0, 116.8, 115.7, 115.3 (d, $J = 21.5$ Hz), 70.6, 70.6, 70.6, 70.6, 70.6, 70.5, 70.5, 70.2, 70.0, 69.2, 56.1, 51.5, 50.9, 39.2, 36.1, 29.6, 28.8, 26.1, 25.4, 15.9; $^{19}$F-NMR (471 MHz, CDCl$_3$): $\delta$ –107.2, –158.3; $^{11}$B-NMR (160 MHz, CDCl$_3$): $\delta$ 2.15; IR (thin film): $\nu$ 3284, 2864, 1697, 1632, 1228, 1091 cm$^{-1}$; ESI-HRMS calcd for C$_{44}$H$_{59}$BF$_2$N$_7$O$_{10}$ [M + H]$^+$ 894.4387, found 894.4380; $[\alpha]_D^{25}$: +3.4 (c = 1.15, CHCl$_3$).

Synthesis of N-(32-(4-(10-(4-fluorobenzoyl)-10-fluoro-9-oxa-10a-aza-10-boraphenanthren-3-yl)-1H-1,2,3-triazol-1-yl)-3,6,9,12,15,18,21,24,27-decaoxadotriacontyl)-6-((4R,5S)-5-methyl-2-oxoimidazolidin-4-yl)hexanamide (55)

To a round-bottom flask were added 46 (32.0 mg, 102 $\mu$mol, 1.50 equiv) and 32-azido-3,6,9,12,15,18,21,24,27,30-decaoxadotriacontan-1-amine (40 $\mu$L, 90% purity, 68 $\mu$mol, 1.0 equiv). The solids were suspended in CH$_3$CN (1.5 mL) and aqueous buffer pH 9.0 (0.5 mL, prepared as describe above). The mixture was stirred at rt for 4 h and 27 (26.0 mg, 75 $\mu$mol, 1.10 equiv) was added, followed by copper sulfate pentahydrate (17.0 mg, 68 $\mu$mol, 1.00 equiv), tris((1-benzyl-1H-1,2,3-triazol-4-yl)methyl)amine (TBTA, 7.2 mg, 14 $\mu$mol, 0.20 equiv) and sodium ascorbate (55 mg, 270 $\mu$mol, 4.00 equiv). The solution was stirred at rt for 2 h and filtered. The filtrate was purified by preparative reverse-phase HPLC (YMC C18 column, gradient of 20-95% CH$_3$CN/H$_2$O + 0.1% TFA for 28 min, $t_R = 22.3$ min). The corresponding peak was collected, diluted with brine, extracted with
CH$_2$Cl$_2$, dried with Na$_2$SO$_4$, filtered and evaporated in vacuo to give 55 as a sticky yellow solid (28.1 mg, 38% over two steps). $^1$H-NMR (600 MHz, CDCl$_3$): $\delta$ 8.65 (d, $J = 1.7$ Hz, 1H), 8.60 (s, 1H), 8.44 (d, $J = 6.3$ Hz, 1H), 8.22 (dd, $J = 8.6$, 5.8 Hz, 2H), 7.97 (dd, $J = 6.3$, 1.7 Hz, 1H), 7.92 (dd, $J = 8.1$, 1.6 Hz, 1H), 7.35 (ddd, $J = 8.5$, 7.2, 1.6 Hz, 1H), 7.10 (t, $J = 8.7$ Hz, 2H), 7.00 (dd, $J = 8.3$, 1.2 Hz, 1H), 6.95 (ddd, $J = 8.2$, 7.1, 1.2 Hz, 1H), 6.44 (t, $J = 5.4$ Hz, 1H), 5.34 (br, 1H), 4.76 (br, 1H), 4.68-4.60 (m, 2H), 3.95 (t, $J = 4.9$ Hz, 2H), 3.81 (p, $J = 6.7$ Hz, 1H), 3.70-3.49 (m, 39H), 3.44-3.38 (m, 2H), 2.16 (t, $J = 7.4$ Hz, 2H), 1.63 (p, $J = 7.2$ Hz, 2H), 1.52-1.19 (m, 6H), 1.10 (d, $J = 6.5$ Hz, 3H); $^{13}$C-NMR (151 MHz, CDCl$_3$): $\delta$ 227.6, 173.1, 165.4 (d, $J = 252.5$ Hz), 163.6, 155.1 (d, $J = 4.8$ Hz), 150.1, 144.5, 143.2, 142.8 (d, $J = 5.6$ Hz), 136.5, 134.5, 131.3 (d, $J = 8.2$ Hz), 125.8, 125.5, 120.7, 120.3, 119.0, 116.8, 115.7, 115.3 (d, $J = 21.5$ Hz), 70.7, 70.7, 70.6, 70.6, 70.6, 70.3, 70.0, 69.2, 56.1, 51.5, 50.9, 39.3, 36.2, 29.6, 28.9, 26.1, 25.4, 15.9; $^{19}$F-NMR (471 MHz, CDCl$_3$): $\delta$ –107.3, –158.4; $^{18}$B-NMR (160 MHz, CDCl$_3$): $\delta$ 1.96; IR (thin film): $\nu$ 3319, 2866, 1698, 1632, 1228, 1100 cm$^{-1}$; ESI-HRMS calcd for C$_{52}$H$_{74}$BF$_2$KN$_7$O$_{14}$ [M + K]$^+$ 1108.4995, found 1100.4998; $[\alpha]_D^{25}$: +1.5 (c = 1.40, CHCl$_3$).

Synthesis of N-(4-azidobutyl)-6-((4R,5S)-5-methyl-2-oxoimidazolidin-4-yl)hexanamide (82)

To a round-bottom flask containing a solution of 74 (244.2 mg, 1.0 mmol, 1.00 equiv) in THF (2 mL) was added hydrazine monohydrate (260 $\mu$L, 5.0 mmol, 5.00 equiv). The solution was stirred at 60 °C for 3 h, cooled to rt, diluted with CH$_2$Cl$_2$, filtered and extracted with 0.2 M aqueous HCl. The acidic aqueous extracts were basified with 1 M aqueous NaOH till pH >14, extracted with CH$_2$Cl$_2$, dried with Na$_2$SO$_4$, filtered and concentrated in the rotavap (complete drying of the sample was avoided).

A separate round-bottom flask was charged with 46 (350.0 mg, 1.1 mmol, 1.10 equiv). The solid was dissolved in CH$_3$CN (5 mL) and aqueous buffer pH 9.0 (2 mL, prepared as describe above), followed by addition of the concentrated CH$_2$Cl$_2$ extracts from above. The solution was stirred at rt overnight, diluted with CH$_2$Cl$_2$, washed with H$_2$O/brine and extracted with CH$_2$Cl$_2$/MeOH (100:5). The organic extracts were collected, dried with Na$_2$SO$_4$, filtered and evaporated in vacuo. The residue was purified by column chromatography on silica gel (eluting with CH$_2$Cl$_2$/MeOH = 100:8) to give 82 as a white solid (196.4 mg, 63%). m.p. 54-56 °C; $^1$H-NMR (500 MHz, CD$_3$OD): $\delta$ 3.83 (p, $J = 6.7$ Hz, 1H), 3.71 (q, $J = 7.2$ Hz, 1H), 3.37-3.29 (m, 2H), 3.21 (t, $J = 6.3$ Hz, 2H), 2.21 (t, $J = 7.4$ Hz, 2H), 1.96.
1.69-1.55 (m, 6H), 1.54-1.27 (m, 6H), 1.12 (d, $J = 6.4$ Hz, 3H); $^{13}$C-NMR (126 MHz, CD$_3$OD): $\delta$ 176.2, 166.2, 57.4, 52.7, 52.1, 39.7, 37.0, 30.7, 30.2, 27.7, 27.3, 27.2, 26.9, 15.6, 15.6; IR (thin film): $\nu$ 3278, 2093, 1702, 1642, 1538, 1444, 1259 cm$^{-1}$; ESI-HRMS calcd for C$_{14}$H$_{26}$N$_{6}$NaO$_2$ [M + Na]$^+$ 333.2009, found 333.2015; $[\alpha]_D^{25}$: +8.2 ($c = 0.90$, CH$_3$OH).

**Synthesis of N-(4-(4-(10-(4-fluorobenzoyl)-10-fluoro-9-oxa-10a-aza-10-boraphenanthren-3-yl)-1H-1,2,3-triazol-1-yl)butyl)-6-((4R,5S)-5-methyl-2-oxoimidazolidin-4-yl)hexanamide (56)**

A round-bottom flask was charged with 27 (34.7 mg, 0.10 mmol, 1.00 equiv), 82 (31.3 mg, 0.10 mmol, 1.0 equiv), copper sulfate pentahydrate (25.0 mg, 0.10 mmol, 1.00 equiv) and tris((1-benzyl-1H-1,2,3-triazol-4-yl)methyl)amine (TBTA, 10.0 mg, 0.02 mmol, 0.20 equiv). The solids were suspended in CH$_3$CN/H$_2$O (1:1, 5 mL) and sodium ascorbate (80 mg, 0.40 mmol, 4.00 equiv) was added. The reaction was stirred at rt for 3h and filtered. The filtrate was purified by column chromatography on silica gel (eluting with CH$_2$Cl$_2$/MeOH = 100:10) to give 56 as a yellow solid (60.3 mg, 92%). m.p. 108-113 °C (decomp.); $^1$H-NMR (600 MHz, CDCl$_3$): $\delta$ 8.59 (br s, 1H), 8.45 (br, 1H), 8.42 (d, $J = 5.8$ Hz, 1H), 8.24 (dd, $J = 8.6, 5.8$ Hz, 2H), 7.94 (br, 1H), 7.89 (d, $J = 7.9$ Hz, 1H), 7.33 (t, $J = 7.5$ Hz, 1H), 7.12 (t, $J = 8.7$ Hz, 2H), 6.98 (dd, $J = 8.2, 1.0$ Hz, 1H), 6.90 (t, $J = 7.4$ Hz, 1H), 6.22 (br s, 1H), 5.68 (br s, 1H), 4.71 (br s, 1H), 4.46 (t, $J = 6.7$ Hz, 2H), 3.81 (p, $J = 6.7$ Hz, 1H), 3.70-3.59 (m, 1H), 3.35-3.25 (m, 2H), 2.17 (t, $J = 7.2$ Hz, 2H), 2.03-1.90 (m, 2H), 1.68-1.59 (m, 2H), 1.57-1.50 (m, 2H), 1.48-1.20 (m, 6H), 1.08 (d, $J = 6.5$ Hz, 3H); $^{13}$C-NMR (151 MHz, CDCl$_3$): $\delta$ 228.1 (br), 173.6, 165.5 (d, $J = 252.9$ Hz), 163.8, 155.1 (d, $J = 4.7$ Hz), 150.1, 144.3, 143.3, 142.8 (d, $J = 5.6$ Hz), 136.5, 134.7, 131.3 (d, $J = 9.0$ Hz), 125.9, 124.5, 120.8, 120.3, 118.9, 116.7, 115.8, 115.4 (d, $J = 21.5$ Hz), 56.1, 51.6, 50.1, 38.3, 36.1, 29.6, 28.6, 27.4, 26.7, 25.9, 25.4, 15.9; $^{19}$F-NMR (471 MHz, CDCl$_3$): $\delta$ $-106.9, -158.2$; $^{11}$B-NMR (160 MHz, CDCl$_3$): $\delta$ 2.02; IR (thin film): $\nu$ 3285, 2934, 1697, 1633, 1229, 1119, 1054 cm$^{-1}$; ESI-HRMS calcd for C$_{34}$H$_{36}$BF$_2$N$_7$NaO$_4$ [M + Na]$^+$ 680.2945, found 680.2946; $[\alpha]_D^{25}$: +6.1 ($c = 0.52$, CHCl$_3$).

The calculation of the statistical distribution of the combinations of starting materials with streptavidin (Figure 4.10) was calculated by using the kinetics modelling software Tenua. To simplify the estimations, the association was assumed to be irreversible ($k_{off} = 0$).
Figure 6.1. Distribution of different association complexes of streptavidin (S) to starting material A.

The corresponding individual equations and rate constants were introduced in the software for a number of equivalencies to produce the following plots:

The distributions shown above were subjected to a similar statistical treatment with starting material B. From the distribution plots, the following matching and mismatching distributions were calculated:

Calculated statistical distribution of streptavidin combinations
Expression and purification of streptavidin mutants

Streptavidin mutants were expressed and purified by following the literature procedures.\textsuperscript{33,34} Plasmids pET21a-Streptavidin-Glutamate_Tag and pET21a-Streptavidin-Dead Aspartate loop were purchased from Addgene (plasmids #46367 and #46368 respectively). The Glu6 tag was deleted from the former plasmid by PCR, employing 5'-TAATAAAAGCTTGGCCGCGG-3' and 5'-GGAACAGCGACTGGTTC-3' as the corresponding primers. Expressed denatured monomers were combined in a 1:1 ratio and refolded. Tetramers were purified by FPLC anion-exchange chromatography using a MonoQ anion-exchange column (eluting with Tris buffer 20 mM, pH 8.0, linear gradient of 0–1 M NaCl over 100 CV, as described). The corresponding peaks were collected and spin-filtered into bufferaq. pH 6.0 (25 mM, prepared by dissolving citric acid monohydrate (663 mg, 3.2 mmol) and sodium citrate anhydrous (5.63 g, 21.8 mmol) in distilled water (1 L); the final pH was adjusted using 1 M NaOH and HCl solutions). Aliquots of each peak were added equal volumes of 2x Laemmli buffer and incubated at rt (to avoid streptavidin to unfold). Samples were analyzed by SDS-PAGE (using a 7.5% precast gel) and visualized by staining with CBB. Both the elution chromatogram and the gel were in agreement with the literature (Figure 6.2).\textsuperscript{34}

![Image](image.png)

**Figure 6.2.** a. FPLC trace of refolded streptavidins. b. SDS-PAGE image of the refolded mixture of streptavidins and the separated individual peaks (after CBB staining and destaining). The possible tetramers are illustrated as combinations of active monomers (light blue indented boxes) and D4-tagged, dead monomers (grey boxes).
S45A and D128A mutations were introduced in the Glu6-deleted plasmid corresponding to the active monomer by PCR using the following primers:

S45A_F: TACCTACGAAgccGCTGTTGGTAAC
S45A_R: CCGGTCAGAGCACCGTCA

D128A_F: GGTTGGTCACgccACCTTCACCA
D128A_R: AGGGTGGATTCCACGCG

S45A and D128 active monomers were expressed and refolded to the tetravalent, fully active tetramer form by following the literature procedure. The mutants were purified by FPLC anion-exchange chromatography using a MonoQ anion-exchange column (eluting with Tris buffer 20 mM, pH 8.0, linear gradient of 0–1 M NaCl over 100 CV, as described). The corresponding peaks were collected and spin-filtered into buffer aq. pH 6.0 (25 mM, prepared by dissolving citric acid monohydrate (663 mg, 3.2 mmol) and sodium citrate anhydrous (5.63 g, 21.8 mmol) in distilled water (1 L); the final pH was adjusted using 1 M NaOH and HCl solutions). Aliquots of each peak were added equal volumes of 2x Laemmli buffer and incubated at rt (to avoid streptavidin to unfold). Samples were analyzed by SDS-PAGE (using a 7.5% precast gel) and visualized by staining with CBB.

S45A mutant: **ESI-HRMS** calcd for $\text{C}_{590}\text{H}_{892}\text{N}_{164}\text{O}_{192}\text{S}$ 13385.4991, found 13385.4825 (deconvoluted).

D128A mutant: **ESI-HRMS** calcd for $\text{C}_{589}\text{H}_{892}\text{N}_{164}\text{O}_{191}\text{S}$ 13357.5042, found 13357.4953 (deconvoluted).
Representative procedure G for the kinetics measurement of the fluorescence turn-on reaction of 42 with 39

In a 96-well plate, aqueous buffer pH 6.0 (153.8 µL, 25 mM, prepared by dissolving citric acid monohydrate (663 mg, 3.2 mmol) and sodium citrate anhydrous (5.63 g, 21.8 mmol) in distilled water (1 L); the final pH was adjusted using 1 M NaOH and HCl solutions) was added into three wells. Freshly deprotected 42 from 48 (22.0 µL, 1.00 nmol, 1.00 equiv) was added from stock solution (45.4 µM in aqueous citrate buffer pH 6.0 prepared as described above, the concentration was measured by UV-Vis at 425 nm using the extinction coefficient \( \varepsilon_{425} = 41.500 \text{ M}^{-1} \text{ cm}^{-1} \)) into each well, followed by expressed tetravalent streptavidin (20.2 µL, 0.50 nmol, 0.50 equiv) added from stock solution (24.7 µM in aqueous buffer pH 6.0). After incubation for 5 minutes, acylboronate 39 (4.0 µL, 2.00 nmol, 2.00 equiv) was added from stock solution (500 µM in 1:1 CH\(_3\)CN / aqueous buffer pH 6.0) and fluorescence monitored at \( \lambda_{\text{ex}} = 430 \text{ nm}, \lambda_{\text{em}} = 485 \text{ nm} \) (final volume per well 200 µL).

To measure the maximum achievable conversion, the following control was carried out in every experiment: in separate wells containing aqueous buffer pH 6.0 (174.0 µL), 42 (22.0 µL, 1.00 nmol, 1.00 equiv) from the same stock solution (45.4 µM) was added followed by 1a (4.0 µL, 200 nmol, 200 equiv) from stock solution (50 mM in 1:1 CH\(_3\)CN / aqueous buffer pH 6.0).

Conversion values were extrapolated from the following formula:

\[
\%\text{conv} = \frac{100 \cdot (F_t - F_0)}{(F_{\text{max}} - F_0)}
\]

\( F_t \) is the fluorescence value at each time interval \( t \), \( F_0 \) is the corresponding fluorescence at time zero. \( F_{\text{max}} \) is the fluorescence output from the full conversion control experiments using 1a.

Conv values obtained via the formula above were plotted over time, as follows:
The last point after the kinetics measurement was analyzed by LC-MS (Figure 6.3, below). Only byproducts 49 and 50 (peak 1), product 32 (peak 2) and starting material 42 (peak 3) could be detected at 425 nm.

**LC-MS chromatogram of the last point from the reaction of 42 with 39 templated by tetravalent streptavidin (plot shown above)**

**Figure 6.3.** LC-MS analysis of the reaction of 32 (5.0 μM) with 39 (2.00 equiv) in the presence of tetravalent S (0.50 equiv) in aqueous buffer pH 6.0. Peak 1 mass correspond to byproducts 49 [M–H]− and 50 [M–H]−. Peak 2 shows the mass of product 32 [M+Na]+. Peak 3 correspond to 42 [M+H]+.
Figure 4.12 experiments

Following Representative procedure G, the conversion over time graphs shown below were generated for a number of reaction conditions (displayed on each graph). Each plot is shown as mean values of three experiments with the corresponding error bars.
Following Representative procedure G, the conversion over time graphs shown below were generated for a number of tested streptavidin mutants (displayed on each graph). Each plot is shown as mean values of three experiments with the corresponding error bars.
Figure 4.15 experiments

Following Representative procedure G, the conversion over time graphs shown below were generated for a number of tested streptavidin mutants (displayed on each graph). Each plot corresponds to single run experiments.
Figure 4.16 experiments

Following Representative procedure G, conversion over time was measured for studies on streptavidin equivalents loading. At 6 h time point, conversion was plotted over S equivalents to generate the graphs below. Each plot corresponds to single run experiments.

- **Conditions**
  - Tetravalent S
  - Conv at t = 6 h

- **Conditions**
  - 1,2-Divalent S
  - Conv at t = 6 h

- **Conditions**
  - 1,3— and 1,4-Divalent S
  - Conv at t = 6 h
Following Representative procedure G, conversion over time was measured for studies on acylboronate 39 equivalents loading. Conversion was plotted over t to generate the graphs below. Each plot corresponds to single run experiments.
Following Representative procedure G, the conversion over time graphs shown below were generated for reactions using starting materials equipped with different linkers (displayed on each graph) in the presence of tetravalent streptavidin (S, 0.50 equiv). Each plot is shown as mean values of three experiments with the corresponding error bars.
Following Representative procedure G, the conversion over time graphs shown below were generated using **42** (5.0 μM), **39** (2.0 equiv) and tetravalent **S** (0.50 equiv) in aqueous buffer of different pH values (displayed on each graph). Each plot is shown as mean values of three experiments with the corresponding error bars.
Figure 4.21 experiments

Following Representative procedure G, the conversion over time graphs shown below were generated using 42 (0.50 – 5.0 μM), 39 (10.0 equiv) and tetravalent S (0.50 equiv) in aqueous buffer at pH 6.0. Each plot is shown as mean values of three experiments with the corresponding error bars.
Preparation of complex 57

Hydroxylamine 42 (7.50 nmol, 1.00 equiv, 129 μL from 58.0 μM stock solution in aqueous buffer pH 6.0) was added into a solution of 1,2-divalent streptavidin (7.50 nmol, 1.00 equiv, 300 μL from 1.42 mg/mL stock solution in aqueous buffer pH 6.0). The solution was mixed gently by pipetting. The mixture was purified by FPLC anion-exchange chromatography (eluting with Tris buffer 20 mM, pH 8.0, linear gradient of 0–1 M NaCl over 100 CV). The corresponding peak (showing absorption at 280, 425 and 500 nm, see Figure XX below) was collected and concentrated by spin filtration (MWCO 10 kDA) replacing the elution buffer to aqueous buffer pH 6.0.

Figure XX. FPLC chromatogram of isolated complex 57 (monitoring at 280, 425 and 500 nm).

Preparation of complex 58

Hydroxylamine 42 (7.50 nmol, 1.00 equiv, 129 μL from 58.0 μM stock solution in aqueous buffer pH 6.0) was added into a solution of a mixture of 1,3- and 1,4-divalent streptavidins (7.50 nmol, 1.00 equiv, 68.6 μL from 6.21 mg/mL stock solution in aqueous buffer pH 6.0). The solution was mixed gently by pipetting. The mixture was purified by FPLC anion-exchange chromatography (eluting with Tris buffer 20 mM, pH 8.0, linear gradient of 0–1 M NaCl over 100 CV). The
corresponding peak (showing absorption at 280, 425 and 500 nm, see Figure 6.1 below) was collected and concentrated by spin filtration (MWCO 10 kDA) replacing the elution buffer to aqueous buffer pH 6.0.

**Figure 6.1.** FPLC chromatogram of isolated complex 58 (monitoring at 280, 425 and 500 nm).

### Conversion over time plot of the reaction of 57 with 39

Carried out by following Representative procedure G using 57 complex (5.0 μM) and 39 (2.00 equiv) or KAT 1a (2.00 equiv) in the presence or absence of biotin (5.00 equiv). The corresponding conversion over time plots are shown as mean values of three experiments with the corresponding error bars.
Non-linear model fit parameters of Figure 4.25

Conversion points from the plots on Figure 4.25 (experimental details described above) were fitted into a biexponential non-linear equation (Equation 1, below). Fitting curves are shown as thin black lines, and each specific fit equation displayed on the corresponding plot.

Equation 1

\[
\% \text{ conv} = \frac{100 \cdot [32]}{[42]_0} = F \cdot \left[ 1 - e^{-k^T \cdot t} \right] + (100 - F) \cdot \left[ 1 - e^{-k^B \cdot [39]_0 \cdot t} \right]
\]

\[F = \text{initial fraction of productive complex} = \frac{100 \cdot [\text{Productive complex}]_0}{[42]_0}\]

\[k^T = \text{first order rate constant of templated amide formation (s}^{-1})\]

\[k^B = \text{pseudo-first order background rate constant (M}^{-1} \text{ s}^{-1})\]

\[[39]_0 \gg [42]_0\]

Assumed irreversible binding of 42 and 39 to S
Synthesis of 10-(4-formylbenzoyl)-3-ethynyl-10-fluoro-9-oxa-10a-aza-10-boraphenanthrene (59)

Analogous to acylboronate 27, compound 59 was synthesized according to a modification of our reported procedure. A Schlenk flask was charged with 26 (195 mg, 1.00 mmol, 1.00 equiv) and the atmosphere replaced with N₂ gas. CH₃CN (1.0 mL) and reagent 68 (400 µL, 1.40 mmol,
1.40 equiv) were added sequentially and the mixture stirred at 60 °C for 1 h. The reaction was allowed to cool to rt and the solvent was evaporated to dryness. The residue was redissolved in CH$_3$CN (2 mL). The solution was transferred to a round-bottom flask containing a suspension of KAT 1f (288 mg, 1.20 mmol, 1.20 equiv) in CH$_3$CN (3 mL), followed by the addition of TMSCl (131 µL, 1.00 mmol, 1.00 equiv). The suspension was stirred at rt for 3 h, neutralized with saturated aqueous NaHCO$_3$ and extracted with CH$_2$Cl$_2$. The organic extracts were collected, dried with Na$_2$SO$_4$, filtered and evaporated in vacuo (bath temperature < 30 °C). The residue was purified by column chromatography on silica gel (eluting with EtOAc/hexanes = 2:3) to give 59 as a crystalline yellow solid (218 mg, 61%). m.p. 168–171 °C (decomp.); $^1$H-NMR (600 MHz, CDCl$_3$): δ 10.10 (s, 1H), 8.44 (dd, J = 6.1, 0.7 Hz, 1H), 8.30 (d, J = 7.8 Hz, 2H), 8.11 (d, J = 1.4 Hz, 1H), 7.96 (d, J = 8.5 Hz, 2H), 7.74 (dd, J = 8.0, 1.6 Hz, 1H), 7.58 (dd, J = 6.2, 1.6 Hz, 1H), 7.38 (ddd, J = 8.3, 7.2, 1.6 Hz, 1H), 7.02 (ddd, J = 8.3, 1.2, 0.4 Hz, 1H), 6.97 (ddd, J = 8.2, 7.2, 1.3 Hz, 1H), 3.69 (s, 1H); $^{13}$C-NMR (151 MHz, CDCl$_3$): δ 229.0 (br), 192.4, 155.2 (d, J = 5.1 Hz), 150.0, 143.6, 142.4 (d, J = 6.5 Hz), 138.5, 137.3, 135.3, 129.9, 129.1 (d, J = 1.6 Hz), 125.6, 125.2, 123.2, 121.1, 120.6, 116.0, 87.8, 79.7; $^{19}$F-NMR (471 MHz, CDCl$_3$): δ –159.1; $^{11}$B-NMR (160 MHz, CDCl$_3$): δ 2.03; IR (thin film): ν 3274, 2116, 1701, 1627, 1608, 1539, 1496, 1411, 1204, 1148 cm$^{-1}$; ESI-HRMS calcd for C$_{21}$H$_{13}$BFNNaO$_3$ [M + Na]$^+$ 380.0869, found 380.0877.
7.5 References


Chapter 7. Experimental Section


NMR Spectra
Potassium 4-\((N\text{-propargylcarbamoyl})\text{benzoyl trifluoroborate}\) (1l)

\(^1\text{H NMR (600 MHz, acetone-}d_6\))

![NMR Spectrum](image)

\(^{13}\text{C NMR (150 MHz, acetone-}d_6\))

![NMR Spectrum](image)
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$^{19}$F NMR (471 MHz, acetone-$d_6$)

$^{11}$B NMR (160 MHz, acetone-$d_6$)
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N-(2-Phenylethyl)-4-fluorobenzamide (3a).

$^1$H NMR (400 MHz, CDCl$_3$)

$^{13}$C NMR (100 MHz, CDCl$_3$)
$^{19}$F NMR (377 MHz, CDCl$_3$)
N-(2,2,2-Trifluoroethyl)-4-fluorobenzamide (3b).

$^1$H NMR (400 MHz, CDCl$_3$)

$^{13}$C NMR (100 MHz, CDCl$_3$)
$^{19}$F NMR (377 MHz, CDCl$_3$)
(S)-N-(4-Fluorobenzoyl)phenylalanine methyl ester (3c).

$^1$H NMR (400 MHz, CDCl$_3$)

$^{13}$C NMR (100 MHz, CDCl$_3$)
$^{19}$F NMR (377 MHz, CDCl₃)
(S)-N-(4-Fluorobenzoyl)serine methyl ester (3d).

$^1$H NMR (400 MHz, CDCl$_3$)

$^{13}$C NMR (100 MHz, CDCl$_3$)
$^{19}\text{F NMR (377 MHz, CDCl}_3\text{)}$
2-(4-Fluorobenzamido)-2-deoxy-1,3,4,6-tetra-O-acetyl-\(\alpha\)-D-glucopyranose (\(\alpha\)-3e).

\(^1\)H NMR (400 MHz, CDCl\(_3\))

\(^{13}\)C NMR (100 MHz, CDCl\(_3\))
$^{19}$F NMR (377 MHz, CDCl$_3$)
2-(4-Fluorobenzamido)-2-deoxy-1,3,4,6-tetra-O-acetyl-β-D-glucopyranose (β-3e).

$^1$H NMR (400 MHz, CDCl$_3$)

$^{13}$C NMR (100 MHz, CDCl$_3$)
$^{19}$F NMR (377 MHz, CDCl$_3$)
N-(2-(4-Hydroxyphenyl)ethyl)-4-fluorobenzamide (3f).

$^1$H NMR (400 MHz, DMSO-$d_6$)

$^{13}$C NMR (100 MHz, DMSO-$d_6$)
$^{19}$F NMR (377 MHz, DMSO-d6)
$N$-(2-Oxo-2-phenylethyl)-4-fluorobenzamide (3g).

$^1$H NMR (400 MHz, CDCl$_3$)

$^{13}$C NMR (100 MHz, CDCl$_3$)
$^{19}$F NMR (400 MHz, CDCl$_3$)
4-(4-Fluorobenzamido)methylbenzoic acid (3h).

$^1$H NMR (400 MHz, DMSO-$d_6$)

$^{13}$C NMR (400 MHz, DMSO-$d_6$)
$^{19}$F NMR (377 MHz, DMSO-$d_6$)
N-(2-Dimethylaminoethyl)-4-fluorobenzamide (3i).

$^1$H NMR (400 MHz, (CD$_3$)$_2$CO)

$^{13}$C NMR (100 MHz, (CD$_3$)$_2$CO)
\(^{19}\text{F NMR (377 MHz, (CD}_3\text{)}_2\text{CO)}\)
N-(Piperidin-4-ylmethyl)-4-fluorobenzamide (3j).

$^1$H NMR (400 MHz, CDCl$_3$)

$^{13}$C NMR (100 MHz, CDCl$_3$)
$^{19}\text{F NMR (377 MHz, CDCl}_3\text{)}$
N-(2-(2,5-dioxo-2,5-dihydro-1H-pyrrrol-1-yl)ethyl)-4-fluorobenzamide (3k).

\(^1\)H NMR (400 MHz, CDCl\(_3\))

\(^{13}\)C NMR (100 MHz, CDCl\(_3\))
$^{19}\text{F NMR (377 MHz, CDCl}_3\text{)}$
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$N$-(4-Nitrophenyl)-4-fluorobenzamide (3l).

$^1$H NMR (400 MHz, CDCl$_3$)

$^{13}$C NMR (100 MHz, CDCl$_3$)
$^{19}\text{F NMR (377 MHz, CDCl}_3\)
N-(2-Pyridyl)-4-fluorobenzamide (3m).

$^1$H NMR (400 MHz, CDCl$_3$)

$^{13}$C NMR (100 MHz, CDCl$_3$)
$^{19}$F NMR (377 MHz, CDCl$_3$)
1-(6-(4-Fluorobenzamido)-9H-purin-9-yl)-1-deoxy-2,3,5-tri-O-acetyladenosine trifluoroacetate salt (3n).

$^1$H NMR (400 MHz, CDCl$_3$)

$^{13}$C NMR (100 MHz, CDCl$_3$)
$^{19}$F NMR (377 MHz, CDCl$_3$)
N-(2-Phenylethyl)-4-methoxybenzamide (3o)

$^1$H NMR (400 MHz, CDCl$_3$)

$^{13}$C NMR (100 MHz, CDCl$_3$)
N-(2-Phenylethyl)-2-picolinamide (3p)

$^1$H NMR (400 MHz, CDCl$_3$)

$^{13}$C NMR (100 MHz, CDCl$_3$)
N-(2-Phenylethyl)-3-furamide (3q).

$^1$H NMR (400 MHz, CDCl$_3$)

$^{13}$C NMR (100 MHz, CDCl$_3$)
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N-(2-Phenylethyl)-4-phenylbutanamide (3r).

$^1$H NMR (400 MHz, CDCl$_3$)

$^{13}$C NMR (100 MHz, CDCl$_3$)
N-(2-Phenylethyl)-4-formylbenzamide (3s).

$^1$H NMR (400 MHz, CDCl$_3$)

$^{13}$C NMR (100 MHz, CDCl$_3$)
Pentafluorophenyl 4-(N-(2-phenylethyl)carbamoyl)benzoate (3t).

$^1$H NMR (600 MHz, CDCl$_3$)

$^{13}$C NMR (150 MHz, CDCl$_3$)
\[ ^{13}\text{C}\{^{19}\text{F}\} \text{ NMR (500 MHz, CDCl}_3 \} \]

\[ ^{19}\text{F} \text{ NMR (377 MHz, CDCl}_3 \} \]
3-((7-(Diethylamino)-2-oxo-2H-chromen-4-yl)methoxy)-N-(2-phenylethyl)benzamide (3u).

$^1$H NMR (400 MHz, CDCl$_3$)

$^{13}$C NMR (100 MHz, CDCl$_3$)
2-(6-(Diethylamino)-3-(diethyliminio)-3H-xanthen-9-yl)-5-(N-(2-(2-(2-(N-(2-phenylethyl)carbamoyl)benzamido)ethoxy)ethoxy)ethyl)sulfamoyl)benzenesulfonate (3v).

$^1$H NMR (400 MHz, CDCl$_3$)

$^{13}$C NMR (100 MHz, CDCl$_3$)
4-(3-Azidopropoxy)-N-(2-phenylethyl)benzamide (3w).

$^1$H NMR (400 MHz, CDCl$_3$)

$^{13}$C NMR (100 MHz, CDCl$_3$)
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N-(2-phenylethyl)-4-(2-trimethylsilylethynyl)benzamide (3x).

$^1$H NMR (400 MHz, CDCl$_3$)

$^{13}$C NMR (100 MHz, CDCl$_3$)
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\[ \text{N-(2-Phenylethyl)-N'-propargylterephthalimide (3y).} \]

\[ {^1}H \text{ NMR (400 MHz, DMSO-}d_6) \]

\[ {^{13}}C \text{ NMR (100 MHz, DMSO-}d_6) \]
N-(4-Fluorobenzoyl)-4-fluorobenzamide (5a).

$^1$H NMR (400 MHz, CDCl$_3$)

$^{13}$C NMR (100 MHz, CDCl$_3$)
$^{19}\text{F NMR (371 MHz, CDCl}_3\text{)}$
N-Benzoyl-4-fluorobenzamide (5b).

$^1$H NMR (400 MHz, CDCl$_3$)

$^{13}$C NMR (100 MHz, CDCl$_3$)
$^{19}$F NMR (377 MHz, CDCl$_3$)
(S)-Methyl 2-(9H-fluoren-9-ylmethoxycarbonylamino)-5-(4-fluorobenzamido)-5-oxopentanoate (5c).

$^1$H NMR (400 MHz, CDCl$_3$)

$^{13}$C NMR (100 MHz, CDCl$_3$)
$^{19}$F NMR (377 MHz, CDCl$_3$)
tert-Butyl N-4-fluorobenzoylcarbamate (5d).

$^1$H NMR (400 MHz, CDCl$_3$)

$^{13}$C NMR (100 MHz, CDCl$_3$)
$^{19}$F NMR (282 MHz, CDCl$_3$)
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*N-(4-Fluorobenzoyl)-N'-methylurea (5e).

$^1$H NMR (400 MHz, DMSO-$d_6$)

$^{13}$C NMR (100 MHz, DMSO-$d_6$)
$^{19}$F NMR (377 MHz, CDCl₃)
$N$-((2,5-Dioxoimidazolidin-4-yl)carbamoyl)-4-fluorobenzamide (5f).

$^1$H NMR (400 MHz, DMSO-$d_6$)

$^{13}$C NMR (100 MHz, DMSO-$d_6$)
$^{19}$F NMR (282 MHz, DMSO-$d_6$)
$N$-Methanesulfonyl-4-fluorobenzamide (5g).

$^1$H NMR (400 MHz, (CD$_3$)$_2$CO)

$^{13}$C NMR (100 MHz, (CD$_3$)$_2$CO)
\(^{19}\text{F NMR (377 MHz, (CD}_3\text{)}_2\text{CO)}\)
\(N^\alpha\)-Benzoyl-\(N^\omega\)-(4-fluorobenzoyl)-L-arginine ethyl ester (5h).

\(^1\)H NMR (400 MHz, CDCl\(_3\))

\(^{13}\)C NMR (100 MHz, CDCl\(_3\))
$^{19}\text{F NMR (377 MHz, CDCl}_3\text{)}$
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N-(E)-Cinnamoyl-4-fluorobenzamide (5i).

$^1$H NMR (400 MHz, CDCl$_3$)

$^{13}$C NMR (100 MHz, CDCl$_3$)
$^{19}$F NMR (377 MHz, CDCl$_3$)
** tert-Butyl 4-((4-fluorobenzoyl)carbamoyl)piperidine-1-carboxylate (5j).**

**$^1$H NMR (400 MHz, CDCl$_3$)**

![1H NMR spectrum](image1)

**$^{13}$C NMR (100 MHz, CDCl$_3$)**

![13C NMR spectrum](image2)
$^{19}$F NMR (377 MHz, CDCl$_3$)
N-Benzoyl-4-phenylbutanamide (5k)

\(^1\)H NMR (400 MHz, CDCl\(_3\))

\(^{13}\)C NMR (100 MHz, CDCl\(_3\))
Ethyl 4-(benzoylcarbamoyl)benzoate (5l)

$^1$H NMR (400 MHz, CDCl$_3$)

$^{13}$C NMR (100 MHz, CDCl$_3$)
N-Benzoyl-4-(2-trimethylsilylethynyl)benzamide (5m).

$^1$H NMR (400 MHz, CDCl$_3$)

$^{13}$C NMR (100 MHz, CDCl$_3$)
Pentafluorophenyl 4-(N-benzoylcarbamoyl)benzoate (5n).

$^1$H NMR (600 MHz, CDCl$_3$)

$^{13}$C NMR (150 MHz, CDCl$_3$)
$^{13}$C $^{19}$F NMR (150 MHz, CDCl$_3$)

$^{19}$F NMR (377 MHz, CDCl$_3$)
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4-(3-Azidopropoxy)-N-benzyolbenzamide (5o).

$^1$H NMR (400 MHz, CDCl$_3$)

$^{13}$C NMR (100 MHz, CDCl$_3$)
3-((7-(Diethylamino)-2-oxo-2H-chromen-4-yl)methoxy)-N-benzoylbenzamide (5p).

$^1$H NMR (400 MHz, CDCl$_3$)

$^{13}$C NMR (100 MHz, CDCl$_3$)
Nα-Benzoyl-Nα-α'-bis(4-fluorobenzoyl)-L-arginine ethyl ester (6).

$^1$H NMR (400 MHz, CDCl$_3$)

$^{13}$C NMR (100 MHz, CDCl$_3$)
$^{19}F$ NMR (377 MHz, CDCl$_3$)
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\(N^\alpha\)-(4-fluorobenzoyl)-L-lysine ethyl ester trifluoroacetate salt (7).

\(^1\)H NMR (400 MHz, CDCl\(_3\))

\[^{13}C\) NMR (100 MHz, CDCl\(_3\))
$^{19}\text{F NMR (371 MHz, CDCl}_3\text{)}$
N-(4-Fluorobenzoyl)morpholine (8).

$^1$H NMR (400 MHz, CDCl$_3$)

$^{13}$C NMR (100 MHz, CDCl$_3$)
$^{19}$F NMR (371 MHz, CDCl$_3$)
$N$-Butyl-4-fluorobenzamide (9)

$^1$H NMR (400 MHz, CDCl$_3$)

$^{13}$C NMR (100 MHz, CDCl$_3$)
$^{19}\text{F NMR (371 MHz, CDCl}_3)$
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$N^2(C^1)'-(4-(N\text{-propargylcarbamoyl})benzoyl)\text{streptomycin sesquisulfate (11a).}$

**1H NMR (600 MHz, CD$_3$OD)**

**$^{13}$C NMR (150 MHz, CD$_3$OD)**
N\textsuperscript{6}(C\textsuperscript{3'})-(4-(N-propargylcarbamoyl)benzoyl)streptomycin sesquisulfate (11b).

\textsuperscript{1}H NMR (600 MHz, CD\textsubscript{3}OD)

\textsuperscript{13}C NMR (150 MHz, CD\textsubscript{3}OD)
*N-(4-(N-Propargylcarbamoyl)benzoyl)gentamicin C₁.*

$^1$H NMR (600 MHz, CD$_3$OD)

$^{13}$C NMR (150 MHz, CD$_3$OD)
tert-Butyl-\(N\)-(7-diethylamino-2-oxo-2\(H\)-chromene-3-carboxamidoethyl)carbamate (64).

\(^1\)H NMR (500 MHz, CDCl\(_3\))

\(^{13}\)C NMR (126 MHz, CDCl\(_3\))
7-Diethylamino-2-oxo-2H-chromene-3-carboxamidoethylammonium trifluoroacetate (20)

$^1$H NMR (500 MHz, CD$_3$OD)

$^{13}$C NMR (126 MHz, CD$_3$OD)
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\textit{N-\textit{tert}-Butoxycarbonyl-O-\textit{(N’-methyl-N’-propargylcarbamoyl)}hydroxylamine (18)}

$^1\text{H NMR (500 MHz, CDCl}_3$)

\begin{figure}
\centering
\includegraphics[width=0.8\textwidth]{hnmr_spectrum.png}
\caption{$^1\text{H NMR (500 MHz, CDCl}_3$)}
\end{figure}

$^{13}\text{C NMR (126 MHz, CDCl}_3$)

\begin{figure}
\centering
\includegraphics[width=0.8\textwidth]{cnmr_spectrum.png}
\caption{$^{13}\text{C NMR (126 MHz, CDCl}_3$)}
\end{figure}
Ethyl 4-(\(N\)-tert-butoxycarbonyl-\(N\)\(^{N'}\)-methyl-\(N\)'-propargylcarbamoyloxy)amino)butanoate (65)

\(^1\)H NMR (500 MHz, CDCl\(_3\))

\(^{13}\)C NMR (126 MHz, CDCl\(_3\))
4-(N-tert-Butoxycarbonyl-N-(N’-methyl-N’-propargylcarbamoyloxy)amino)butanoic acid (19)

$^1$H NMR (500 MHz, CDCl$_3$)

$^{13}$C NMR (126 MHz, CDCl$_3$)
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\[ N-(7\text{-Diethylamino-2-oxo-2H-chromene-3-carboxamidoethylcarbamoyl})propyl-N\text{-tert-butoxycarbonyl-O-(N'\text{-methyl-N'}\text{-propargylcarbamoyl})hydroxylamine (21)} \]

\[ ^1H\text{ NMR (500 MHz, CDCl}_3\) \]

\[ ^{13}C\text{ NMR (126 MHz, CDCl}_3\) \]
2-(2-Hydroxyphenyl)pyridine-4-carboxaldehyde (66)

$^1$H NMR (500 MHz, CDCl$_3$)

$^{13}$C NMR (126 MHz, CDCl$_3$)
2-(2-Hydroxyphenyl)-4-ethynylpyridine (26)

$^1$H NMR (400 MHz, CDCl$_3$)

$^{13}$C NMR (126 MHz, CDCl$_3$)
10-(4-Fluorobenzoyl)-3-ethynyl-10-fluoro-9-oxa-10a-aza-10-boraphenanthrene (27)

\( ^1H\) NMR (600 MHz, CDCl$_3$)

\( ^{13}C\) NMR (151 MHz, CDCl$_3$)
$^{19}$F NMR (471 MHz, CDCl$_3$)

$^{11}$B NMR (160 MHz, CDCl$_3$)
$N$-(2-(4-(4-Fluorobenzamido)butanamido)ethyl)-7-(diethylamino)-2-oxo-2$H$-chromene-3-carboxamide (32)

$^1$H NMR (500 MHz, CDCl$_3$)

$^{13}$C NMR (126 MHz, CDCl$_3$)
$^{19}\text{F NMR (471 MHz, CDCl}_3\text{)}$
N-(11-(4-(4-Fluorobenzoyl)-10-fluoro-9-oxa-10a-aza-10-boraphenanthren-3-yl)-1H-1,2,3-triazol-1-yl)-3,6,9-trioxaundecyl)adamantane-1-carboxamide (30)

$^1$H NMR (600 MHz, CDCl$_3$)

$^{13}$C NMR (150 MHz, CDCl$_3$)
$^{19}$F NMR (470 MHz, CDCl$_3$)

$^{11}$B NMR (160 MHz, CDCl$_3$)
N-(20-Azido-3,6,9,12,15,18-hexaoxaecosyl)adamantane-1-carboxamide (29)

$^1$H NMR (500 MHz, CDCl$_3$)

$^{13}$C NMR (126 MHz, CDCl$_3$)
\[ N-(20-(4-(4-\text{Fluorobenzoyl})-10\text{-fluoro-9-oxa-10a-aza-10-boraphenanthren-3-yl})-1H-1,2,3-\text{triazol}-1\text{-yl})-3,6,9,12,15,18\text{-hexaoxaicosyl})\text{adamantane-1-carboxamide (31)} \]

\( ^1\text{H NMR} \ (600 \text{ MHz, CDCl}_3) \)

\( ^{13}\text{C NMR} \ (150 \text{ MHz, CDCl}_3) \)
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$^{19}$F NMR (470 MHz, CDCl$_3$)

$^{11}$B NMR (160 MHz, CDCl$_3$)
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**N-(11-Azido-3,6,9-trioxaundecyl)-6-((4R,5S)-5-methyl-2-oxoimidazolidin-4-yl) hexanamide (S25)**

$^1$H NMR (500 MHz, CDCl$_3$)

$^{13}$C NMR (126 MHz, CDCl$_3$)
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*N*-(7-Diethylamino-2-oxo-2*H*-chromene-3-carboxamidoethylcarbamoyl)propyl) -*N*-tert-butoxycarbonyl-*O*-(N'*-(1-11-(5-([3aS,4S,6aR]-2-oxohexahydro-1*H*-thieno[3,4-d]imidazol-4-yl]pentanamido)-3,6,9-trioxadecyl)-1*H*-1,2,3-triazol-4-yl)methyl)-*N*'methylcarbamoyl)hydroxylamine (36)

$^1$H NMR (500 MHz, CDCl$_3$)

$^{13}$C NMR (126 MHz, CDCl$_3$)
$N$-(7-Diethylamino-2-oxo-2-$H$-chromene-3-carboxamidoethylcarbamoyl)propyl) -$N$-tert-butoxycarbonyl-$O$-($N'$-1-(11-(6-((4$R,5S$)-5-methyl-2-oximidazolidin-4-yl)hexanamido)-3,6,9-trioxoauandecyl)-1$H$-1,2,3-triazol-4-yl)methyl)-$N'$-methylcarbamoyl) hydroxylamine (37)

$^1$H NMR (500 MHz, CDCl$_3$)

$^{13}$C NMR (126 MHz, CDCl$_3$)
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\[ N\text{-}(11\text{-}(4\text{-}(4\text{-Fluorobenzoyl})\text{-}10\text{-fluoro-9-oxa-10a-aza-10-boraphenanthren-3-yl})\text{-}1H\text{-}1,2,3\text{-triazol-1-yl})\text{-}3,6,9\text{-trioxaundecyl})\text{-}5\text{-}[\text{((3aS,4S,6aR)}\text{-}2\text{-oxohexahydro-1H-thieno[3,4-d]imidazol-4-yl]}\text{]pentanamide (38)} \]

\[ ^1\text{H NMR (600 MHz, CDCl}_3] \]

\[ ^{13}\text{C NMR (151 MHz, CDCl}_3] \]
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$^{19}$F NMR (471 MHz, CDCl$_3$)

![$^{19}$F NMR spectrum](image)

$^{11}$B NMR (160 MHz, CDCl$_3$)

![$^{11}$B NMR spectrum](image)
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\( N-(11-(4-(4-Fluorobenzoyl)-10-fluoro-9-oxa-10a-aza-10-boraphenanthren-3-yl)-1H-1,2,3-triazol-1-yl)-3,6,9-trioxaundecyl)-6-((4R,5S)-5-methyl-2-oxoimidazolidin-4-yl)hexanamide (39) \)

\(^1\)H NMR (600 MHz, CDCl\(_3\))

\[^{13}\text{C}\) NMR (151 MHz, CDCl\(_3\))
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$^{19}$F NMR (471 MHz, CDCl$_3$)

$^{11}$B NMR (160 MHz, CDCl$_3$)
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$N^\alpha$-(4-Dimethylaminophenylazo)benzenesulfonyl-$N^\varepsilon$-tert-butoxycarbonyl-L-lysine methyl ester (69).

$^1$H NMR (500 MHz, CDCl$_3$)

$^{13}$C NMR (126 MHz, CDCl$_3$)
\(N^\alpha-(4\text{-Dimethylaminophenylazo})\text{-benzenesulfonyl})-N^\varepsilon\text{-tert-butoxycarbonyl-L-lysine } (43)\).  

\(^1\text{H} \text{NMR (500 MHz, CD}_3\text{OD)}\)

\(^{13}\text{C} \text{NMR (126 MHz, CD}_3\text{OD)}\)
(S)-N-(11-Azido-3,6,9-trioxaundecyl)-2-(4-dimethylaminophenylazo)benzenesulfonamido)-6-(tert-butoxycarbonylamino) hexanamide (45)

$^1$H NMR (500 MHz, CDCl$_3$)

$^{13}$C NMR (126 MHz, CDCl$_3$)
(S)-N-(20-Azido-3,6,9,12,15,18-hexaoxaeicosyl)-2-(4-dimethylaminophenylazo)benzenesulfonamido)-6-(tert-butoxycarbonylamino)hexanamide (72)

$^1$H NMR (500 MHz, CDCl$_3$)

$^{13}$C NMR (126 MHz, CDCl$_3$)
(S)-N-(32-Azido-3,6,9,12,15,18,21,24,27,30-decaoxadotriacontyl)-2-(4-dimethylaminophenylazo) benzenesulfonamido)-6-(tert-butoxycarbonylamino)hexanamide (73)

$^1$H NMR (500 MHz, CDCl$_3$)

$^{13}$C NMR (126 MHz, CDCl$_3$)
N-(4-Azidobutyl)phthalimide (74)

$^1$H NMR (500 MHz, CDCl$_3$)

$^{13}$C NMR (126 MHz, CDCl$_3$)
\((S)-N-(4-Azidobutyl)-2-(4-dimethylaminophenylazo)benzenesulfonamido)-6-(tern-butoxycarbonylamino) hexanamide (75)\)

\(^1\)H NMR (500 MHz, CDCl\(_3\))

\[^{13}\text{C} \text{NMR (126 MHz, CDCl}_3\))\]
(S)-N-(11-Azido-3,6,9-trioxaundecyl)-2-(4-dimethylaminophenylazo)benzenesulfonamido)-6-(6-((4R,5S)-5-methyl-2-oxoimidazolidin-4-yl)hexanamido)hexanamide (47)

$^1$H NMR (500 MHz, CD$_3$OD)

$^{13}$C NMR (126 MHz, CD$_3$OD)
**(S)-N-(20-Azido-3,6,9,12,15,18-hexaoxaecosyl)-2-(4-dimethylaminophenylazo)benzenesulfonamido)-6-((4R,5S)-5-methyl-2-oximidazolidin-4-yl)hexanamido)hexanamide (76)**

$^{1}$H NMR (500 MHz, CDCl$_3$)

$^{13}$C NMR (126 MHz, CDCl$_3$)
(S)-N-(32-Azido-3,6,9,12,15,18,21,24,27,30-decaoxadotriacontyl)-2-(4-dimethylaminophenylazo) benzenesulfonamido)-6-(6-((4R,5S)-5-methyl-2-oxoimidazolidin-4-yl)hexanamido)hexanamide (77)

$^1$H NMR (500 MHz, CDCl$_3$)

$^{13}$C NMR (126 MHz, CDCl$_3$)
**(S)-N-(4-Azidobutyl)-2-(4-dimethylaminophenylazo)benzenesulfonamido)-6-(6-((4R,5S)-5-methyl-2-oxoimidazolidin-4-yl)hexanamido)hexanamide (78)

\(^1\)H NMR (500 MHz, CD\(_3\)OD)

\[^{13}\text{C} \text{NMR (126 MHz, CD}_3\text{OD)}\]
Boc-hydroxylamine 48

$^{1}$H NMR (500 MHz, CDCl$_3$)

$^{13}$C NMR (126 MHz, CDCl$_3$)
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Boc-hydroxylamine 79

$^1$H NMR (500 MHz, CDCl$_3$)

$^{13}$C NMR (126 MHz, CDCl$_3$)
Boc-hydroxylamine 80

$^1$H NMR (500 MHz, CDCl$_3$)

$^{13}$C NMR (126 MHz, CDCl$_3$)
Boc-hydroxylamine 81

$^1$H NMR (500 MHz, CDCl$_3$)

$^{13}$C NMR (126 MHz, CDCl$_3$)
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\[ N-(20-(4-(10-(4-\text{Fluorobenzoyl})-10-\text{fluoro}-9-\text{oxa}-10\text{a}-\text{aza}-10\text{-boraphenanthren}-3\text{-yl})-1\text{H}-1,2,3\text{-triazol-1-yl})-3,6,9,12,15,18\text{-hexaoxaecosyl})-6-((4R,5S)-5-\text{methyl}-2-\text{oxoimidazolidin-4-yl})\text{hexanamide (54)} \]

\[ ^1\text{H NMR (600 MHz, CDCl}_3\text{)} \]

\[ ^{13}\text{C NMR (151 MHz, CDCl}_3\text{)} \]
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$^{19}$F NMR (471 MHz, CDCl$_3$)

$^{11}$B NMR (160 MHz, CDCl$_3$)
N-(32-(4-(10-(4-Fluorobenzoyl)-10-fluoro-9-oxa-10a-aza-10-boraphenanthren-3-yl)-1H-1,2,3-triazol-1-yl)-3,6,9,12,15,18,21,24,27-decaoxadotriacontyl)-6-((4R,5S)-5-methyl-2-oxoimidazolidin-4-yl)hexanamide (55)

$^1$H NMR (600 MHz, CDCl$_3$)

$^{13}$C NMR (151 MHz, CDCl$_3$)
$^{19}$F NMR (471 MHz, CDCl$_3$)

$^{11}$B NMR (160 MHz, CDCl$_3$)
N-(4-(4-(10-(4-Fluorobenzoyl)-10-fluoro-9-oxa-10a-aza-10-boraphenanthren-3-yl)-1H-1,2,3-triazol-1-yl)butyl)-6-((4R,5S)-5-methyl-2-oxoimidazolidin-4-yl)hexanamide (56)

\(^1\)H NMR (600 MHz, CDCl\(_3\))

\(^{13}\)C NMR (151 MHz, CDCl\(_3\))
$^{19}$F NMR (471 MHz, CDCl$_3$)

$^{11}$B NMR (160 MHz, CDCl$_3$)
10-(4-Formylbenzoyl)-3-ethynyl-10-fluoro-9-oxa-10a-aza-10-boraphenanthrene (59)

$^1H$ NMR (600 MHz, CDCl$_3$)

$^{13}C$ NMR (151 MHz, CDCl$_3$)
$^{19}$F NMR (471 MHz, CDCl$_3$)

$^{11}$B NMR (160 MHz, CDCl$_3$)