Molecular Engineering of Synthetic Amino Acids for the Mechanistic Study of Dipole Effects on Intramolecular Charge Transfer

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by

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My Mother: Gloria M. Larsen
My Dearly Departed Father: Frederick N. Larsen
My Sister: Laura A. Gutierrez.

My Uncle: Joe Perez
My Dearly Departed Great Uncle: Benjamin Rojas

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ABSTRACT OF THE DISSERTATION

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Doctor of Philosophy, Graduate Program in Bioengineering
University of California, Riverside, December 2016
Dr. Valentine I. Vullev, Chairperson

Photoconversion efficiency is primarily impaired by interfacial charge recombination. Natural light harvesting systems address this challenge by incorporating molecular electrets. (Electrets are the electrostatic analogues of magnets, i.e., they possess ordered dipole moments.) Protein alpha helices are the best examples of molecular electrets since the co-directionally ordered amide and hydrogen bonds naturally generate a permanent electric dipole.

Although these local electric fields generated by the protein dipoles provide a means for “steering” electron transduction, they cannot be directly incorporated as electronic material because: 1) they mediate electron transfer via tunneling limiting its efficiency to about 2 nm, 2) they have large band gaps and inaccessible redox states (i.e., they can accept or donate electrons only under extreme potentials that can result in their
degradation), and 3) their conformations are immensely sensitive to non-native environments.

Therefore, a significant part of my studies focused on developing a library of non-native aromatic beta-amino acid residues, namely, derivatives of the anthranilic acid. I designed the aromatic moieties to provide an electronic framework that supports hole hopping resulting in conjugates that capture the benefits of natural systems. Furthermore, altering the substituents on the distal sites of these residues allows for adjusting their reduction potentials over a range of one volt.

Electrochemical and computational examination revealed the structure-function relationship between the position and strength of the electron donating substituent. These findings provided the criteria for generating a long-lived radical cations, which is essential for long-range hole transfer. Namely, for stable radical cations of the anthranilamide non-native residues: 1) their reduction potential must be under about 1.5 V vs. S.C.E., and 2) the electron spin density should not extend over the C-terminal amide.

To test the validity of these criteria and to expand our set of non-native residues, I developed a sub-set of *ad hoc* hole-transfer amino acids. Based on a fluorinated aminoanthranilamide template, each residue contains both, an electron withdrawing and donating group. The spin-density-distribution and electrochemical analysis revealed that while the amine at 5th position ensures spin-density distribution extending over the N-terminal (rather than C-terminal) amide, the fluorine atom at position 4 induces a 200-mV
positive shift in the reduction potentials. Additionally, regio-selective nucleophilic aromatic substitution of a difluoronitrobenzoic acid provides a straightforward synthetic route for making these residues.

Overall, the most significant contributions from my doctoral research are: (1) the design and development of a library of synthetic amino acids with a wide range of reduction potentials; (2) the determination of the effects of location and electron donating strength on the stability of anthranilamide radical cations; and (3) the implantation of nucleophilic aromatic substitution to synthesize residues for hole hopping.
# Table of Contents

List of Figures ......................................................................................................................... xii  
List of Schemes ....................................................................................................................... xiv
List of Tables .............................................................................................................................. xv  
List of Charts ............................................................................................................................ xvi
List of Abbreviations ................................................................................................................... xvii

Chapter 1: Introduction: Bioinspired Approach to Improve Solar Energy Conversion ..... 1  
  Solar energy’s capability ........................................................................................................... 2  
  Nature’s ancient answer .......................................................................................................... 3  
  Our approach .......................................................................................................................... 4  
  In summary ............................................................................................................................ 8  
  References ............................................................................................................................... 9

Chapter 2: Building Blocks for Bioinspired Electrets: Molecular-Level Approach to  
  Materials for Energy and Electronics .................................................................................... 14  
    Abstract .............................................................................................................................. 15  
    Introduction ....................................................................................................................... 16  
    Results ............................................................................................................................... 20  
    Reduction Potentials ......................................................................................................... 23  
    Photophysical Properties ................................................................................................. 25  
    Permanent electric dipoles and distribution of the frontier orbitals ................................ 29  
    Discussion .......................................................................................................................... 31  
    Conclusion .......................................................................................................................... 34  
    References .......................................................................................................................... 36  
    Supplemental Information ................................................................................................. 52  
    Supplemental References .................................................................................................. 74

Chapter 3: What Makes Oxidized N-Acylanthranilamides Stable? ................................. 75  
    Abstract .............................................................................................................................. 76  
    Introduction ....................................................................................................................... 77  
    Results ............................................................................................................................... 79  
    Discussion .......................................................................................................................... 82  
    Experimental Methods ..................................................................................................... 84  
    References .......................................................................................................................... 90  
    Supplemental Information ............................................................................................... 106

Chapter 4: Fluorinated Aminoanthranilamides: How to Make Them and Why They Are  
  Important? ............................................................................................................................... 111  
    Abstract .............................................................................................................................. 112  
    Introduction ....................................................................................................................... 113
Results ........................................................................................................................................... 117
References .................................................................................................................................... 121
Discussion ..................................................................................................................................... 118
Supplemental Information .............................................................................................................. 128
Reference ...................................................................................................................................... 127
List of Figures

Chapter 1
Figure (1-1) Polypeptide alpha-helix .................................................................11
Figure (1-2) Bioinspired molecular electrets and the origin of their electric dipole ..........12
Figure (1-3) Frontier orbitals of an anthranilamide trimer ........................................13

Chapter 2
Figure (2-1) Bioinspired molecular electret composed of anthranilamide residues ......44
Figure (2-2) Anthranilamide residues ........................................................................45
Figure (2-3) Solvent dependence of the electrochemical potentials of the anthranilamide residues ........................................................................................................46
Figure (2-4) UV/visible absorption and emission spectra of anthranilamide residues for various solvent media ..........................................................................................47
Figure (2-5) Absorption and emission properties of the anthranilamide residues ......48
Figure (2-6) HOMOs and LUMOs of Ant, Met, 4Pip and 5Pip for the gas phase (GP) and for acetonitrile (MeCN), obtained from DFT calculations ......................................49
Figure (2S-1) Cyclic voltammograms and their 1st and 2nd derivatives used for extracting half-wave reduction potentials, $E^{(1/2)}$, of the residues exhibiting reversible (e.g., 5Hxm) and irreversible (e.g., Hox) electrochemical oxidation (100 mM NBu$_4$PF$_6$ in DCM). .....69
Figure (2S-2) Overlays of relaxed ground-state structures of the eight Aa residues in the gas phase, in DCM, and in MeCN, obtained from DFT calculations .........................70
Figure (2S-3) HOMOs and LUMOs of Ant, Met, Hox and Dmx for the gas phase (GP) and for acetonitrile (MeCN), obtained from DFT calculations ...................................71
Figure (2S-4) HOMOs and LUMOs of the amine-reprivatized residues for the gas phase (GP) and for acetonitrile (MeCN), obtained from DFT calculations ....................71

Chapter 3
Figure (3-1) Cyclic voltammograms of Aa residues with electron-donating side chains.... ........................................................................................................................................98
Figure (3-2) Electron spin density of the radical cations of the Aa residues

Figure (3-3) 4-piperidinyl Aa residue with a piperidine-capped C-terminus, 4PipC-Pip

Figure (3S-1) $^1$H 1D NMR spectrum of 2-nitro-5-(piperidin-N-yl) benzoic acid (CDCl$_3$)

Figure (3S-2) 1D NMR spectra, (a) $^1$H and (b) $^{13}$C, of (2-nitro-4-(piperidin-N-yl)-benzoyl) piperidine (CDCl$_3$)

Figure (3S-3) 1D NMR spectra, (a) $^1$H and (b) $^{13}$C, of 4PipC-Pip (CDCl$_3$)

Chapter 4

Figure (4-1) Aa bioinspired molecular electrets with the dipole originating from ordered amide bonds and polarization upon hydrogen bonding.

Figure (4-2) NMR analysis of the preparation of fluorinated aminoanthranilamide precursors.

Figure (4-3) Comparison between the electronic properties of fluorinated.
List of Schemes

Chapter 2
Schematic (2-1) Syntheses of the 2-nitrobenzoic acid precursors for (a) 5Pip, (b) 4Pip, (c) 5Hxm, (d) 4Hxm, and (e) Hox .......................................................... 51
Schematic (S2-1) Synthesis of the anthranilamides from the corresponding 2-nitrobenzoic acids. ........................................................................................................ 73

Chapter 3
Schematic (3S-1) Synthesis of 4PipC-Pip ................................................................ 107

Chapter 4
Schematic (4-1) Synthesis of fluorinated Aa residues ............................................ 127
List of Tables

Table (2-1) Electronic characteristics of the anthranilamide residues ...............................50

Table (3-1) Half-wave reduction potentials of the oxidation of $N$-acyl Aa residues, $Aa^+ + e^- \rightleftharpoons Aa$ .................................................................................................................................103

Table (4-1) Half-wave reduction potentials for the oxidation of the fluorinated Aa residues ........................................................................................................................................126
List of Charts

Chart (3-1) Bioinspired Molecular Electrets and Their Anthranilic Residues ...............104
### List of Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aa</td>
<td>Anthranilamide</td>
</tr>
<tr>
<td>CHCl₃</td>
<td>Chloroform</td>
</tr>
<tr>
<td>Ceₐ</td>
<td>Electrolyte Concentration</td>
</tr>
<tr>
<td>CT</td>
<td>Charge Transfer</td>
</tr>
<tr>
<td>CV</td>
<td>Cyclic Voltammetry</td>
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<tr>
<td>D</td>
<td>Debye</td>
</tr>
<tr>
<td>DCM</td>
<td>Dichloromethane</td>
</tr>
<tr>
<td>DFNBA</td>
<td>4,5-difluoro-2-nitrobenzoic acid</td>
</tr>
<tr>
<td>DFT</td>
<td>Density Functional Theory</td>
</tr>
<tr>
<td>DNA</td>
<td>Deoxyribonucleic acid</td>
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<tr>
<td>E₀₀</td>
<td>zero-to-zero energies</td>
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<td>eV</td>
<td>Electron Volt</td>
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<tr>
<td>EDG</td>
<td>Electron Donating Group</td>
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</table>
ε : Dielectric Constant

$^{19}$F NMR: Fluorine Nuclear Magnetic Resonance

Fmoc: Fluorenlymethyloxycarbonyl

$h^+$: Hole

HOMO: Highest Molecular Orbital

LE: Locally Excited

LUMO: Lowest Molecular Orbital

MeCN: Acetonitrile

M: Molarity

NOESY: Nuclear Overhauser Effect Spectroscopy (2D NMR)

NMR: Nuclear Magnetic Resonance

PhCN: Benzonitrile

PC: Propylene Carbonate

PNA: Peptide Nucleic Acid

SCE: Standard Calomel electrode

SDD: Spin-Density Distribution

tBoc: tert-butyloxy carbonyl

UV: Ultraviolet

V: Volt
Chapter 1

Introduction

Bioinspired Approach to Improve Solar Energy Conversion
Solar energy’s capability

For more than 2 billions years, solar energy has sustained the life on earth after the occurrence of photosynthesis. The energy reaching the earth’s surface from solar radiation has an average flux of about 160 W m$^{-2}$, making solar energy one of our best resources to power the plant.$^{1,2}$ Because this celestial radiation would provide the needed energy at rates that are orders of magnitude larger than the ones from other carbon-neutral energy sources, the ability to efficiently harnessing and utilize our sun’s radiation has been one of the major global scientific endeavors of the 21st century.$^{1,2}$

Currently, solar energy is captured in photovoltaic (PV) devices, which implement a p-n junction for separating a photoexcited electron from a hole. As light strikes the PV device, it gives energy for the electrons to transfer from the valence to the conduction band of the device, leaving holes in the valence band. Electrons cascade down energetically and physically move to the n-side of the junction and similarly the holes move to the p-side of the junction. In the case of donor-acceptor systems, with discretely defined orbitals, rather than bands, the photoinduced charge separation (CS) follows the same principles. When the donor is photoexcited, it forms a locally excited (LE) state, in which, an electron from its HOMO is moved to its LUMO. Since the frontier orbitals of the donor lie energetically above those of the acceptor, the electron from the LUMO of the donor moves to the LUMO of the acceptor, generating a CS state, i.e., a radical cation of the donor and a radical anion of the acceptor. Conversely, when the acceptor gets excited to its LE state, and electron from the HOMO of the donor moves to the HOMO of
the acceptor, forming the same CS state as when the excitation goes to the donor. All these photoinduced charge-transfer (CT) processes represent the most important step of converting light into electrical energy.

While in the intermediate CT states, there are two outcomes that can take place: either the formed positive and negative charges will migrate away from one another generating electric current, or a more probable charge recombination (CR) releasing the harvested energy in the form of heat. The higher tendency for CR to occur is the result of an intrinsic electron-hole local Columbic attraction. Therefore, the ability to suppress this natural recombination tendency and to control the charge transfer process is a key requirement for improving light harvesting efficiencies.

**Nature’s ancient energy answer**

Fortunately, nature has provided the excellent example of efficient light harvesting through the highly evolved Photosystems I and II. Through the perfect application of the principles of physics and chemistry, these natural systems show a superb example for efficient light harvesting. One of the tools used to achieve such high energy-conversion efficiencies is the implementation of molecular electrets. Electrets contain co-directionally ordered permanent electric dipoles and can be considered electrostatic analog of magnets.3-6

Protein alpha-helices are the best examples of molecular electrets and contain ordered amide linkages and hydrogen-bonding network, generating intrinsic dipole moments of about 5 Debyes per residue and electric fields of Gigavolts per meter.7,8
Although the intrinsic dipoles of these biopolymers can rectify electron transfer and aid ion transport,\textsuperscript{9-11} the direct use of protein helices as electronic materials is challenging because of: (1) their large band gaps; (2) inability to efficiently mediate electron transfer beyond 2 nm; and (3) conformational sensitivity. In relevance to (2), the limits are posed by the inability to inject electrons or take electrons (inject holes) in protein backbones. Hence, they cannot store/host charges and charges can only move through them via quantum mechanical tunneling.

Despite the fact that these highly evolved, natural structures cannot be directly incorporated into our current energy conversion devices, they do offer insight into successful methods for controlling energy-conversion. Since local electric fields provide a means for “steering” electron transduction, the use of molecular electrets presents an important paradigm in energy science and engineering.

**Our approach**

We undertake a bioinspired approach in the design of molecular electrets that possess all advantages that protein helices have to offer (i.e., ordered amide and hydrogen bonds that generate intrinsic dipoles of about 4 Debyes per residue).\textsuperscript{12,13} Unlike their biological macromolecular counterparts, the bioinspired electrets can store charges (electrons or holes --- electrochemical oxidation), hence we hypothesize they will be able to mediate efficient long-range charge transfer along their backbones. Composed of anthranilamides, our system contains aromatic moieties directly linked with ordered amide and hydrogen bonds that provide an extended $\pi$-conjugation essential for long-
range CT (Figure 1-2). The extended conformation of the anthranilamide chain is supported by a hydrogen-bonding network, securing the dipole generating orientation.

As shown from previous work in our group, similar to protein helices, anthranilamides possess large intrinsic dipoles resulting from ordered amide and hydrogen bonds.\textsuperscript{12,13} (Hence, they are referenced as “bioinspired.”\textsuperscript{12-14}). The local fields from the dipolar bonds induce a “cascade” energy profile in the CT pathways along electrets with identical redox moieties. (Figure 1-3) The length of the CT pathways can be easily adjusted by increasing or decreasing the number of identical residues within the oligomers. This feature is unique to this system. Typically, moieties with different redox properties have to be added to extend cascade CT pathways. We demonstrate that the electrets rectify the rates of charge transfer to an auxiliary electron acceptors attached to either of their termini.\textsuperscript{15} Furthermore, monitoring the formation of radical ions with femtosecond transient absorption spectroscopy indicated that charges do reside on the electrets, which is essential for long-range charge transfer to be attainable via hopping mechanism.

Similar to the design of the natural amino acids, the non-native bioinspired molecular electret also contains features which allows structural and electronic versatility. In living organisms, the varying combinations of the 20 native amino acids results in countless proteins with a range of functionalities and structures. The native residues contain a single side chain, R, altering of which allows for achieving this proteomic diversity. As an alternative, anthranilamides, composing the molecular electrets, contain
two side chains, \( R_1 \) and \( R_2 \), which allow for the electronic properties of the moieties in the bioinspired systems to be tuned. Substitution with an electron donating or withdrawing groups onto the distal positions of the aromatic ring, \( R_1 \) and \( R_2 \), allows for adjusting the electrochemical potential (and thus, the energy levels of the frontier orbitals) of an anthranilamide. As a principle focus of my Ph.D. studies, I was involved in the design and synthesis of a library of non-native amino acid residue. Through this work, I found that the electronic properties also depend on the exact placement of theses group, i.e., \( R_1 \) vs. \( R_2 \) side chains. This regio-dependency offers an access to much larger diversity in anthranilamide residues than in native-type amino acids with single side chains. Synthesis of a set of different anthranilamide residues arranged in various sequences can provide unlimited possibilities for exploring a wide range of CT paradigms (Chapter 2).\(^\text{16}\)

For energy applications where hole transfer is desired, the CT moiety must form a stable radical cation without undergoing oxidative degradation. Therefore, I was involved in an extensive electrochemical and computational study analyzing how to suppress oxidative degradation and stabilize radical cations of these anthranilamide molecular electrets. We found two requirements for generating a long-lived radical cation within these non-native amino acids: (1) the reduction potentials for oxidizing anthranilamide residues must be under about 1.4 V vs SCE and (2) the electron spin density distribution of the radical cations of the oxidized residues must not extent over their C-termini. For (2), attaching a strong electron donating group \textit{para} to the N-terminal
amine drastically changes the distribution of the positive charge to be primarily over the N-terminal amide and avoids extending onto the C-terminus. For example, placing a strong electron donating group on the 4th position vs. the 5th position results in a reduction potential below 1.4 vs. SCE but generates a radical cation whose electron spin density distributes over the C-terminus causing oxidative degradation (Chapter 3).\(^{17}\)

These findings provide me with the requirements needed for designing a new set of *ad hoc* hole-transfer molecular electrets. Based on a fluorinated aminoanthranilamide templet, each residue was designed to contain both, an electron-withdrawing and electron-donating group as substituents. The presence of a strong electron donating amine as an \(R_2\) side chain ensures chemical reversibility in the measured voltammograms. Conversely, the presence of the electronegative fluorine next to the amine causes a 200-mV positive shift in the reduction potentials. Computational analysis reveals that the presence of the fluorine atom does not disrupt the charge distribution allowing the spin density distribution to extend over the N-terminus and not over the C-terminus. These new anthranilamide derivatives manifest reduction potentials similar to those of 4 amino anthranilamide moieties but with the ability to form stable radical cations. Additionally, regio-selective nucleophilic aromatic substitution of the starting material, 4,5-difluoro-2-nitrobenzoic acid provides a straightforward synthetic route. The ability to selectively substitute the only the 5th-position fluorine atom \(1\) yields solely the derivative which will produce an stable radical cation, \(2\) avoids the generation of oppositely substituted (and irreversibly oxidizable) side products that would be difficult to separate due to
similar chromatographic retention factors, and (3) renders the 4th position fluorine atom non-reactive, thus, preventing the formation of disubstituted side products. These findings from my research allow for the development of robust synthetic procedures, which produce residues with the desired electronic framework and also provide unique NMR “thumbprints” essential for monitoring the reaction progress. (Chapter 4, submitted manuscript).

In summary, the most important contributions from my doctoral work are: (1) the design and development of a library of synthetic amino acid with a wide range of reduction potentials; (2) the determination of the effects of position and electron-donating strength of the side chains on the stability of anthranilamide radical cations, important for the design of stable residues that can mediate hole hopping; and (3) the design and synthesis of the fluorinated aminoanthranilamides using highly regio-selective nucleophilic aromatic substitution.
References


Figure 1.1 Polypeptide alpha-helix (a) ribbon representation; (b) ball-and-sticks model; and (c) direction of the intrinsic dipole moment of the helix. (The hydrogen bonds supporting the secondary confirmation are shaded in red)
Figure 1-2. Bioinspired molecular electrets and the origin of their electric dipole from: (1) the ordered amide-bond dipoles (solid arrows); and (2) the shift in electron density from O to H upon hydrogen bonding (hallow arrows)
Figure 1-3. Frontier orbitals of an anthranilamide trimer.
Chapter 2

Building Blocks for Bioinspired Electrets: Molecular-Level Approach to Materials for Energy and Electronics
Abstract

In biology, an immense diversity of protein structural and functional motifs originates from only 20 native amino acids arranged in various sequences. Is it possible to attain the same diversity in electronic materials based on organic macromolecules composed of non-native residues with different characteristics? This publication describes the design, preparation and characterization of non-native aromatic β-amino acid residues, i.e., derivatives of anthranilic acid, for polyamides that can efficiently mediate hole transfer. Chemical derivatization with three types of substituents at two positions of the aromatic ring allows for adjusting the energy levels of the frontier orbitals of the anthranilamide residues over a range of about one electron volt. Most importantly, the anthranilamide residues possess permanent electric dipoles, adding to the electronic properties of the bioinspired conjugates they compose, making them molecular electrets.
Introduction

Charge transfer (CT) drives almost any phenomenon known to us from a molecular to macroscopic scale. At a cellular level, CT is responsible for a range of chemical and biochemical transformations, and is essential for life on Earth to exist [1-5]. In addition to its vital role in living systems, CT resides at the heart of energy conversion, transduction and storage [6-13], and provides signal transduction for devices integral to our modern lifestyles [14, 15]. For more than a century, the key importance of CT has sustained the ever-growing scientific interest in it.

Among the four fundamental forces in the universe, electromagnetic interactions are the second strongest, weaker only than the nuclear strong force [16]. Unlike nuclear forces, however, electromagnetism prompts long-range interactions making it deterministic for condensed matter [17]. Even minute displacement of charges can result in macroscopically observable phenomena [18-28]. Similarly, with the development of metamaterials, controlling nanometer-scale CT allows for an emergence of unprecedented properties.

The value of the capability to control CT at molecular and nanometer scales cannot be overstated. The utility of local electric fields, generated from molecular and macromolecular dipoles, for ion transport and electron transfer is paramount for living systems [29-31]. Hence, molecular electrets present an important paradigm for guiding CT. (Dipole-polarization electrets are the electrostatic analogues of magnets, i.e., they possess co-directionally ordered electric dipole moments.)
Protein helices represent one of the best examples for molecular electrets, and their electronic properties are essential for various processes in biology [29, 30]. Biomimetic systems, based on polypeptide helices comprising native α-amino acids, rectify the directionality of CT [32-36]. These protein structures, however, possess drawbacks that are inherent to electret materials. Electrets are dielectrics and they may not contain free charge carriers. Free-moving charges in the electret or in the surrounding media would screen the dipole-generated fields eliminating the dipole effect. Hence, electron tunneling is representative of the prevalent mechanism of CT mediated by biological and biomimetic polypeptide structures [37-43]. Tunneling, however, limits the distance of efficient CT to about 2 nm [37, 38]. Sites where charges can temporarily reside (such as redox active cofactors, nucleotides, or amino-acid side chains) can greatly extend the CT distance beyond the 2-nm tunneling limit [5, 44]. Good electronic coupling between a sequence of redox moieties ensures pathways for efficient multiple electron tunneling short steps allowing long-range CT to occur, i.e., long-range electron or hole hopping [5, 44].

To address some of the challenges with polypeptide biological and biomimetic structures, we have undertaken a bioinspired approach to designing molecular electrets in the search of properties that are beyond what natural systems can offer [45-48]. Composed of anthranilamide (Aa) residues, the bioinspired electrets possess ordered amide and hydrogen bonds that, similar to the ordered peptide bonds in protein helices, generate an axial electric dipole (Figure 2-1). In addition to determining that these
oligomers of aromatic ortho-amino acids are indeed molecular electrets [46], we also demonstrated that the Aa dipole rectifies charge transfer [45]. Furthermore, derivatizing an anthranilamide with a secondary amine as R₂ at the 5th position (Figure 2-1), yields a residue that not only is a good electron donor, but also can host a positive charge for seconds as evident from the reversible electrochemical oxidation [45]. The ability to accommodate positive charges is a promising feature for mediating CT via hole (h⁺) hopping.

Chemical derivatization provides a means to widely diversify the electronic properties of the Aa residues. With all trans amide bonds, the Aa oligomers assume an extended conformation, which also is key for their intrinsic dipole. To maintain this extended conformation, it is essential to prevent steric hindrance between residues proximal within an oligomer sequence. Therefore, only the distal sites, i.e., the 4th and 5th position in the aromatic rings of the residues (corresponding to R₁ and R₂, respectively, on Figure 1), are available for chemical derivatization.

Each of the 20 native amino acids differs from the rest by only a single side chain. The side chains of these α-L-amino acid residues govern the conformational folds, and overall the protein structural and functional features. Conversely, the non-native anthranilic residues have two side chains (R₁ and R₂, Figure 2-1) that can be used for adjusting their electronic properties. To explore the potential of these bioinspired molecular electrets as CT-controlling materials it is paramount to design a set of non-native Aa residues with diverse electronic characteristics.
Aromatic poly- and oligo-amides, such as Huc’s foldamers [49-51], provide incomparable venues for exploring biological types of structural motifs, the diversity of which expands beyond what the natural systems can offer [52-56]. Indeed, the rich π-conjugation of such foldamers governs their immensely promising CT characteristics [57]. Our focus, conversely, is on extended aromatic structures, such as Aa oligomers (Figure 1) [46, 48, 58], which are not truly foldamers. While the common theme as electrets with extended conformations and large intrinsic dipoles is conserved in the Aa structures, alterations of the side chains (R₁ and R₂, Figure 2-1) provides venues for achieving diversity in the electronic properties of such aromatic poly and oligo-amides.

Herein, we demonstrate the preparation and characterization of eight non-native Aa residues with their N- and C-termini capped as alkyl amides (Figure 2-2). In addition to the basic anthranilamide residue (Ant) where R₁ = R₂ = H, we investigate Aa derivatives with three types of electron-donating substituents for R₁ and R₂: alkyl (i.e., methyl), alkyoxyls (i.e., methoxy and hexyloxy groups) and amines (i.e., piperidinyl and hexylmethylamine). Electrochemical and spectroscopy studies allowed for estimating the energy levels of the frontier orbitals of these Aa residues. Density functional theory (DFT) calculations yielded information about the intrinsic electric dipoles of the Aa derivatives and provided visualization of their highest occupied and lowest unoccupied molecular orbitals (HOMO and LUMO, respectively).

In agreement with the contribution from the amide and hydrogen bonds [48], the magnitudes of the dipole moments of the Aa residues exceed 4 D. The chemical
derivatization with the three listed electron-donating groups at the two positions of the Aa aromatic ring (i.e., the 4th and 5th) allow for adjusting the reduction potentials of the Aa oxidation over the range of about 1 V. This range is quite significant: adjusting the HOMO energy levels over 1 eV provides an incomparable means for modulating the hole-transfer pathways along sequences composed of such Aa residues.

In addition, the results demonstrate that the electronic properties of Aa depend on both the type of substituted groups used and the exact position of these groups. That is, an electron-donating group (EDG) as a substituent at the 4th and 5th positions can yield three distinct Aa residues with different electronic properties. A residue with \( R_1 = \text{EDG} \) and \( R_2 = \text{H} \) has different properties from a residue with \( R_1 = \text{H} \) and \( R_2 = \text{EDG} \) that also differs from a residue with \( R_1 = \text{EDG} \) and \( R_2 = \text{EDG} \). This feature demonstrates diversity in electronic characteristics that can be achieved via permutations within a single Aa residue, something that native amino acids with single side chains cannot offer. It illustrates some of the advantages of bioinspired over biomimetic approaches [47].

**RESULTS**

**Preparation of the anthranilamide residues**

As polypeptide conjugates of aromatic non-native β-amino acids, we build the Aa oligomers from their C- to their N-termini [46]. Each Aa residue is added to the sequence as a 2-nitrobenzoic acid derivative [45, 46]. A selective reduction of the nitro group to amine prepares the thus added N-terminal residue for the next amide-coupling step [46].
The use of “traditional” synthetic protocols, where each residue is introduced as an Nβ-Fmoc or Nβ-tBoc anthranilic acid derivative, renders negligible to no yields. The electron-withdrawing nitro group at ortho position ensures the electrophilicity of the carbonyl carbon of the activated carboxylate that is needed for coupling it with the N-terminal amine. The N-terminal anthranilic amines are weak nucleophiles due to the neighboring electron-withdrawing carbonyls.

To attain a diversity of Aa residues, we focus on the preparation of a variety of 4- and 5- derivatives of the 2-nitrobenzoic acid as precursors for non-native Aa residues. Three types of substituents at R1 and R2 positions (Figure 1-1) allow for producing electron-rich Aa residues with a wide distribution of the energy levels of their frontier orbitals: (1) strong electron-donating groups, dialkylamines; (2) moderately strong electron-donating groups, alkoxyls; and (3) a weak electron-donating group, methyl. Starting with fluoro-derivatives, nucleophilic aromatic substitution allow for introducing amines as substituents at the 4th and 5th positions of the 2-nitrobenzoic acid. In the 5-fluoro-2-nitrobenzoic acid, the C-F bond at the para position in relevance to the nitro group is quite polarized, making that carbon susceptible to the attack from an amine nucleophile. Using a cyclic secondary amine, i.e., piperidine, requires relatively short reaction times under conventional heating to produce in quantitative yields the nitrobenzoic precursor for the 5Pip residue (Scheme 2-1a) [45]. In the 4-fluoro-2-nitrobenzoic acid, the C-F bond is not as polarized due to the meta (rather than para) position of the nitro group. For the precursor for the 4Pip residue, therefore, the same
nucleophilic aromatic substitution with the 4-fluoro-2-nitrobenzoic acid requires longer reaction times for attaining similar yields (Scheme 2-1b).

A synthetic challenge arises when the nucleophiles are non-cyclic secondary amines. Entropic restrictions decrease the nucleophilic reactivity of dialkylamines as the length of their chains increases [59]. Under conventional heating, the substitution of fluorine in 4-fluoro-2-nitrobenzoic acid with hexylmethylamine leads to negligible yields even when the reaction proceeds for unreasonably long periods of time (Scheme 2-1c).

Utilizing microwave radiation as a heat source allows for addressing this challenge. Although still debated, microwave heating may enhance reaction rates via the entropy components of their activation energies [60, 61], making it appropriate for overcoming the limitations imposed by amine nucleophiles with long alkyl chains. Employing microwave heating, indeed, allows us to develop procedures that lead to completion of the syntheses of the hexylmethylamino precursors for 4Hxm and 5Hxm (Figure 2-2) within reasonable time durations (Scheme 2-1c,d).

The starting material for the Hox precursor is 5-hydroxy-2-nitrobenzoic acid, the carboxylate and the phenolate of which are indiscriminately strong nucleophiles readily producing the dialkyl derivatives. An extra hydrolysis step leads to the Hox nitrobenzoic precursor (Scheme 2-1e). Coupling of the 2-nitrobenzoic acids with 1-hexylamine, followed by selective reduction of the nitro group to amine and another amide coupling produces the eight Aa residues [45].
**Reduction potentials**

Electrochemical studies allow for quantifying the propensity of the Aa residues to serve as electron donors and to potentially mediate hole transfer. The reduction potentials of the oxidation of the Aa residues (i.e., $E_{Aa^*}$, $Aa^{*+} + e^- \rightarrow Aa$) provide a means for quantifying their electron-donating capabilities [62], and for estimating the energy levels of their HOMOs [63].

To elucidate the media effects on the electronic properties, we focus on the dependence of the Aa electrochemical potentials on the solvent polarity [64-68]. Our selection includes five aprotic solvents with different polarity that have electrochemical windows extending over the expected potentials needed for the oxidation of the Aa residues: chloroform (CHCl₃), dichloromethane (DCM), benzonitrile (PhCN), acetonitrile (MeCN), and propylene carbonate (PC).

As a representation of the standard electrode potentials, $E^{(0)}$, the half-wave potentials, $E^{(1/2)}$, are readily obtained from cyclic voltammetry (CV). For reversible electrochemical oxidation, $E^{(1/2)}$ represents the average between the peak potentials of the anodic and the cathodic waves. If the lifetimes of the radical cations, Aa**, generated on the surface of the working electrode do not extend over milliseconds and seconds (the time scales of CV), the cathodic wave becomes undetectable. For such electrochemically irreversible oxidation, the potential at the inflection point of the rise of the anodic wave provides an estimate for $E^{(1/2)}$ (see Supplementary Material). 5Hxm, 5Pip, and Dmx manifest electrochemically reversible oxidation indicating that these residues produce
radical cations with pronounced stability.

While electrochemical measurements require media with high electrolyte concentrations, it is the information for $E^{(0)}$ in neat solvents that is directly relevant to spectroscopy and computational data [65]. From the dependence of the measured $E_{Aa^-/Aa}^{(1/2)}$ on the electrolyte concentration, $C_{el}$, therefore, we extrapolate the values of the reduction potentials of the residues for zero electrolyte concentration, i.e., the $Aa$ potentials for neat solvents, $E_{Aa^-/Aa}^{(G_{w=0})}$ (Figure 2-3a) [65].

Based on the Born solvation energy [69], a linear correlation between $E_{Aa^-/Aa}^{(G_{w=0})}$ and the inverse dielectric constant of the neat solvents, $\varepsilon^{-1}$, reveals the effects of the media polarity on the electrochemical properties of each $Aa$ residue (Figure 3b,c). As expected, an increase in the solvent polarity (i.e., a decrease in $\varepsilon^{-1}$) shifts the potentials to less positive values, elevating the energy levels of the HOMOs, and improving the capabilities of the residues as electron donors (Figure 2-3b,c). The stabilization of the radical cations, $Aa^+$, by polar media accounts for this negative shifts of the measured potentials.

For each solvent, an increase in the electron-donating strength of the substituents, from methyl to amines, causes negative shifts in the reduction potentials. The potential of the best electron donor, 5Hxm, is about 1 V more negative than that of Ant (Figure 2-3b, Table 2-1). This finding is consistent with our theoretical predictions that placing dialkylamine at the 5th position of anthranilamides elevates the energy levels of their HOMOs with about 1 eV [48].
While the amine-derivatized Aa residues are the best electron donors, moving the amine substituents from the 4th to the 5th position causes another negative shift (of about 0.2 – 0.4 V) in the potentials (Figure 2-3b,c). The alkylamines in 5Pip and 5Hxm are \textit{para}-oriented to the electron-donating N-terminal amide and \textit{meta}-oriented to the electron-withdrawing C-terminal amide. This \textit{para}-orientation between the two electron-donating groups in 5Pip and 5Hxm can account for the more negative values of their potentials in comparison with 4Pip and 4Hxm.

This “reinforcement” from two electron-donating groups \textit{para}-positioned to each other can account for stabilizing the radical cations, \textit{Aa}**, and the reversible electrochemical oxidation of 5Pip and 5Hxm. This argument should hold also for the other electron-donating groups at the 5th position. While Dmx manifests reversible oxidation, however, the cyclic voltammograms of Hox and Met exhibit irreversible behavior. Despite the stabilization that 5-methyl and 5-hexyloxy groups might provide to the racial cations of Met and Hox, respectively, their reduction potentials appear positive enough to irreversibly cleave the amide bonds attached to the aromatic rings [70].

\textbf{Photophysical properties}

While electrochemical analysis provides information about the capabilities of the Aa residues to serve as electron donors and hole transducers, optical spectroscopy reveals complementary features about the energetics of the Aa frontier orbitals. In particular, UV/visible absorption and fluorescence spectroscopy provide a means for estimating the
zero-to-zero energies, $E_{00}$. $E_{00}$ represents optical HOMO-LUMO gaps, which in molecular photophysics can be viewed as the optical band gaps of these Aa building blocks for organic materials.

The eight Aa residues absorb in the UV spectral region and fluoresce with substantial quantum yields, ranging between about 0.1 and 0.3 (Figure 2-4) The wavelength where the intensity-normalized absorption and emission spectra cross provides a means for estimating $E_{00}$ (Figure 2-4c) [71-74]. For the Aa residues, $E_{00}$ ranged from about 3 to 3.7 eV (Figure 2-5c). The capability of the alkoxy and the dialkylamine substituents to extend the $\pi$-conjugation of the aromatic rings leads to a decrease in $E_{00}$ that is consistent with narrowing the HOMO-LUMO gaps of the residues. This effect, however, was pronounced only for strong electron-donating substituents placed at the para position to the N-terminal amides (5Pip and 5Hxm vs. 4Pip and 4Hxm, Figure 2-5).

Similar comparison for the alkoxy-derivatized residues reveals that the spectral features of Hox are red-shifted compared to these of Dmx (Figure 2-5). This red spectral shifts for Hox vs. Dmx, are most likely due to the methoxy group at the 4th position of Dmx. An electron-donating group at the 4th position appears to cause blue spectral shifts. Even strong electron-donating groups, such as amines, placed at the 4th position, i.e., 4Hxm and 4Pip, result in Aa residues with spectral features similar to those of Met and Ant (Figure 2-5).
While $E_{00}$ depends on the $R_1$ and $R_2$ substituents, the solvent polarity has insignificant to no effect on the spectral properties of the Aa residues (Figure 2-4, 2-5). This lack of substantial solvatochromism is consistent with our previous experimental observations and theoretical findings for Ant and 5Pip derivatives [45, 46]. The anthranilamides are, indeed, polar molecules. The observed lack of significant solvatochromism, therefore, suggests that photoexcitation of the Aa residues does not substantially alter their polarity. That is, the permanent ground-state dipoles have dominating effect on the ground- and excited-state polarity of the Aa conjugates.

Another feature revealed by the emission spectra of the Aa residues is their propensity to aggregate. As expected from our previous studies [46], two of the residues, Ant and Met, exhibit fluorescence bands with two peaks (Figure 2-4b), the ratios between which are concentration dependent. We ascribe the red-shifted peak, the intensity of which increases with an increase in concentration, to aggregates that form at the excited and/or the ground state [46, 75-80]. The trends from this assignment of the fluorescence spectra indicate that the chlorinated hydrocarbons, such as DCM, tend to suppress aggregation (Figure 2-4b).

In addition, 4Pip and 4Hxm also aggregate at $\mu$M concentrations when dissolved in some of the tested organic solvents (Figure 2-4b). This finding was somewhat surprising because the residues with identical alkyl chains attached to them, 5Pip and 5Hxm, did not manifest detectable aggregation even at concentrations reaching 1 mM.
These findings show that the position of substituents with alkyl chains (i.e., $R_1$ vs $R_2$) pronouncedly affects the aggregation propensity of the Aa derivatives. To confirm this trend, other two residues, Hox and Dmx, that also contain alkyl chains at the 5th, also show a single fluorescence peak, indicating that they do not manifest detectable aggregation in the tested organic solvents (Figure 2-4b).

Comparison between 4Pip and 4Hxm reveals an important trend about the dependence of the aggregation propensity on the structure of the substituents. Both residues aggregate when dissolved in most organic solvents at $\mu$M concentrations. In chlorinated solvents, however, while 4Pip exists as a monomer, 4Hxm manifests some propensity for aggregation (Figure 2-4b). Both residues contain secondary amines at the 4th positions of their aromatic rings. Both substituents contain more than five carbons in their alkyl chains (piperidinyl for 4Pip, and hexyl and methyl for 4Hxm, Figure 2-2). In fact, 4Hxm has a longer chain than 4Pip. Contrarily to correlating improved solubility with increased length of alkyl substituents, however, 4Hxm has a larger propensity for aggregation than 4Pip. In the piperidinyl substituents more carbons are located closer to the aromatic ring than in hexylmethyamine. This increase in the volume of the solvation cavity immediately next to the aromatic moieties (that may drive aggregation) appears to have dominant effect on improving the residue solubility. This trend indicates that long linear chains, such as hexyls, do not improve the solubility in organic solvents to the extent that branched substituents, such as piperidinyl, do.
Permanent electric dipoles and distribution of the frontier orbitals

Ab initio computational studies provide further key information about the electronic properties of the Aa residues and the dependence of these properties on the media polarity. Ground-state DFT calculations at the B3LYP/6-311+G(d,p) level [81-83] performed using Gaussian 09 [84] revealed that the HOMOs and LUMOs of all eight Aa residues are predominantly localized on their aromatic rings (Figure 2-6). For the residues with no substituents or with relatively weak electron-donating R1 and R2 groups, i.e., for Ant, Met, Hox and Dmx, the HOMOs extend over the N-terminal amide bond, while the LUMOs tend to delocalize over both amides (Figure 2-6). Placing a strong electron-donating group on the 5th position (i.e., R2 = dialkylamine) amplifies these orbital-delocalization trends (see 5Pip on Figure 2-6, and 5Hxm in the Supplementary Material). Conversely, placing the same strong electron-donating groups on the 4th position shifts the delocalization of the HOMOs to the C-terminal amides (see 4Pip Figure 2-6, and 4Hxm in the Supplementary Material).

To account for the solvent effects on the electronic properties of the Aa residues, we introduced DCM and MeCN to the calculations using a polarizable continuum model [85-87]. Inclusion of the solvents had no visible effect on the distribution of frontier orbitals (Figure 2-6). Most importantly, the optimized structures of the eight Aa residues remain practically identical when varying the solvent media (see Supplementary Material). While the anthranilamides are polar molecules, principally because of the amide dipoles, these computational findings suggest that the preferential Aa
conformation, with trans amides, is not affected by changes in the solvent polarity.

The permanent electric dipoles are the most important feature of the Aa residues, making them promising building blocks for electrets. The predicted dipole moment of Ant is about 4.7 D (Table 2-1), which is consistent with the contributions of 1.9 D from each of the two amides and of 0.9 D from the polarization due to the hydrogen bonding (Figure 2-1) [48]. Adding methyl to the 5th position, such as in Met, slightly enhances the dipole (Table 2-1), which is consistent with the 5-methyl-induced polarization of the aromatic ring co-directionally with the total dipole from the amide and hydrogen bonds. This enhancement effect on the Aa dipole is even more pronounced for Hox, 5Hxm and 5Pip, where R2 electron-donating groups have mesomeric, rather than inductive, effects on the aromatic ring (Table 2-1). Conversely, when an electron-donating group is at the 4th position, such as in 4Hxm and 4Pip, the substituent-induced polarization of the aromatic ring has a diminishing effect on the total residue dipole (Table 2-1).

Solvent polarity further enhances the magnitude of the Aa dipoles (Table 2-1). Because the electronic and structural properties of the Aa residues have a negligible dependence on the media, this polarity-driven enhancement can be attributed to the effect of the Aa dipoles on the solvent itself. As we have shown for simple aliphatic amides, such dipole enhancement results from the Onsager fields inside the solvation cavities of the solutes [66, 88]. The Aa dipoles polarize the solvent media in the proximity to the solvation cavities. This polarization increases the displacements between the centers of the positive and negative charges of the solvated Aa molecules, increasing the
magnitudes of their total dipole moments.

**DISCUSSION**

The solvent effect on the permanent molecular dipoles has important implications on the CT properties of the anthranilamides. Others and we have shown that an increase in solvent polarity diminishes the dipole effects on CT [32, 33, 45], which is attributed to the screening of the dipole-generated electric field by the surrounding polar media. Concurrently, the media polarity enhances the dipole-generated field inside a solvated molecule [66]. Conversely, for the reported solvent effects on dipole-mediated CT, the electron donor and acceptor are linked in a manner that places both or either of them outside the solvation cavity containing the groups generating the dipole fields. That is, the sources of the permanent dipoles are frequently polypeptide helices, and the redox moieties involved in the CT are located at certain distance from the helix backbones, linked to side chains of residues composing the polypeptide. Thus, while the electron-tunneling pathways may transverse through cavities with solvent-induced enhancement of dipolar fields, the solvation of the acceptor and/or of the donor, outside these cavities, principally affects the CT kinetics.

As an alternative to protein-derived structures, anthranilamide molecular electrets have the structural and electronic features for exploration of solvation dependence of dipole-mediated CT. The aromatic moieties, providing sites for charge hopping, are directly linked via amides that are responsible for the permanent dipole moments. Hence, the
intertwining of the charge-hopping sites and the dipolar groups generating the fields situates them within the same solvation cavity.

While the dipole-generated fields of such electrets can guide the CT processes, the ability to tune energetics along the CT pathways should not be undermined. The electron-donating substituents stabilize positive charges, $h^+$, injected in the aromatic residues, as evident from the negative shifts in the reduction potentials of Aa oxidation when $R_1$ and $R_2$ are changed from hydrogen to alkyloxys and to amines (Table 2-1). The ability to adjust the Aa reduction potentials over a range of 1 V shows a key advantage of anthranilamide CT electrets illustrating their promising potential and utility.

Frequently, potential wells with depth in the order of a few hundred millielectronvolts are responsible for undesired charge-trapping and charge-recombination, decreasing performance efficiencies of materials and devices [89-93]. For Aa residues, only a single change in the position of an amine from $R_2$ to $R_1$ lowers their HOMO energy with about 0.3 eV, which exceeds the thermal energy, $k_B T$, by more than an order of magnitude. Overall, the magnitude of the substituent effects on the electronic properties of the Aa residues is comparable with the energetics that governs CT processes responsible for the performance of electronic materials and devices.

Considering such applications, three of the residues, Dmx, 5Hxm and 5 Pip, appear to have the most desirable characteristics. First, they all exhibit reversible electrochemical oxidation making them excellent sites for $h^+$ hopping. Indeed, the potentials of Hox, Met and Ant are positive enough to cause oxidative cleavage of the
amide bonds [70] that sustain the structural integrity of the macromolecules these residues may compose. Therefore, their use may be limited to introducing them as tunneling barriers on the CT pathways. Conversely, 4Hxm and 4Pip present a curious case. Their reduction potentials do not appear positive enough to induce amide oxidation. Can 4Hxm and 4Pip, then, mediate $h^+$ hopping without oxidative cleavage? The cyclic voltammetry results indicate that 4Hxm$^{+\cdot}$ and 4Pip$^{+\cdot}$ have lifetimes much shorter than hundreds of milliseconds, making the cathodic waves undetectable. If the life of these radical cations, however, is longer than a few nanoseconds, 4Pip and 4Hxm still can be viable sites for $h^+$ hopping that does not cause irreversible damage of the molecular structures.

Second, 5Hxm, 5Pip and Dmx do not aggregate at hundreds of $\mu$M concentrations. This feature is important not only for solution phase studies, but also for processing materials composed of these residues. In addition to these three residues, the lack of aggregation propensities of Hox can also prove beneficial for macromolecular designs. While electronically Hox residues may be used solely for adding tunneling barriers along CT pathways, introducing Hox to anthranilamide macromolecules will improve their solubility in organic media. Again, 4Hxm and 4Pip appear to have an outlier-like behavior. The six-carbon alkyl chains of their $R_1$ substituents do not eliminated their propensity for aggregation. Polarizable chlorinated solvents appear to at least partially suppress the aggregation of the 4-amino residues. 4Hxm and 4Pip, however, tend to aggregate when dissolved in other organic solvents.
Overall, 5Hxm, 5Pip, Dmx and Hox appear as most viable building blocks for molecular electrets that can readily mediate efficient long-range CT. Indeed, this analysis is based on conjugates composed of single Aa residues. Although the electronic properties of anthranilamide oligomers have negligible dependence on the number of residues [46], the single-residue findings should be viewed as important guidelines, rather than strict rules. Therefore, we cannot rule out the potential utility of 4Hxm and 4Pip. The irreversible electrochemical oxidation and the aggregation propensity of 4Hxm and 4Pip are not truly desirable features. Before these two residues are tested as building blocks of Aa oligomers, it will be premature to decide how adverse these features may prove. Conversely, the energy levels of their HOMOs of the 4-amino Aa residues are located between of the HOMOs of Dmx and 5Pip, making 4Hxm and 4Pip still attractive candidates for the exploration of the diversity of the CT molecular electrets.

In living organisms, each of the 20 native amino acids has quite a difference relative abundance in the known proteins they compose [94]. Similarly, in the design of CT molecular electrets, not all the Aa residues need to be equally present. Some residues, such as 5Hxm and Hox, may play principal role in determining the structural and electronic characteristics of the molecular electrets. Other residues, such as Ant and Met, may be used scarcely as “dopants.”

**Conclusions**

Their permanent dipole moments make anthranilamides attractive candidates for charge-transfer systems. Combinations of three types of electron-donating substituents at
two possible positions yield a set of non-native Aa residues with diverse electronic properties. In proteomics, permutations using 20 native amino acids lead to countless structure-function relationships. We believe that, in a similar manner, the herein described non-native Aa residues are key building blocks for countless macromolecular systems with a wide range of unexplored electronic features.
References


Figures

Figure 2-1. Bioinspired molecular electret composed of anthranilamide residues (---Aa(i)- Aa(j)- Aa(k)- Aa(l)- Aa(m)----) and the origin of its electric dipole from the ordered orientation of the amide linkers and the polarization of the hydrogen bonds.
Figure 2-2. Anthranilamide residues.
Figure 2-3. Solvent dependence of the electrochemical potentials of the anthranilamide residues. (a) Dependence of the half-wave potentials of the Aa residues on the electrolyte concentration, \( C_{el} \) (for DCM in the presence of (C4H9)4NPF6 as electrolyte). Extrapolation to zero electrolyte concentration from exponential data fits provides the estimates for the reduction potentials of the residues in neat solvents. (b,c) Dependence of the extrapolated potentials for neat solvents on the media dielectric characteristics obtained from measurements for five different solvents: propylene carbonate, PC (\( \sum^{-1} = 0.016 \)); acetonitrile, MeCN (\( \sum^{-1} = 0.027 \)); benzonitrile, PhCN (\( \sum^{-1} = 0.040 \)); dichloromethane, DCM (\( \sum^{-1} = 0.11 \)); and chloroform (\( \sum^{-1} = 0.21 \)).
Figure 2-4. UV/visible absorption and emission spectra of anthranilamide residues for various solvent media: propylene carbonate (PC), acetonitrile (MeCN), benzonitrile (PhCN), dichloromethane (DCM), chloroform (CHCl₃), and cyclohexane (CH). (a) Absorption spectra of the eight residues for MeCN. (b) Fluorescence spectra of the residues recorded for MeCN and DCM ($\lambda_{ex} = 310$ nm; each fluorescence spectrum was normalized by $\times (1 - 10^{-\delta(\lambda_{ex})^{-1}}$). (c) Absorption and fluorescence spectra for Hox in the different solvents ($\lambda_{ex} = 310$ nm; each fluorescence spectrum was normalized to the height of the red-most band of the corresponding absorption spectrum; except for PC, the baselines of the spectra are elevated from 0 for improved visualization; the arrows point to wavelength, $\lambda_{00}$, of crossing point between the two spectra that is used for calculating the zero-to-zero energy, $E_{00} = h c / \lambda_{00}$).
Figure 2-5. Absorption and emission properties of the anthranilamide residues. (a) Wavelengths of the maxima of the most red-shifted bands of the absorption spectra of the eight residues for different solvents: propylene carbonate (PC), acetonitrile (MeCN), benzonitrile (PhCN), dichloromethane (DCM), chloroform (CHCl₃), and cyclohexane (CH). (b) Wavelengths of the maxima of the fluorescence spectra of the eight residues in different solvents ($\lambda_{ex} = 310$ nm). For each residue that, due to aggregation, exhibits two fluorescence bands, the wavelength of the blue-shifted maximum is reported. (c) Zero-to-zero energy values of the anthranilamide residues, extracted from wavelength where the normalized absorption and emission spectra cross (Figure 4).
Figure 2-6. HOMOs and LUMOs of Ant, Met, 4Pip and 5Pip for the gas phase (GP) and for acetonitrile (MeCN), obtained from DFT calculations. For the computational studies, the alkyl chains at the C- and N-termini were truncated to C₂H₅. The residues are displayed with their N-termini oriented to the left and the C-termini – to the right. (See the Supplementary Material for the HOMOs and LUMOs of all eight residues.)

Tables
Table 2-1. Electronic characteristics of the anthranilamide residues.\textsuperscript{a}

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<th>R\textsubscript{1}</th>
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<th>E\textsuperscript{c}/V vs. SCE</th>
<th>(E_{00})\textsuperscript{d}/eV</th>
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\textsuperscript{a}From experimental and theoretical studies of the eight residues where R\textsubscript{1} and R\textsubscript{2} correspond to the 4\textsuperscript{th} and 5\textsuperscript{th} positions, respectively, in the aromatic rings (Figure 1, 2). \textsuperscript{b}Dipole moments are obtained from DFT calculations for gas phase (vacuum) and for structures where the solvents, DCM and MeCN, are implemented as dielectric continua. The orientation of the molecular dipoles is from the N to the C-termini of the anthranilamide residues. \textsuperscript{c}Reduction potentials for the residue oxidation, i.e., Aa\textsuperscript{+} + e\textsuperscript{−} \rightarrow Aa, for neat solvents obtained from extrapolation of half-wave potentials to zero electrolyte concentration (Figure 3a). \textsuperscript{d}The zero to zero energy from the crossing point of the normalized absorption and fluorescence spectra (Figure 4).
Scheme 2-1. Syntheses of the 2-nitrobenzoic acid precursors for (a) 5Pip, (b) 4Pip, (c) 5Hxm, (d) 4Hxm, and (e) Hox.
Supplementary Material
(Chapter 2)
Building Blocks for Bioinspired Electrets: Molecular-Level Approach to Materials for Energy and Electronics (Supplementary Material)

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Experimental

Materials
5-fluoro-2-nitrobenzoic acid (1), 4-fluoro-2-nitrobenzoic acid (3), 5-hydroxy-2-nitrobenzoic acid (7), 4,5-dimethoxy-2-nitrobenzoic acid (16), 5-methyl-2-nitrobenzoic acid (18), and 2-nitrobenzoic acid (20), and hexanoic anhydride were purchased from TCI America. Tin (II) chloride dihydrate (SnCl₂·2H₂O), N,N’-Diisopropylcarbodiimide (DIC, 99%), N-hydroxysuccinimide (NHS, 98%), N,N-dimethylacetamide (anhydrous), cesium carbonate (Cs₂CO₃, 99.995%), N,N-diisopropylethylamine (DIPEA, 99.5%), 4-(dimethylamino)pyridine (DMAP, 99%), zinc (purum, powder), ammonium formate, triethylamine (Et₃N), tetrabutylammonium hexafluorophosphate, N-hexylmethylamine,
and n-hexylamine were purchased from Sigma-Aldrich. All other reagents (including HPLC grade, spectroscopic grade and anhydrous solvents) were purchased from Fisher Scientific. 2-nitro-5-(piperidin-N-yl)benzoic acid (2), N-hexyl-2-nitro-5-(piperidin-N-yl)benzamide (10), and 2-hexanamido-N-hexyl-5-(piperidin-N-yl)benzamide (5Pip) were prepared following protocols that we have previously described [S1].

General synthesis information

Proton ($^1$H) NMR spectra were recorded at 400 MHz at ambient temperature using degassed CDCl$_3$ as solvent. $^{13}$C NMR spectra were recorded at 100 MHz at ambient temperature with CDCl$_3$ as solvent. Chemical shifts are reported in parts per million relative to CDCl$_3$ ($^1$H, $\delta = 7.241; ^{13}$C, $\delta = 77.233$). Data for $^1$H NMR are reported as follows: chemical shift, integration, multiplicity ($s =$ singlet, $d =$ doublet, $t =$ triplet, $q =$ quartet, $p =$ pentaplet/quintet, $m =$ multiplet), integration and coupling constants. All $^{13}$C NMR spectra were recorded with complete proton decoupling. The microwave-mediated reactions were carried out in 5-ml microwave reaction vials at atmospheric pressure in a microwave reactor, Discover CEM (CEM Corporation, Matthews, NC, USA), at a constant temperature with a preset upper limit of the radiation power. High-resolution mass-spectra were obtained on a Q-TOF mass spectrometer. Analytical thin layer chromatography (TLC) was performed using 0.25 mm silica gel 60-F plates. Flash chromatography was performed using 60 Å, 32–63 μm silica gel. Yields refer to chromatographically pure materials, unless otherwise stated.

2-nitro-4-(piperidin-N-yl)benzoic acid (4) (Scheme 2S-3b)
4-fluoro-2-nitrobenzoic acid (3) (740 mg, 4 mmol) and piperidine (2 ml, 20 mmol) were mixed in a 50 ml flask equipped with a water-cooled condenser and immersed in a temperature-controlled oil bath. The mixture was refluxed at 105 °C for 10 h. After cooling to room temperature, the reaction solution was diluted with 100 ml DCM, and washed with 1M HCl (100 ml × 3) and with brine (100 ml × 3). The organic layer was collected, dried over Na₂SO₄, and concentrated in vacuo to produce yellow powder (976 mg, 3.9 mmol, 97%) of 2-nitro-4-(piperidin-1-yl)benzoic acid (4): ¹H-NMR (400 MHz, CDCl₃) δ/ppm: 7.84 (1 H, d, J = 9.2 Hz), 6.87 (2 H, m), 3.38 (4 H, m), 1.67 (6 H, s); ¹³C-NMR (400 MHz, CDCl₃) δ/ppm: 168.94, 154.18, 153.38, 133.58, 114.46, 107.88, 48.61, 25.35, 24.28; HRMS m/z calculated for C₁₂H₁₈N₃O₄⁺ (M + NH₄)⁺ 268.1292, found 268.1304 (M + NH₄)⁺.

5-(hexyl(methyl)amino)-2-nitrobenzoic acid (5) (Scheme 2S-3d)

5-fluro-2-nitrobenzoic acid (1) (185 mg, 1 mmol) and N-hexylmethylamine (384 µl, 2.5 mmol) were mixed and heated in a microwave reactor at 100 °C (power ≤ 100 W) for 1 hr. After cooling, the reaction mixture was diluted with 50 ml DCM and sequentially washed with 5% HCl, brine, and MilliQ. The organic phase was dried over anhydrous Na₂SO₄ and concentrated in vacuo. Purification using flash chromatography (stationary phase: silica gel; eluent gradient from 100% hexanes to 30% DCM in hexanes with 1% acetic acid added to all eluent solvents) to produce yellow powder (275 mg, 0.98 mmol, 98%) of 5-(hexyl(methyl)amino)-2-nitrobenzoic acid (5): ¹H-NMR (400 MHz, CDCl₃) δ/ppm: 11.08 (1 H, s), 8.00 (1 H, d, J = 9.4 Hz), 6.69 (1 H, d, J = 2.8), 6.61 (1 H, dd, J₁ =
9.4 Hz, $J_2 = 2.8$ Hz), 3.41 (2 H, t, $J = 7.6$ Hz), 3.07 (3 H, s), 1.60 (2 H, p, $J = 6.5$ Hz), 1.3 (6 H, m), 0.87 (3 H, t, $J = 6.9$ Hz); $^{13}$C-NMR (400 MHz, CDCl$_3$) $\delta$/ppm: 173.43, 152.78, 133.79, 131.64, 127.44, 111.43, 110.10, 52.99, 39.09, 31.75, 27.01, 26.80, 22.77, 14.18; HRMS m/z calculated for C$_{14}$H$_{21}$N$_2$O$_4$ (M + H)$^+$ 281.1496, found 281.1499 (M + H)$^+$.

4-(hexyl(methyl)amino)-2-nitrobenzoic acid (6) (Scheme 2S-3c)

Starting with 4-fluro-2-nitrobenzoic acid (3) (555 mg, 3 mmol) and N-hexylmethylamine (910 µl, 6 mmol) the procedure was similar to that for 5, but the microwave heating time was increased to 4 h. Purification following the procedure for 5 produced yellow powder (480 mg, 1.7 mmol, 57%) of 4-(hexyl(methyl)amino)-2-nitrobenzoic acid (6): $^1$H-NMR (400 MHz, CDCl$_3$) $\delta$/ppm: 7.84 (1 H, d, $J = 8.8$ Hz), 6.66 (1 H, dd, $J_1 = 8.9$ Hz, $J_2 = 2.6$ Hz), 6.64 (1 H, d, $J = 2.5$), 3.36 (2 H, t, $J = 7.6$ Hz), 3.02 (3 H, s), 1.57 (2 H, p, $J = 7.2$ Hz), 1.3 (6 H, m), 0.87 (3 H, t, $J = 6.9$ Hz); $^{13}$C-NMR (400 MHz, CDCl$_3$) $\delta$/ppm: 169.33, 153.57, 153.79, 133.58, 112.14, 107.59, 105.51, 52.86, 38.86, 31.76, 26.88, 26.80, 22.79, 14.19; HRMS m/z calculated for C$_{14}$H$_{20}$N$_2$NaO$_4$ (M + Na)$^+$ 303.1315, found 303.1324 (M + Na)$^+$.

Hexyl 5-(hexyloxy)-2-nitrobenzoate (8) (Scheme 2S-3e)

5-hydroxy-2-nitrobenzoic acid (7) (915 mg, 5 mmol), (3.26 g, 10 mmol) cesium carbonate and 1-iodohexane (1.62 ml, 11 mmol) were dissolved in 100 ml anhydrous N,N-dimethylacetamide and flushed with nitrogen. The reaction immediately turned yellow and the mixture was kept at 150 °C for 3 h. Upon cooling, the reaction mixture was suspended in DCM, washed with acidic and basic aqueous solutions, and dried over
anhydrous Na$_2$SO$_4$ to produce slightly yellow oil. Purification using flash chromatography (stationary phase: silica gel and a 10% sodium carbonate; eluent gradient: from 100% hexanes to 30% DCM in hexanes) afforded 934 mg (2.66 mmol, 97% yield) of hexyl 5-(hexyloxy)-2-nitrobenzoate (8): $^1$H-NMR (400 MHz, CDCl$_3$) δ/ ppm: 7.93 (1 H, d, $J$ = 9.0 Hz), 6.97 (1 H, d, $J$ = 2.6 Hz), 6.86 (1 H, dd, $J_1$ = 9.0 Hz, $J_2$ = 2.7 Hz), 4.27 (2 H, t, $J$ = 6.8 Hz), 3.99 (2 H, t, $J$ = 6.5 Hz), 1.75 (2 H, p, $J$ = 7.0 Hz), 1.66 (2 H, p, $J$ = 7.3 Hz), 1.40 (2 H, m), 1.3 (10 H, m), 0.83 (6 H, tt, $J$ = 7.0 Hz); $^{13}$C-NMR (400 MHz, CDCl$_3$) δ/ppm: 166.27, 163.10, 139.79, 131.63, 126.66, 115.84, 114.69, 69.28, 66.72, 31.51, 31.45, 28.91, 28.31, 25.60, 25.56, 22.61, 22.58, 14.03; HRMS m/z calculated for C$_{19}$H$_{30}$NO$_5$+ (M + H)$^+$ 352.2118, found 352.2118 (M + H)$^+$.

5-(hexyloxy)-2-nitrobenzoic acid (9) (Scheme 2S-3e)

8 (934 mg, 2.66 mmol) was dissolved in 2 ml ethanol and while stirring, 1 ml of 3 M KOH in ethanol was added drop-wise. The basified solution was heated to 60 °C. The progress of the reaction was monitored with TLC. After the complete consumption of the starting material, the reaction solution was allowed to cool to room temperature and quenched by slowly adding it to a mixture of DCM and 5% aqueous HCl. The organic phase was collected, washed with MilliQ water, dried over anhydrous Na$_2$SO$_4$, and concentrated in vacuo to produce white solid (640 g, 2.4 mmol, 92%) of 5-(hexyloxy)-2-nitrobenzoic acid (9): $^1$H-NMR (400 MHz, CDCl$_3$) δ/ppm: 8.01 (1 H, d, $J$ = 9.1 Hz), 7.13 (1 H, d, $J$ = 2.7 Hz), 7.02 (1 H, dd, $J_1$ = 9.1 Hz, $J_2$ = 2.7 Hz), 4.05 (2 H, t, $J$ = 6.5 Hz), 1.81 (2 H, p, $J$ = 7.1 Hz), 1.45 (2 H, p, $J$ = 7.1 Hz), 1.32 (4 H, m), 0.89 (3 H, t, $J$ = 6.9 Hz).
Hz); $^{13}$C-NMR (400 MHz, CDCl$_3$) $\delta$/ppm: 170.89, 163.12, 140.09, 130.38, 126.81, 116.74, 114.92, 69.49, 31.60, 29.00, 25.69, 22.71, 14.15; HRMS m/z calculated for C$_{13}$H$_{18}$NO$_5$ $^+/(M + H)^+$ 268.1179, found 268.1192 ($M + H)^+$.

N-hexyl-2-nitro-4-(piperidin-N-yl)benzamide (11) (Scheme 2S-1a)

DIC (467 µl, mmol) was added to 10 ml ice-chilled DMF solution of 4 (250 mg, 1 mmol) and NHS (230 mg, 2 mmol). After stirring the mixture for 2 h at 0 °C, n-hexylamine (400 µl, 3 mmol) was added drop-wise. The solution was stirred at 0 °C for additional 0.5 h, allowed to warm up to room temperature, and stirred for an additional hour. The thus obtained viscous reaction mixture was diluted with DCM, washed with 5% HCl and MilliQ water, and dried over anhydrous N$_2$SO$_4$. The DCM was removed under reduced pressure to produce yellow solid. Purification using flash chromatography (stationary phase: silica gel; eluent gradient: from 100 % hexanes to 50 % ethyl acetate in hexanes) afforded 250 mg yellow powder (0.75 mmol, 75%) of N-hexyl-2-nitro-4-(piperidin-N-yl)benzamide (11): $^1$H-NMR (400 MHz, CDCl$_3$) $\delta$/ppm: 7.17 (2 H, m), 6.85 (1 H, dd, $J_1$ = 8.6 Hz, $J_2$ = 2.6 Hz), 5.46 (1 H, t, $J = 5.5$ Hz), 3.20 (6 H, m), 1.59 (6 H, m), 1.46 (2 H, p, $J = 6.6$ Hz), 1.22 (6 H, m), 0.81 (3 H, t, $J = 6.9$ Hz); $^{13}$C-NMR (400 MHz, CDCl$_3$) $\delta$/ppm: 166.62, 152.28, 148.61, 129.51, 120.85, 117.79, 109.56, 48.96, 40.14, 31.54, 29.26, 26.62, 25.22, 24.08, 22.59, 14.06; HRMS m/z calculated for C$_{18}$H$_{28}$N$_3$O$_3$ $^+/(M + H)^+$ 334.2125, found 334.2139 ($M + H)^+$.

N-hexyl-5-(hexyl(methyl)amino)-2-nitrobenzamide (12) (Scheme 2S-1a)
Applying the procedure for 11 to a crude 5 (210 mg, 0.75 mmol) plus an additional hexane wash of the final solid product afforded 242 mg of yellow solid (0.66 mmol, 89%) of 12: ^1^H-NMR (400 MHz, CDCl₃) δ/ppm: 8.00 (1 H, d, J = 9.4 Hz), 6.54 (1 H, dd, J₁ = 9.4 Hz, J₂ = 2.9 Hz), 6.47 (1 H, d, J = 2.9 Hz), 5.73 (1 H, t, J = 5.6 Hz), 3.38 (4 H, m), 3.03 (3 H, s), 1.58 (4 H, m), 1.3 (12 H, m), 0.86 (6 H, tt); ^1^C-NMR (400 MHz, CDCl₃) δ/ppm: 166.42, 152.94, 136.55, 133.27, 127.69, 110.76, 110.11, 52.87, 40.47, 38.97, 31.75, 31.67, 29.34, 27.00, 26.82, 26.79, 22.76, 14.22, 14.18; HRMS m/z calculated for C₂₀H₃₃N₃NaO₃⁺ (M + Na)⁺ 386.2414, found 386.2421 (M + Na)⁺.

N-hexyl-4-(hexyl(methyl)amino)-2-nitrobenzamide (13) (Scheme 2S-1a)

Applying the procedure for 11 to a crude 6 (210 mg, 0.75 mmol), using EDC instead of DIC, and adding a washing step of the final solid product with hexanes afforded 94 mg yellow powder (0.26 mmol, 13%) of 13: ^1^H-NMR (400 MHz, CDCl₃) δ/ppm: 7.27 (1 H, d, J = 8.7 Hz), 7.01 (1 H, d, J = 2.6 Hz), 6.70 (1 H, dd, J₁ = 8.7 Hz, J₂ = 2.6 Hz), 5.95 (1 H, t, J = 5.4 Hz), 3.32 (4 H, m), 2.95 (3 H, s), 1.53 (4 H, m), 1.27 (12 H, m), 0.85 (6 H, tt); ^1^C-NMR (400 MHz, CDCl₃) δ/ppm: 166.84, 150.35, 149.19, 129.72, 118.66, 114.53, 106.45, 52.64, 40.33, 38.63, 31.75, 31.65, 29.50, 26.78, 26.66, 22.73, 14.22, 14.18; HRMS m/z calculated for C₂₀H₃₄N₃O₃⁺ (M + H)⁺ 364.2595, found 364.2613 (M + H)⁺.

N-hexyl-5-hexyloxy-2-nitrobenzamide (14) (Scheme 2S-1a)

Applying the procedure for 11 to 9 (534 mg, 2 mmol) afforded 423 mg of white solid (1.2 mmol, 60%) of 14: ^1^H-NMR (400 MHz, CDCl₃) δ/ppm: 7.98 (1 H, d, J = 9.1 Hz), 6.86 (1 H, dd, J₁ = 9.1 Hz, J₂ = 2.7 Hz), 6.80 (1 H, d, J = 2.7 Hz), 6.09 (1 H, t, J = 5.6 Hz), 3.99
(2 H, t, J = 6.5 Hz), 3.32 (2 H, q, J = 6.8 Hz), 1.76 (2 H, p, J = 7.0 Hz), 1.55 (2 H, p, J = 7.3 Hz), 1.41 (2 H, m), 1.3 (10 H, m), 0.86 (6 H, tt); $^{13}$C-NMR (400 MHz, CDCl$_3$) δ/ppm: 166.9, 163.5, 138.6, 135.9, 127.17, 115.22, 114.32, 69.3, 40.42, 31.62, 31.59, 29.26, 29.01, 26.75, 25.68, 22.70, 14.17, 14.15; HRMS m/z calculated for C$_{19}$H$_{31}$N$_2$O$_4$+ (M + H)$^+$ 351.2278, found 351.2281 (M + H)$^+$. 

N-hexyl-4,5-dimethoxy-2-nitrobenzamide (16) (Scheme 2S-1a)

A mixture of 4,5-dimethoxy-2-nitrobenzoic acid (15) (454 mg, 2 mmol) and 2 ml thionyl chloride was refluxed at 70 °C for 2 hours. The thionyl chloride was evaporated out and a solution of 490 mg DMAP, 10 ml n-hexylamine and 15 ml of 1,2-dimethoxyethane was added and refluxed at 85 °C for 3 hours. The reaction was slowly poured in 200 mL of 5% HCl and allowed stay overnight. The formed precipitate was collected by vacuum filtration to afford 492 mg of white solid (1.6 mmol, 80%) of 16: $^1$H-NMR (400 MHz, CDCl$_3$) δ/ppm: 7.57 (1 H, s), 6.85 (1 H, s), 6.76 (1 H, t, J = 5.3 Hz), 3.95 (3 H, s), 3.94 (3 H, q, J = 7 Hz), 2.33 (2 H, t, J = 7.6 Hz), 1.60 (2 H, p, J = 7.3 Hz), 1.55 (2 H, p, J = 7.3 Hz), 1.3 (6 H, m), 0.87 (3 H, t, J = 6.9 Hz); $^{13}$C-NMR (400 MHz, CDCl$_3$) δ/ppm: 166.92, 153.68, 149.49, 138.76, 127.9, 110.45, 107.39, 56.85, 56.73, 40.65, 31.67, 29.34, 26.82, 22.76, 14.23; HRMS m/z calculated for C$_{15}$H$_{23}$N$_2$O$_5$+ (M + H)$^+$ 311.1601, found 311.1611 (M + H)$^+$. 

N-hexyl-5-methyl-2-nitrobenzamide (18) (Scheme 2S-1a)

Applying the procedure for 11 to 5-methyl-2-nitrobenzoic acid (17) (181 mg, 1 mmol), and using EDC (430 mg, 2.24 mmol) and DIC (470 µl, 3 mmol) instead of only DIC,
afforded 56 mg of white solid (0.21 mmol, 21%) of 18: $^1$H-NMR (400 MHz, CDCl$_3$) δ/ppm: 7.89 (1 H, d, $J = 8.4$ Hz), 7.27 (1 H, dd, $J_1 = 8.4$ Hz, $J_2 = 1.5$ Hz), 7.21 (1 H, d, $J = 1.5$ Hz), 6.02 (1 H, s), 3.35 (2 H, q, $J = 7.1$ Hz), 2.40 (3 H, s), 1.56 (2 H, p, $J = 7.2$ Hz), 1.3 (6 H, m), 0.86 (3 H, t, $J = 6.8$ Hz); $^{13}$C-NMR (400 MHz, CDCl$_3$) δ/ppm: 166.96, 145.45, 144.08, 133.52, 130.73, 129.46, 124.72, 40.44, 31.64, 29.36, 26.76, 22.73, 21.54, 14.19; HRMS m/z calculated for C$_{14}$H$_{21}$N$_2$O$_3$+ (M + H)$^+$ 265.1547, found 265.1556 (M + H)$^+$.

N-hexyl-2-nitrobenzamide (20) (Scheme 2S-1a)

Applying the procedure for 11 to 2-nitrobenzoic acid (19) (501 mg, 3 mmol) afforded 548 g of white solid (2.19 mmol, 73%) of 20: $^1$H-NMR (400 MHz, CDCl$_3$) δ/ppm: 7.90 (1 H, dd, $J_1 = 8.1$ Hz, $J_2 = 1.1$ Hz), 7.55 (1 H, td, $J_1 = 7.5$ Hz, $J_2 = 1.2$ Hz), 7.46 (1 H, td, $J_1 = 7.8$ Hz, $J_2 = 1.4$ Hz), 7.37 (1 H, dd, $J_1 = 7.5$ Hz, $J_2 = 1.4$ Hz), 6.40 (1 H, t, $J = 6.0$ Hz), 3.28 (2 H, td, $J_1 = 7.1$ Hz, $J_2 = 6.1$ Hz), 1.51 (2 H, p, $J = 7.2$ Hz), 1.26 (6 H, m), 0.84 (3 H, t, $J = 6.9$ Hz); $^{13}$C-NMR (400 MHz, CDCl$_3$) δ/ppm: 166.61, 146.49, 133.69, 133.24, 130.30, 128.84, 124.44, 40.36, 31.58, 29.22, 26.67, 22.67, 14.14; HRMS m/z calculated for C$_{13}$H$_{19}$N$_2$O$_3$+ (M + H)$^+$ 251.1396, found 251.1393 (M + H)$^+$.

2-hexanamido-N-hexyl-4-(piperidin-N-yl)benzamide or hexyl N-hexanoyl-4-(piperidin-N-yl)anthranilamide (4Pip) (Scheme 2S-1b,c)

In a 50-ml flask equipped with a water-cooled condenser, 11 (400 mg, 1.2 mmol) and SnCl$_2$·2H$_2$O (4-to-5 fold molar excess) were suspended in 3 ml EtOH and purged with nitrogen [S2]. The mixture was refluxed at 80 °C and the progress of the reaction was
monitored with TLC. After complete reduction of the nitro group (usually about 3 h), the ethanol was removed under reduced pressure and 3 ml DMF was added to the reaction solid under nitrogen. Upon suspending the solid, 0.4 ml hexanoyl anhydride was added drop-wise. After stirring for 15 min, 0.25 ml Et3N was added drop-wise and the mixture was allowed to stir for 4 h. The reaction mixture was dissolved in 50 ml DCM, washed with a saturated aqueous solution of Na2CO3 and dried over anhydrous Na2SO4. Purification using flash chromatography (stationary phase: silica gel; eluent gradient: from 100 % hexanes to 50 % ethyl acetate in hexanes) to afford 103 mg of a white solid (0.26 mmol, 21%) of 4Pip: 1H-NMR (400 MHz, CDCl3) δ/ppm: 11.73 (1 H, s), 8.28 (1 H, d, J = 2.6 Hz), 7.29 (1 H, d, J = 9.0 Hz), 6.39 (2 H, m), 3.31 (2 H, q, J = 6.7 Hz), 3.22 (4 H, m), 2.33 (2 H, t, J = 7.6 Hz), 1.66 (2 H, p, J = 7.5 Hz), 1.55 (8 H, m), 1.27 (10 H, m), 0.83 (6 H, tt, J1 = 7.0, J2 = 6.4 Hz); 13C-NMR (400 MHz, CDCl3) δ/ppm: 172.58, 169.20, 154.20, 142.05, 127.82, 108.41, 108.30, 105.81, 48.78, 39.94, 38.79, 31.62, 31.46, 29.72, 26.81, 25.50, 25.30, 24.46, 22.67, 22.53, 14.11, 14.03; HRMS m/z calculated for C24H39N3O2+(M)+ 401.3037, found 401.3021 (M)+.

2-hexanamido-N-hexyl-5-(hexyl(methyl)amino)benzamide or hexyl N-hexanoyl-5-(hexyl(methyl)amino)anthranilamide (5Hxm) (Scheme 2S-1b,c)

Applying the procedure for 4Pip to crude 12 (203 mg; 0.550 mmol), and using 1,2-dimethoxy ethane instead of EtOH for the SnCl2 reduction step, afforded 69 mg white solid (0.159 mmol, 29%) of 5Hxm: 1H-NMR (400 MHz, CDCl3) δ/ppm: 10.16 (1 H, s), 8.23 (1 H, d, J = 9.0 Hz), 6.79 (1 H, dd, J1 = 9.0 Hz, J2 = 2.7 Hz), 6.67 (1 H, d, J = 2.7 Hz).
Hz), 6.30 (1 H, s), 3.38 (2 H, q, \( J = 6.7 \) Hz), 3.24 (2 H, t, \( J = 7.5 \) Hz), 2.87 (3 H, s), 2.32 (2 H, t, \( J = 6.9 \) Hz), 1.67 (2 H, p, \( J = 7.1 \) Hz), 1.60 (2 H, p, \( J = 7.4 \) Hz), 1.51 (2 H, p, \( J = 6.5 \) Hz), 1.3 (16 H, m), 0.87 (9 H, m); \(^{13}\)C-NMR (400 MHz, CDCl\(_3\)) \( \delta /ppm: 172.02, 169.74, 145.40, 128.57, 123.83, 123.75, 116.54, 110.21, 53.48, 40.23, 38.39, 31.89, 31.68, 31.62, 29.67, 27.00, 26.86, 26.60, 25.66, 22.84, 22.78, 22.62, 14.21, 14.16, 14.09; HRMS m/z calculated for C\(_{26}\)H\(_{44}\)N\(_3\)O\(_2\) (M – H)\(^+\) 430.3428, found 430.3429 (M – H)\(^+\).

2-hexanamido-N-hexyl-4-(hexyl(methyl)amino)benzamide or hexyl N-hexanoyl-4-(hexyl(methyl)amino)anthranilamide (4Hxm) (Scheme 2S-1b,c)

Applying the procedure for 5Hxm to 13 (60 mg; 0.164 mmol) produced 20 mg of 4Hxm (0.046 mmol; 28%): \(^1\)H-NMR (400 MHz, CDCl\(_3\)) \( \delta /ppm: 11.76 (1 H, s), 8.14 (1 H, d, \( J = 1.7 \) Hz), 7.26 (1 H, d, \( J = 8.9 \) Hz), 6.27 (1 H, dd, \( J_1 = 9.0 \) Hz, \( J_2 = 2.0 \) Hz), 6.02 (1 H, s), 3.34 (4 H, m), 2.97 (3 H, s), 2.38 (2 H, t, \( J = 7.7 \) Hz), 1.71 (2 H, p, \( J = 7.6 \) Hz), 1.57 (4 H, p, \( J = 7.1 \) Hz), 1.3 (16 H, m), 0.85 (9 H, m); \(^{13}\)C-NMR (400 MHz, CDCl\(_3\)) \( \delta /ppm: 172.75, 169.44, 152.27, 142.36, 127.84, 105.56, 103.15, 52.63, 40.01, 39.01, 31.89, 31.74, 31.62, 29.92, 29.87, 27.09, 26.94, 26.92, 25.48, 22.84, 22.80, 22.65, 14.24, 14.17; HRMS m/z calculated for C\(_{26}\)H\(_{46}\)N\(_3\)O\(_2\) (M + H)\(^+\) 432.3585, found 432.3626 (M + H)\(^+\).

2-hexanamido-N-hexyl-5-hexyloxybenzamide or hexyl N-hexanoyl-5-hexyloxyanthranilamide (Hox) (Scheme 2S-1b,c)

Under nitrogen, 14 (424 mg, 1.2 mmol), ammonium formate (760 mg, 12 mmol) and zinc dust (434 mg, 6.7 mmol) were suspended in 2 ml 1,2-dimethoxyethane and stirred for 6 hours [S3]. The reaction mixture was filtered and the filtrate was diluted with DCM,
washed 1% HCl and MilliQ water. The DCM was removed \textit{in vacuo} and 2 mL of DMF was added under nitrogen. Sequentially, hexanoic anhydride (0.23 ml, 1 mmol) and Et$_3$N (0.14 ml, 1 mmol) were added drop-wise and the mixture was stirred at room temperature for 2 hours. The reaction mixture was diluted with 50 ml ethylacetate, washed with 5% HCl and MilliQ water, and dried over Na$_2$SO$_4$, and concentrated \textit{in vacuo}. Purification using flash chromatography (stationary phase: silica gel; eluent gradient: from 100 % hexanes to 50 % ethyl acetate in hexanes) afforded 205 mg white solid (0.49 mmol, 40%) of Hox: $^1$H-NMR (400 MHz, CDCl$_3$) $\delta$/ppm: 10.56 (1 H, s), 8.28 (1 H, d, $J = 9.1$ Hz), 6.91 (1 H, d, $J = 2.8$ Hz), 6.86 (1 H, dd, $J_1 = 9.1$ Hz, $J_2 = 2.8$ Hz), 6.79 (1 H, t, $J = 5.2$ Hz), 3.84 (2 H, t, $J = 6.6$ Hz), 3.32 (2 H, q, $J = 6.7$ Hz), 2.28 (2 H, t, $J = 7.7$ Hz), 1.65 (4 H, m), 1.55 (2 H, p, $J = 7.3$ Hz), 1.3 (16 H, m), 0.84 (9 H, t, $J = 6.5$ Hz); $^{13}$C-NMR (400 MHz, CDCl$_3$) $\delta$/ppm: 172.15, 168.88, 154.44, 132.19, 123.13, 122.86, 117.11, 113.52, 68.57, 40.21, 38.35, 31.70, 31.63, 31.53, 29.53, 29.35, 26.83, 25.80, 25.48, 22.70, 22.52, 14.13, 14.05; HRMS m/z calculated for C$_{25}$H$_{42}$N$_2$NaO$_3$\textsuperscript{+} (M + Na)$^+$ 441.3088, found 441.3109 (M + Na)$^+$.

2-hexanamido-N-hexyl-4,5-dimethoxybenzamide or hexyl N-hexanoyl-4,5-dimethoxyanthranilamide (Dmx) (Scheme 2S-1b,c)

Applying the procedure for 5Hxm to 16 (156 mg, 0.5 mmol) produced 20 mg of Dmx (0.053 mmol; 11%): $^1$H-NMR (400 MHz, CDCl$_3$) $\delta$/ppm: 11.32 (1 H, s), 8.35 (1 H, s), 6.88 (1 H, s), 6.40 (1 H, t, $J = 5.3$ Hz), 3.85 (3 H, s), 3.82 (3 H, s), 3.34 (2 H, q, $J = 6.8$ Hz), 2.33 (2 H, t, $J = 7.6$ Hz), 1.67 (2 H, p, $J = 7.5$ Hz), 1.55 (2 H, p, $J = 7.4$ Hz), 1.3 (10
H, m), 0.85 (3 H, t, J = 6.5 Hz), 0.84 (3 H, t, J = 6.7 Hz); $^{13}$C-NMR (400 MHz, CDCl$_3$) δ/ppm: 172.41, 168.91, 152.36, 144.00, 135.71, 111.70, 109.76, 104.84, 56.64, 56.11, 40.23, 38.64, 31.63, 31.51, 29.70, 26.84, 25.36, 22.70, 22.55, 14.14, 14.07; HRMS m/z calculated for C$_{21}$H$_{34}$N$_2$NaO$_4$+ (M + Na)$^+$ 401.2411, found 401.2426 (M + Na)$^+$.

2-hexanamido-N-hexylbenzamide or hexyl N-hexanoylanthranilamide (Ant) (Scheme 2S-1b,c)

Applying the procedure for 5Hxm to 20 (250 mg, 1 mmol) produced 28 mg of Ant (0.09 mmol, 9% yield): $^1$H-NMR (400 MHz, CDCl$_3$) δ/ppm: 11.00 (1 H, s), 8.56 (1 H, d, J = 8.8 Hz), 7.42 (2 H, m), 7.01 (1 H, s), 6.31 (1 H, s), 3.40 (2 H, q, J = 6.7 Hz), 2.36 (2 H, t, J = 7.6 Hz), 1.70 (2 H, p, J = 7.5 Hz), 1.60 (2 H, p, J = 7.5 Hz), 1.3 (10 H, m), 0.88 (6 H, t, J = 7.0 Hz); $^{13}$C-NMR (400 MHz, CDCl$_3$) δ/ppm: 172.46, 169.21, 139.75, 132.58, 126.51, 122.72, 121.72, 120.83, 40.27, 38.69, 31.68, 31.59, 29.86, 26.87, 25.48, 22.78, 22.61, 14.22, 14.15; HRMS m/z calculated for C$_{19}$H$_{30}$N$_2$NaO$_2$+ (M + Na)$^+$ 341.2199, found 341.2196 (M + Na)$^+$.

2-hexanamido-N-hexyl-5-methylbenzamide or hexyl N-hexanoyl-5-methylanthranilamide (Met) (Scheme 2S-1b,c)

Applying the procedure for 5Hxm to 18 (264 mg, 1 mmol) produced 86 mg of the final Met (0.26 mmol; 25%): $^1$H-NMR (400 MHz, CDCl$_3$) δ/ppm: 10.85 (1 H, s), 8.44 (1 H, dd, $J_1$ = 8.5 Hz, $J_2$ = 2.8 Hz), 7.23 (1 H, d, J = 8.6 Hz), 7.18 (1 H, d, J = 1.9 Hz), 6.24 (1 H, s), 3.39 (2 H, q, J = 7.2 Hz), 2.35 (2 H, t, J = 7.6 Hz), 2.29 (3 H, s), 1.70 (2 H, p, J = 7.5 Hz), 1.60 (2 H, p, J = 7.4 Hz), 1.3 (10 H, m), 0.88 (3 H, t, J = 6.5 Hz), 0.87 (3 H, t, J
$^{13}$C-NMR (400 MHz, CDCl$_3$) $\delta$/ppm: 172.44, 169.29, 137.03, 133.07, 132.35, 126.95, 121.80, 121.09, 40.25, 38.56, 34.28, 31.67, 31.56, 29.64, 26.86, 25.50, 22.74, 22.57, 14.19, 14.11; HRMS m/z calculated for C$_{20}$H$_{32}$N$_2$NaO$_2^+$ (M + Na)$^+$ 355.2356, found 355.2371 (M + Na)$^+$.

**Methods**

**UV/visible absorption and emission spectroscopy**

Steady-state absorption spectra were recorded in a transmission mode using a JASCO V-670 spectrophotometer (Tokyo, Japan); and steady-state emission spectra were measured, also in a transmission mode, with a FluoroLog-3 spectrofluorometer (Horiba-Jobin-Yvon, Edison, NJ, USA) as previously reported [S4, S5].

**Electrochemical measurements**

Cyclic voltammetry was conducted using Reference 600$^{\text{TM}}$ Potentiostat/Galvanostat/ZRA (Gamry Instruments, PA, U.S.A.), equipped with a three-electrode cell, as previously described [S6]. The half-wave potentials, $E^{(1/2)}$, were determined from the midpoints between the cathodic and anodic peak potentials for reversible oxidation; and from the inflection points of the anodic waves for irreversible oxidation (Figure S1). Specifically, the anodic and cathodic peak potentials, $E_a$ and $E_c$, respectively, were determined from the zero points of the first derivatives of the voltammograms, i.e., the potentials where $\frac{\partial I}{\partial E} = 0$ at $\frac{\partial E}{\partial t} = \text{constant}$ (Figure 1S). For reversible oxidation $E^{(1/2)} = \frac{(E_a + E_c)}{2}$. For irreversible oxidation, $E^{(1/2)}$ was estimated from the inflection point of the rise of the anodic wave, i.e., from the zero point of the second derivative, $\frac{\partial^2 I}{\partial E^2} = 0$ at $\frac{\partial E}{\partial t} =$
constant (Figure 1S). The second derivatives of reversible voltammograms show that the inflection-point potentials are quite close to the mid-points between $E_a$ and $E_c$, ensuring the reliability for the estimates of $E^{(1/2)}$ from the inflection points of irreversible voltammograms. The voltammograms showing reversible oxidation were recorded at a scan rate of 200 mV/s. When the oxidation was irreversible, the voltammograms were recorded at scan rate between 20 and 50 mV/s. From the dependence of $E^{(1/2)}$ on the electrolyte concentration, the potentials for neat solvents were estimated from extrapolations to zero (Figure 1a) [S6, S7].

Computational methods

Geometries were optimized using Gaussian 09 both in the gas phase and with implicit polarizable continuum solvent models for dichloromethane (DCM) and acetonitrile (MeCN). All optimizations were performed using B3LYP with a 6-311+G(d,p) basis and a pruned (99,590) “ultrafine” integration grid. The inclusion of a solvent has minimal impact on the final optimized geometries, as shown in Figure S2, which superimposes the gas phase optimized geometries with the structures optimized in DCM and MeCN for each of the Aa residues. Likewise, the inclusion of solvent effects has no qualitative impact on the HOMO charge distribution, as shown in Figures S3 and S4. We therefore only report results for the gas phase and MeCN calculations for the HOMO/LUMO figures.

Figures 4, S3 and S4 illustrate the charge distribution in the HOMO and LUMO for each of the residues in both the gas phase and in MeCN. These figures clearly indicate a
pronounced shift in the electron density of the HOMO for both the 4Hxm and 4Pip species relative to the other anthranilamides. Table 1 provides the magnitude of the dipole moments for each residue computed from the optimized structures for the gas phase, DCM and DCM.
Supplementary Figures

Figure 2S-1. Cyclic voltamograms and their 1st and 2nd derivatives used for extracting half-wave reduction potentials, $E^{(1/2)}$, of the residues exhibiting reversible (e.g., 5Hxm) and irreversible (e.g., Hox) electrochemical oxidation (100 mM NBu$_4$PF$_6$ in DCM).
Figure 2S-2. Overlays of relaxed ground-state structures of the eight Aα residues in the gas phase, in DCM, and in MeCN, obtained from DFT calculations. For each residue, the three structures in the different media show a good overlap, suggesting for negligible solvent effect on the anthranilamide conformations. For online viewing, red is for the structures in the gas phase, green is for the structures in DCM, and blue s for the structures in MeCN. For the computational studies, the alkyl chains at the C- and N-termini were truncated to C2Hs.
Figure 2S-3. HOMOs and LUMOs of Ant, Met, Hox and Dmx for the gas phase (GP) and for acetonitrile (MeCN), obtained from DFT calculations. For the computational studies, the alkyl chains at the C- and N-termini were truncated to C\textsubscript{2}H\textsubscript{5}. The residues are displayed with their N-termini oriented to the left and the C-termini – to the right.
Figure 2S–4. HOMOs and LUMOs of the amine-reprivatized residues for the gas phase (GP) and for acetonitrile (MeCN), obtained from DFT calculations. For the computational studies, the alkyl chains at the C- and N-termini were truncated to C$_2$H$_5$. The residues are displayed with their N-termini oriented to the left and the C-termini – to the right.
Supplementary Schemes

**Scheme 2S-1.** Synthesis of the anthranilamides from the corresponding 2-nitrobenzoic acids.

(a) DIC, HNS, C₆H₄NH₂, DMF, r.t.; (b) SnCl₂, H₂CO(CH₂)₂OCH₃, reflux; or Zn, NH₄(HCO₂), MeOH, r.t. (the produced amines were detected with TLC and HRMS, but not isolated); (c) (C₅H₁₀CO)₃O, Et₃N, DMF, r.t.
Supplemental References


Chapter 3

What Makes Oxidized N-Acylanthranilamides Stable?
ABSTRACT

Oligoamides composed of anthranilic acid derivatives present a promising choice for mediating long-range charge transfer and controlling its directionality. Hole hopping, modulated by the anthranilamide (Aa) permanent dipoles, provides a plausible means for such rectified long-range charge transduction. All aliphatic and most aromatic amides, however, decompose upon oxidation, rendering them unacceptable for hole-hopping pathways. We, therefore, employ electrochemical and computational analysis to examine how to suppress oxidative degradation and stabilize the radical cations of N-acylated Aa derivatives. Our findings reveal two requirements for attaining long-lived radical cations of these aromatic amides: (1) keeping the reduction potentials for oxidizing the Aa residues under about 1.4 V vs. SCE; and (2) adding an electron-donating group para to the N-terminal amide of the aromatic ring, which prevents the electron spin density of the radical cation from extending over the C-terminal amide. These findings provide essential information for the design of hole-transfer amides.
Introduction
As the electrostatic analogues of magnets, dipole-polarization electrets contain polar groups with their electric dipole moments arranged in a co-directional manner.\textsuperscript{1-6} The electric fields originating from such ordered dipoles can pronouncedly affect charge-transfer processes.\textsuperscript{7-9} However, because mobile charge carriers can readily redistribute and screen the fields from permanent dipoles, all electrets are dielectrics, unable to efficiently mediate long-range electron transduction.

Protein helices are some of the best known macromolecular electrets with permanent dipoles of about 2 to 5 D per residue originating from ordered amide and hydrogen bonds.\textsuperscript{10-13} As a result, the electric fields in the vicinity of these macromolecular structures can amount to MV/m and GV/m, capable of rectifying electron transfer\textsuperscript{14-16} and facilitating ion transport.\textsuperscript{17,18} Along their backbones, however, proteins mediate electron transfer via tunneling, which cannot be efficient at distances exceeding about 2 nm.\textsuperscript{19-24} Conversely, multiple short tunneling steps along arrays of cofactors can provide a means for efficient long-range electron transfer (i.e., via electron hopping), as observed for photosynthesis and respiration protein assemblies.\textsuperscript{25-28} Similarly, DNA and PNA strands, comprising arrays of nucleotides with relatively low reduction potentials, provide pathways for efficient long-range hole hopping at distances considerably exceeding 2 nm.\textsuperscript{29,30}
Combining favorable electret and charge-transfer features of biological and biomimetic systems, oligomers of anthranilic acid derivatives possess large permanent dipole moments and have the potential to mediate charge hopping.2-4,9,31 These bioinspired molecular electrets are polypeptides composed of non-native aromatic β-amino acids with dipoles originating from ordered amide and hydrogen bonds (Chart 3-1a).3,4 The aromatic residues, directly linked with amide bonds, provide π-conjugation extending over the anthranilamide (Aa) backbones, which can serve as pathways for efficient long-range electron or hole transfer. Even a single N-acylated Aa residue pronouncedly rectifies charge transfer: accelerating photoinduced charge separation, while impeding charge recombination.9 These properties make the anthranilic molecular electrets promising candidates for electronics and energy applications.32-36

A key feature of the anthranilic acid residues is their two side chains, R1 and R2 (Chart 3-1a), which provide an important means for tuning their electronic properties. In analogy with the structural and functional diversity of proteins attained by the side chains of the native amino acids combined in different sequences, the two side chains of the anthranilic residues are a key means for pursuing a wide variety of electronic functionalities for these bioinspired molecular electrets.

Focusing on Aa residues for hole transfer, we select three types electron-donating substituents as side chains: alkyl, alkyloxy and amine groups (Chart 3-1b). In order to be feasible for mediating hole hopping, Aa residues should be able to sustain positive charges without undergoing oxidative degradation. We use the reversibility of
electrochemical oxidation, estimated from cyclic voltammetry (CV), to examine if the radical cations of the different Aa residues are sufficiently stable. The CV results for the Aa residues reveal a cutoff reduction potential, $E_{co}$, that varies slightly for different solvent media: i.e., all the tested amides undergoing oxidation at potentials more positive than $E_{co}$ manifest irreversibility. Conversely, not all Aa residues with reduction potentials more negative than $E_{co}$ undergo reversible oxidation. Spin-density distribution of the radical cations, obtained from DFT calculations, correlates the extension of the positive charge over the C-terminal amide with the irreversibility of the oxidation behavior, which can prove to be an important predictive tool for the design of aromatic amides for hole-transfer organic materials.

**Results**

Varying the electron-donating substituents allows us to adjust the reduction potentials of the oxidation of the Aa residues over a range of about 1 V (Table 3-1). As expected, an increase in the solvent polarity causes a negative shift in the reduction potentials, i.e., making the oxidation more favorable (Table 3-1).\(^{37-43}\) Furthermore, it is important not only what the substituent is, but also what its position is in the aromatic ring. Comparing 4Hxm and 5Hxm, for example, reveals that moving the amine from position 4 to position 5 (i.e., from $R_1$ to $R_2$) lowers the reduction potential with more than 200 mV (Table 3-1). The other two amine-derivatized residues, 4Pip and 5Pip, exhibit the same trend.

While the wide tunability of the electronic properties of the Aa residue is their most attractive property, most of them exhibit irreversible electrochemical oxidation,
suggesting for the formation of radical cations with relatively short lifetimes even when
in aprotic solvents (Figure 1a). Indeed, all aliphatic and many aromatic amides exhibit
such chemical irreversibility due to electrochemical oxidative degradation.\textsuperscript{44,45} As
expected, increasing the electron-donating strength of the substituents causes a
substantial negative shift in the Aa reduction potentials (Figure 3-1, Table 3-1). As this
negative shift becomes significant enough, the Aa voltammograms start to exhibit
reversibility (Figure 3-1a,b). While the residue with a single alkyloxy group (Hox)
exhibits irreversible oxidation, an addition of a second alkyloxy (as in Dmx) results in
100-mV negative shift and reversible voltammograms (Figure 3-1a). Similarly, replacing
the alkyloxy in Hxm with an amine (as in 5Hxm and 5Pip) results not only in half-a-volt
negative shift in the reduction potential, but also in reversible oxidation behavior (Figure
3-1a,b).

These findings suggest for a cutoff potential, $E_{co}$, of the chemical reversibility of Aa
electrochemical oxidation that is lower than the reduction potential of Hox and higher
than that of Dmx. If the formation of the radical cations requires potentials more positive
than $E_{co}$, the conditions are oxidative enough to cause degradation, most likely a cleavage
of the amide bonds, which is a common outcome from electrochemical oxidation of such
aromatic conjugates.\textsuperscript{45} Therefore, to attain chemical reversibility, and sufficient stability
of radical cations, such as of Dmx, 5Pip and 5Hxm, the oxidation should be at reduction
potentials more negative than $E_{co}$.
Strictly speaking, Dmx, 5Pip and 5Hxm exhibit quasi-reversible oxidation. While the peaks of the anodic and cathodic currents on their voltammograms are practically the same, the separation between the anodic and the cathodic peak potentials ranges between 70 and 100 mV when recorded at 0.1 V s\(^{-1}\). This separation between the peak potentials increases with increasing the scan rate. This observation indicates that the interfacial charge-transfer rates are slower than the mass transport rates of Aa and Aa\(^+\) toward and away from the electrode surface. That is the oxidation is electrochemically irreversible and chemically reversible. Therefore, we report the reduction potentials as half-wave potentials, \(E^{(1/2)}\). Nevertheless, even such quasi-reversible behavior is indicative for relatively stable long-lived radical cations of Dmx, 5Pip and 5Hxm.

For reversible and quasi-reversible cyclic voltammograms, the midpoint between the anodic and cathodic peak potentials (\(E_a\) and \(E_c\), respectively) provides an estimate for \(E^{(1/2)}\) (Figure 3-1c,d). Conversely, for irreversible behavior, where the cathodic peak is not apparent and \(E_c\) cannot be determined, the first inflection point of the anodic wave provides an acceptable estimate for \(E^{(1/2)}\). In fact, the potentials at the inflection points of reversible waves, determined from the second derivatives of the voltammograms (i.e., \(E\) where \(\frac{\partial^2 I}{\partial E^2} = 0\)), are quite close to \(E^{(1/2)}\) obtained from \((E_a + E_c) / 2\) (Figure 3-1c,d). This observation justifies the use of the inflection points for estimating half-wave potentials from irreversible cyclic voltammograms.

The electrochemical reduction potentials of the Aa residues manifest dependence on the concentration of the supporting electrolyte, \(C_{el}\). Decreasing \(C_{el}\) causes a positive shift in
Figure 3-1b). This concentration dependence of the reduction potentials allows for extrapolation of $E^{1/2}$ to $C_{el} = 0$, i.e., obtaining $E^{1/2}$ for neat solvents (Table 3-1),\textsuperscript{37,38} which have significant relevance to charge-transfer studies.\textsuperscript{9,37}

Keeping $E^{1/2} < E_{co}$ to attain reversible or quasi-reversible oxidation proves successful for Dmx, 5Pip and 5Hxm. Placing a strong electron-donating group on the 4\textsuperscript{th} rather than the 5\textsuperscript{th} position of the Aa aromatic ring (as in 4Hxm and 4Pip) results in $E^{1/2} < E_{co}$ but does not provide reversibility of the oxidation (Figure 1a). $E^{1/2}$ of 4Hxm and 4Pip is about 100 to 400 mV more negative than $E^{1/2}$ of Dmx (Table 3-1), and yet unlike Dmx, 4Hxm and 4Pip undergo irreversible oxidation. What is the reason for this discrepancy and what compromises the stability of the radical cations of the 4-amino Aa derivatives?

To elucidate the reason for this discrepancy with the irreversible oxidation of 4Pip and 4Hxm, we resort to computational analysis utilizing density functional theory (DFT) calculations, and focusing on the distribution of the electron spin density of the radical cations of the Aa residues. The positive charge of the radical cation of the residue without any substituents, Ant\textsuperscript{•+}, spreads over the aromatic ring, extending mostly over the N-terminal amide and to a lesser extent over to the nitrogen of the C-terminal amide (Figure 3-2). Electron-donating groups at the 5\textsuperscript{th} position enhance this electron spin-density distribution extending over the N-terminal and receding from the C-terminal amide. It is an especially pronounced effect for the strong electron-donating groups of the 5-amino Aa\textsuperscript{•+} derivatives, 5Hxm\textsuperscript{•+} and 5Pip\textsuperscript{•+} (Figure 3-2). Conversely, placing the same strong electron-donating groups on the 4\textsuperscript{th} position, as in 4Pip\textsuperscript{•+} and 4Hxm\textsuperscript{•+}, drastically changes
the distribution of the positive charge, as apparent from the comparisons of 4Pip$^{\text{+}}$ vs. 5Pip$^{\text{+}}$, and 4Hxm$^{\text{+}}$ vs. 5Hxm$^{\text{+}}$ (Figure 3-2). In the radical cations of 4Hxm and 4Pip, the electron spin-density extends predominantly over the C-terminal amide, rather than the N-terminal one (Figure 3-2).

**Discussion**

Cleavage of the amide bonds (C-C(O)NHC, CC(O)-NHC, or CC(O)NH-C) is the most likely outcome from the oxidative decomposition of aromatic amides. Concurrently, the principal difference between the radical cations of the 4-amino derivatives and the rest of the Aa$^{\text{+}}$ is the distribution of the positive charge over the C-termianl amides (Figure 3-2). Therefore, it appears that while the N-terminal Aa amide requires $E > E_{co}$ for irreversible cleavage, the C-terminal Aa amide is susceptible to milder oxidizing conditions at $E < E_{co}$.

Extending the positive charge over the C-terminal amide can lead to its deprotonation, providing a plausible route for the observed decomposition of the oxidized 4-amino Aa residues. To examine the importance of the C-amide deprotonation, we prepared a derivative of 4Pip with a tertiary C-amide, i.e., 4Pip$_{C-Pip}$ (Figure 3-3a). The spin-density distribution of 4Pip$_{(T)C-Pip}$ (Figure 3-3b) is practically the same as the one for 4Pip$_{(T)}$ (Figure 3-2), indicating that adding the piperidine ring to the C-terminus does not noticeably alter the electronic structure of the radical cation. Conversely, the cyclic voltammograms of 4Pip$_{C-Pip}$ for DCM (a relatively non-polar solvent with pronounced polarizability) manifest a small cathodic peak when the sweep is reversed (Figure 3-3c).
In comparison, the cyclic voltammograms for 4Pip and 4Hxm do not show detectable cathodic peaks (Figure 3-1). This feature indicates that while 4PipC-Pip, which lacks a C-terminal amide hydrogen, still shows chemical irreversibility, the irreversibility is partially suppressed. Thus, we can conclude that most plausibly the deprotonation of the C-terminal amide represents only one of the routes responsible for the oxidative degradation of the 4-amino Aa residues.

To attain reversible or quasi-reversible electrochemical oxidation of N-acylated anthranilamides and stabilize their radical cations, it is essential to lower their reduction potentials by adding electron-donating substituents. Meanwhile, to prevent steric hindrance between the residues in the Aa oligomers, we focus on substituents at the 4th and the 5th distal position of the Aa aromatic ring, corresponding to the R1 and R2 side chains, respectively (Chart 3-1a). Making the aromatic ring sufficiently electron rich by adding electron-donating groups, however, is a necessary but not a sufficient condition for stabilizing the Aa radical cations. Placing electron-donating groups on the 5th position (as R2 side chains) is another requirement for ensuring chemical reversibility of the electrochemical oxidation and long-lived Aa•+. While these findings are key specifically for the anthranilic molecular electrets (Chart 3-1a), they underline important considerations for the design of aromatic amides for electronic and energy applications where hole transfer is crucial.
EXPERIMENTAL METHODS

The eight residues, \( N_C \)-hexyl \( N_2 \)-hexanoyl(\( xyz \))anthranilamide (where for Ant, \((xyz)\) is blank; for Met, \((xyz) = -5\)-methyl; for Hox, \((xyz) = -5\)-hexyloxy; for Dmx, \((xyz) = -4,5\)-dimethoxy; for 4Hxm, \((xyz) = -4\)-(hexyl(methyl)amino); for 5Hxm, \((xyz) = -5\)-(hexyl(methyl)amino); for 4Pip, \((xyz) = -4\)-(piperidin-N-yl); and for 5Pip, \((xyz) = -5\)-(piperidin-N-yl)) are prepared as previously described.\(^9,39\) Adopting the same procedures, we prepared 4Pip\(_{C-Pip}\) in five synthetic steps from commercially available starting materials: 4-fluoro-2-nitrobenzoic acid, piperidine, and hexanoic anhydride.

\[\text{(2-nitro-4-(piperidin-N-yl)-benzoyl)piperidine (precursor for 4Pip}_{C-Pip}\).\] 2-nitro-4-(piperidin-N-yl)-benzoic acid (125 mg, 0.5 mmol), prepared as previously described,\(^31,39\) was placed in a 100-ml Schlenk tube equipped with a magnetic stir bar. While purging with argon, 5 ml dry DCM and 3 drops of DMF were added, and the reaction vessel was immersed in a dry-ice/acetone bath. Oxalyl chloride (130 µl, 1.5 mmol) was slowly added and the reaction mixture was allowed to gradually warm up to room temperature. The progress of the reaction was monitored using TLC of the reaction mixture treated with methanol and within 30 min the conversion was quantitative. The liquid, including the left over oxalyl chloride, was removed \textit{in vacuo}, followed by several addition and removal of small portions (5 ml) of DCM. The thus dried reaction mixture was dissolved in dry 5 ml DCM, and piperidine (150 µL, 1.5 mmol) was slowly added to it while purged with argon. The Schlenk tube was immersed in a dry-ice/acetone bath and
pyridine (60 µl, 0.75 mmol) was slowly added to it. The reaction mixture was allowed to warm up to room temperature and was stirred for an additional hour. The solution was diluted with 25 ml of DCM, and washed with 5% HCL (100 ml × 2) and with brine (100 ml). The organic layer was collected, dried over Na₂SO₄, and concentrated in vacuo. The product was purified using flash chromatography (column, 1” internal diameter, was packed with silica gel in hexanes, 6” to 8” height of the packed stationary phase). Isocratic elution at 70% ethyl acetate and 30% hexanes produced the fraction of interest (as monitored with TLC) to afford, after drying, yellow solid (75 mg, 0.24 mmol, 48%) of (2-nitro-4-(piperidin-N-yl)-benzoyl)piperidine: ¹H-NMR (400 MHz, CDCl₃) ℧/ppm: 7.56 (1 H, d, J = 2.4 Hz), 7.15 (1 H, d, J = 8.5 Hz), 7.08 (1 H, dd, J₁ = 8.6 Hz, J₂ = 2.4 Hz), 3.71 (2 H, s), 3.27 (4 H, t, J = 5.1 Hz), 3.17 (2 H, t, J = 5.5 Hz), 1.66 (10 H, m), 1.45 (2 H, s); ¹³C-NMR (400 MHz, CDCl₃) ℧/ppm: 167.22, 152.22, 146.75, 128.81, 122.14, 120.25, 110.19, 49.46, 48.19, 42.98, 26.17, 25.45, 25.42, 24.77, 24.28; HRMS m/z calculated C₁₇H₂₃N₃O₃⁺ (M + H)⁺ 318.1818, found 318.1827 (M + H)⁺.

(N-hexanoyl-2-amino-4-(piperidin-N-yl)-benzoyl)piperidine (4PipC-Pip). (2-nitro-4-(piperidin-N-yl)-benzoyl)piperidine (75 mg, 0.24 mmol) and Co₂(CO)₈ (149 mg, 0.47 mmol) were added to a 100-ml pressure tube. While purging with argon, 5 ml of 1,2-dimethoxyethane and 2 drops of DI water were added. While mixing, the pressure tube was immersed in a temperature-controlled oil bath. The mixture was heated to 90 °C and stirred for an hour. The reaction mixture was filtered; the filtrate was collected, diluted
with 25 ml DCM, and washed with water (100 ml). The organic layer was collected, dried over Na₂SO₄, and concentrated in vacuo. While purging with argon, the resulting solid was transferred into a Schlenk tube using 5 ml dry DCM; hexanoic anhydride (163 μL, 0.708 mmol) was added, and the solution was cooled on a dry-ice/acetone bath. After adding pyridine (38 μl, 0.47 mmol), the solution was allowed to warm up to room temperature and stirred for additional 1.5 h. The reaction solution was diluted with 25 ml DCM, and washed with an aqueous solution of Na₂CO₃ (100 ml × 2) and with brine (100 mL). The organic layer was collected, dried over Na₂SO₄, concentrated in vacuo, and purified using flash chromatography (column, 1” internal diameter, was packed with silica gel in hexanes, 6” to 8” height of the packed stationary phase). Isocratic elution at 70% ethyl acetate and 30% hexanes produced the fraction of interest. After further wash with Na₂CO₃ solution and drying, the solvent was removed in vacuo to produce white solid (37 mg, 0.096 mmol, 40%) of 4Pip-C-Pip: ¹H-NMR (400 MHz, CDCl₃) δ/ppm: 9.56 (1 H, s), 8.00 (1 H, d, J = 2.4 Hz), 7.04 (1 H, d, J = 8.7 Hz), 6.50 (1 H, dd, J₁ = 8.7 Hz, J₂ = 2.3 Hz), 3.53 (4 H, s), 3.24 (4 H, t, J = 5.1 Hz), 2.32 (2 H, t, J = 7.5 Hz), 1.68 (8 H, m), 1.56 (6 H, m), 1.32 (4 H, m), 0.87 (3 H, t, J = 6.8 Hz); ¹³C-NMR (400 MHz, CDCl₃) δ/ppm: 172.11, 170.52, 153.45, 139.60, 129.19, 112.27, 108.92, 108.06, 49.35, 38.42, 31.52, 26.44, 25.73, 25.39, 24.84, 24.56, 14.14; HRMS m/z calculated C₉₃H₃₅N₃O₂⁺ (M + H)⁺ 386.2807, found 386.2821 (M + H)⁺.
Cyclic voltammetry is conducted using Reference 600™ Potentiostat/Galvanostat/ZRA (Gamry Instruments, PA, U.S.A.), connected to a three-electrode cell, at scan rates of 20 to 500 mV s\(^{-1}\), as previously described\(^{37,38}\). Anhydrous aprotic solvents with different polarity, chloroform (CHCl\(_3\)), dichloromethane (DCM), and acetonitrile (MeCN), are employed with different concentrations of tetrabutylammonium hexafluorophosphate (NBu\(_4\)PF\(_6\)) as supporting electrolyte. Prior to recording each voltammogram, the sample was extensively purged with argon while maintaining its volume of 5 ml by adding more of the anhydrous solvent.

For each Aa residue and each solvent, a set of voltammograms is recorded where the electrolyte concentration is increased from 25 mM to 200 mM in steps of 25 mM. The half-wave potentials, \(E^{(1/2)}\), are determined from the midpoints between the cathodic and anodic peak potentials for reversible or quasi-reversible oxidation; and from the inflection points of the anodic waves for irreversible oxidation (Figure 3-1c,d). The anodic and cathodic peak potentials, \(E_a\) and \(E_c\), respectively, are determined from the zero points of the first derivatives of the voltammograms, i.e., the potentials where \(\partial I/\partial E = 0\) at \(\partial E/\partial t = \text{constant}\) (Figure 3-1c,d). The inflection points are determined from the zero point of the second derivatives of the voltammograms, \(\partial^2 I/\partial E^2 = 0\) at \(\partial E/\partial t = \text{constant}\) (Figure 1c,d).

The second derivatives of reversible and quasi-reversible voltammograms show that the inflection-point potentials are quite close to the mid-points between \(E_a\) and \(E_c\), ensuring
the reliability for the estimates of $E^{(1/2)}$ from the inflection points of irreversible voltammograms. To correct for potential drifts in the reference electrode (which is SCE, connected with the cell via a salt bridge) ferrocene was used as a standard ($E^{(1/2)} = 0.45 \pm 0.01$ V vs. SCE for MeCN, 100 mM NBu$_4$BF$_4$). Voltammograms of the standard are recorded before and after each set of measurements. From the dependence of $E^{(1/2)}$ on the electrolyte concentration, the potentials for each neat solvents are estimated from extrapolations to zero (Table 3-1).

The $N$-acylated Aa residues (Chart 3-1b) are modeled using density functional theory (DFT). For simplicity, the aliphatic chains are truncated to two carbons. The DFT calculations are performed at the B3LYP/6-311+G(d,p) level$^{9,39,46,49-51}$ for the gas phase using Gaussian 09.$^{52}$ Spin-unrestricted calculations are used for radical-cation (doublet state) modeling.
REFERENCES


FIGURES
Figure 3-1. Cyclic voltammograms of Aa residues with electron-donating side chains (Chart 1), for dichloromethane (DCM) with NBu$_4$PF$_6$ as a supporting electrolyte at different concentration, $C_{el}$. (a) Voltammograms of six Aa residues with manifesting oxidation at different potentials (DCM; $C_{el}$ = 200 mM). (b) Voltammograms of 5Hxm for DCM in the presence of different electrolyte concentrations. (inset: the first oxidation waves recorded at different $C_{el}$.) (c,d) Extracting half-wave potentials, $E^{(1/2)}$, from voltammograms with Faradaic currents comparable to the Ohmic and capacitance currents (DCM; $C_{el}$ = 200 mM). For Dmx, when the anodic and cathodic peaks are apparent, $E^{(1/2)} = (E_a + E_c) / 2$, where $E_a$ and $E_c$ are the anodic and cathodic peak potentials, respectively, determined from the zero points of the first derivative, i.e., at $E$ where $\delta I/\delta E = 0$. For 4Hxm, when only the cathodic peak is not present, $E^{(1/2)}$ is extracted from the first inflection point of the anodic wave, i.e., at $E$ where $\delta^2 I/\delta E^2 = 0$. Scan rates: 0.02 V s$^{-1}$ for the irreversible voltammograms (4Pip, 4Hxm, Hox, Met, and Ant); and 0.05 V s$^{-1}$ for the quasi-reversible ones (5Pip, 5Hxm, and Dmx).
**Figure 3-2.** Electron spin density of the radical cations of the Aa residues (black – excess spin up, i.e., radical cation; and white, excess spin down). For each of the computations, the long alkyl chains of the structures are truncated (T) to ethyls. Truncation and conformational changes of the alkyl chains do not alter the spin-density distribution. Even switching an amide from *trans* to *cis* does not cause noticeable changes on the distribution over the reset of the structure (compare the two bottom *cis-trans*, Aa\(^{(T)}_{ct}•^+\), structures with the corresponding *trans-trans*, Aa\(^{(T)}_{tt}•^+\), ones
Figure 3-3. 4-piperidinyl Aa residue with a piperidine-capped C-terminus, 4Pip-C-Pip. (a) Structure of 4Pip-C-Pip. (b) Electron spin density of 4Pip-C-Pip•+. (c) Cyclic voltammograms of 4Pip-C-Pip for DCM with NBu4PF6 as a supporting electrolyte at different concentration, C_{el} (scan rate: 0.05 V s^{-1}). The value of E^{1/2} extrapolated to zero electrolyte concentration, i.e., for neat DCM, is 1.09 ± 0.02 V vs. SCE.
Table 3-1. Half-wave reduction potentials of the oxidation of \(N\)-acyl Aa residues, \(Aa^{\text{+} +} + e^- \rightleftharpoons Aa\).

<table>
<thead>
<tr>
<th>-R(_1)</th>
<th>-R(_2)</th>
<th>(E^{(1/2)}) / V vs. SCE (^a)</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td>CHCl(_3) (^b)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>((\Sigma = 4.8))</td>
</tr>
<tr>
<td>Ant</td>
<td>-H</td>
<td>2.01 ± 0.26</td>
</tr>
<tr>
<td>Met</td>
<td>-H</td>
<td>2.05 ± 0.29</td>
</tr>
<tr>
<td>Hox</td>
<td>-H</td>
<td>1.60 ± 0.03</td>
</tr>
<tr>
<td>Dmx</td>
<td>-OCH(_3)</td>
<td>1.54 ± 0.08</td>
</tr>
<tr>
<td>4Pip</td>
<td>-N(CH(_2)(_2))</td>
<td>1.29 ± 0.12</td>
</tr>
<tr>
<td>5Pip</td>
<td>-H</td>
<td>1.16 ± 0.04</td>
</tr>
<tr>
<td>4Hxm</td>
<td>-N(CH(_3)(_3))C(_6)H(_13)</td>
<td>1.42 ± 0.03</td>
</tr>
<tr>
<td>5Hxm</td>
<td>-H</td>
<td>0.93 ± 0.01</td>
</tr>
</tbody>
</table>

\(^a\) For Dmx, 5Pip and 5Hxm, \(E^{(1/2)}\) are estimated as the average of the anodic and the cathodic potentials, i.e., \(E^{(1/2)} = (E_a + E_c) / 2\) (Figure 1). For Ant, Met, Hox, 4Pip and 4Hxm, \(E^{(1/2)}\) is obtained from the inflexion points of the anodic waves (Figure 1). \(^b\) From the dependence of \(E^{(1/2)}\) on \(C_e\) for each solvent, the values of \(E^{(1/2)}\), reported in this table, are from extrapolations to zero electrolyte concentration.\(^{37-39}\) (\(\Sigma\) – static dielectric constant, i.e., relative permittivity at zero frequency, of each solvent; CHCl\(_3\) – chloroform; DCM – dichloromethane; and MeCN – acetonitrile)
Chart 3-1. Bioinspired Molecular Electrets and Their Anthranilic Residues.$^a$
(a) Molecular electrets composed of anthranilic acid residues and the origin of their permanent electric dipole from ordered amide bonds and a co-directional shift in the electron density (from O to H) upon hydrogen bonding. (b) $N$-acylated anthranilamide residues with electron-donating substituents as side chains, $R_1$ and $R_2$, and $n$-alkyl chains at their N- and C-termini ($R_N = C_5H_{11}$; $R_C = C_6H_{13}$).
What Makes Oxidized N-Acylantranilamides Stable? (Supporting Information)

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Supplemental Schemes

Scheme 3S-1. Synthesis of 4PipC-Pip.
Figure 3S-1. $^1$H 1D NMR spectrum of 2-nitro-5-(piperidin-N-yl) benzoic acid (CDCl$_3$).
Figure 3S-2. 1D NMR spectra, (a) $^1$H and (b) $^{13}$C, of (2-nitro-4-(piperidin-N-yl)-benzoyl) piperidine (CDCl$_3$).
Figure 3S-3. 1D NMR spectra, (a) $^1$H and (b) $^{13}$C, of 4Pip$_{eq}$ (CDCl$_3$).
Chapter 4

Fluorinated Aminoanthranilamides: How to Make Them and Why They Are Important?
ABSTRACT: The ability to control charge transfer at molecular and nanometer scales represents the ultimate level of electronic mastery, and its impacts cannot be overstated. As electrostatic analogues of magnets, electrets possess ordered electric dipoles that present key paradigms for directing transduction of electrons and holes. Here, we describe the design and development of fluorinated aminoanthranilamides, derivatives of non-native aromatic beta-amino acids, for building blocks of hole-transfer molecular electrets. The reported here highly regio-selective nucleophilic aromatic substitution of a difluorinated nitrobenzoic acid provides the underpinnings for an array of unprecedented anthranilamide structures. Spin-density-distribution and electrochemical analyses reveal that fluorine induces a 200-mV positive shift in reduction potentials without compromising the stability of the oxidized residues, making them invaluable building blocks for hole-transfer systems. These findings open unexplored routes to new amino-acid structures, setting a foundation for bringing principles of proteomics to designs of charge-transfer systems.
Introduction

Vital processes in biology, such as photosynthesis and cellular respiration, rely on relays of change-transfer (CT) steps. The ability of these natural systems to sustain high-potential states for CT with quantitative efficiencies serves as an inspiration for the design of electronic and energy-converting systems. Indeed, optimizing the CT processes mediated in materials and devices is key for the paths to sustainable energy infrastructures.

The effects of dipoles play a defining role in a myriad of biological processes, aiding ion transport and guiding the directionality of electron transfer (ET). Local electric fields from molecular dipoles can pronouncedly affect CT via the Franck-Condon contribution to its kinetics. Therefore, molecular electrets provide a key means for controlling the rates and efficiencies of CT. While protein helices are some of the best known molecular electrets, they cannot efficiently mediate CT beyond inherent electron tunneling limits of 2 – 2.5 nm. Conversely, another class of biomacromolecules, polynucleotides, offers routes for long-range CT via hole ($h^+$) hopping, i.e., via a series of short ET steps along the HOMOs of a sequence of electron-rich moieties. The universality of these two CT paradigms is illustrated through biomimetics involving polypeptide helices directly incorporated for CT rectification, and foldamers of π-stacked aromatic moieties mediating DNA-type long-range $h^+$ hopping. To combine these two beneficial features, we design bioinspired molecular electrets based on anthranilamide (Aa) templates (Figure 4-1a). Similar to protein helices, ordered amide
and hydrogen bonds embedded within the backbone generate a permanent electric dipole. The linked aromatic moieties provide an electronic framework to support long-range CT resulting in a design that captures the benefits of natural molecular electrets and the efficiency of biological ET.

For $h^+$-transfer electrets, we develop non-native amino acids modified with various electron-donating groups (Figure 4-1b). By altering the electron-donating strength of these groups we adjust the reduction potentials of the residues over a range of 1 V (Figure 4-1b). Additionally, altering the position of the substituents, i.e., $R_1$ vs. $R_2$ (Figure 4-1a), provides a means for fine tuning of the potentials, e.g., 4Hxm vs. 5Hxm (Figure 4-1b).

For successful mediation of $h^+$ transfer, the CT electrets should form stable radical cations, i.e., they should not undergo oxidative degradation when $h^+$ is located on their residues. By examining the chemical reversibility of electrochemical oxidation of the Aa residues we have determined two key requirements for attaining stable radical cations, Aa$: (1) the reduction potential should be kept under 1.5 V vs. SCE in order to prevent inherent oxidative degradation of the amides; and (2) the spin-density distribution (SDD) of the radical cations should not extend over the C-terminal amide. For example, 4-amino Aa residues, 4Pip and 4Hxm, irreversibly oxidize at about 0.9 to 1 V vs. SCE.

Conversely, the analogous 5-amino Aa residues, 5Pip and 5Hxm, oxidize reversibly at about 0.7 to 0.9 V vs. SCE (Figure 4-1b). Both types of residues oxidize at
potentials smaller than 1.5 V vs. SCE. However, the positioning of the electron-donating
groups of the 4-amino Aa residues (as R$_1$) causes an expansion of the Aa$^{+*}$ SDD over their
C-terminal amides.$^8$ To test the validity of these design principles and more importantly,
to expand the set of Aa residues that are desirable for $h^+$-transfer applications, we aim at
Aa derivatives that have reduction potentials similar to those of 4-amino Aa moieties and
the Aa$^{+*}$ stability of the 5-amino Aa residues. To achieve this goal, we: (1) keep a strongly
electron-donating bonds embedded within the backbone generate a permanent electric
dipole. The linked aromatic moieties provide an electronic framework to support long-
range CT resulting in a design that captures the benefits of natural molecular electrets and
the efficiency of biological ET.

For $h^+$-transfer electrets, we develop non-native amino acids modified with
various electron-donating groups (Figure 4-1b).$^8$ By altering the electron-donating
strength of these groups we adjust the reduction potentials of the residues over a range of
1 V (Figure 4-1b).$^{8a}$ Additionally, altering the position of the substituents, i.e., R$_1$ vs. R$_2$
(Figure 4-1a), provides a means for fine tuning of the potentials, e.g., 4Hxm vs. 5Hxm
(Figure 4-1b).$^8$

For successful mediation of $h^+$ transfer, the CT electrets should form stable radical
cations, i.e., they should not undergo oxidative degradation when $h^+$ is located on their
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Aa$^{+*}$: (1) the reduction potential should be kept under 1.5 V vs. SCE in order to prevent
inherent oxidative degradation of the amides; and (2) the spin-density distribution (SDD) of the radical cations should not extend over the C-terminal amide.\(^8\) For example, 4-amino Aa residues, 4Pip and 4Hxm, irreversibly oxidize at about 0.9 to 1 V vs. SCE. Conversely, the analogous 5-amino Aa residues, 5Pip and 5Hxm, oxidize reversibly at about 0.7 to 0.9 V vs. SCE (Figure 4-1b). Both types of residues oxidize at potentials smaller than 1.5 V vs. SCE. However, the positioning of the electron-donating groups of the 4-amino Aa residues (as \(R_1\)) causes an expansion of the Aa\(^{\ddagger}\) SDD over their C-terminal amides.\(^8\)

To test the validity of these design principles and more importantly, to expand the set of Aa residues that are desirable for \(h^+\)-transfer applications, we aim at Aa derivatives that have reduction potentials similar to those of 4-amino Aa moieties and the Aa\(^{\ddagger}\) stability of the 5-amino Aa residues. To achieve this goal, we: (1) keep a strongly electron-donating group, i.e., an amine, at position 5 to ensure SDD over the N-terminal amide, rather than the C-terminal one; and (2) place an electronegative atom, i.e., fluorine, at position 4 which causes a positive shift in the reduction potentials making them comparable to those of the 4-amino Aa residues. Our findings show that the fluorine at position 4 does not noticeably perturb the SDD of the 5-amino Aa\(^{\ddagger}\), and confirming the predictive power of our design principles, the 5-amino-4-fluoro Aa residues reversibly oxidize at potentials similar to those of the 4-amino Aa derivatives.

Because we synthesize Aa oligomers from their C- to their N-termini by adding each residue as a conjugate of 2-nitrobenzoic acid,\(^4d\) facile preparation of 5-amino-4-
fluoro-2-nitrobenzoic acids is essential for ensuring building blocks for fluorinated Aa molecular electrets. An important finding, which is responsible for the success of this synthesis, is that a commercially available 4,5-difluoro-2-nitrobenzoic acid (DFNBA) undergoes selective nucleophilic aromatic substitution at position 5 (Scheme 4-1). Once an electron-donating amine replaces the fluorine at position 5, it decreases the electrophilicity of the carbon at position 4, making the substitution of the second fluorine impossible.

**Results**

Under neat conditions, with exception to dihexylamine, the substitution reaction proceeds cleanly to completion with quantitative yields. $^{19}$F and $^1$H NMR spectroscopy demonstrates the strict selectivity of this reaction when the corresponding amine is used as a solvent (Figure 4-2a-c). For some small amines, this substitution occurs even at room temperature but requires hours and days for completion. Additionally, the solubility of DFNBA into the amine appears to be a rate-limiting process. Conversely, microwave heating leads to completion of the reactions within a few minutes (Figure 4-2a,b). Prolonging the microwave radiation to a few hours does not lead to a second substitution of the fluorine at position 4. Furthermore, the NMR spectra of the reaction mixtures do not show noticeable impurities within the first 15 minutes of microwave heating.

These results demonstrate the robustness of this nucleophilic aromatic substitution and its immense practicality for preparing the building blocks for fluorinated Aa electrets.
These findings prove to be encouraging for scaling up and provide directions for developing facile gram-quantity high-yield synthetic procedures using conventional heating (Figure 4-2c).

**Discussion**

Increasing the size of the alkyl chains of the amine slows down the reaction, making microwave heating a key requirement. For dihexylamine, while after 4 hours of microwave radiation there is still noticeable amount of unreacted DFNBA, after 1 hour – the $^{19}$F NMR spectrum already shows the presence of impurities (Figure 4-2d). While this finding shows that making Fdx presents challenges, it also demonstrates a microwave procedure for attaining fluorinated Aa residues with long alkyl chains attached to the amine substituent, which may prove useful for lipid types of self-assemblies.

Two-dimensional NMR analysis reveals that the substitution, indeed, occurs at position 5. NOESY of $(\text{NO}_2)$Fpi shows a correlation of the more shielded of the two aromatic protons ($\delta \approx 7$ ppm) with the amide proton, $a_N$. Hence, we assign the 7-ppm doublet to the Aa proton at position 6, i.e., $a_6$, and the other aromatic doublet, at $\sim$7.6 ppm, – to $a_3$ (Figure 4-2e). The correlation of $a_6$ with the piperidine protons and the lack of through-space correlations detected for the other aromatic proton, $a_3$, confirm that the amine is located at position 5 (Figure 4-2e).

All 5-amino-4-fluoro Aa residues exhibit the same electronic properties and electrochemical behavior. While the fluorine at position 4 mesomerically provides *para*
activation for electrophiles, the dominating effect of the electron-donating group at position 5 ensures that the SDD does not extend over the C-terminal amide (Figure 4-3a). Concurrently, the first electrochemical oxidation step of all 5-amino-4-fluoro Aa residues manifests chemical reversibility (Figure 4-3b), suggesting for the formation of relatively stable Aa$^{\text{•+}}$. These findings further confirm the validity of predicting the stability of Aa radical cations from their SDD patterns.\textsuperscript{8c}

Despite its mesomeric electron-donating properties, the strong inductive electron-withdrawing character of fluorine causes ~200-mV positive shift in $E_{\text{Aa}^+/\text{Aa}}^{(1/2)}$. Namely, this electronegative substituent makes the potentials of the fluorinated 5-amino Aa residues resemble those of the 4-amino derivatives (Table 4-1, Figure 4-1b).

Solvent polarity and electrolyte concentration (especially for less polar media) have a notable effect on the reduction potentials (Table 4-1, Figure 4-3b), as one would expect.\textsuperscript{8a, 8c,10} The exact structure of the aliphatic amine substituent at position 5, however, negligibly affects the values of $E_{\text{Aa}^-/\text{Aa}}^{(1/2)}$ (Table 4-1). For example, changing one of the amine chains (R”, Scheme 4-1) from proton (Fhx) to methyl (Fmx) to hexyl (Fdx) causes small fluctuations in $E_{\text{Aa}^-/\text{Aa}}^{(1/2)}$ that are well within the uncertainty of the experimental error (Table 4-1). This finding demonstrates the sole importance of the amine nitrogen, rather than of the aliphatic chains attached to it, for the electronic properties of the residues. Thus, the side chains of the amine, i.e., R’ and R” (Scheme 4-1), provide a handle for controlling the solubility and the self-assembly propensity of
the Aa molecular electrets (Figure 4-1a) in a manner decoupled from their electronic properties.

The use of a small electronegative substituent, such as fluorine, is instrumental for adjusting reduction potentials with up to 200 mV. It translates to about $8 \times k_B T$, considering $F E^{(1/2)}$, which is a substantial contribution to CT driving forces, $-\Delta G^{(0)}$. Such adjustments offer an additional means for controlling the CT properties of Aa molecular electrets. Furthermore, the fluorine does not compromise the stability of the oxidized Aa residues. The strongly electron-donating $R_2$ group (Figure 4-1a) dominates the definition of favorable Aa$^{+}$ SDD. Thus, the fluorinated amino Aa residues present an important set of building blocks for hole-transfer molecular electrets. While the preparation of regioisomerically pure aromatic conjugates with multiple substituents is challenging, the highly regioselective nucleophilic aromatic substitution of the difluoro starting material is a breakthrough making the pursuit of these molecular designs practical. The approach for achieving targeted electronic characteristics, and for developing reliable and robust synthetic procedures for facile preparation of these fluorinated conjugates, presents universality that can prove broadly applicable for advancing organic electronics and developing energy materials.
REFERENCES


Figure 4-1. (a) Aa bioinspired molecular electrets with the dipole originating from ordered amide bonds and polarization upon hydrogen bonding. (b) Aa residues for $h^+$-transfer electrets with electron-donating $R_1$ and $R_2$ groups. The listed values, $(E^{1/2}_\text{DCM}, E^{1/2}_\text{MeCN})$, are for $E^{1/2}$ vs. SCE for $Aa^+ + e^- \rightleftharpoons Aa$. (* irreversible oxidation)
Figure 4-2. NMR analysis of the preparation of fluorinated aminoanthranilamide precursors. (a) $^{19}$F and (b) $^1$H NMR spectra (DMSO-d$_6$) of a reaction mixture of DFNBA (1 mmol) and piperidine for the synthesis of (NO$_2$)Fpi(CO$_2$H) after 2.5 min of microwave radiation (80 W, 80 °C), showing quantitative conversion to the product and the left over piperidine. (c) $^1$H NMR spectrum (CDCl$_3$) of the crude product from gram-quantity synthesis of (NO$_2$)Feb(CO$_2$H), starting with 40 mmol DFNBA, after 20 hours of conventional heating in a pressure tube. (d) $^{19}$F NMR spectra (DMSO-d$_6$) following the synthesis of (NO$_2$)Fdx(CO$_2$H), starting with 1 mmol DFNBA, using microwave radiation (80 W, 80 °C). Inset: time progress of the change in the relative abundance of the DFNBA starting material (sm) and the product (pr) extracted from the integrated values of the NMR signals, i.e., $R_{sm} = [sm] / ([pr] + [sm])$ and $R_{pr} = [pr] / ([pr] + [sm])$. (e) NOESY spectrum of (NO$_2$)Fpi (CDCl$_3$) demonstrating that the nucleophilic aromatic substitution occurs at position 5 of DFNBA.
Figure 4-3. Comparison between the electronic properties of fluorinated (Fpi) and non-fluorinated (5Pip and 4Pip) piperidin-1-yl Aa residues. (a) Spin density distribution in the radical cations calculated for Aa•+ with truncated C- and N-terminal alkyls (black – excess spin up). (b) Cyclic voltammograms of 5Pip, 4Pip and Fpi recorded for different solvents (DCM and MeCN) at different electrolyte concentration (C_e). (electrolyte, NBu₄PF₆; scan rate, 50 mV s⁻¹)
Tables

Table 4-1. Half-wave reduction potentials for the oxidation of the fluorinated Aa residues.

<table>
<thead>
<tr>
<th></th>
<th>DCM  b</th>
<th>MeCN b</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fhx</td>
<td>1.10 ± 0.05</td>
<td>0.91 ± 0.02</td>
</tr>
<tr>
<td>Fmx</td>
<td>1.12 ± 0.03</td>
<td>0.95 ± 0.06</td>
</tr>
<tr>
<td>Fdx</td>
<td>1.04 ± 0.02</td>
<td>0.90 ± 0.01</td>
</tr>
<tr>
<td>Feb</td>
<td>1.03 ± 0.03</td>
<td>0.90 ± 0.02</td>
</tr>
<tr>
<td>Fpi</td>
<td>1.08 ± 0.10</td>
<td>0.96 ± 0.02</td>
</tr>
</tbody>
</table>

a The half-wave potentials, $E^{(1/2)}$, are obtained from cyclic voltammograms using the first derivatives, $\partial I / \partial E$, for reversible oxidation.\textsuperscript{3a,8c} 
b The potentials for the neat solvents, dichloromethane (DCM) and acetonitrile (MeCN), were obtained from extrapolation to zero electrolyte concentrations, i.e., $C_{el} = 0$, from data plots of $E^{(1/2)}$ vs $C_{el}$\textsuperscript{10}.
Scheme 4-1. Synthesis of fluorinated Aa residues.

(i) HN(R’)R”, microwave or conventional heating; (ii) 1) (COCl)$_2$, DCM, DMF, \(-78 \, ^\circ\text{C} \rightarrow \text{r.t.};$
2) H$_2$NR$, DCM, \(-78 \, ^\circ\text{C} \rightarrow \text{r.t.};$
(iii) H$_2$, Pd/C, EtAc, r.t.; (iv) R$_2$-C(O)Cl, DCM, pyridine, \(-78 \, ^\circ\text{C} \rightarrow \text{r.t.}$
Supporting Information

Fluorinated Aminoanthranilamides: How to Make Them and Why Are They Important?

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1. Materials

1.1. General methods

All chemicals were used as received unless otherwise specified. The reported $^1$H NMR, $^{13}$C NMR, $^{19}$F NMR, and NOESY spectra were recorded on a 400 MHz spectrometer. $^1$H chemical shifts (δ) are reported in ppm relative to CHCl$_3$ in CDCl$_3$ (δ = 7.24 ppm) and DMSO-d$_5$ in DMSO-d$_6$ (δ = 2.50 ppm); $^{13}$C δ are reported in ppm relative to CDCl$_3$ (δ = 77.23 ppm) and DMSO-d$_6$ (δ = 39.51 ppm); and $^{19}$F δ are reported in ppm relative to an internal standard, trifluorotoluene (C$_6$H$_5$CF$_3$, δ = – 63.90 ppm), that was added in mM quantities only to samples for $^{19}$F NMR analyses. Data for $^1$H NMR and $^{19}$F NMR are reported as follows: chemical shift, integration, multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, p = pentet/quintet, h = hextet/sextet, e = eptet(from επτά)/heptet, m = multiplet), and coupling constants. All $^{13}$C NMR spectra were recorded with complete
proton decoupling; nevertheless, fluorine causes splitting in the signals of five of the aromatic carbons (all but the one \textit{para} to the fluorine), and sometime of the two carbons directly attached to the amine nitrogen (\textit{ortho} to the fluorine) due to inherent long-range $^{19}\text{F} - ^{13}\text{C}$ coupling. High-resolution mass spectrometry (HRMS) was performed using Agilent LCTOF (6200) mass spectrometer (Agilent Technologies, Santa Clara, CA). Analytical thin layer chromatography was performed using 0.25 mm silica gel 60-F plates. Flash chromatography was performed using 60 Å, 32–63 μm silica gel.

1.2. Synthesis of the 5-amino-4-fluoro-2-nitrobenzoic acid derivatives, (NO$_2$)$_2$Aa(CO$_2$H) ((i), Scheme 4S-1)

![Chemical Structure](image)

5-(butyl(ethyl)amino)-4-fluoro-2-nitrobenzoic acid, (NO$_2$)$_2$Feb(CO$_2$H) (microwave procedure). 4,5-Difluoro-2-nitrobenzoic acid, DFNBA (203 mg, 1 mmol) and N-ethylbutylamine (820 µl, 6 mmol) were slowly mixed in a glass microwave vial, forming a dark brown solution. The mixture was microwaved for 70 min at 80 watts, 80 °C. The progress of the reaction was monitored using TLC, and in some occasions, using $^{19}$F NMR. After cooling to room temperature, the mixture was diluted with DCM (50 ml) and
washed with 2% HCl (3×50 ml). The organic layer was collected, dried over anhydrous Na₂SO₄ and concentrated in vacuo to produce 273 mg yellow powder (0.96 mmol, 96% yield) of (NO₂)Feb(CO₂H). ¹H-NMR (CDCl₃) δ/ppm: 10.36 (1H, s (broad)), 7.75 (1H, d, J = 14.4 Hz), 6.84 (1H, d, J = 8.6 Hz), 3.46 (2H, q, J = 6.2 Hz), 3.37 (2H, t, J = 7.3 Hz), 1.61 (2H, p, J = 7.7 Hz), 1.35 (2H, h, 7.5 Hz), 1.23 (3H, t, J = 7.0 Hz), 0.95 (3H, t, J = 7.3 Hz); ¹³C-NMR (CDCl₃) δ/ppm: 172.17, 150.56 (d, J = 252 Hz), 142.49 (d, J = 7.4 Hz), 134.67 (d, J = 8.8 Hz), 126.73, 115.0 (d, J = 5.9 Hz), 114.64 (d, J = 29.0 Hz), 52.28 (d, J = 5.9 Hz), 47.52 (d, J = 5.9 Hz), 30.36, 20.32, 14.05, 13.30; HRMS m/z calculated for C₁₃H₁₈FN₂O₄⁺ (M+H)⁺ 285.1251, found 285.1245 (M+H)⁺.

(scaled-up, conventional-heating procedure) 8.16 g of DFNBA (40.2 mmol) was placed in a glass pressure tube. While purging with argon, 16.6 ml of N-Ethylbutylamine (121 mmol) was added to the pressure tube. The pressure tube was sealed with a screw cap and immersed in an oil bath with a temperature regulator. The temperature was raised to 110 °C and kept for 20 hrs. The reaction mixture was allowed to cool to room temperature, diluted with 5% HCl, and extracted with DCM. The organic layers were combined, dried over anhydrous Na₂SO₄, and the solvents were evaporated in vacuo to afford 11.1 g light brown solid (39 mmol, 97% yield) of (NO₂)Feb(CO₂H), confirmed with NMR (Figure 2c) and HRMS. For further purification, 5.2 g of the crude solid was placed in a round bottom flask; DCM (15 ml) was added to it and the mixture was sonicated for 5 minutes until becoming clear. The solution was diluted with 300 ml
hexanes and kept in a refrigerator overnight, which led to the formation of yellow crystalline precipitate. The mixture was allowed to reach room temperature and the precipitate was collected using vacuum filtration and dried to afford 4.46 g light brown solid.

4-fluoro-2-nitro-5-(piperidin-1-yl)benzoic acid, (NO$_2$)Fpi(CO$_2$H). DFNBA (203 mg, 1 mmol) and piperidine (590 µl, 6 mmol) were slowly mixed in a glass microwave vial, forming a dark brown solution. The mixture was microwaved for 1 h at 80 watts, 80 °C. The progress of the reaction was monitored using TLC, and in some occasions, using $^{19}$F NMR. After cooling to room temperature, the mixture was diluted with DCM (50 ml) and washed with 2% HCl (3×50 ml). The organic layer was collected, dried over anhydrous Na$_2$SO$_4$ and concentrated in vacuo to produce a dark yellow oil. Purification was performed using flash chromatography: stationary phase – silica gel; eluent gradient – from 100% hexanes to 80% hexanes / 20% ethyl acetate. After the 80%/20% ratio was reached, 1% acetic acid was added to the solvent mixture to elute the product. The collected fraction was concentrated in vacuo, washed with dionized water (50 ml), dried over anhydrous Na$_2$SO$_4$ and the rest of the organic solvents were removed in vacuo to
afforded 260 mg yellow powder (0.97 mmol, 97% yield) of (NO$_2$)Fpi(CO$_2$H). $^1$H-NMR (CDCl$_3$) $\delta$/ppm: 7.72 (1H, d, $J = 12.9$ Hz), 7.04 (1H, d, $J = 8.2$ Hz), 3.28 (4H, t, $J = 5.4$ Hz), 1.72 (4H, m), 1.65 (2H, m); $^{13}$C-NMR (CDCl$_3$) $\delta$/ppm: 170.79, 153.20 (d, $J = 253$ Hz), 145.06 (d, $J = 7.2$ Hz), 137.83 (d, $J = 8.4$ Hz), 126.26 (d, $J = 3.6$ Hz), 117.67 (d, $J = 5.4$ Hz), 113.56 (d, $J = 27.6$ Hz), 50.99 (d, $J = 5.4$ Hz), 25.76, 24.04; HRMS m/z calculated for C$_{12}$H$_{14}$FN$_2$O$_4$ (M+H)$^+$ 269.0938, found 269.0926 (M+H)$^+$.

4-fluoro-5-(hexylamino)-2-nitrobenzoic acid, (NO$_2$)Fhx(CO$_2$H). DFNBA (203 mg, 1 mmol) and 1-hexylamine (790 $\mu$l, 6 mmol) were slowly mixed in a glass microwave vial, forming a dark brown solution. The mixture was microwaved for 1 h at 80 watts, 80 °C. The progress of the reaction was monitored using TLC. After cooling to room temperature, the mixture was diluted with DCM (50 ml) and washed with 2% HCl (3×50 ml). The organic layer was collected, dried over anhydrous Na$_2$SO$_4$ and concentrated in vacuo to produce a dark orange-red oil. Recrystallization from hexanes resulted in 200 mg orange solid (0.70 mmol, 70% yield) of (NO$_2$)Fhx(CO$_2$H). $^1$H-NMR (DMSO-d$_6$) $\delta$/ppm: 7.86 (1H, d, $J = 12.1$ Hz), 7.20 (1H, t, $J = 5.0$ Hz), 6.78 (1H, d, $J = 8.6$ Hz), 3.23 (2H, t, $J = 6.6$ Hz), 1.55 (2H, p, $J = 7.0$ Hz), 1.29 (6H, m), 0.86 (3H, t, $J = 6.6$ Hz); $^1$H-NMR
(CDCl₃) δ/ppm: 7.78 (1H, d, J = 11.4 Hz), 6.77 (1H, d, J = 7.9 Hz), 3.26 (2H, t, J = 7.2 Hz), 1.68 (2H, p, J = 7.3 Hz), 1.40 (2H, p, J = 7.5 Hz), 1.33 (4H, m), 0.89 (3H, t, J = 7.0 Hz); ¹³C-NMR (CDCl₃) δ/ppm: 171.7, 149.44 (d, J = 246 Hz), 142.17 (d, J = 11.8 Hz), 134.17 (d, J = 7.6 Hz), 128.78, 111.87 (d, J = 24.0 Hz), 109.55 (d, J = 4.5 Hz), 43.42, 31.62, 29.16, 26.78, 22.75, 14.19; HRMS m/z calculated for C₁₃H₁₆FN₂O₄⁻ (M-H)⁻ 283.1094, found 283.1089 (M-H)⁻.

4-fluoro-5-(hexyl(methyl)amino)-2-nitrobenzoic acid, (NO₂)Fmx(CO₂H). Employing the procedure for (NO₂)Fpi(CO₂H), while using N-hexylmethylamine (910 µl, 6 mmol) instead of piperidine, resulted in 290 mg yellow powder (0.97 mmol, 97 % yield) of (NO₂)Fmx(CO₂H). ¹H-NMR (CDCl₃) δ/ppm: 7.75 (1H, d, J = 14.4 Hz), 6.83 (1H, d, J = 8.2 Hz), 3.43 (2H, t, J = 7.8 Hz), 3.08 (3H, d, J = 2 Hz), 1.61 (2H, p, J = 6.8 Hz), 1.29 (6H, s), 0.87 (3H, t, J = 6.6 Hz); ¹³C-NMR (CDCl₃) δ/ppm: 172.36, 150.69 (d, J = 250 Hz), 143.61 (d, J = 7.4 Hz), 134.98 (d, J = 8.1 Hz), 126.71, 115.01 (d, J = 5.2 Hz), 114.22 (d, J = 28.0 Hz), 55.01 (d, J = 7.4 Hz), 40.29, 31.67, 27.97, 26.60, 22.72, 14.13; HRMS m/z calculated for C₁₄N₂O₄FH₂₀⁺ (M+H)⁺ 299.1407, found 299.1401 (M+H)⁺.
5-(dihexylamino)-4-fluoro-2-nitrobenzoic acid, (NO$_2$)F$_{dx}$(CO$_2$H). Employing the procedure for (NO$_2$)Fpi(CO$_2$H), while using dihexylamine (1.4 ml, 6 mmol) instead of piperidine, 4-hour microwave heating, and a gradient to 80% ethyl acetate instead of 20% for the flash chromatography, resulted in 290 mg yellow powder (0.79 mmol, 79% yield) of (NO$_2$)F$_{dx}$(CO$_2$H). $^1$H-NMR (DMSO-d$_6$) $\delta$/ppm: 7.87 (1H, d, $J = 14.8$ Hz), 6.89 (1H, d, $J = 9.0$ Hz), 3.39 (4H, t, $J = 7.4$ Hz), 1.53 (4H, p, $J = 6.8$ Hz), 1.26 (12H, m), 0.85 (6H, t, $J = 6.6$ Hz); $^1$H-NMR (CDCl$_3$) $\delta$/ppm: 11.15 (1H, s(broad)), 7.74 (1H, d, $J = 14.0$ Hz), 6.82 (1H, d, $J = 8.5$Hz), 3.37 (4H, t, $J = 7.7$ Hz), 1.60 (4H, p, $J = 7.1$ Hz), 1.29 (12H, m), 0.88 (6H, t, $J = 6.9$ Hz); $^{13}$C-NMR (CDCl$_3$) $\delta$/ppm: 172.34, 150.53 (d, 251 Hz), 142.60 (d, 8.8 Hz), 134.63 (d, 8.8 Hz), 126.81, 115.13 (d, 5.9 Hz), 114.65 (d, 24.5 Hz), 53.17 (d, 5.9 Hz), 31.10, 28.12, 26.74, 22.78, 14.19; HRMS m/z calculated for C$_{19}$H$_{30}$F$_{2}$N$_{2}$O$_4$+ (M+H)$^+$ 369.2190, found 369.2184 (M+H)$^+$.

1.3. NMR analysis of the progress of the (NO$_2$)Aa(CO$_2$H) synthesis (Figure 2a-d)

In addition to TLC, we used $^{19}$F and $^1$H NMR to monitor the progress of the nucleophilic aromatic substitution of DFNBA and the corresponding amine. For each sample, a drop of the stirred reaction mixture was transferred into an NMR tube and diluted with about 1
ml DMSO-d6. To the samples for \(^{19}\text{F}\) NMR, \(C_6H_5CF_3\) was added for an internal reference. \(^{19}\text{F}\) NMR spectra show if the starting material, DFNBA, or the product, \((\text{NO}_2)\text{Aa(CO}_2\text{H)}\), is present. We could not observe extra \(^{19}\text{F}\) signals at early time that we could ascribe to the fluoride replaced by the amine. Since we use glass vessels for the reactions, we believe that most likely Si from the class surface reacts with the produced \(\text{F}^-\), capturing it and forming \(\text{SiF}_4\), which is a gas. While for most of the amines, the reaction appeared to be completed within a few minutes of microwave radiation, we always observe traces (< 1%) of starting material, DFNBA, within the first 15 – 30 min. When conducting the same tests at room temperature (e.g., for pyridine and \(N\)-methylbutylamine as solvents), the reaction mixture contains undissolved solid material ascribed to DFNBA. NMR tests of the clear brown reaction mixture, however, tend to show only the presence of the product and the amine. It leads us to believe, that dissolving DFNBA in the amine is a rate-limiting process; and once the difluoro reagent is dissolved it undergoes relatively fast nucleophilic aromatic substitution. Indeed, overexposure of the DFNBA/amine mixture to microwave radiation leads to formation of side products. Conversely, separation of DFNBA from the formed \((\text{NO}_2)\text{Aa(CO}_2\text{H)}\) tends to be challenging, even chromatographically. Most of the impurities from the prolonged reaction times proved to be separable from the product. Therefore, we aimed at conditions where the removal of the starting material was practically complete without compromising the yields \((\text{NO}_2)\text{Aa(CO}_2\text{H)}\) due to prolonged heating. \(N\)-ethylbutylamine and \(N\)-methylbutylamine prove most promising as nucleophiles under neat (solvent-free) conditions where the final
crude products are readily isolated in good purity without requiring column chromatography for their isolation.

1.4. Synthesis of the 5-amino-4-fluoro-N-hexyl-2-nitrobenzamide derivatives, 
(NO$_2$)Aa ((ii), Scheme 4S-1)

5-(butyl(ethyl)amino)-4-fluoro-N-hexyl-2-nitrobenzamide, (NO$_2$)Feb. (NO$_2$)Feb(CO$_2$H) (142 mg, 0.5 mmol) was placed in a baked round bottom flask with a stir bar, and blanked with N$_2$. Anhydrous DCM (3.5 ml) and 5 drops of amine-free dry DMF were added, and the reaction was cooled down in a dry ice/acetone bath. While stirring, oxalyl chloride (130 µl, 1.5 mmol) was added drop-wise and allowed to react for 30 min. The progress of the reaction was monitored using TLC, i.e., a drop of the reaction was quenched with dry methanol to form methyl ester that shows distinctly different R$_f$ values than the starting material. After the completion of the reaction, the mixture was concentrated in vacuo followed by resuspension in dry DCM (3×2.5 ml) and dried in vacuo. 1-Hexylamine (460 µl, 3.5 mmol) dissolved in dry DCM (3.5 ml) was blanketeted with N$_2$ and cooled in a dry ice/acetone bath. The carboxylic chloride was suspended in 5
ml dry DCM and added drop-wise to the cold amine solution. The reaction mixture was allowed to slowly reach room temperature. The progress of the reaction was monitored with TLC. Upon completion, the reaction mixture was dissolved in 45 ml DCM, washed with 2% HCl (3×25 ml), dried over anhydrous Na₂SO₄, and concentrated in vacuo to afford dark yellow solid. Purification using flash chromatography (stationary phase: silica gel; eluent gradient: from 100 % hexanes to 60 % hexanes / 40% ethyl acetate) produced 180 mg yellow solid (0.48 mmol, 96%) of (NO₂)Feb.

1H-NMR (CDCl₃) δ/ ppm: 7.77 (1H, d, J = 14.8 Hz), 6.61 (1H, d, J = 8.8 Hz), 5.72 (1H, t, J = 5.5 Hz), 3.41 (4H, m), 3.33 (2H, t, J = 7.8 Hz), 1.58 (4H, h, J = 7.1 Hz), 1.31 (8H, m), 1.19 (3H, t, J = 7.1 Hz), 0.92 (3H, t, J = 7.3 Hz), 0.87 (3H, t, J = 6.9 Hz); ¹³C-NMR (CDCl₃) δ/ ppm: 167.37, 149.93 (d, J = 248 Hz), 142.70 (d, J = 7.4 Hz), 133.57 (d, J = 7.4 Hz), 132.2, 114.43, 52.24 (d, J = 5.9 Hz), 47.49 (d, J = 5.9 Hz), 40.56, 31.66, 30.31, 29.31, 26.80, 22.76, 20.29, 14.21, 14.02, 13.30; HRMS m/z calculated for C₁₉H₃₁FN₃O₃⁺ (M+H)⁺ 368.2349, found 368.2344 (M+H)⁺.

4-fluoro-N-hexyl-2-nitro-5-(piperidin-1-yl)benzamide, (NO₂)Fpi. Employing the procedure for (NO₂)Feb, while starting with (NO₂)Fpi(CO₂H) (134 mg, 0.5 mmol), and using 7 ml dry DCM for making the acyl chloride, and a flash chromatography gradient
to hexanes to 80% hexanes / 20% ethyl acetate, resulted in 160 mg yellow solid (0.45 mmol, 89%) of (NO₂)Fpi. ¹H-NMR (CDCl₃) δ/ppm: 7.75 (1H, d, J = 13.4 Hz), 6.78 (1H, d, J = 8.3 Hz), 5.81 (1H, t, J = 5.5 Hz), 3.37 (2H, q, J = 6.9 Hz), 3.25 (4H, t, J = 5.4 Hz), 1.72 (4H, m), 1.62 (4H, m), 1.32 (6H, m), 0.86 (3H, t, J = 6.4 Hz); ¹³C-NMR (CDCl₃) δ/ ppm: 167.11, 152.52 (d, J = 251 Hz), 145.73 (d, J = 5.9 Hz), 136.25 (d, J = 5.9 Hz), 117.16, 113.90, 113.64, 51.06 (d, J = 5.9 Hz), 40.59, 31.64, 28.30, 26.60, 25.92, 24.22, 22.75, 14.19; HRMS m/z calculated for C₁₈H₂₇FN₃O₃⁺ (M+H)⁺ 352.2036, found 352.2031 (M+H)⁺.

4-fluoro-N-hexyl-5-(hexylamino)-2-nitrobenzamide, (NO₂)Fhx. Employing the procedure for (NO₂)Feb, while starting with (NO₂)Fhx(CO₂H) (142 mg, 0.5 mmol), and using 7 ml dry DCM for making the acyl chloride, 760 µl 1-hexylamine (5.75 mmol), and a flash chromatography gradient to hexanes to 70% hexanes / 30% ethyl acetate, resulted in 95 mg yellow solid (0.46 mmol, 92%) of (NO₂)Fhx. ¹H-NMR (CDCl₃) δ/ ppm: 7.78 (1H, d, J = 9.2 Hz), 7.41 (1H, t, J = 4.9 Hz), 7.32 (1H, d, J = 7.0 Hz), 6.26 (1H, d, J = 4.5 Hz), 3.30 (2H, t, J = 6.9 Hz), 3.10 (2H, t, J = 7.0 Hz), 1.55 (2H, h, J = 7.2 Hz), 1.44 (2H, p, J = 6.4 Hz), 1.28 (12H, m), 0.82 (6H, t, J = 7.0 Hz); ¹³C-NMR (CDCl₃) δ/ ppm: 165.12,
157.38 (d, $J = 254$ Hz), 144.89 (d, $J = 5.9$ Hz), 136.42 (d, $J = 14.7$ Hz), 130.50, 127.89, 112.95 (d, $J = 26.5$ Hz), 52.00, 39.74, 31.70, 31.55, 31.47, 29.21, 26.68, 26.39, 22.67, 22.61, 14.12, 14.04; HRMS $m/z$ calculated for $\text{C}_{19}\text{H}_{29}\text{FN}_{3}\text{O}_{3}^-$ (M-H)$^-$ 366.2193, found 366.2198 (M-H)$^-$. 

![Image](image.png)

**4-fluoro-N-hexyl-5-(hexyl(methyl)amino)-2-nitrobenzamide, (NO$_2$)Fmx.** Employing the procedure for (NO$_2$)Feb, while starting with (NO$_2$)Fmx(CO$_2$H) (285 mg, 1 mmol), and using 170 $\mu$l oxalyl chloride (2 mmol) and 7 ml dry DCM for making the acyl chloride, and 620 $\mu$l 1-hexylamine (4.7 mmol) with 5 ml dry DCM for the amide coupling, resulted in 324 mg yellow solid (0.84 mmol, 84%) of (NO$_2$)Fmx. $^1$H-NMR (CDCl$_3$) $\delta$/ppm: 7.75 (1H, d, $J = 14.8$ Hz), 6.59 (1H, d, $J = 8.6$ Hz), 6.09 (1H, t, $J = 5.5$ Hz), 3.36 (4H, m), 3.01 (3H, d, $J = 2.0$ Hz), 1.57 (4H, p, $J = 7.2$ Hz), 1.28 (12H, m), 0.83 (6H, t, $J = 6.8$ Hz); $^{13}$C-NMR (CDCl$_3$) $\delta$/ppm: 167.32, 150.11 (d, $J = 259$ Hz), 143.90 (d, $J = 7.4$ Hz), 134.02, 132.12, 114.74 (d, $J = 4.6$ Hz), 114.25 (d, $J = 27.5$ Hz), 55.07 (d, $J = 8.1$ Hz), 40.60, 40.34, 31.72, 31.67, 29.33, 28.02, 26.83, 26.65, 22.77, 14.22; HRMS $m/z$ calculated for $\text{C}_{20}\text{H}_{34}\text{FN}_{3}\text{O}_{3}^+$ (M+H)$^+$ 383.2584, found 383.2532 (M+H)$^+$. 

135
5-(dihexylamino)-4-fluoro-N-hexyl-2-nitrobenzamide. (NO$_2$)Fdx. Employing the procedure for (NO$_2$)Feb, while starting with (NO$_2$)Fdx(CO$_2$H) (250 mg, 0.67 mmol), and using 260 µl oxalyl chloride (3 mmol) and 7 ml dry DCM for making the acyl chloride, 930 µl 1-hexylamine (7 mmol) with 7 ml dry DCM for the amide coupling, and a flash chromatography gradient to hexanes to 80% hexanes / 20% ethyl acetate, resulted in 264 mg yellow solid (0.59 mmol, 87%) of (NO$_2$)Fdx. $^1$H-NMR (CDCl$_3$) δ/ppm: 7.79 (1H, d, $J = 14.9$ Hz), 6.60 (1H, d, $J = 8.7$ Hz), 5.63 (1H, t, $J = 5.6$ Hz), 3.42 (2H, q, $J = 6.8$ Hz), 3.33 (4H, t, $J = 7.5$ Hz), 1.59 (6H, m), 1.30 (18H, m), 0.87 (9H, t, $J = 6.8$ Hz); $^{13}$C-NMR (CDCl$_3$) δ/ppm: 167.40, 149.79 (d, $J = 248$ Hz), 142.78 (d, $J = 6.6$ Hz), 133.35 (d, $J = 7.7$ Hz), 132.16, 114.68, 114.40, 53.16 (d, $J = 5.6$ Hz), 40.51, 31.67, 29.30, 28.08, 26.80, 26.72, 22.76, 14.20, 14.16; HRMS m/z calculated for C$_{25}$H$_{43}$FN$_3$O$_3$ $^+$ (M+H) 452.3288, found 452.3250 (M+H)$^+$. 

1.5. Synthesis of the 5-amino-4-fluoro-N-hexyl-2-(alkaneamido)benzamide derivatives, Aa ((iii) and (iv), Scheme 4S-1)
5-(butyl(ethyl)amino)-4-fluoro-N-hexyl-2-(2-propylpentanamido)benzamide,  Feb. (NO\textsubscript{2})Feb (165 mg, 0.44 mmol) was suspended in ethyl acetate with 40 mg Pd/C. The mixture was stirred overnight under a hydrogen atmosphere at room temperature. The completion of the reduction led to a color change from yellow to colorless and appearance of blue fluorescence, which was monitored using TLC. Pd/C was filtered out and the ethyl acetate removed in vacuo. The solid was resuspended in dry DCM (5 ml), blanked with continuous flow of nitrogen and pyridine (2 ml) was added. The mixture was placed in a dry ice/acetone bath for 10 minutes and 2,2-di-\textit{n}-propylacetyl chloride (150 µl, 0.875 mmol) was added drop-wise while stirring. The reaction was allowed to reach room temperature and upon completion, as monitored using TLC, diluted in 25 ml DCM and washed with 2% HCl (2×25) and Brine (25 ml). The organic layer was collected, dried over Na\textsubscript{2}SO\textsubscript{4}, concentrated in vacuo. Purification using flash chromatography, stationary phase: silica gel; eluent gradient: from 100 % hexanes to 80 % hexanes / 20% ethyl acetate afforded 14 mg (0.04 mmol, 9%) of Feb. \textsuperscript{1}H-NMR (CDCl\textsubscript{3}) δ/ppm: 11.01 (1H, s), 8.38 (1H, d, \textit{J} = 14.8 Hz), 7.12 (1H, d, \textit{J} = 7.8 Hz), 6.16 (1H, s), 3.39 (2H, q, \textit{J}_{1} = 6.7 Hz), 3.13 (2H, q, \textit{J}_{1} = 7.0 Hz), 3.05 (2H, t, \textit{J}_{1} = 7.8 Hz), 2.26 (1H, e, \textit{J} = 4.7 Hz), 1.60 (4H, m), 1.43 (4H, m), 1.38 (8H, m), 1.25 (4H, m), 1.02 (3H, t, \textit{J}
= 7.0), 0.87 (9H, t, J = 7.0 Hz); $^{13}$C-NMR (CDCl$_3$) $\delta$/ppm: 175.55, 168.70, 158.93 (d, J = 250 Hz), 136.31 (d, J = 11.0 Hz), 131.91 (d, J = 7.7 Hz), 122.74 (d, 5.8 Hz), 116.93, 110.34 (d, J = 26.9 Hz), 53.03, 49.33, 48.13, 40.38, 35.51, 31.70, 29.71, 29.55, 26.86, 22.75, 20.96, 20.79, 14.30, 14.19; HRMS m/z calculated for C$_{27}$H$_{47}$FN$_3$O$_2$ (M+H)$_+$ 464.3652, found 464.3709 (M+H)$_+$.

4-fluoro-N-hexyl-5-(piperidin-1-yl)-2-(2-propylpentanamido)benzamide (Fpi).

Employing the procedure for Feb, while starting with (NO$_2$)Fpi (150 mg, 0.43 mmol) and using 110 µl 2,2-di-n-propylacetyl chloride (1.3 mmol), resulted in 65 mg (0.15 mmol, 36%) of Fpi. $^1$H-NMR (CDCl$_3$) $\delta$/ppm: 10.93 (1H, s), 8.39 (1H, d, J = 15.2 Hz), 7.02 (1H, d, J = 8.4 Hz), 6.29 (1H, t, J = 5.6 Hz), 3.39 (2H, td, $J_1$ = 7.2 Hz, $J_2$ = 5.8 Hz), 2.97 (4H, t, J = 5.2 Hz), 2.25 (1H, e, J = 4.9 Hz), 1.72 (4H, p, J = 5.2 Hz), 1.61 (6H, m), 1.43 (4H, m), 1.32 (8H, m), 0.88 (9H, td, $J_1$ = 7.2 Hz, $J_2$ = 4.7 Hz); $^{13}$C-NMR (CDCl$_3$) $\delta$/ppm: 175.42, 168.87, 157.93 (d, J = 251 Hz), 136.65 (d, J = 8.8 Hz), 135.28 (d, J = 14.7 Hz), 117.54 (d, 5.9 Hz), 117.22, 110.26 (d, J = 29.5 Hz), 52.50, 49.30, 40.35, 35.51, 31.67, 29.71, 26.83, 26.22, 24.25, 22.72, 20.93, 14.28, 14.19; HRMS m/z calculated for C$_{26}$H$_{43}$FN$_3$O$_2$ (M+H)$_+$ 448.3339, found 448.3383 (M+H)$_+$. 
Employing the procedure for Feb, while starting with (NO\textsubscript{2})Fhx (70 mg, 0.19 mmol) and using 50 µl 2,2-di-n-propylacetetyl chloride (0.3 mmol), resulted in 65 mg (0.14 mmol, 74%) of Fhx. \textsuperscript{1}H-NMR (CDCl\textsubscript{3}) δ/ppm: 11.50 (1H, s), 8.60 (1H, d, \(J = 12.9\) Hz), 7.46 (1H, d, \(J = 7.5\) Hz), 7.23 (1H, t, \(J = 5.7\) Hz), 6.72 (1H, q, \(J = 4.1\) Hz), 3.29 (2H, m), 3.08 (2H, m), 2.31 (1H, e, \(J = 4.8\) Hz), 1.57 (4H, m), 1.39 (4H, m), 1.29 (8H, m), 1.22 (8H, m), 0.88 (12H, t, \(J = 7.1\) Hz); \textsuperscript{13}C-NMR (CDCl\textsubscript{3}) δ/ppm: 176.86, 167.85, 162.03 (d, \(J = 198\) Hz), 141.25 (d, \(J = 11.80\) Hz), 127.73, 123.55 (d, \(J = 14.74\) Hz), 116.76, 108.78 (d, \(J = 28.0\)), 51.12, 40.44, 39.68, 35.43, 31.68, 31.63, 31.51, 29.49, 26.86, 26.56, 22.74, 22.68, 20.92, 14.27; HRMS \textit{m/z} calculated for \textit{C}\textsubscript{27}H\textsubscript{46}FN\textsubscript{3}O\textsubscript{2} (M-H)\textsuperscript{−} 462.3501, found 462.3526 (M-H)\textsuperscript{−}.

Employing the procedure for Feb, while starting with (NO\textsubscript{2})Fmx (310 mg, 0.81 mmol)
and using 300 µl 2,2-di-n-propylacetyl chloride (1.75 mmol), resulted in 284 mg (0.60 mmol 74%) of Fmx. $^1$H-NMR (CDCl$_3$) δ/ppm: 10.94 (1H, s), 8.36 (1H, d, $J = 15.6$ Hz), 7.07 (1H, d, $J = 9.0$ Hz), 6.29 (1H, t, $J = 6.0$ Hz), 3.38 (2H, q, $J = 5.8$ Hz), 3.04 (2H, t, $J = 7.8$ Hz), 2.78 (3H, s), 2.26 (1H, e, $J = 4.8$ Hz), 1.61 (4H, m), 1.42 (4H, m), 1.31 (8H, m), 1.24 (8H, m), 0.86 (12H, m); $^{13}$C-NMR (CDCl$_3$) δ/ppm: 176.30, 166.93, 157.49 (d, $J = 253$ Hz), 143.05 (d, $J = 12.16$ Hz), 125.04, 121.06 (d, $J = 10.1$ Hz), 117.14, 110.13 (d, $J = 26.5$), 58.42 (d, $J = 2.9$), 49.43, 44.82, 40.60, 35.32, 31.62, 31.20, 29.37, 26.80, 26.17, 25.69, 22.67, 22.52, 20.87, 14.21, 14.16, 13.99; HRMS m/z calculated for C$_{28}$H$_{49}$FN$_3$O$_2$ $^+$ (M+H)$^+$ 478.3809, found 478.3859, (M+H)$^+$.

![Diagram](image)

5-(dihexylamino)-4-fluoro-2-hexanamido-N-hexylbenzamide (Fdx).$^2$ (NO$_2$)Fdx (70 mg, 0.155 mmol) was suspended in 2 ml of dimethoxyethane and SnCl$_2$·2H$_2$O (210 mg, 0.93 mmol) was added. Under argon, the reaction was refluxed at 75 °C for 24 hours while stirring. The reduction led to a color change from yellow to colorless and a new fluorescent spot on TLC. Upon completion of the reduction, the reaction mixture was brought to room temperature. Hexanoic anhydride (54 µl, 0.23 mmol) and triethylamine (33 µl, 0.23 mmol) were added and the reaction was stirred for 3 hours. The mixture was diluted with 10 ml DCM and washed with saturated aqueous solution of NaHCO$_3$ (3×10

140
ml). The organic layer was collected, dried over anhydrous Na$_2$SO$_4$ and concentrated in vacuo. Purification using flash chromatography on silica gel (100% hexanes to 90% hexanes 10% ethyl acetate) produced 10 mg (0.02 mmol, 13%) Fdx. $^1$H-NMR (CDCl$_3$) $\delta$/ppm: 10.91 (1H, s), 8.33 (1H, d, $J$ = 15.2 Hz), 6.96 (1H, d, $J$ = 8.4 Hz), 6.11 (1H, t, $J$ = 5.6 Hz), 3.38 (2H, q, $J$ = 6.1 Hz), 3.03 (4H, t, $J$ = 7.5 Hz), 2.33 (2H, t, $J$ = 7.6 Hz), 1.68 (2H, p, $J$ = 7.5 Hz), 1.59 (2H, p, $J$ = 7.3 Hz), 1.38 (4H, m), 1.30 (10H, m), 1.22 (12H, m), 0.84 (12H, m); $^{13}$C-NMR (CDCl$_3$) $\delta$/ppm: 172.16, 168.80, 158.73 (d, $J$ = 252 Hz), 135.01 (d, $J$ = 12.3 Hz), 133.53 (d, $J$ = 8.7 Hz), 120.30 (d, $J$ = 5.5 Hz), 116.83, 110.32 (d, $J$ = 27.9 Hz), 53.35, 40.28, 38.57, 31.88, 31.66, 31.56, 29.67, 26.99, 26.85, 25.47, 22.83, 22.76, 22.59, 14.20, 14.13; HRMS $m$/z calculated for C$_{31}$H$_{53}$FN$_3$O$_2$ $^-$ (M-H)$^-$ 518.4116, found 518.4126 (M-H)$^-$.  

2. Methods  

2.1. Electrochemical measurements  

Cyclic voltammetry is conducted using Reference 600 Potentiostat/Galvanostat/ZRA (Gamry Instruments, PA, U.S.A.), connected to a three-electrode cell, at scan rates of 50 mV s$^{-1}$, as previously described.$^3$ Anhydrous solvents are employed for the sample preparation, with different concentrations of tetrabutylammonium
hexafluorophosphate (NBu₄PF₆) as supporting electrolyte. Prior to recording the voltammograms, the samples are extensively purged with argon while maintaining constant volume by adding more of the anhydrous solvent. For each sample and each solvent, a set of voltammograms is recorded where the electrolyte concentration is increased from 25 mM to 200 mM in steps of 25 mM. The half-wave potentials, $E^{(1/2)}$, are determined from the midpoints between the anodic and cathodic peak potentials for reversible or quasireversible oxidation. The anodic and cathodic peak potentials are determined from the zero points of the first derivatives of the voltammograms, that is, the potentials where $\partial I/\partial E = 0$ at $\partial E/\partial t =$ constant.²⁻⁴ To correct for potential drifts in the reference electrode (which is SCE, connected with the cell via a salt bridge), ferrocene was used as a standard ($E^{(1/2)} = 0.45 \pm 0.01$ V vs. SCE for MeCN, 100 mM NBu₄BF₄).⁵ Voltammograms of the standard are recorded before and after each set of measurements. From the dependence of $E^{(1/2)}$ on the electrolyte concentration, the potential for each neat solvents are estimated from extrapolation to zero electrolyte concentration (Figure 4-2b).²ᵃ,⁵⁻⁶
Supplemental References


