

生体内反応模倣型の物質生産を指向した
電気化学的ペプチド合成法の開発

**Bioinspired Electrochemical Peptide Synthesis
for Greener Production**

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略語表

DMF: *N,N*-dimethylformamide

DMA: *N,N*-dimethylacetamide

THF: tetrahydrofuran

c-Hex: cyclohexane

PG: protecting group

Fmoc: 9-fluorenylmethyloxycarbonyl

Boc: *tert*-butoxycarbonyl

^tBu: *tert*-butyl

Trt: trityl

Pbf: 2,2,4,6,7-pentmethyl-2,3-dihydrobenzofuran-5-sulfonyl

DBU: 1,8-diazabicyclo[5.4.0]undec-7-ene

DMAP: 4-dimethylaminopyridine

DIPCI: *N,N'*-diisopropylcarbodiimide

DIPEA: *N,N*-diisopropylethylamine

COMU:

(1-cyano-2-ethoxy-2-oxoethylideneaminoxy)dimethylaminomorpholinocarbenium
hexafluorophosphate

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1. 序論：ペプチド医薬品への期待と生産における課題

1-1. 分子量による医薬品の分類と各医薬品の特徴

有機合成化学や生物工学の発展は多様な化合物の生産を可能にし、様々な医薬品の開発につながっている。現在、医薬品は分子のサイズや特徴に着目して、低分子医薬品、中分子医薬品、抗体医薬品の3種類に大別されている。低分子医薬品は分子量 500 程度までの医薬品を指し、その長所として化学合成による大量生産や経口投与が可能である点が挙げられる。その一方、分子サイズが小さいことによって標的以外にも作用し、副作用を引き起こしやすいという短所がある。この短所を克服するのが抗体医薬品であり、タンパク質間相互作用を利用した厳密な分子認識を利用することで高い標的特異性を発揮し、副作用が小さい点が長所となっている。しかし、分子量が数万以上にもなる抗体を生産するには遺伝子工学的技術が必要となり、大量生産が難しく、生産コストが大きい点が短所となっている。また、抗体医薬品は経口投与では体内で分解や変性が起こってしまうため、血管を介して直接投与する必要がある。以上のように、低分子医薬品と抗体医薬品はそれぞれ補完的な長所を有する一方、短所の克服には至っていない。この課題を解決するうえで、中分子医薬品が注目を集めるようになった。中分子医薬品は、分子量が 500 から 5000 程度と化学合成可能な範囲であるとともに、低分子医薬品よりも化学構造に多様性を付与することが可能となるため、高い標的特異性を有し、大量生産可能な医薬品となることが期待されている (Figure 1)。

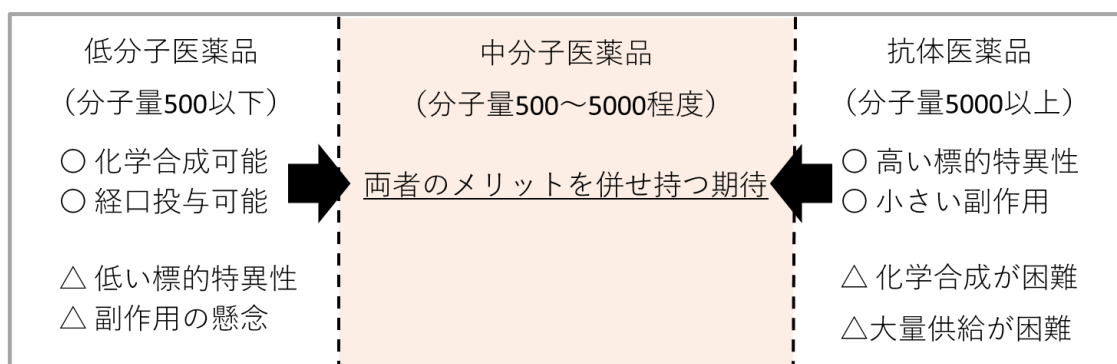


Figure 1. 医薬品の分類とそれぞれの特徴

1-2. 中分子医薬品候補としてのペプチド

中分子医薬品の候補化合物群として、ペプチドが注目を集めている¹。ペプチド医薬品は、主にホルモンや成長因子、神経伝達物質、イオンチャネルのリガンド、抗炎症因子として機能する。作用機構はタンパク質医薬品や抗体医薬品と類似しており、細胞表面のレセプターに高い親和性・特異性で結合し、細胞内反応を引き起こす。

初めてペプチド医薬品が上市されたのは1923年のインスリンであり、2018年までに60以上のペプチドが医薬品として承認されている。さらに、2018年時点で150以上のペプチドに対して臨床試験が行われ、200以上のペプチドが前臨床試験に供されている¹。医薬品市場においてもペプチド医薬品の存在感は増しており、2019年の売上額は2013年の2倍となる700億ドルとなっている^{1(a)}。構造設計や合成・修飾、薬効評価などペプチド医薬品開発にかかわる技術の発展も相まって、難病治療に有効なペプチド医薬品開発への期待はますます高まっている。

1-3. 現在主流となっているペプチド合成とその課題

ペプチド医薬品開発のうち、ペプチドの化学合成において中心的な役割を果たしてきたのは Merrifield によって開発された固相合成法である(Figure 2)²。この方法では、不溶性の樹脂にアミノ酸を担持し、N末端側の保護基の脱保護および縮合反応を繰り返すことでペプチド鎖を伸長する。伸長中のペプチドは不溶性樹脂に担持されている一方、他の試薬は液相中に存在するため、樹脂の洗浄を行うだけで精製操作が完了する。

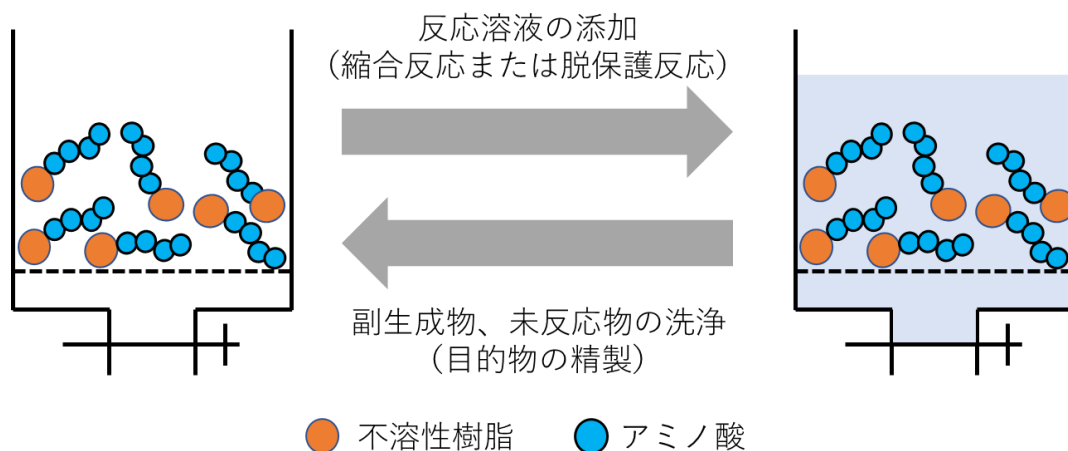
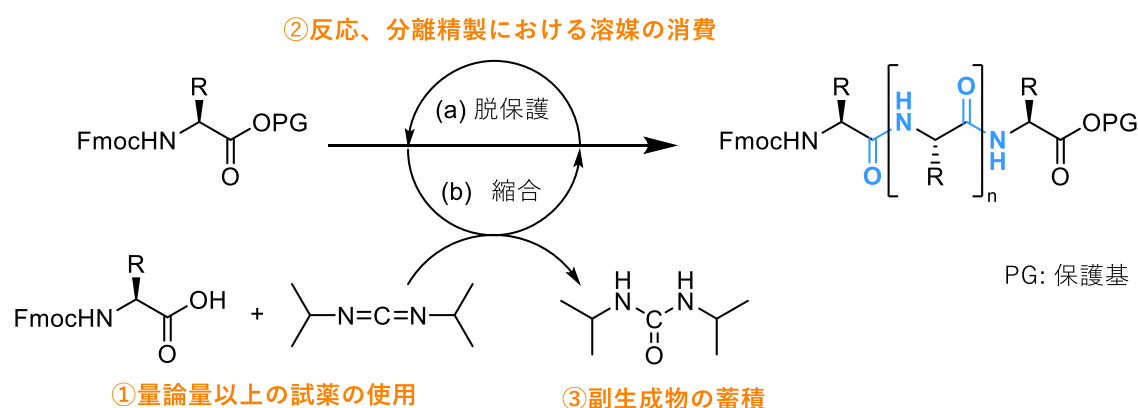


Figure 2. 固相合成法の概要

したがって、液相ペプチド合成法では避けられなかった、各反応後の分液操作やカラムクロマトグラフィーといった精製操作が必要なくなり、簡便な操作で所望のペプチドを合成できるようになった。最近では自動合成装置の開発も進み、フロー合成法との組み合わせによって 100 以上のアミノ酸からなるペプチドを数時間で合成することも可能となり³、必要なペプチドを高速で簡便に生産するという観点で、固相合成法は発明から 50 年以上たった今日でもペプチド合成の有力な手段となっている。

しかし、近年では限りある資源の効率的な活用や、環境保全・改善に貢献できる生産プロセスの開発が産業で重要視されるようになり、固相合成法をはじめとした従来のペプチド合成においても、改善が求められている。具体的には、従来の合成法では 1 kg の生理活性を有するペプチドを生産する際に 3,000~15,000 kg の廃棄物が生じるとされており、廃棄物量削減を実現するペプチド合成系の開発が求められている⁴。この目的を達成するうえで解決すべき主な課題として、①化学量論量以上の試薬の使用、②反応、分離精製における溶媒の消費、③反応後に生じる縮合剤由来の廃棄物の蓄積が挙げられる (Scheme 1)^{4d}。



Scheme 1. 従来のペプチド合成法における問題点

①については、固相合成法において特に大きな問題である。固相合成法での反応は、不溶性の樹脂に担持したペプチドと溶液中に存在する試薬間での不均一系で行われるため、液相で反応を行う場合と比較して反応効率が低くなる。そのため、液相合成法と比べてより過剰量の試薬を加える必要があり、反応後に生じる廃棄物量増加につながっている。②については、固相、液相両方に共通する課題である。固相合成においては、精製操作の簡便化には成功しているものの、反

応・精製では多くの溶媒を消費しているうえ、頻用されるのは環境負荷が大きいとされる DMF や DMA である。一方、液相合成法では分液操作やカラムクロマトグラフィーによる精製操作で固相合成法以上に多くの溶媒を消費している。③についてはペプチド合成全体における課題である。縮合剤は、ペプチド結合形成反応を効率的に進行させる試薬であり、従来のペプチド合成においては欠かせない試薬となっている。しかし、反応を完結させるためには化学量論量以上用いる必要があり、反応後には同当量の副生成物が生じる。副生成物は目的物との分離が容易となるように、水に溶解やすく回収が難しいため、アミノ酸を伸長するごとに廃棄物として蓄積していくのが現状である。また、ペプチド合成で用いられる縮合剤のなかでも、反応効率およびラセミ化抑制能が高いものは、原料から複数のステップを経て合成されるため、縮合反応は化学エネルギー消費の観点からもロスが多い過程といえる。

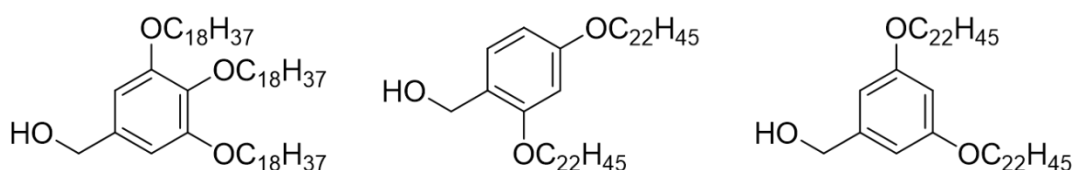
1-4. ペプチド合成で生じる廃棄物量削減への取り組み

①、②の問題解決に関しては、液相ペプチド合成法が見直され、成果を上げている⁵。1-3 で述べたように、反応効率の高さや反応に使用する試薬量の観点では液相合成法の方が有利であり、精製操作の簡便化が達成できれば固相合成法よりも廃棄物量の小さいペプチド合成法になりうる。このような背景で開発されたのが、ペプチドの溶解性をコントロールすることのできる担体である。初めに報告されたのは、ポリエチレングリコールなど、可溶性のポリマーを担体として用いたペプチド合成法である^{5c}。ポリマーに担持されているアミノ酸・ペプチドは DMF、MeCN、CH₂Cl₂、トルエンのような溶媒には溶解する一方で、水やイソプロピルアルコール中では沈殿するため、反応を液相中で行った後に貧溶媒中で沈殿させることにより、濾過操作のみで目的のペプチドを精製することができる。この特徴を生かし、最長で 10 残基のアミノ酸からなるペプチドの合成を達成した。しかし、ポリマーの分子量にばらつきがあることが原因で、MS や TLC 等を用いた反応追跡が困難になるという難点が指摘された。

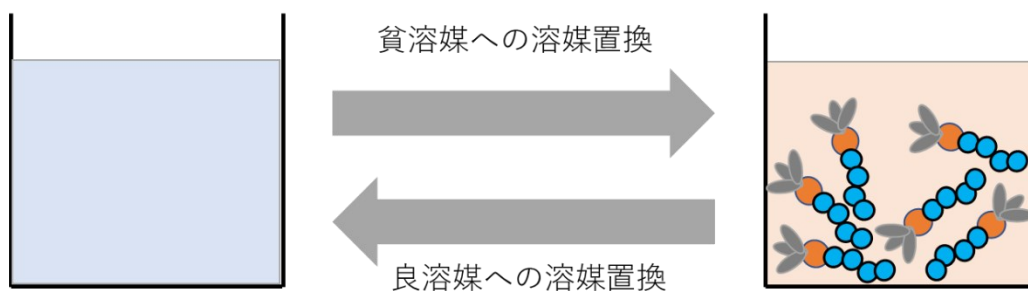
そこで新たな解決策として提案されたのが、ポリマーよりも分子量が小さい単一化合物を担体とするペプチド合成である。民秋らの報告^{5d}を参考に、千葉・岡田らは長鎖アルキル鎖を有するベンジルアルコールをカルボキシ基の保護基とするペプチド合成法を開発した(Figure 3)^{5e-l}。現在、このベンジルアルコールは可溶性疎水性タグと呼ばれ、有用性が広く認められている。可溶性疎水性タグで保護されたアミノ酸・ペプチドは THF や CH₂Cl₂、*c*-Hex には溶解する一方、



MeCN や DMF、MeOH といった溶媒には溶解せずに沈殿するため、可溶性ポリマーを担体として用いた場合と同様に濾過操作を行うだけで目的のペプチドを精製できる。さらに、保護基が単一化合物であるため、TLC や MS、NMR によって解析も行うことができる。つまり、可溶性疎水性タグを用いたペプチド合成法は、液相合成法のメリットを保持しつつ、固相合成法のメリットを利用できるペプチド合成法といえる。

(a) 可溶性疎水性タグ



(b) 可溶性担体によるペプチドの溶解性のコントロール



 可溶性疎水性担体  アミノ酸



in THF or *c*-Hex



in MeCN

Figure 3. 可溶性疎水性担体を用いた液相合成法の概要

現在もこの方法は改良が進められており、分液操作のみでペプチドを合成できる方法も開発され⁶、更なる使用試薬・溶媒量の削減が目指されている。また、可溶性疎水性タグの報告を発端として、様々な可溶性担体が開発されており、その例として味の素（株）が報告した Ajiphase[®]法が挙げられる(Figure 4)⁷。近年では、ケイ素原子を含む可溶性担体も報告され、ペプチドの溶解性を大きく向上させる機能があることが明らかとなっている⁸。以上のように、可溶性担体を利用したペプチド液相合成法の開発により、従来の液相合成法の課題であった精製操作の煩雑さを解決しつつ、使用溶媒量の削減に成功している。現在では、使用する溶媒当たりの生産性向上を目指し、より溶解性の高い可溶性担体の開発や合成プロセス検討が進められている。

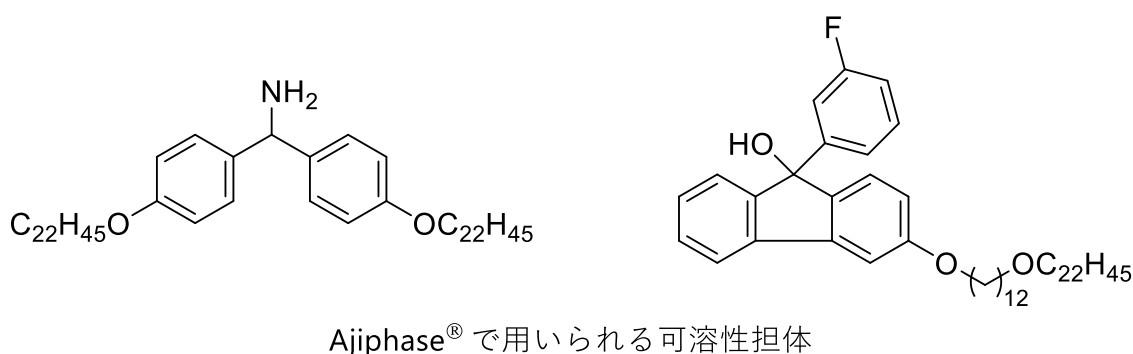
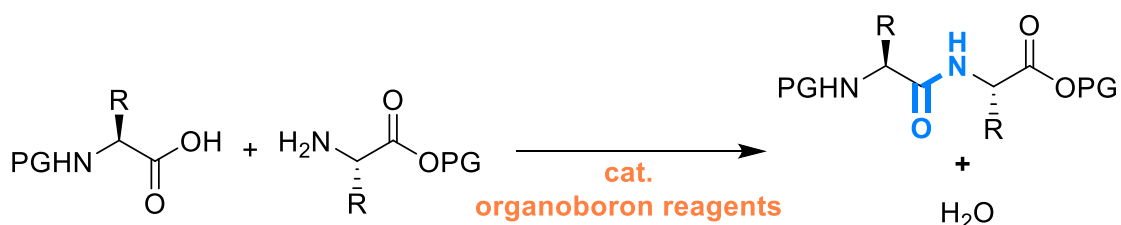


Figure 4. 可溶性担体の具体例

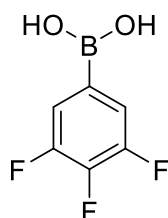
③の課題に対する解決策として主に挙げられるのは、(i)ペプチド結合形成反応を進行させることが可能な触媒の開発や、(ii)縮合剤の触媒化もしくはリサイクルである。

現在主流となっているのは(i)であり、化学量論量以上を要する縮合剤を使わずに、触媒量の試薬によってペプチド合成を実現するという戦略である(Scheme 2)。特徴としては、触媒量の試薬で反応を促進できることに加えて、副生成物がH₂Oのみである点が挙げられる。1996年に山本らが触媒量のボロン酸を用いた

アミド結合形成反応を報告して以来^{9a}、より高効率で反応を進行させることができるホウ素触媒の開発が進められている。近年では、ジペプチド合成だけでなくオリゴペプチド合成への応用も可能な触媒も報告されており^{9f,g}、より穏和な条件下で効率的に反応を行うことができる触媒開発が目指されている。

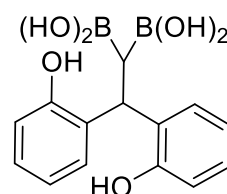


(a) ボロン酸



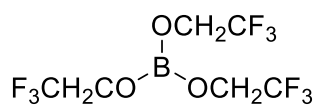
(山本 *et al.*)

(b) ジボロン酸



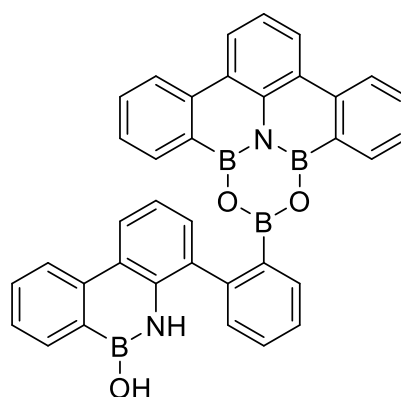
(竹本 *et al.*)

(c) ボロン酸エステル



(Sheppard *et al.*)

(d) 1,3-dioxa-5-aza-2,4,6-triborinane (DATB)



(柴崎 *et al.*)

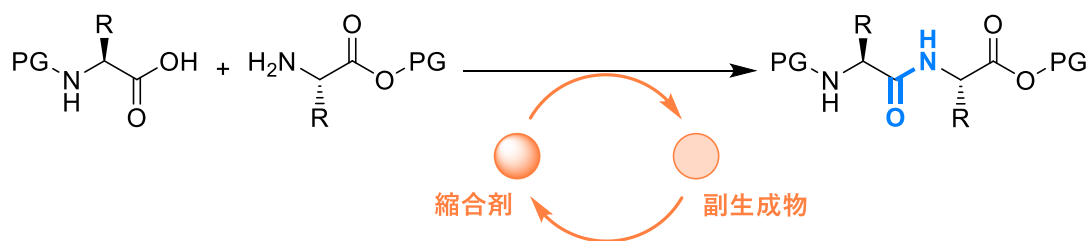
Scheme 2. 触媒的ペプチド合成の概要とホウ素触媒の例

(ii)は、効率的にペプチド結合形成反応を進行させることができるという縮合剤の特性を活かしつつ、縮合剤の使用によって生じる廃棄物の量を削減するこ

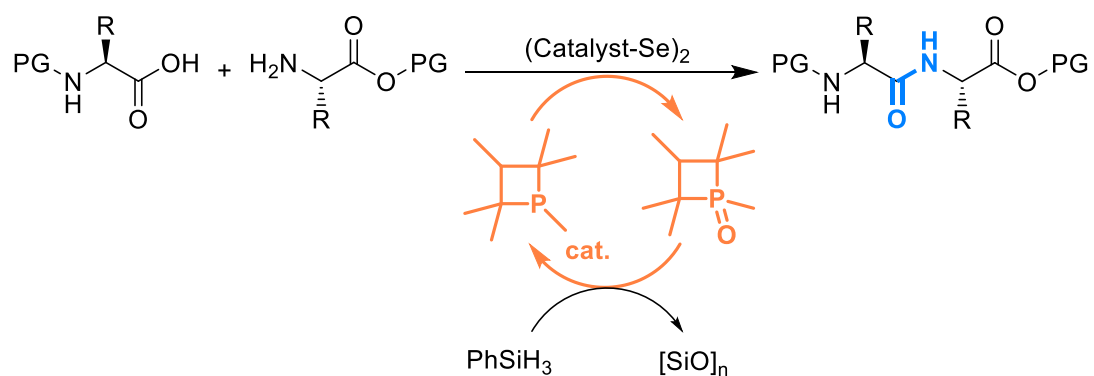
とを目指す戦略である(Scheme 3)。具体的な合成プロセスの設計は、縮合剤由来の副生成物を反応系中で縮合剤へと再生し、触媒量の縮合剤でペプチド合成を行っていくか、反応後の混合物中から副生成物を回収し、縮合剤へと変換する反応に供するかである。しかし、この戦略は従来の縮合剤から生じる副生成物の反応性の低さや回収の難しさのために、ほとんど未開拓の状況であった^{4c}。この状況を打開策するべく、反応系中での再生が可能な試薬や、反応後の副生成物の回収・再生が従来よりも容易になる試薬を用いたペプチド合成系の開発が進められ、(ii)の実現可能性が示唆されるようになった。その例として、2022年にAroraらが開発した触媒量のホスフェタンを縮合剤として利用する合成系^{10a}や、同年Wang, Sunらによって報告された5-nitro-4,6-dithiocyanatopyrimidine (NDTP)^{10b}を用いた合成系が挙げられる。Aroraらはジセレニド触媒を酸化剤とし、ホスフェタンを活性化することでペプチド結合形成反応を行うことに成功している。ホスフェタンはフェニルシラン(PhSiH₃)によって還元されることで再度縮合剤として機能するため、縮合剤の触媒化に成功している。また、Wang, SunらはNDTPを新規縮合剤として利用し、従来の縮合剤に匹敵する反応効率でペプチド合成を達成するとともに、反応後に生じた副生成物を回収して再度縮合剤へと変換することで、廃棄物量削減が可能であることを示すことに成功している。

これらの成果と並び、本研究では縮合剤由来の廃棄物量削減を実現する新規ペプチド合成法を確立することを目的として、電気とトリフェニルホスフィン(Ph₃P)を組み合わせた生体内反応模倣型のペプチド合成法の開発に取り組んだ。

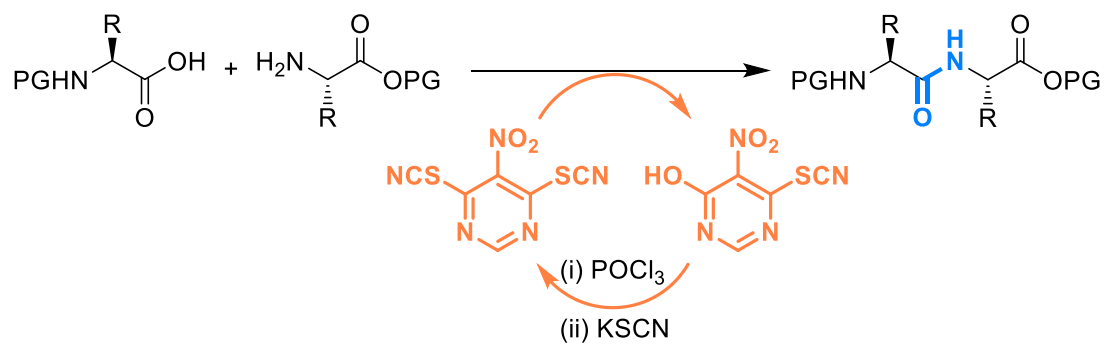
(a) 縮合剤の触媒化またはリサイクルが可能なペプチド合成



(b) 系中で縮合剤を再生するペプチド合成 (Arora *et al.*)



(c) 副生成物を回収して縮合剤へ再生するペプチド合成 (Wang, Sun *et al.*)



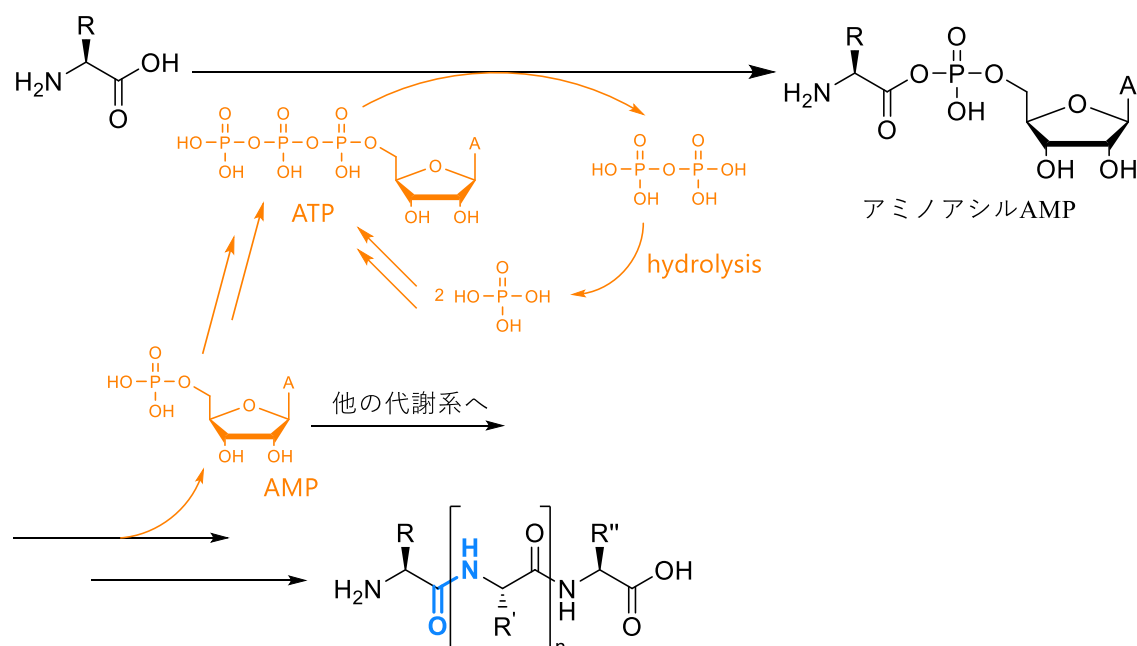
Scheme 3. 縮合剤を再利用するペプチド合成系の概要と具体例

2. 本論：有機電解反応を利用したペプチド合成法の開発

2-1 緒言：縮合剤のリサイクルが可能なペプチド合成法の設計

2-1-1 生体内のペプチド合成系

縮合剤のリサイクルが可能なペプチド合成法の設計指針として、本研究では生体内でのペプチド合成におけるアミノ酸のカルボキシ基活性化段階とその後続反応に着目した(Scheme 4)。生体内でのカルボキシ基活性化は ATP を用いたアミノアシル AMP 合成によって行われる。アミノアシル AMP 生成の際に生じる副生成物はピロリン酸であり、アミノアシル AMP が反応した後に生じる副生成物は AMP である。ピロリン酸は生成後に速やかに加水分解されてリン酸 2 分子となり、再び ATP 合成の原料となる。また AMP は ATP 合成の原料になるか、他の代謝経路に組み込まれて物質合成に利用される。つまり、生体内ではカルボキシ基活性化および後続反応で生じる副生成物が他の物質生産に利用されており、副生成物が廃棄物とならない物質生産システムが構築されている。



Scheme 4. 生体内でのカルボン酸の活性化およびペプチド結合形成反応

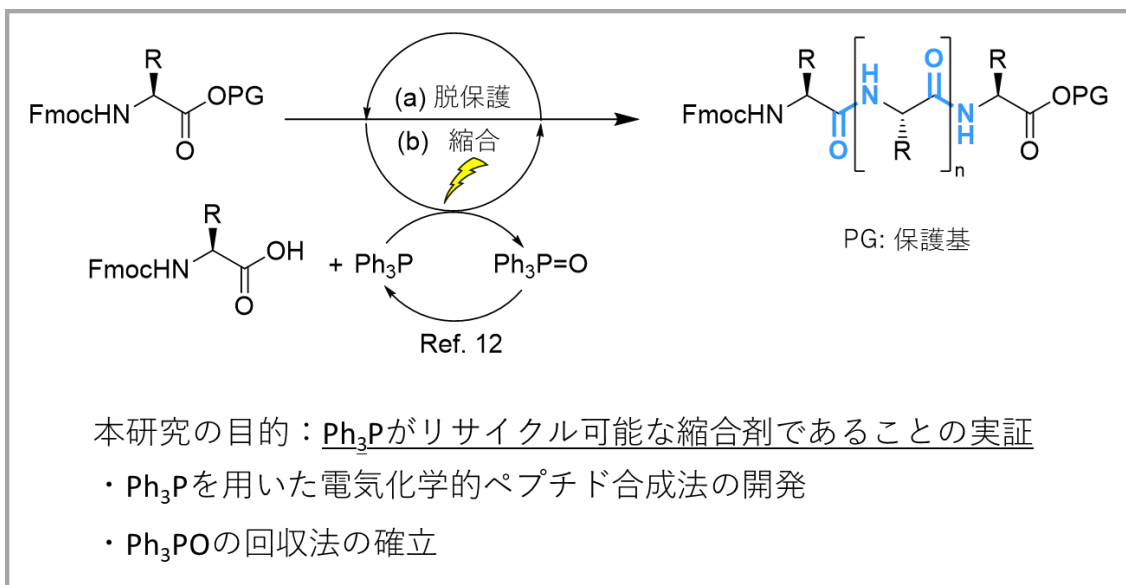
また、生体内でのカルボキシ基の活性化は、リン原子の特性を活かした、化学反応の観点からも理にかなった仕組みになっている。リン原子は酸素原子との親和性が非常に高く、リン原子と酸素原子間での二重結合形成によって C-O 結合切断を起し、種々の反応を進行させることが知られている。生体内ペプチド

合成においても同様で、P=O 結合形成による AMP の脱離が駆動力となり、アミノアシル AMP 合成以降の反応を進行させることが可能となっている。

2-1-3 生体内ペプチド合成系を模倣したペプチド化学合成系の設計

現在、廃棄物削減を目指すペプチド合成法の開発は、ペプチド結合形成触媒の開発が中心となっており、反応における使用試薬量の削減が目指されている。その一方、生体内のペプチド合成系のように副生成物が廃棄物とならない化学合成系を確立することができれば、大量に廃棄物が生じるという課題に対して新たな解決策を提案できると考えた。これを達成するうえで、リン化合物、特に Ph_3P を縮合剤とするペプチド合成系の開発は有力な戦略であると考えた。これまでに、 Ph_3P を酸化して生じる求電子種が縮合剤として機能し、アミド・ペプチド結合形成反応を進行させることが報告されている¹¹。また、反応後に生じる副生成物はトリフェニルホスフィンオキシド (Ph_3PO)であり、結晶性が高いため従来の縮合剤由来の副生成物よりも回収が簡便になることが期待される。さらに、 Ph_3PO から Ph_3P への変換は様々な方法で達成されており、高収率かつ大スケールで還元できる反応が報告されている¹²。これらを踏まえ、本研究では Ph_3P を用いたペプチド合成法と、反応後の混合物中から Ph_3PO を回収する方法を確立することで、 Ph_3P を用いたペプチド合成法では縮合剤由来の廃棄物削減が可能であることの実証を目指すこととした。

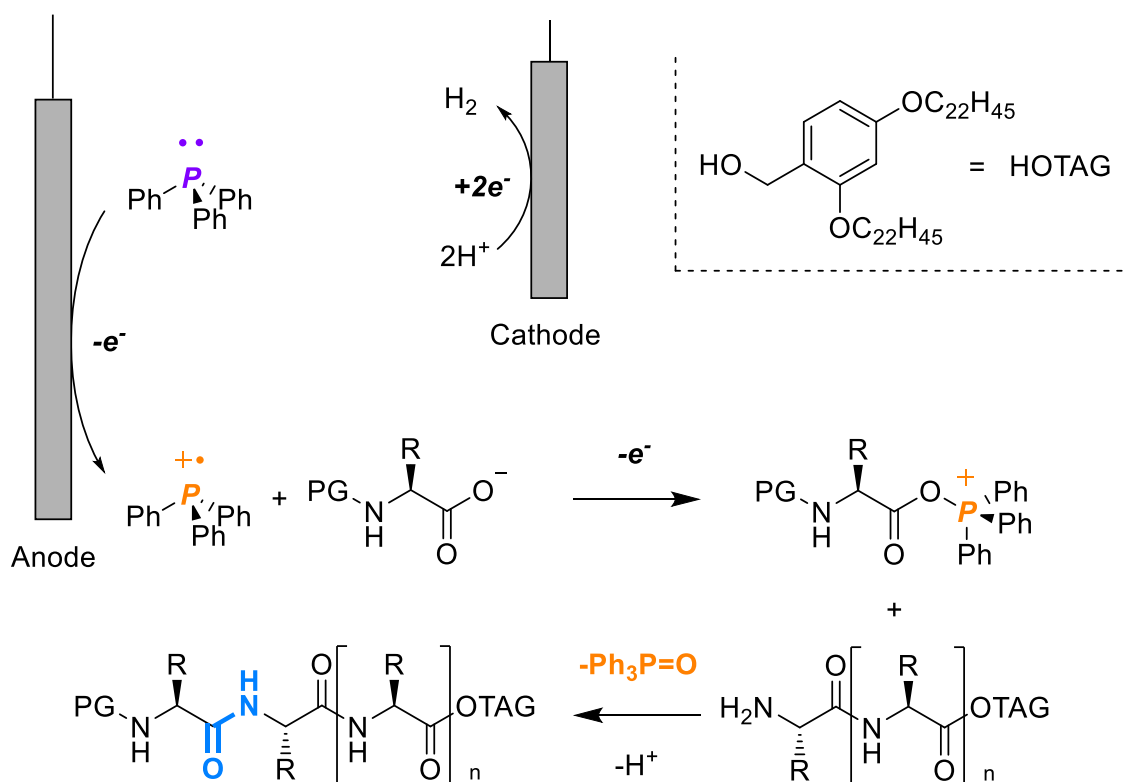
Ph_3P を酸化する過程については、有機電解反応での陽極酸化を利用することとした^{12c}。これにより、化学酸化剤を使うことなく Ph_3P を活性化できるようになり、化学酸化剤の使用量削減が可能となることに加えて活性化に必要な化学エネルギーを電気エネルギーに代替することが可能となる¹³。また、千葉らが開発してきた可溶性疎水性タグをカルボキシ基の保護基とし、反応後の精製操作の簡便化を図った。これにより、濾過操作のみでペプチドの精製が可能となることに加え、極性溶媒に溶解している Ph_3PO との分離も完了し、続いて行う Ph_3PO の精製操作の簡便化も期待される。



Scheme 5. 本研究で目指すペプチド合成系

2-1-4 ペプチド結合形成反応の設計

陽極酸化と Ph_3P を組み合わせたペプチド結合形成反応は Scheme 6 のように進行すると考えられる。まず、陽極上で Ph_3P が一電子酸化を受け、トリフェニルホスフィンラジカルカチオン ($\text{Ph}_3\text{P}^{\bullet+}$) が生成する。 $\text{Ph}_3\text{P}^{\bullet+}$ はカルボン酸と反応し、続く一電子酸化を経て活性カルボン酸中間体が生成する。この中間体に対してアミンが求核攻撃し、四面体中間体から Ph_3PO が脱離することでペプチド結合が形成される。陽極では Ph_3P および中間体の酸化が行われる一方で、陰極ではカルボン酸及び中間体由来のプロトンの還元が行われ、水素が生成する。



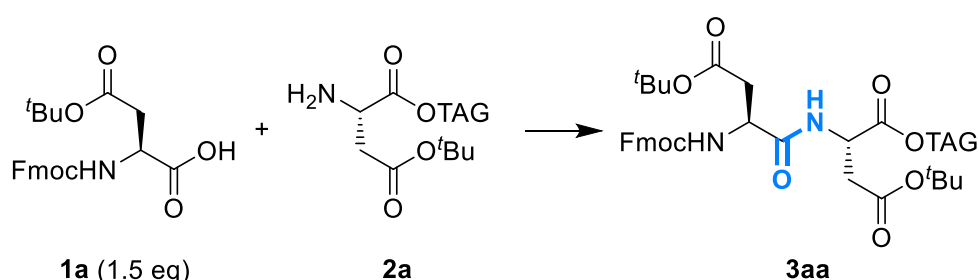
Scheme 6. 電気化学的ペプチド合成の推定反応機構

2-2 反応条件の最適化

Fmoc-Asp(O^tBu)-OH (**1a**)と H_2N -Asp(O^tBu)-TAG (**2a**)によるジペプチド合成をモデル反応として、電解質溶液の最適化を行った(Table 1)。**2a**を基準として、 Ph_3P を2.0当量、塩基として2,6-lutidineを3.0当量、0.50 Mの電解質を用いて4.8 F/molを通電し、電流密度は 0.67 mA/cm^2 とした。溶媒を CH_2Cl_2 としたとき、**2a**は消失し収率92%で目的物が得られた。一方、THFを溶媒として用いた場合には、収率が大きく減少し(19%)、未反応の**2a**が残存していた。また、*c*-HexとMeCNを用いた二相系溶媒中でも**2a**は消失し、**3aa**を収率95%で与えた。この

反応系においては、*c*-Hex と MeCN は攪拌を行わないと混合せず、可溶性疎水性タグで保護された **2a** は *c*-Hex に選択的に溶解する一方、支持電解質や **1a** のような極性の高い試薬は MeCN 層に溶解する。反応中は攪拌することで、均一系溶媒同様に反応を行うことができる。反応後、**3aa** は **2a** と同様に *c*-Hex 層に選択的に溶解しているため、*c*-Hex を用いて抽出操作を行うことで粗精製を行うことができる。Ph₃PO は MeCN 層に溶解するため、抽出段階でペプチドと Ph₃PO を分離できるようになり、より簡便な精製操作が可能となった (Figure 5)。

Table 1. 電解ペプチド結合形成反応の最適化



Conditions: Ph₃P (2.0 eq), 2,6-lutidine (3.0 eq), supporting electrolyte (0.05 M), platinum electrodes, 2.0 mA, 4.8 F/mol, rt, undivided cell.

Entry ^a	Electrolyte Solution	Yield (%) ^a
1	Bu ₄ NClO ₄ /CH ₂ Cl ₂	92
2	Bu ₄ NClO ₄ /THF	19
3	Bu ₄ NClO ₄ / <i>c</i> -Hex/MeCN	95 ^b
4	LiClO ₄ / <i>c</i> -Hex/MeCN	86
5	NaClO ₄ / <i>c</i> -Hex/MeCN	- ^c
6	KClO ₄ / <i>c</i> -Hex/MeCN	55

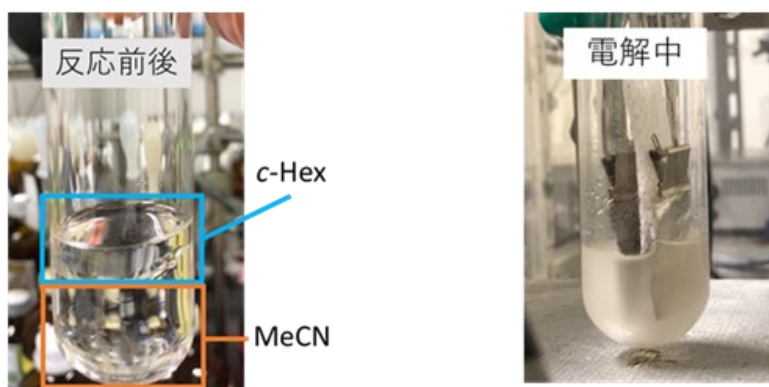
^aDetermined by NMR analysis. ^bIsolated yield.

^c Severe gelation was observed before the reaction.

また、Entry 3-6 では、支持電解質のカウンターカチオンの影響を検証した。LiClO₄ を用いた場合、収率が 86% に低下した (Entry 3)。また、NaClO₄ を用いると、通電前に白色の不溶物が生じて攪拌が困難となり、反応に供することができなかった (Entry 4)。**1a** と混合すると不溶物が生じたことから、Fmoc-Asp(O^tBu)-O⁻Na⁺ の生成が原因であると考えられる。KClO₄ を用いた際は、不溶物は生じな

かったものの、反応溶液の粘性の増加が見られ、収率は 55%まで低下した。これらの検討から、金属カチオンは **1a** と相互作用し、反応性を低下させることが示唆された。一方で、テトラブチルアンモニウムカチオンは立体障害が大きく、カルボキシ基との相互作用が小さいことから **1a** の反応性への影響が小さいことが考えられる。以上の結果から Bu_4NClO_4 を最適な支持電解質とし、Entry 3 の条件を最適条件として以後の検討を行った。

(a) c-Hex-MeCN二相系での電解ペプチド結合形成反応



(b) 反応後の目的のペプチド精製操作

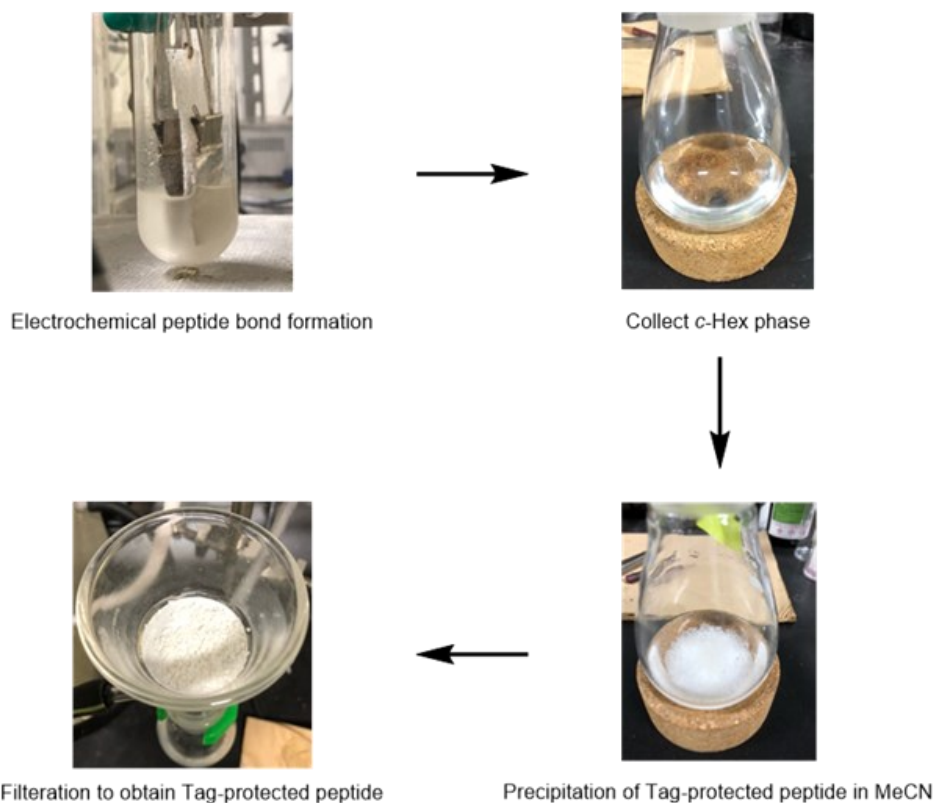


Figure 5. 最適条件下での電解ペプチド結合形成反応と精製操作

2-3 ペプチドの精製および Ph₃PO 回収プロセスの最適化

Entry 3 の条件で反応を行った後、**3aa** を精製した際の濾液 (MeCN) と反応後に *c*-Hex 層と分離した MeCN 層を合わせ、主に Ph₃PO、Bu₄NClO₄、Fmoc-Asp(O^tBu)-OH、2,6-lutidine で構成される混合物中から Ph₃PO を精製する方法の確立に取り組んだ。

まず、活性炭を用いて構造不明の色素化合物を除去し、濾過操作により無色の MeCN 溶液を得た。次に、MeCN をトルエンに置換することで、Bu₄NClO₄ のみを白色固体として析出させ、濾過操作によって定量的かつ高純度で回収した。濾過操作後のトルエン溶液に対し、DBU および piperidine を加えることで Fmoc 基の脱保護を行って H₂N-Asp(O^tBu)-OH を生成させ、水溶性を向上させた。最後に、塩酸溶液を用いて分液操作を行うことで、アスパラギン酸及び 2,6-lutidine を除去し、有機層を濃縮した後に *c*-Hex-EtOAc 混合溶媒で再結晶を行うことで、Ph₃PO を 91% で回収することに成功した。また、Bu₄NClO₄、Ph₃PO とともに高純度で回収できたことを ¹H NMR で確認した(Figure 6, 7)。

2-2 および 2-3 の検討から、陽極酸化によって Ph₃P を活性化して縮合剤として利用する反応は、アミノ酸同士の反応にも利用可能で、また反応後の混合物から Ph₃PO を高純度で回収することができたことから、Ph₃P を利用した電気化学的ペプチド合成は、縮合剤由来の副生成物が廃棄物とならないペプチド合成系となりうることを実証できた。

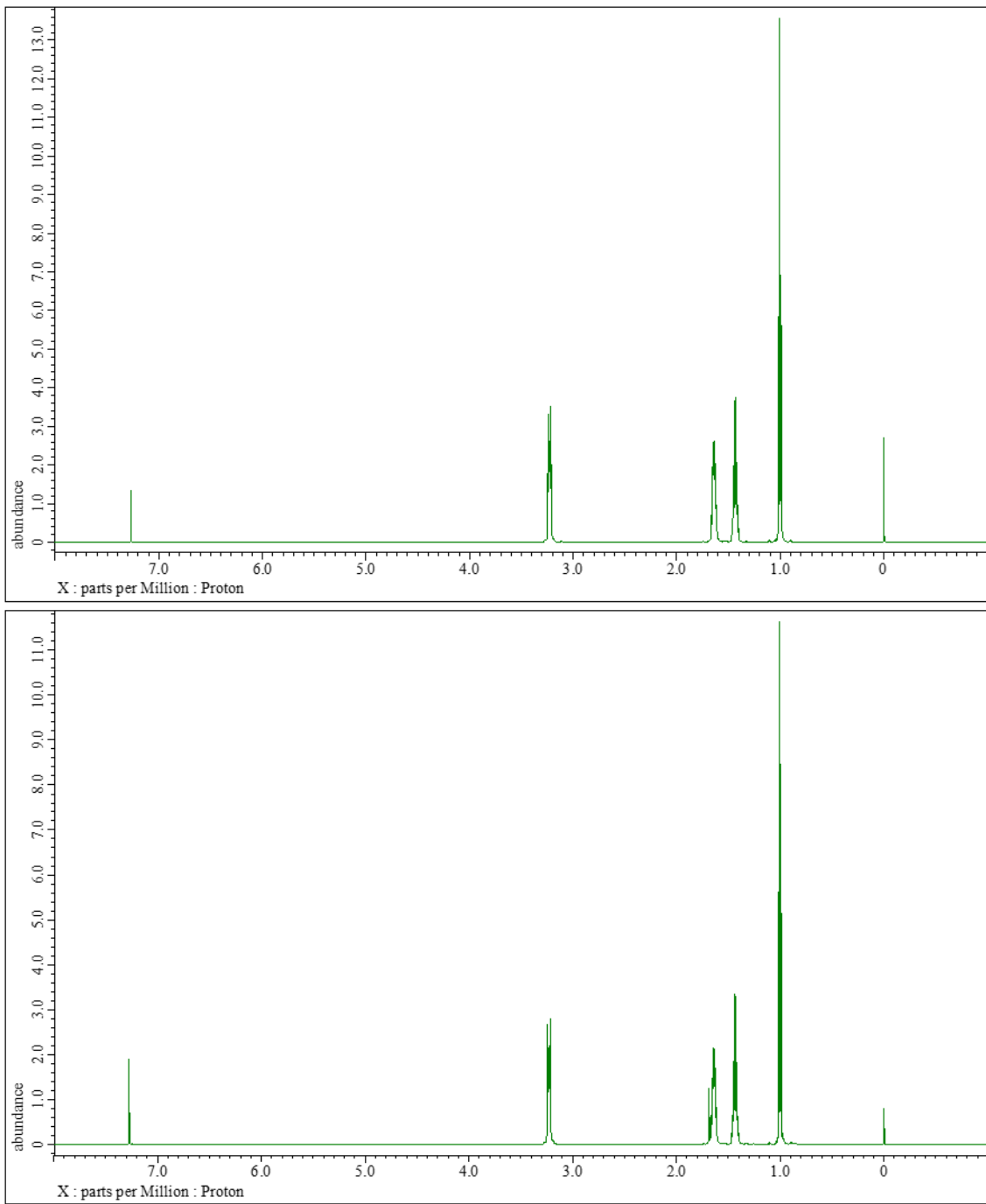


Figure 6. 標品の Bu_4NClO_4 (上)と精製後の Bu_4NClO_4 (下)の ^1H NMR の比較

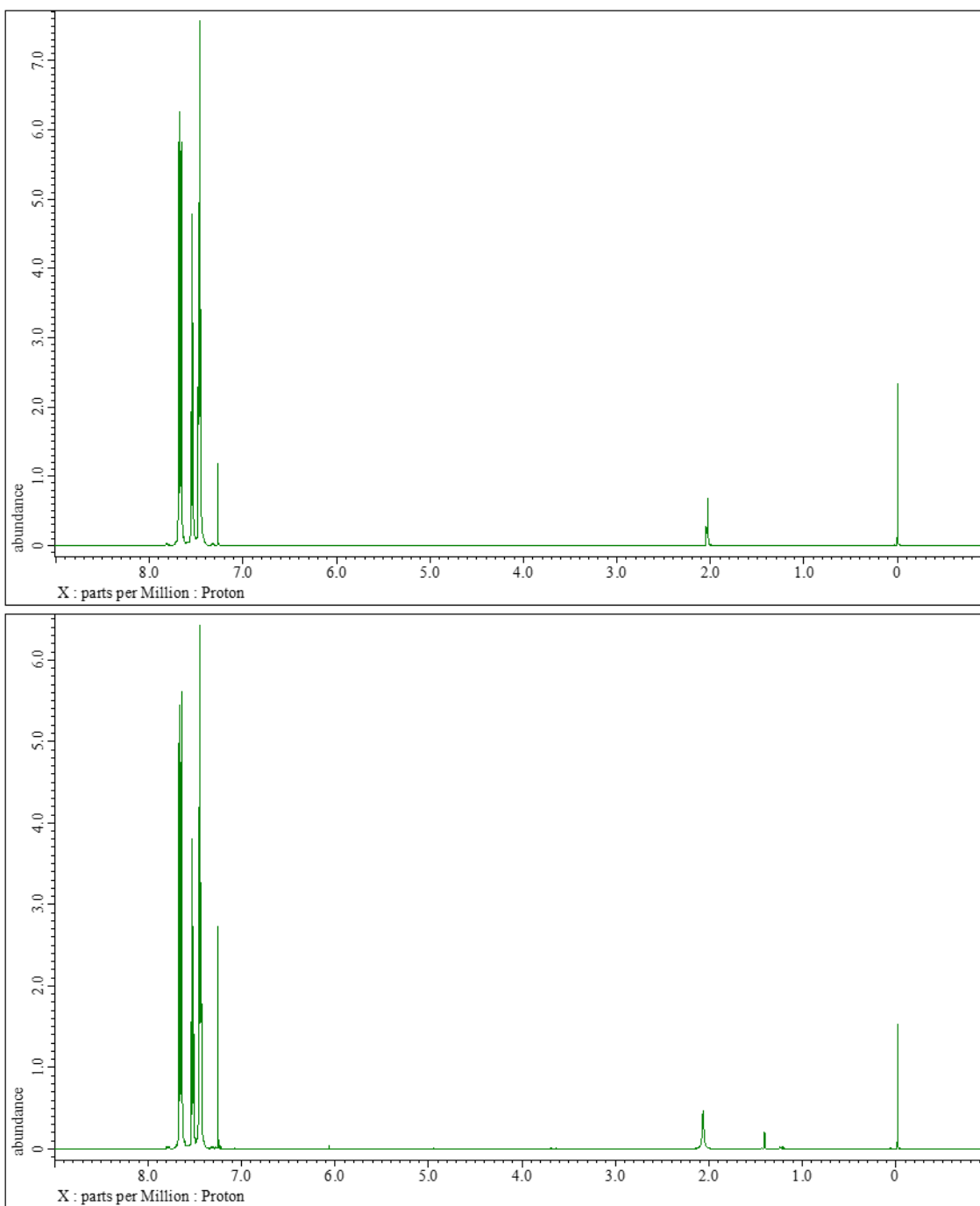


Figure 7. 標品の Ph_3PO (上)と精製後の Ph_3PO (下)の ^1H NMR の比較

2-4 電気化学的ペプチド結合形成反応の適用範囲の探索

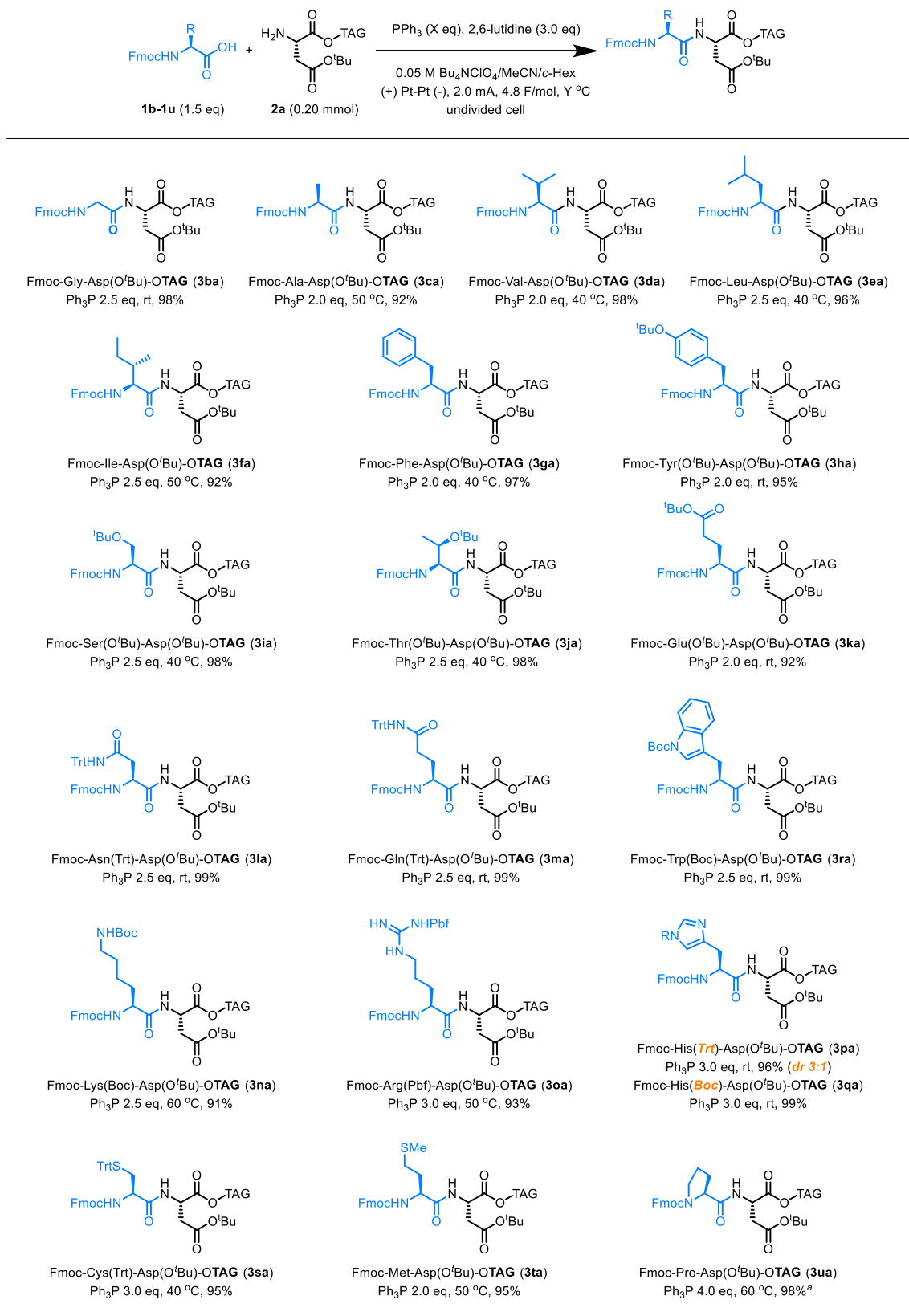
Ph₃P を用いた電気化学的ペプチド合成法では、副生成物の再利用が可能であることを確認したうえで、本法が **1a**、**2a** 以外のアミノ酸に対しても適用可能か検討した。純度の高いペプチドを大量合成するには、各反応が完結していることが前提となるため、各アミノ酸について反応完結に至る条件の再検討を行った。なお、本研究における反応の完結は、通電後の TLC 解析で H₂N-X-Tag (**2a-2u**) のスポットが消失し、すべて目的物に変換されていることを指す。

はじめに、Fmoc 基でアミノ基を保護したアミノ酸の適用範囲探索を行った (Table 2)。Fmoc-Gly-OH (**1b**) を用いた際には、Ph₃P を 2.5 当量とすることで反応が完結し、目的のジペプチドが収率 98% で得られた。また、側鎖が炭化水素基のみで構成されているアミノ酸 (**1c-1g**) については、まず Fmoc-Ile-OH (**1f**) に対して **2-3** での最適条件を適用した。その結果、通電の途中で溶液のゲル化がみられ、さらに反応は完結していなかった。ゲル化による攪拌効率低下が反応効率の低下につながったと考えられ、加熱によってゲルが解消されることを見出したため、加熱条件下で電解反応を行って反応効率の向上を図った。40 °C に昇温した場合、ゲル化は改善されたものの TLC によって原料の残存を確認したため、反応温度を 50 °C、Ph₃P を 2.5 当量として通電を行った。その結果、反応は完結し、目的のペプチドが収率 92% で得られた。この結果を踏まえて Fmoc-Ala-OH (**1c**)、Fmoc-Val-OH (**1d**)、Fmoc-Leu-OH (**1e**)、Fmoc-Phe-OH (**1g**) に対しては加温しながら電解反応を行った。温度や Ph₃P の当量についてはさらなる検討を要したが、ゲル化を抑制して反応を完結させることに成功し、目的のジペプチドが高収率で得られた。Fmoc-Tyr(O^tBu)-OH (**1h**) を用いた際は、**2-3** で見出した最適条件下で反応が完結した (収率 95%)。Fmoc-Ser(O^tBu)-OH (**1i**) と **2a** の反応においては、室温下でゲル化による反応効率の低下が見られ、**2a** の残存を確認した。そこで 40 °C に加熱したうえで Ph₃P を 2.5 当量、**1i** を 2.0 当量用いたところ、反応の完結を確認するとともに、目的のジペプチドが収率 98% で得られた。この条件は **1i** と同様に側鎖のヒドロキシ基が *tert*-ブチル基で保護された Fmoc-Thr(O^tBu)-OH (**1j**) に対しても適用可能だった (収率 98%)。**1a** 同様に側鎖に *tert*-ブチルエステル基を有する Fmoc-Glu(O^tBu)-OH (**1k**) と **2b** による縮合反応は、**1a** と同条件で完結し、目的のジペプチドを収率 92% で与えた。また、側鎖にアミド基を有する Fmoc-Gln(Trt)-OH (**1m**) を **2-3** で見出した最適条件に供したところ、溶液のゲル化は見られなかったが、反応の完結には至らなかった。そこで、Ph₃P

を 2.5 当量用いたところ、**2a** がすべて **3ma** に変換されたことを確認し (収率 99%)、この条件は Fmoc-Asn(Trt)-OH (**1l**) (収率 99%)、Fmoc-Trp(Boc)-OH (**1r**) に対しても適用可能だった (収率 99%)。Fmoc-Lys(Boc)-OH (**1n**) を用いた場合は、室温条件下ではゲル化が見られ、反応は完結していなかった。60 °C に加熱するとゲル化が解消されたため、60 °C に加熱して Ph₃P を 2.5 当量用いたところ、反応が完結して目的物が 91% で得られた。また Fmoc-Arg(Pbf)-OH (**1o**) を室温条件下で電解反応に供したところ、**1n** を用いた場合と同様にゲル化が見られ、**2a** の残存を確認した。この結果を受け、50 °C に加熱し、Ph₃P を 3.0 当量用いることで、反応を完結させることができた (収率 93%)。もう一つの塩基性アミノ酸であるヒスチジンをを用いた縮合反応においては、エピメリ化の問題に直面した。Fmoc-His(Trt)-OH (**1p**) を用いた場合、室温条件下でゲル化は見られなかったが、Ph₃P を 2.5 当量用いた条件では反応が完結しなかった。そこで Ph₃P を 3.0 当量を増やして電解反応に供したところ、反応は完結したが、25% のエピメリ化を NMR で確認した。これは側鎖のイミダゾール部が塩基としてはたらいだためであると考え¹⁴、イミダゾール部の N^ε の保護基をより電子求引性の高い Boc 基 (**1q**) に変えて塩基性の低下を図った。この結果、エピメリ化を起こすことなく反応を進行させることができた (収率 99%)。続いて、側鎖に酸化を受けやすい硫黄原子を有するアミノ酸について検討を行った。Fmoc-Cys(Trt)-OH (**1s**) については 40 °C に加熱し、Ph₃P を 3.0 当量用いることで反応完結に至り、目的のジペプチドが収率 95% で得られた。Fmoc-Met-OH (**1t**) では、室温条件下での溶液のゲル化及び **2a** の残存を確認したため、50 °C に加熱して反応に供することで、反応を完結させることができた (収率 95%)。**1s**、**1t** のどちらを用いた場合にも硫黄原子の酸化は見られず、目的のジペプチドを高収率で得ることができた。環状アミノ酸である Fmoc-Pro-OH (**1u**) では反応効率が大きく低下し、**2-3** で見出した最適条件下では収率 39% (1,1,2,2-テトラクロロエタンを内標とした NMR による収率計算) であった。温度や試薬の当量の再検討を行った結果、60 °C に加熱したうえで、Ph₃P を 4.0 当量、**1u** を 2.5 当量、支持電解質として NaClO₄ を用いることで反応の完結を確認し、収率 98% で **3ua** が得られた。

以上の結果から、本法は生体内のペプチドを構成する 20 種の基本的なアミノ酸すべての Fmoc 保護体に適用可能であり、アミノ酸側鎖の官能基による副反応を起こすことなく目的のジペプチドが得られることが明らかになった。

Table 2. Fmoc アミノ酸の適用範囲探索

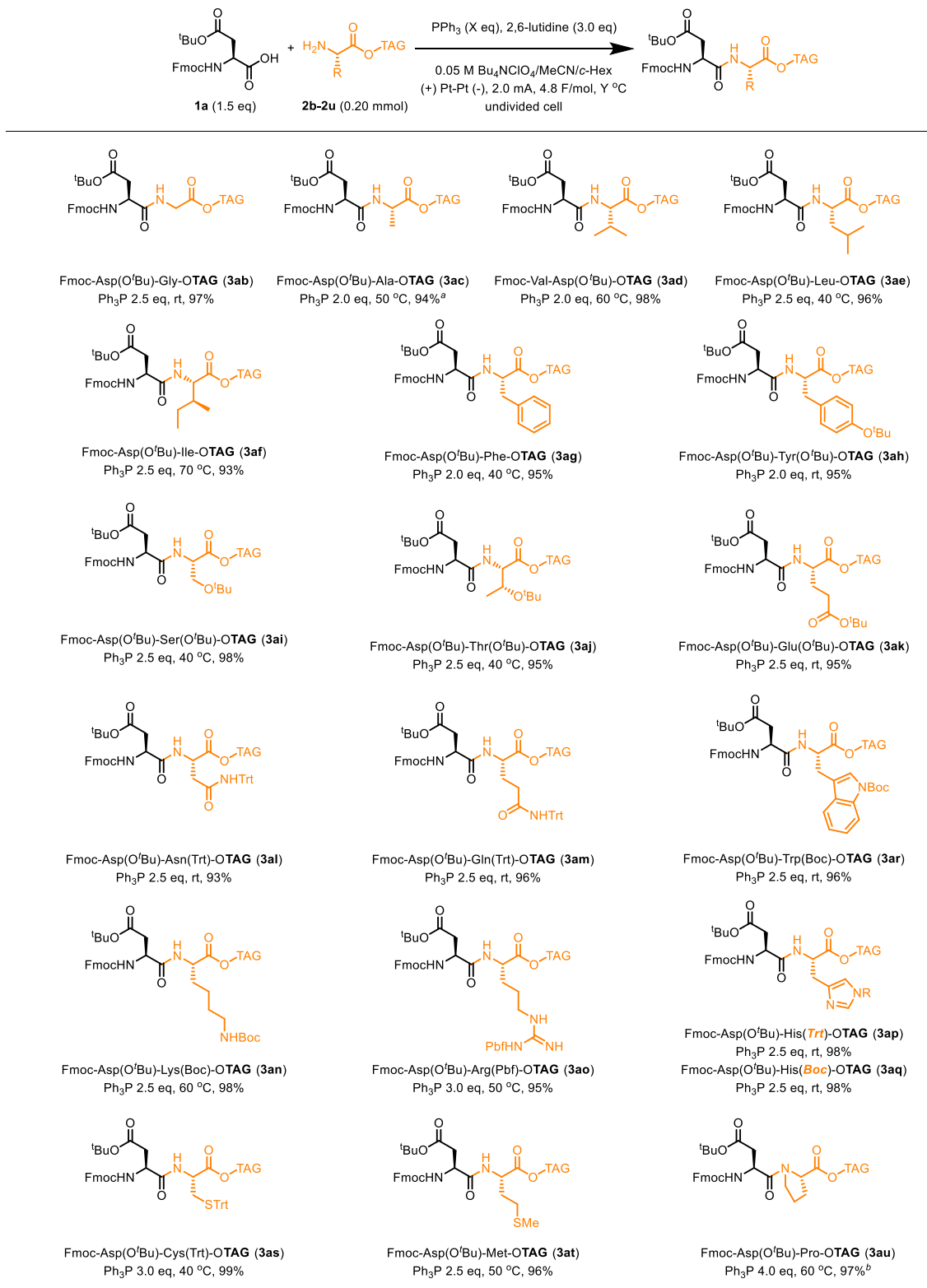


^aNaClO₄ was used instead of Bu₄NClO₄ and 6.0 F/mol was passed.

次に、可溶性疎水性タグで保護したアミノ酸の適用範囲検討を行った(Table 3)。反応条件は、Fmoc アミノ酸 (**1a-1u**)の適用範囲探索で見出した条件を、**1b-1u** と同じ側鎖を有する求電子剤 (**2b-2u**)それぞれに適用した。H₂N-Ala-Tag (**2c**)を反応に供した際には、**1a** を 2.0 当量にする必要があった。また、H₂N-Val-Tag (**2d**)、H₂N-Ile-Tag (**2f**)を用いた際にはゲル化を防ぐために更なる昇温が必要となり、それぞれ 60 °C、70 °C に加熱することで反応が完結し、目的のジペプチドが高収率で得られた。H₂N-Glu(O^tBu)-Tag (**2k**)を用いた際は、Ph₃P を 2.5 当量に増やすことで反応が完結し、**3ak** が収率 95%で得られた。H₂N-His(Trt)-TAG (**2p**)、Fmoc-His(Boc)-OH (**2q**)については Ph₃P を 3.0 当量から 2.5 当量に減らしても反応が完結することが明らかになり、どちらを用いても目立ったエピメリ化は見られなかった。H₂N-Met-TAG (**2t**)を用いた場合、2.5 当量の Ph₃P を要した(収率 98%)。H-Pro-OTAG (**2u**)を用いる際は、Fmoc-Asp(O^tBu)-O⁻Na⁺の生成を防ぐために支持電解質を NaClO₄ から Bu₄ClO₄ に変更したが、反応は完結して目的物 **3au** が 97% で得られた。その他のアミノ酸については、反応条件の変更なく反応が完結し、目的のジペプチドを高収率で得ることができた。

以上の検討結果から、本法は生体内のペプチドを構成する 20 種の基本的なアミノ酸すべてに適用可能であることが明らかになった。また、アミノ酸側鎖の官能基の保護基は従来のペプチド合成で用いられている保護基が適用可能であることも示された。したがって、本法で合成したペプチドの脱保護は、従来のペプチド合成で適用されている酸性条件に供することによって可能であることが示唆された。

Table 3. TAG アミノ酸の適用範囲探索



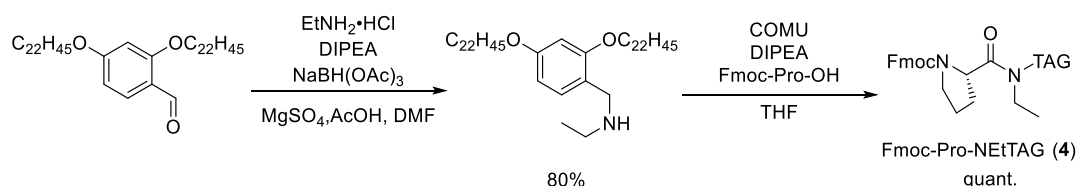
^a2.0 eq of **1a** was used., ^b2.5 eq of **1a** was used and 6.0 F/mol of electricity was passed.

2-5 電気化学的ペプチド結合形成反応を応用したオリゴペプチド合成

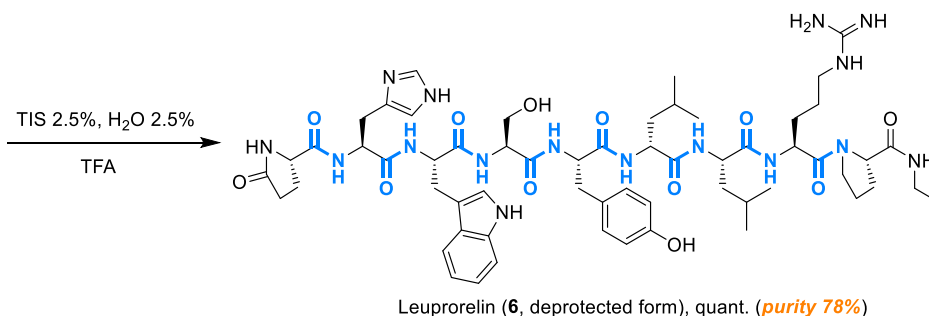
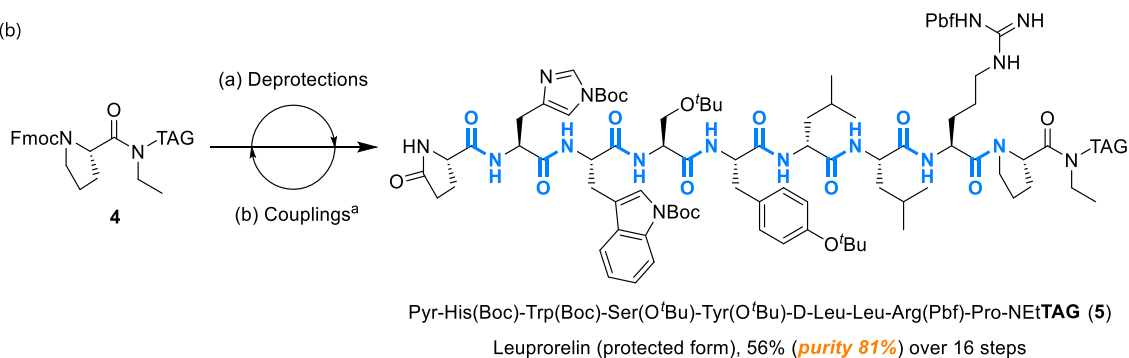
Ph_3P を用いた電気化学的ペプチド結合形成反応が、20種の基本的なアミノ酸すべてに適用可能であることを確認し、目的のジペプチドが高収率で得られたので、続いてオリゴペプチド合成への応用を試みた(Scheme 7)。モデルペプチドは、アミノ酸 9 残基から構成され、すでに乳がんや前立腺がんの治療薬として用いられている leuprorelin¹⁵ とした。

Leuprorelin の C 末端側がエチルアミドとなっていることに着目し、出発物は酸による全脱保護後に直接エチルアミド体が得られる **4** とした。**4** は可溶性疎水性タグのアルデヒド体を還元的アミノ化に供してエチルアミノ体を合成しその後 Fmoc-Pro-OH と縮合することで得られた(Scheme 7(a))。

(a)



(b)



a: Conditions: (a) DBU (1.3 eq), piperidine (1.5 eq), rt, THF; (b) PPh_3 (3.0 eq), 2,6-lutidine (3.0 eq), 40 °C, 0.05 M Bu_4NCl_4 /c-Hex/MeCN, platinum electrodes, 2.0 mA, 4.8 F/mol.

Scheme 7. (a) 出発物質の合成、(b) Leuprorelin の電気化学的合成

電解ペプチド結合形成反応については、通電途中でのゲル化による反応効率低下を防ぐために 40 °C に昇温することとし、Scheme 7 に示した条件を基本条件とした。しかし、1 段階目の Fmoc-Arg(Pbf)-OH と **4** の条件では反応が完結しなかったため、各試薬の当量および通電量、温度の検討を行った。その結果、Fmoc-Arg(Pbf)-OH を 3.5 当量、Ph₃P を 5.0 当量用いて通電量を 6.0 F/mol とし、60 °C に加熱することで反応の完結を確認した。3 残基目の Fmoc-Leu-OH から 5 残基目の Fmoc-Tyr(O^tBu)-OH までの伸長は基本条件を適用することで反応が完結したが、6 残基目の Fmoc-Ser(O^tBu)-OH を伸長する際に反応が完結せず、求核剤の残存を確認した。

本研究では、各ステップでの反応を完結させ、純度の高いオリゴペプチドを合成することを目指しているため、Fmoc-Ser(O^tBu)-OH 以降の反応条件を見直したうえで、化合物 **4** から leuprorelin への電解合成をやり直すこととした。反応条件の見直しについては、(i) Fmoc-Ser(O^tBu)-OH と H₂N-Tyr(O^tBu)-OTAG、(ii) Fmoc-Trp(Boc)-OH と H₂N-Ser(O^tBu)-OTAG、(iii) Fmoc-His(Boc)-OH と H₂N-Trp(Boc)-OTAG、(iv) Pyr と H₂N-His(Boc)-OTAG による電解ペプチド結合形成反応を行い、反応が完結する条件を探索した。(i)については、Fmoc-Ser(O^tBu)-OH を 3.0 当量、Ph₃P を 4.0 当量、通電量を 6.0 F/mol とし、60 °C に加熱することで反応が完結した。(iv)については、Pyr を 3.0 当量、Ph₃P 4.0 当量、通電量を 6.0 F/mol とし、60 °C へ昇温することで反応の完結を確認した。(ii)、(iii)については基本条件下で反応が完結した。

これらの検討で見出した反応条件を用いて、化合物 **4** を出発物として Fmoc 基の脱保護および電解ペプチド結合形成反応を繰り返すことで、leuprorelin の保護体を計 16 ステップ、crude 収率 56%で合成することに成功した(Scheme 7(b))。HPLC 解析の結果、純度は 81%となったため、総収率は 45%であった。したがって、各反応段階での平均収率は 95%であり、オリゴペプチド合成であっても反応効率の大幅な低下は見られなかった。最後に、従来のペプチド合成でも行われる酸性条件下での全脱保護を行うことで、leuprorelin が定量的に得られた(Figure 8)。

以上の結果から、本法はジペプチド合成に限らず、オリゴペプチド合成にも適用可能であることが確認できた。

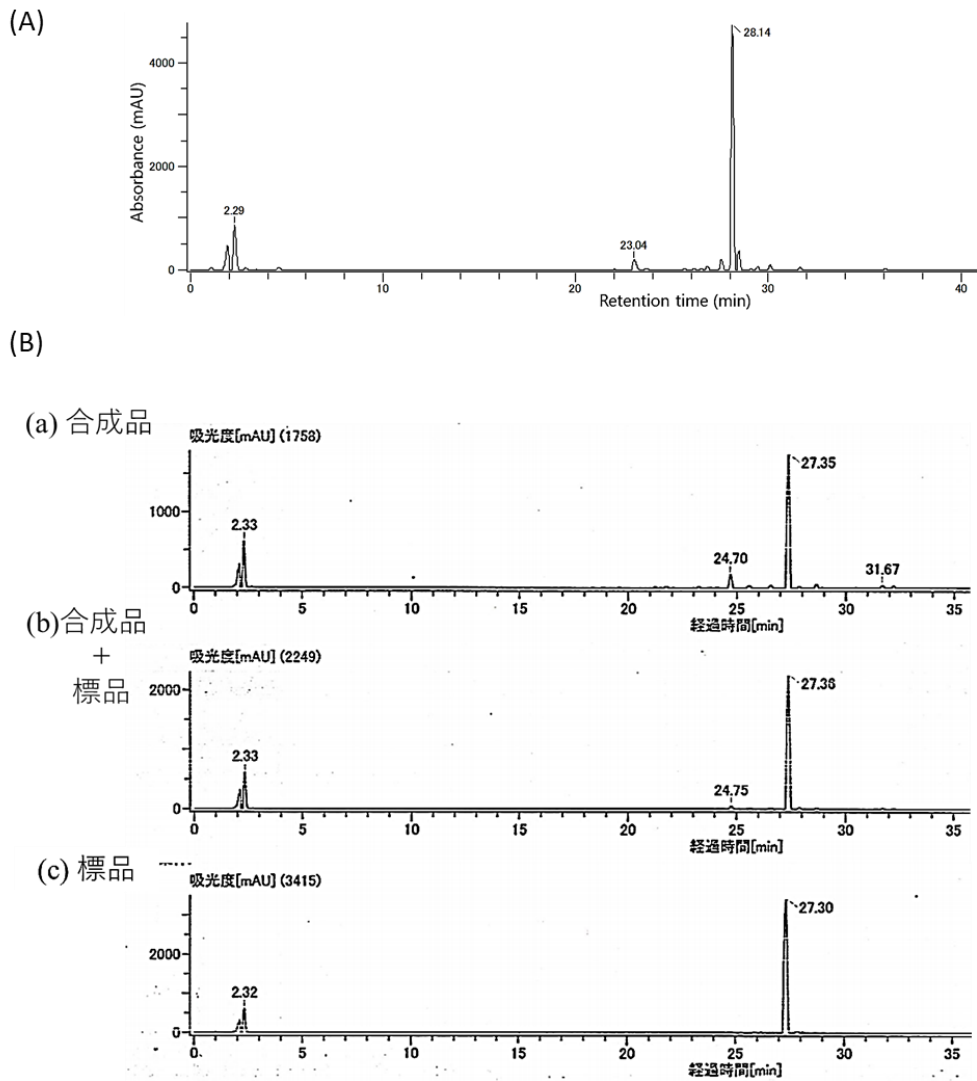


Figure 8. (A) Leuprorelin 保護体の HPLC 解析 (R.T. = 28.14)、(B) 全脱保護後の HPLC 解析

3. 結論・今後の展望

本研究では、従来のペプチド合成法で生じる大量の廃棄物の一因である、縮合剤由来の副生成物が廃棄物とならない新たなペプチド合成法を開発した。具体的には、生体内のペプチド結合形成過程におけるカルボン酸活性化段階および後続反応を参考に、 Ph_3P と有機電解反応を組み合わせたペプチド合成法を開発を行った。反応条件検討の結果、本合成法は生体内のペプチドを構成する基本的なアミノ酸 20 種すべてに適用可能であることが明らかになった。さらに、9 残基のアミノ酸から構成され、すでに医薬品として用いられている leuprorelin の合成にも成功し、オリゴペプチド合成にも応用可能であることが示された。精製段階においては、千葉らが開発してきた可溶性疎水性タグを利用することでペプチド精製の簡便化を達成し、反応後の副生成物である Ph_3PO の回収法も確立した。 Ph_3PO から Ph_3P への変換は、Favre-Réguillon らが開発した 100 g スケールで反応を行える化学還元法¹²ⁱ や、Sevov らが開発したグラムスケールに適用可能な電気化学的還元法^{12k}をはじめとして、様々な手法で達成されている。これを踏まえ、本研究では Ph_3P は電気化学的ペプチド合成法においてリサイクル可能な縮合剤となり、縮合剤由来の廃棄物量削減が可能であることを示すことができた。

縮合剤の触媒化やリサイクル利用という戦略は有望とされながらも、その実例は触媒的ペプチド合成法開発に比べて非常に少ない。その中で、反応条件の検討から精製操作までを行い、一連の合成プロセスを新たな一例として確立、提案できたということで、今後の廃棄物量削減を実現するペプチド合成法の開発の加速に貢献できることを期待している。

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6. 本研究に関する出版物

Shingo Nagahara, Yohei Okada, Yoshikazu Kitano, Kazuhiro Chiba, Biphasic electrochemical peptide synthesis, *Chem. Sci.*, **2021**, *12*, 12911-12917.

7. 使用機器

【核磁気共鳴装置】

日本電子 JNM-ECA 600 (^1H NMR: 600 MHz, ^{13}C NMR: 150 MHz)

日本電子 JNM-ECS 400 (^1H NMR: 400 MHz, ^{13}C NMR: 100 MHz)

内部標準 ^1H -NMR : TMS ($\delta=0.00$) ^{13}C -NMR : CDCl_3 ($\delta=77.03$)

【質量分析計】

日本電子 JMS-T-100LC

【HPLC】

Agilent 1200 Infinity series

【ポテンシヨ/ガルバナスタット】

北斗電光 HABF-501A

【TLC】

Merck precoated silica gel F254 plates (thickness 0.25 mm)

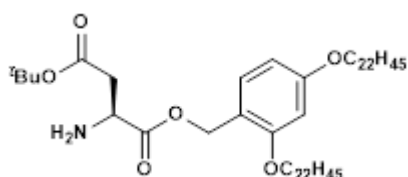
8. 実験項

General Procedure for Synthesis of Tag-Protected Amino Acids

To a solution of tag (0.50 mmol) in DCM (10 mL), Fmoc-protected amino acid (0.75 mmol), DMAP (0.10 mmol) and DIPCI (0.75 mmol) were added. The resulting mixture was stirred for 15 min at r.t. After completion, MeCN was added to reaction solution, and concentrated under vacuum. To remaining solids, MeCN was added and filtered to give soluble tag protected amino acid as white solid quantitatively.

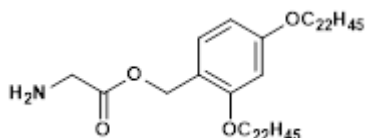
General Procedure for Deprotection of Fmoc Group

To a solution of Fmoc protected tagged amino acid (0.50 mmol) in THF (10 mL), piperidine (74.2 μ L, 0.75 mmol) and DBU (1% v/v) were added. The reaction mixture was stirred for 10 min at r.t. After completion, 6 M HCl aq. was added to the solution to neutralize (pH 7.0), and then MeCN was added followed by concentration under vacuum. To remaining solid, MeCN was added and filtered to give *N*-deprotected amino acid quantitatively as white solid.



H₂N-Asp(O^tBu)-TAG (2a)

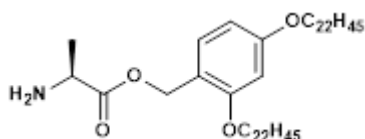
¹H NMR (CDCl₃, 400 MHz) δ 7.19 (1H, d, J = 7.6 Hz), 6.45-6.40 (2H, m), 5.16 (1H, d, J = 11.7 Hz), 5.12 (1H, d, J = 11.7 Hz), 3.93 (4H, t, J = 6.9 Hz), 3.76 (1H, dd, J = 7.6 Hz, 4.8 Hz), 2.72 (1H, dd, J = 16.5 Hz, 4.8 Hz), 2.60 (1H, dd, J = 16.5 Hz, 7.6 Hz), 1.81-1.68 (6H, m), 1.46-1.21 (85H, m), 0.88 (6H, t, J = 6.9 Hz); ¹³C NMR (CDCl₃, 100 MHz) δ 174.5, 170.4, 160.8, 158.4, 131.2, 116.2, 104.5, 99.6, 81.1, 68.1, 62.6, 51.4, 40.1, 31.9, 29.7, 29.6, 29.6, 29.4, 29.4, 29.3, 29.2, 28.0, 26.1, 22.7, 14.1; HRMS (ESI-MS) [M+Na]⁺ calcd for C₅₉H₁₀₉NO₆ 950.8147, found 950.8136.



H₂N-Gly-TAG (2b)

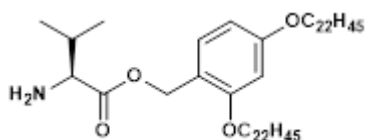
¹H NMR (CDCl₃, 400 MHz) δ 7.20 (1H, d, J = 8.2 Hz), 6.46-6.40 (2H, m), 5.15 (2H, s), 3.98-3.90 (4H, m), 3.51-3.33 (2H, s), 1.81-1.71 (4H, m), 1.63-1.59 (2H, s), 1.49-1.20

(76H, m), 0.88 (6H, t, $J = 6.9$ Hz); ^{13}C NMR (CDCl_3 , 100 MHz) δ 160.9, 158.5, 131.4, 116.2, 104.6, 99.8, 68.1, 62.4, 44.1, 31.9, 29.7, 29.6, 29.4, 29.3, 29.2, 26.1, 22.7, 14.1; HRMS (ESI-MS) $[\text{M}+\text{Na}]^+$ calcd for $\text{C}_{53}\text{H}_{99}\text{NO}_4$ 836.7466, found 836.7483.



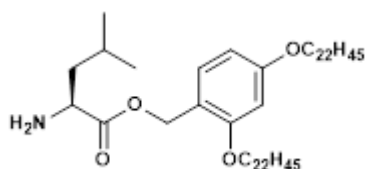
H₂N-Ala-TAG (2c)

^1H NMR (CDCl_3 , 400 MHz) δ 7.19 (1H, d, $J = 8.2$ Hz), 6.46-6.40 (2H, m), 5.14 (1H, d, $J = 11.9$ Hz), 5.10 (1H, d, $J = 11.9$ Hz), 3.94 (4H, t, $J = 6.4$ Hz), 3.54 (1H, q, $J = 6.9$ Hz), 1.82-1.71 (4H, m), 1.68 (2H, s), 1.47-1.21 (79H, m), 0.88 (6H, t, $J = 6.9$ Hz); ^{13}C NMR (CDCl_3 , 100 MHz) δ 176.7, 160.9, 158.5, 131.2, 116.4, 104.5, 99.7, 68.1, 62.4, 50.2, 31.9, 29.7, 29.6, 29.4, 29.3, 29.2, 26.1, 22.7, 20.6, 14.1; HRMS (ESI-MS) $[\text{M}+\text{Na}]^+$ calcd for $\text{C}_{54}\text{H}_{101}\text{NO}_4$ 850.7617, found 850.7603.



H₂N-Val-TAG (2d)

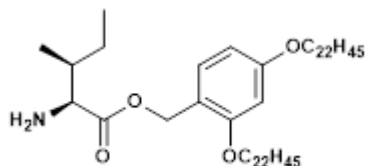
^1H NMR (CDCl_3 , 400 MHz) δ 7.20 (1H, d, $J = 8.7$ Hz), 6.46-6.40 (2H, m), 5.14 (1H, d, $J = 11.9$ Hz), 5.10 (1H, d, $J = 11.9$ Hz), 3.94 (4H, t, $J = 6.4$ Hz), 3.29 (1H, d, $J = 4.6$ Hz), 2.08-1.98 (1H, m), 1.84-1.70 (4H, m), 1.65-1.58 (2H, m), 1.51-1.18 (76H, m), 0.95 (3H, d, $J = 6.9$ Hz), 0.92-0.84 (9H, m); ^{13}C NMR (CDCl_3 , 100 MHz) δ 175.7, 160.9, 158.6, 131.4, 116.4, 104.5, 99.7, 68.1, 62.2, 60.0, 32.1, 31.9, 29.7, 29.6, 29.4, 29.4, 29.3, 29.2, 26.1, 22.7, 19.3, 17.2, 14.1; HRMS (ESI-MS) $[\text{M}+\text{Na}]^+$ calcd for $\text{C}_{56}\text{H}_{105}\text{NO}_4$ 878.7936, found 878.7946.



H₂N-Leu-TAG (2e)

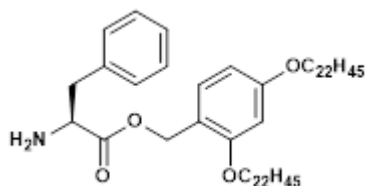
^1H NMR (CDCl_3 , 400 MHz) δ 7.19 (1H, d, $J = 8.7$ Hz), 6.46-6.40 (2H, m), 5.11 (2H, s), 3.94 (4H, t, $J = 6.4$ Hz), 3.46 (1H, dd, $J = 8.7$ Hz, 5.5 Hz), 1.81-1.71 (4H, m), 1.62-1.49 (4H, m), 1.49-1.19 (77H, m), 0.94-0.84 (12H, m); ^{13}C NMR (CDCl_3 , 100 MHz) δ 176.8, 160.8, 158.5, 131.2, 116.4, 104.5, 99.7, 68.1, 62.3, 53.0, 44.0, 31.9, 29.7, 29.6, 29.4,

29.4, 29.3, 29.2, 26.1, 24.7, 23.0, 22.7, 21.9, 14.1; HRMS (ESI-MS) $[M+Na]^+$ calcd for $C_{57}H_{107}NO_4$ 892.8092, found 892.8092..



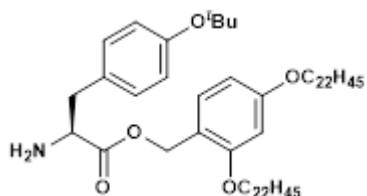
H₂N-Ile-TAG (2f)

¹H NMR (CDCl₃, 400 MHz) δ 7.20 (1H, d, $J = 8.7$ Hz), 6.46-6.40 (2H, m), 5.13 (1H, d, $J = 11.5$ Hz), 5.10 (1H, d, $J = 11.5$ Hz), 3.94 (4H, t, $J = 6.4$ Hz), 3.34 (1H, d, $J = 5.0$ Hz), 1.82-1.68 (5H, m), 1.61 (2H, s), 1.51-1.20 (77H, m), 1.2-1.07 (1H, m), 0.94-0.82 (12H, m); ¹³C NMR (CDCl₃, 100 MHz) δ 175.8, 160.8, 158.5, 131.4, 116.4, 104.5, 99.7, 68.1, 62.1, 59.2, 39.1, 31.9, 29.7, 29.6, 29.4, 29.3, 29.2, 26.1, 24.6, 22.7, 15.7, 14.1, 11.7; HRMS (ESI-MS) $[M+Na]^+$ calcd for $C_{57}H_{107}NO_4$ 892.8092, found 892.8113.



H₂N-Phe-TAG(2g)

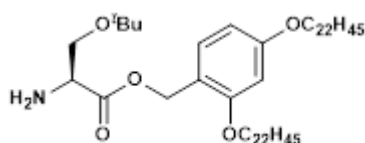
¹H NMR (CDCl₃, 400 MHz) δ 7.29-7.11 (6H, m), 6.47-6.39 (2H, m), 5.14 (2H, s), 3.95 (4H, t, $J = 6.4$ Hz), 3.73 (1H, dd, $J = 7.8$ Hz, 5.0 Hz), 3.08 (1H, dd, $J = 13.7$ Hz, 5.0 Hz), 2.87 (1H, dd, $J = 13.7$ Hz, 7.8 Hz), 1.82-1.72 (4H, m), 1.55 (2H, s), 1.50-1.20 (76H, m), 0.88 (6H, t, $J = 6.9$ Hz); ¹³C NMR (CDCl₃, 100 MHz) δ 175.2, 160.9, 158.6, 137.3, 131.5, 129.4, 128.5, 126.7, 116.2, 104.5, 99.7, 68.1, 62.4, 55.8, 40.9, 31.9, 29.7, 29.6, 29.6, 29.4, 29.4, 29.3, 29.2, 26.1, 22.7, 14.1; HRMS (ESI-MS) $[M+Na]^+$ calcd for $C_{60}H_{105}NO_4$ 926.7936, found 926.7952.



H₂N-Tyr(O'Bu)-TAG (2h)

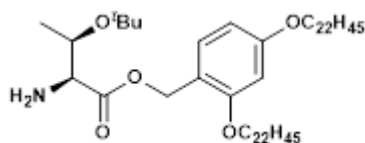
¹H NMR (CDCl₃, 400 MHz) δ 7.16 (1H, d, $J = 8.2$ Hz), 7.03 (2H, d, $J = 8.7$ Hz), 6.87 (2H, d, $J = 8.7$ Hz), 6.46-6.39 (2H, m), 5.13 (2H, s), 3.99-3.90 (4H, m), 3.70 (1H, dd, J

= 7.7 Hz, 5.0 Hz), 3.03 (1H, dd, $J = 13.7$ Hz, 5.0 Hz), 2.83 (1H, dd, $J = 13.7$ Hz, 7.3 Hz), 1.82-1.71 (4H, m), 1.58 (2H, s), 1.48-1.20 (85H, m), 0.88 (6H, t, $J = 7.1$ Hz); ^{13}C NMR (CDCl_3 , 100 MHz) δ 175.17, 160.9, 158.6, 154.2, 132.0, 131.4, 129.8, 124.1, 116.3, 104.5, 99.7, 78.3, 68.1, 62.4, 55.8, 40.2, 31.9, 29.7, 29.4, 29.4, 29.3, 29.2, 28.9, 26.1, 22.7, 14.1; HRMS (ESI-MS) $[\text{M}+\text{Na}]^+$ calcd for $\text{C}_{64}\text{H}_{113}\text{NO}_5$ 998.8511, found 998.8529.



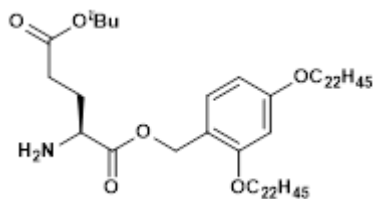
H₂N-Ser(O^tBu)-TAG (2i)

^1H NMR (CDCl_3 , 400 MHz) δ 7.21 (1H, d, $J = 8.3$ Hz), 6.45-6.40 (2H, m), 5.18 (1H, d, $J = 12.4$ Hz), 5.13 (1H, d, $J = 12.4$ Hz), 3.94 (4H, t, $J = 6.9$ Hz), 3.64-3.54 (3H, m), 1.80-1.71 (6H, m), 1.47-1.22 (76H, m), 1.13 (9H, s), 0.88 (6H, t, $J = 6.9$ Hz); ^{13}C NMR (CDCl_3 , 100 MHz) δ 174.2, 160.7, 158.4, 131.0, 116.5, 104.5, 99.6, 73.0, 68.1, 63.7, 62.2, 55.3, 31.9, 29.7, 29.6, 29.6, 29.4, 29.4, 29.3, 29.2, 27.4, 26.1, 22.7, 14.1; HRMS (ESI-MS) $[\text{M}+\text{Na}]^+$ calcd for $\text{C}_{64}\text{H}_{109}\text{NO}_5$ 922.8198, found 922.8188.



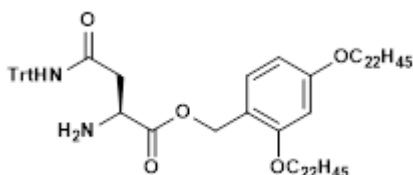
H₂N-Thr(O^tBu)-TAG (2j)

^1H NMR (CDCl_3 , 400 MHz) δ 7.21 (1H, d, $J = 8.3$ Hz), 6.45-6.41 (2H, m), 5.20 (1H, d, $J = 11.7$ Hz), 5.01 (1H, d, $J = 11.7$ Hz), 4.01 (1H, td, $J = 6.2$ Hz, 3.4 Hz), 3.97-3.91 (4H, m), 3.26 (1H, d, $J = 2.8$ Hz), 1.80-1.74 (4H, m), 1.63 (2H, s), 1.47-1.20 (79H, m), 1.12 (9H, s), 0.88 (6H, t, $J = 6.9$ Hz); ^{13}C NMR (CDCl_3 , 100 MHz) δ 174.9, 160.9, 158.5, 131.5, 116.3, 104.5, 99.7, 73.6, 68.5, 68.1, 62.3, 60.7, 31.9, 29.7, 29.6, 29.6, 29.4, 29.4, 29.3, 29.2, 28.5, 26.1, 22.7, 21.0, 14.1; HRMS (ESI-MS) $[\text{M}+\text{Na}]^+$ calcd for $\text{C}_{59}\text{H}_{111}\text{NO}_5$ 936.8354, found 936.8374.



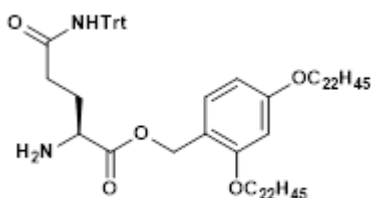
H₂N-Glu(O^tBu)-TAG(2k)

¹H NMR (CDCl₃, 400 MHz) δ 7.19 (1H, d, *J* = 8.7 Hz), 6.45-6.40 (2H, m), 5.13 (2H, s), 3.94 (4H, t, *J* = 6.4 Hz), 3.45 (1H, dd, *J* = 8.4 Hz, 4.8 Hz), 2.33 (2H, t, *J* = 7.3 Hz), 1.80-1.91 (1H, m), 1.87-1.71 (5H, m), 1.60 (2H, s), 1.48-1.18 (85H, m), 0.88 (6H, t, *J* = 6.9 Hz); ¹³C NMR (CDCl₃, 100 MHz) δ 175.7, 172.4, 160.9, 158.5, 131.2, 116.3, 104.5, 99.7, 80.3, 68.1, 62.4, 54.0, 31.9, 31.9, 30.0, 29.7, 29.4, 29.3, 29.2, 28.1, 26.1, 22.7, 14.1; HRMS (ESI-MS) [M+Na]⁺ calcd for C₆₀H₁₁₁NO₆ 964.8304, found 964.8309.



H₂N-Asn(Trt)-TAG (2l)

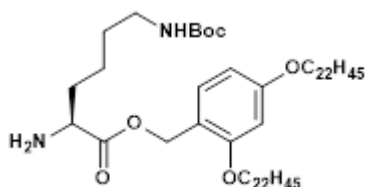
¹H NMR (CDCl₃, 400 MHz) δ 8.72 (1H, s), 7.29-7.17 (15H, m), 7.15 (1H, d, *J* = 8.2 Hz), 6.43-6.37 (2H, m), 5.16 (1H, d, *J* = 11.9 Hz), 5.11 (1H, d, *J* = 11.9 Hz), 3.92 (4H, t, *J* = 6.4 Hz), 3.83 (1H, dd, *J* = 9.6 Hz, 2.8 Hz), 2.68 (1H, dd, *J* = 15.6 Hz, 3.2 Hz), 2.51 (1H, dd, *J* = 16.0 Hz, 9.6 Hz), 1.81-1.70 (4H, m), 1.65 (2H, s), 1.48-1.21 (76H, m), 0.88 (6H, t, *J* = 6.9 Hz); ¹³C NMR (CDCl₃, 100 MHz) δ 174.2, 169.3, 160.9, 158.4, 144.9, 131.2, 128.7, 127.9, 126.9, 115.9, 104.6, 99.7, 70.4, 68.2, 62.8, 51.7, 40.7, 31.9, 29.7, 29.6, 29.4, 29.4, 29.3, 29.2, 26.1, 22.7, 14.1; HRMS (ESI-MS) [M+Na]⁺ calcd for C₇₄H₁₁₆N₂O₅ 1135.8776, found 1135.8770.



H₂N-Gln(Trt)-TAG (2m)

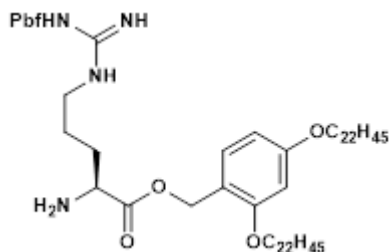
¹H NMR (CDCl₃, 400 MHz) δ 7.29-7.14 (16H, m), 7.05 (1H, s), 6.42-6.36 (2H, m), 5.14 (1H, d, *J* = 11.9 Hz), 5.09 (1H, d, *J* = 11.9 Hz), 3.94-3.84 (4H, m), 3.42 (1H, dd, *J* = 8.2 Hz, 5.0 Hz), 2.46-2.31 (2H, m), 2.11-2.01 (1H, m), 1.89-1.69 (5H, m), 1.64 (2H,

s), 1.50-1.19 (76H, m), 0.88 (6H, t, $J = 6.9$ Hz); ^{13}C NMR (CDCl_3 , 100 MHz) δ 175.5, 171.3, 160.9, 158.4, 144.7, 131.3, 128.7, 127.9, 126.9, 116.2, 104.6, 99.8, 70.4, 68.2, 68.1, 62.5, 53.9, 33.9, 31.9, 30.1, 29.7, 29.7, 29.6, 29.4, 29.4, 29.3, 29.2, 26.1, 26.0, 22.7, 14.1; HRMS (ESI-MS) $[\text{M}+\text{Na}]^+$ calcd for $\text{C}_{75}\text{H}_{118}\text{N}_2\text{O}_5$ 1149.8933, found 1149.8945.



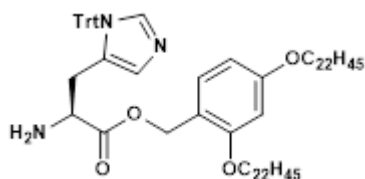
H₂N-Lys(Boc)-TAG (3n)

^1H NMR (CDCl_3 , 400 MHz) δ 7.19 (1H, d, $J = 9.2$ Hz), 6.45-6.41 (2H, m), 5.12 (2H, s), 4.51 (1H, s), 3.94 (4H, t, $J = 6.4$ Hz), 3.42 (1H, dd, $J = 7.7$ Hz, 5.0 Hz), 3.08 (2H, m), 1.81-1.71 (5H, m), 1.58 (2H, s), 1.51-1.21 (90H, m), 0.88 (6H, t, $J = 6.9$ Hz); ^{13}C NMR (CDCl_3 , 100 MHz) δ 176.1, 160.9, 158.5, 155.9, 131.2, 116.4, 104.6, 99.7, 68.1, 62.3, 54.5, 40.4, 34.4, 31.9, 29.7, 29.6, 29.6, 29.4, 29.4, 29.3, 29.2, 28.4, 26.1, 22.9, 22.7, 14.1; HRMS (ESI-MS) $[\text{M}+\text{Na}]^+$ calcd for $\text{C}_{62}\text{H}_{116}\text{N}_2\text{O}_6$ 1007.8726, found 1007.8723.



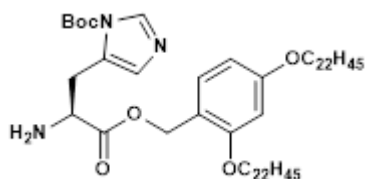
H₂N-Arg(Pbf)-TAG (3o)

^1H NMR (CDCl_3 , 600 MHz) δ 7.17 (1H, d, $J = 8.3$ Hz), 6.44-6.35 (3H, m), 6.31 (2H, s), 5.12 (1H, d, $J = 11.7$ Hz), 5.09 (1H, d, $J = 11.7$ Hz), 3.93 (4H, t, $J = 6.5$ Hz), 3.47-3.43 (1H, m), 3.20-3.06 (2H, m), 2.93 (2H, s), 2.56 (3H, s), 2.50 (3H, s), 2.08 (3H, s), 1.81-1.51 (9H, m), 1.47-1.22 (83H, s), 0.88 (6H, t, $J = 7.2$ Hz); ^{13}C NMR (CDCl_3 , 150 MHz) δ 175.5, 160.9, 158.6, 158.4, 156.3, 138.3, 133.1, 132.3, 131.3, 124.5, 117.4, 116.1, 104.6, 99.7, 86.3, 68.2, 62.5, 53.9, 43.2, 40.7, 31.9, 29.7, 29.7, 29.7, 29.6, 29.5, 29.4, 29.3, 29.2, 28.6, 26.1, 26.0, 25.5, 22.7, 19.3, 17.9, 14.1, 12.5; HRMS (ESI-MS) $[\text{M}+\text{Na}]^+$ calcd for $\text{C}_{70}\text{H}_{124}\text{N}_4\text{O}_7\text{S}$ 1187.9083, found 1187.9077.



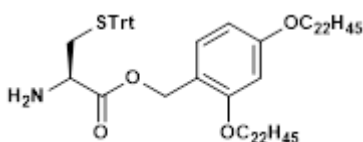
H₂N-His(Trt)-TAG (3p)

¹H NMR (CDCl₃, 400 MHz) δ 7.35 (1H, d, *J* = 1.4 Hz), 7.34-7.28 (9H, m), 7.14-7.09 (6H, m), 7.07 (1H, d, *J* = 8.2 Hz), 6.57 (1H, d, *J* = 1.4 Hz), 6.40 (1H, d, *J* = 2.3 Hz), 6.33 (1H, dd, *J* = 8.2 Hz, 2.3 Hz), 5.13 (1H, d, *J* = 11.9 Hz), 4.97 (1H, d, *J* = 11.9 Hz), 3.95-3.85 (4H, m), 3.77-3.71 (1H, m), 3.00 (1H, dd, *J* = 14.7 Hz, 4.2 Hz), 2.84 (1H, dd, *J* = 14.7 Hz, 7.3 Hz), 1.81-1.70 (6H, m), 1.48-1.20 (76H, m), 0.88 (6H, t, *J* = 6.9 Hz); ¹³C NMR (CDCl₃, 100 MHz) δ 175.1, 160.6, 158.3, 142.4, 138.6, 137.4, 130.9, 129.8, 128.0, 128.0, 119.5, 116.4, 104.5, 99.7, 75.2, 68.1, 68.0, 62.2, 54.9, 33.3, 31.9, 29.7, 29.6, 29.4, 29.3, 29.2, 29.2, 26.1, 26.0, 25.6, 22.7, 14.1; HRMS (ESI-MS) [M+H]⁺ calcd for C₇₆H₁₁₇N₃O₄ 1136.9117, found 1136.9132.



H₂N-His(Boc)-TAG (3q)

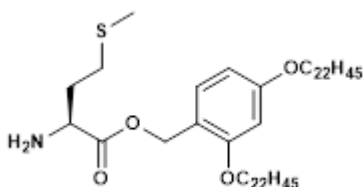
¹H NMR (CDCl₃, 600 MHz) δ 8.00-7.95 (1H, s), 7.18 (1H, d, *J* = 8.3 Hz), 7.13 (1H, s), 6.44-6.40 (2H, m), 5.16 (1H, d, *J* = 12.4 Hz), 5.13 (1H, d, *J* = 12.4 Hz), 3.94 (4H, t, *J* = 6.4 Hz), 3.84 (1H, dd, *J* = 8.3 Hz, 4.8 Hz), 3.04 (1H, dd, *J* = 14.4 Hz, 4.9 Hz), 2.81 (1H, *J* = 14.4 Hz, 8.3 Hz), 1.80-1.73 (4H, m), 1.70 (2H, s), 1.60 (9H, m), 1.47-1.20 (76H, m), 0.88 (6H, t, *J* = 6.9 Hz); ¹³C NMR (CDCl₃, 151 MHz) δ 175.1, 160.8, 158.4, 146.9, 139.7, 136.8, 131.1, 116.3, 114.5, 104.5, 99.7, 85.4, 68.1, 68.1, 62.4, 54.2, 33.1, 31.9, 29.7, 29.7, 29.6, 29.6, 29.4, 29.4, 29.3, 29.2, 27.9, 26.1, 22.7, 14.1; HRMS (ESI-MS) [M+H]⁺ calcd for C₆₂H₁₁₁N₃O₆ 994.8546, found 994.8528.



H₂N-Cys(Trt)-TAG (3s)

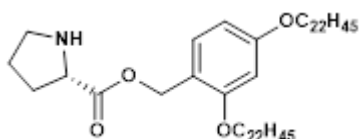
¹H NMR (CDCl₃, 400 MHz) δ 7.42-7.36 (6H, m), 7.27-7.09 (10H, m), 6.43-6.38 (2H, m), 5.07 (2H, m), 3.94 (2H, t, *J* = 6.4 Hz), 3.87 (2H, t, *J* = 6.4 Hz), 3.20 (1H, dd, *J* = 8.2 Hz, 4.6 Hz), 2.56 (1H, dd, *J* = 12.4 Hz, 4.6 Hz), 2.46 (1H, dd, *J* = 12.4 Hz, 7.8 Hz),

1.82-1.66 (4H, m), 1.60-1.18 (78 H, m), 0.88 (6H, t, $J = 6.9$ Hz); ^{13}C NMR (CDCl_3 , 100 MHz) δ 173.8, 160.8, 158.3, 144.6, 131.0, 129.6, 129.6, 127.9, 127.8, 126.7, 126.6, 116.3, 104.5, 99.6, 68.1, 66.8, 62.4, 54.0, 37.0, 31.9, 29.7, 29.4, 29.4, 29.3, 29.2, 26.1, 26.0, 22.7, 14.1; HRMS (ESI-MS) $[\text{M}+\text{Na}]^+$ calcd for $\text{C}_{73}\text{H}_{115}\text{NO}_4\text{S}$ 1124.8439, found 1124.8461.



H₂N-Met-TAG (3t).

^1H NMR (CDCl_3 , 400 MHz) δ 7.20 (1H, d, $J = 8.2$ Hz), 6.45-6.39 (2H, m), 5.16 (1H, d, $J = 11.5$ Hz), 5.11 (1H, d, $J = 11.5$ Hz), 3.94 (4H, t, $J = 6.4$ Hz), 3.60 (1H, dd, $J = 8.2$ Hz, 5.0 Hz), 2.58 (2H, t, $J = 7.3$ Hz), 2.1-1.98 (4H, m), 1.94-1.70 (7H, m), 1.48-1.20 (76H, m), 0.88 (6H, t, $J = 6.6$ Hz); ^{13}C NMR (CDCl_3 , 100 MHz) δ 175.5, 160.9, 158.6, 131.4, 116.2, 104.5, 99.7, 68.1, 62.6, 53.5, 33.8, 31.9, 30.4, 29.7, 29.6, 29.4, 29.4, 29.3, 29.2, 26.1, 22.7, 15.4, 14.1; HRMS (ESI-MS) $[\text{M}+\text{Na}]^+$ calcd for $\text{C}_{56}\text{H}_{105}\text{NO}_4\text{S}$ 910.7657, found 910.7672.

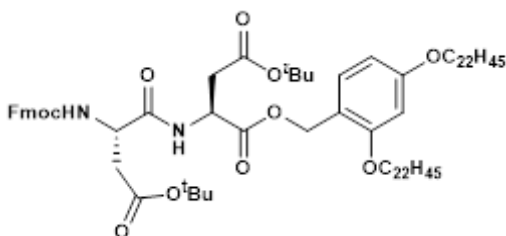


H₂N-Pro-TAG (3u)

^1H NMR (CDCl_3 , 400 MHz) δ 7.19 (1H, d, $J = 8.2$ Hz), 6.45-6.41 (2H, m), 5.15 (1H, d, $J = 11.9$ Hz), 5.11 (1H, d, $J = 11.9$ Hz), 3.94 (4H, t, $J = 6.4$ Hz), 3.77 (1H, dd, $J = 8.8$ Hz, 6.0 Hz), 3.12-3.04 (1H, m), 2.93-2.85 (1H, m), 2.16-2.05 (1H, m), 1.90-1.70 (8H, m), 1.48-1.21 (76H, m), 0.88 (6H, t, $J = 6.7$ Hz); ^{13}C NMR (CDCl_3 , 100 MHz) δ 175.5, 160.9, 158.5, 131.2, 116.4, 104.5, 99.7, 68.1, 62.5, 59.8, 47.0, 31.9, 30.3, 30.0, 29.9, 29.7, 29.6, 29.5, 29.4, 29.3, 29.2, 26.1, 25.4, 22.71, 14.1; HRMS (ESI-MS) $[\text{M}+\text{Na}]^+$ calcd for $\text{C}_{56}\text{H}_{103}\text{NO}_4$ 876.7779, found 876.7798.

General Procedure for Electrochemical Peptide Bond Formation

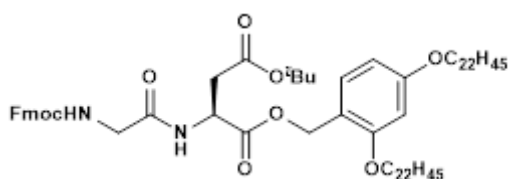
Solvents were purged with Ar gas for 2 min. To a purged solvent, 2,6-lutidine (69.9 μL , 0.60 mmol), electrolyte (0.04 mmol), Ph_3P (0.40-0.80 mmol), Fmoc-protected amino acid (0.30-0.50 mmol), and *N*-deprotected tagged amino acid (0.20 mmol) were added. The solution was electrolyzed at constant current (0.67 mA/cm^2) under Ar at rt-60 $^\circ\text{C}$. After 4.8-6.0 F/mol of electricity based on *N*-deprotected tagged amino acid was passed, the reaction mixture was extracted with *c*-hex (20 mL x 2), and then the solvent was replaced with MeCN. The resulting precipitants were collected by filtration and washed with MeCN to give the peptides.



Fmoc -Asp(OtBu)-Asp(OtBu)-TAG (3aa)

Following the general procedure, 2,6-lutidine (69.9 μL , 0.60 mmol), Bu_4NClO_4 (136.8 mg, 0.40 mmol), Ph_3P (104.9 mg, 0.40 mmol), Fmoc-Asp(OtBu)-OH (123.4 mg, 0.30 mmol), and H_2N -Asp(OtBu)-Tag (185.7 mg, 0.20 mmol) were added to Ar-purged MeCN (8.0 mL)-*c*-hex (4.0 mL). The solution was electrolyzed at constant current (0.67 mA/cm^2) under Ar at room temperature. After 4.8 F/mol of electricity based on H_2N -Asp(OtBu)-Tag was passed, the reaction mixture was extracted with *c*-hex (20 mL x 2), and then the solvent was replaced with MeCN. The resulting precipitants were collected by filtration and washed with MeCN to give the product in 95% yield (251.1 mg) as white solid.

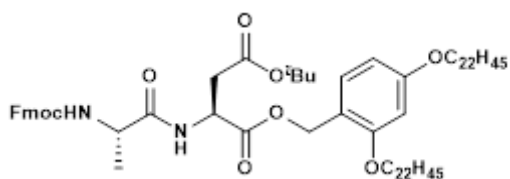
^1H NMR (CDCl_3 , 600 MHz) δ 7.76 (2H, d, $J = 7.6$ Hz), 7.63-7.56 (2H, m), 7.45-7.35 (3H, m), 7.31 (2H, t, $J = 7.6$ Hz), 7.14 (1H, d, $J = 8.3$ Hz), 6.43-6.34 (2H, m), 5.95 (1H, d, $J = 8.3$ Hz), 5.19-5.11 (2H, m), 4.82-4.76 (1H, m), 4.62-4.54 (1H, m), 4.42-4.32 (2H, m), 4.22 (1H, t, $J = 7.6$ Hz), 3.95-3.84 (4H, m), 2.90 (2H, m), 2.72 (1H, dd, $J = 16.5$ Hz, 4.1 Hz), 2.63 (1H, dd, $J = 17.2$, 6.2 Hz), 1.80-1.70 (4H, m), 1.48-1.20 (94H, m), 0.88 (6H, t, $J = 6.9$ Hz); ^{13}C NMR (CDCl_3 , 150 MHz) δ 171.1, 170.4, 170.3, 169.6, 160.8, 158.3, 155.9, 143.9, 143.7, 141.3, 130.9, 127.7, 127.2, 127.1, 125.1, 120.0, 116.0, 104.5, 99.6, 81.8, 81.7, 68.1, 68.1, 67.3, 62.9, 51.1, 49.1, 47.1, 37.6, 37.2, 31.9, 29.7, 29.7, 29.6, 29.6, 29.4, 29.4, 29.3, 29.1, 28.0, 27.9, 26.1, 26.0, 22.7, 14.1; HRMS (ESI-MS) $[\text{M}+\text{Na}]^+$ calcd for $\text{C}_{82}\text{H}_{132}\text{N}_2\text{O}_{11}$ 1343.9723, found 1392.9703.



Fmoc-Gly-Asp(O^tBu)-TAG (3ba)

Following the general procedure, 2,6-lutidine (69.9 μ l, 0.60 mmol), Bu_4NClO_4 (136.8 mg, 0.40 mmol), Ph_3P (131.1 mg, 0.50 mmol), Fmoc-Gly-OH (89.2 mg, 0.30 mmol), and $\text{H}_2\text{N-Asp(O}^t\text{Bu)-Tag}$ (185.7 mg, 0.20 mmol) were added to Ar-purged MeCN (8.0 mL)-*c*-hex (4.0 mL). The solution was electrolyzed at constant current (0.67 mA/cm²) under Ar at room temperature. After 4.8 F/mol of electricity based on $\text{H}_2\text{N-Asp(O}^t\text{Bu)-Tag}$ was passed, the reaction mixture was extracted with *c*-hex (20 mL x 2), and then the solvent was replaced with MeCN. The resulting precipitants were collected by filtration and washed with MeCN to give the product in 98% yield (237.0 mg) as white solid.

¹H NMR (CDCl_3 , 600 MHz) δ 7.76 (2H, d, $J = 7.6$ Hz), 7.66-7.53 (2H, m), 7.40 (2H, t, $J = 7.6$ Hz), 7.31 (2H, t, $J = 7.6$ Hz), 7.16 (1H, d, $J = 8.3$ Hz), 6.86 (1H, d, $J = 6.9$ Hz), 6.45-6.38 (2H, m), 5.55-5.35 (1H, m), 5.21 (1H, d, $J = 11.7$ Hz), 5.14 (1H, d, $J = 11.7$ Hz), 4.86-4.79 (1H, m), 4.39 (2H, d, $J = 6.9$ Hz), 4.22 (1H, t, $J = 7.6$ Hz), 4.01-3.87 (6H, m), 2.93 (1H, dd, $J = 17.2$ Hz, 4.8 Hz), 2.75 (1H, dd, $J = 17.2$ Hz, 4.1 Hz), 1.80-1.72 (4H, m), 1.48-1.21 (85H, m), 0.88 (6H, t, $J = 6.9$ Hz); ¹³C NMR (CDCl_3 , 150 MHz) δ 170.5, 169.9, 168.4, 160.9, 158.4, 156.4, 143.8, 143.8, 141.3, 131.1, 127.7, 127.1, 125.1, 125.1, 120.0, 115.8, 104.6, 99.7, 81.8, 68.2, 68.1, 67.3, 63.1, 48.8, 47.1, 44.3, 37.3, 31.9, 29.7, 29.7, 29.6, 29.4, 29.4, 29.3, 29.1, 27.9, 26.1, 26.0, 22.7, 14.1; HRMS (ESI-MS) $[\text{M}+\text{Na}]^+$ calcd for $\text{C}_{76}\text{H}_{122}\text{N}_2\text{O}_9$ 1229.9043, found 1229.9046.

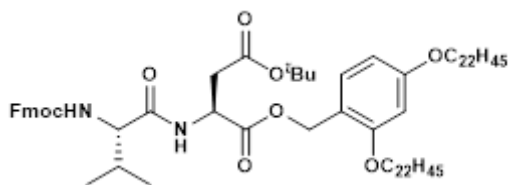


Fmoc-Ala-Asp(O^tBu)-TAG (3ca)

Following the general procedure, 2,6-lutidine (69.9 μ l, 0.60 mmol), Bu_4NClO_4 (136.8 mg, 0.40 mmol), Ph_3P (104.9 mg, 0.40 mmol), Fmoc-Ala-OH (93.4 mg, 0.30 mmol), and $\text{H}_2\text{N-Asp(O}^t\text{Bu)-Tag}$ (185.7 mg, 0.20 mmol) were added to Ar-purged MeCN (8.0 mL)-*c*-hex (4.0 mL). The solution was electrolyzed at constant current (0.67 mA/cm²) under Ar at 50 °C. After 4.8 F/mol of electricity based on $\text{H}_2\text{N-Asp(O}^t\text{Bu)-Tag}$ was passed, the reaction mixture was extracted with *c*-hex (20 mL x 2), and then the solvent

was replaced with MeCN. The resulting precipitants were collected by filtration and washed with MeCN to give the product in 92% yield (225.9 mg) as white solid.

^1H NMR (CDCl_3 , 600 MHz) δ 7.76 (2H, d, $J = 7.6$ Hz), 7.63-7.53 (2H, m), 7.40 (2H, t, $J = 7.6$ Hz), 7.31 (2H, t, $J = 7.6$ Hz), 7.16 (1H, d, $J = 8.3$ Hz), 6.78 (1H, d, $J = 8.3$ Hz), 6.44-6.36 (2H, m), 5.44 (1H, d, $J = 6.2$ Hz), 5.20 (1H, d, $J = 11.7$ Hz), 5.14 (1H, d, $J = 11.7$ Hz), 4.86-4.72 (1H, m), 4.36 (2H, d, $J = 6.9$ Hz), 4.26 (1H, t, $J = 7.3$ Hz), 4.21 (2H, t, $J = 7.2$ Hz), 3.92 (4H, t, $J = 4.8$ Hz), 2.95 (1H, dd, $J = 16.5$ Hz, 4.1 Hz), 2.72 (1H, dd, $J = 16.5$ Hz, 4.1 Hz), 1.80-1.72 (4H, m), 1.47-1.21 (88H, m), 0.88 (6H, t, $J = 7.3$ Hz); ^{13}C NMR(CDCl_3 , 150 MHz) δ 172.0, 170.6, 170.1, 160.9, 158.4, 155.8, 144.0, 143.9, 141.4, 131.2, 127.8, 127.2, 125.2, 120.1, 116.0, 104.6, 100.0, 81.9, 68.2, 68.2, 67.1, 63.1, 50.5, 48.9, 47.2, 37.3, 32.0, 29.8, 29.8, 29.7, 29.5, 29.5, 29.3, 29.2, 28.1, 22.8, 19.2, 14.2; HRMS (ESI-MS) $[\text{M}+\text{Na}]^+$ calcd for $\text{C}_{77}\text{H}_{124}\text{N}_2\text{O}_9$ 1243.9199, found 1243.9199.

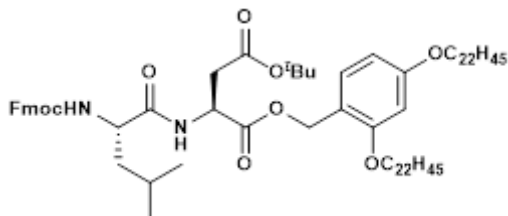


Fmoc-Val-Asp(OtBu)-TAG (3da)

Following the general procedure, 2,6-lutidine (69.9 μl , 0.60 mmol), Bu_4NClO_4 (136.8 mg, 0.40 mmol), Ph_3P (104.9 mg, 0.40 mmol), Fmoc-Val-OH (101.8 mg, 0.30 mmol), and $\text{H}_2\text{N-Asp(OtBu)-Tag}$ (185.7 mg, 0.20 mmol) were added to Ar-purged MeCN (8.0 mL)-*c*-hex (4.0 mL). The solution was electrolyzed at constant current (0.67 mA/cm 2) under Ar at 40 $^\circ\text{C}$. After 4.8 F/mol of electricity based on $\text{H}_2\text{N-Asp(OtBu)-Tag}$ was passed, the reaction mixture was extracted with *c*-hex (20 mL x 2), and then the solvent was replaced with MeCN. The resulting precipitants were collected by filtration and washed with MeCN to give the product in 98% yield (245.7 mg) as white solid.

^1H NMR (CDCl_3 , 600 MHz) δ 7.76 (2H, d, $J = 7.6$ Hz), 7.64-7.56 (2H, m), 7.43-7.37 (2H, m), 7.34-7.28 (2H, m), 7.15 (1H, d, $J = 8.3$ Hz), 6.75 (1H, d, $J = 8.9$ Hz), 6.45-6.35 (2H, m), 5.45 (1H, d, $J = 8.9$ Hz), 5.18 (1H, d, 12.4 Hz), 5.14 (1H, d, 12.4 Hz), 4.89-4.80 (1H, m), 4.40 (1H, dd, $J = 11.0, 7.6$ Hz), 4.33 (1H, dd, $J = 11.0$ Hz, 7.6 Hz), 4.22 (1H, t, $J = 7.6$ Hz), 4.06 (1H, dd, $J = 8.3, 5.5$ Hz), 3.92 (4H, t, $J = 6.5$ Hz), 2.97 (1H, dd, $J = 17.2$ Hz, 4.8 Hz), 2.70 (1H, dd, $J = 17.2$ Hz, 4.1 Hz), 2.15-2.05 (1H, m), 1.81-1.70 (4H, m), 1.45-1.21 (85H, m), 1.01-0.85 (12H, m); ^{13}C NMR(CDCl_3 , 150 MHz) δ 170.7, 170.5, 170.2, 160.9, 158.4, 156.2, 143.9, 143.8, 141.3, 131.2, 127.7, 127.1, 125.2, 125.1, 120.0, 115.8, 104.5, 99.6, 81.9, 68.1, 68.1, 67.1, 63.1, 60.0, 48.6, 47.2, 37.2,

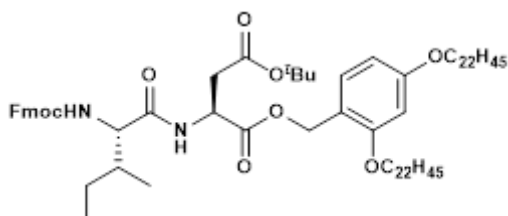
31.9, 31.7, 29.7, 29.7, 29.6, 29.6, 29.4, 29.4, 29.3, 29.1, 28.0, 26.1, 22.7, 19.0, 17.6, 14.1; HRMS (ESI-MS) $[M+Na]^+$ calcd for $C_{79}H_{128}N_2O_9$ 1271.9512, found 1271.9531.



Fmoc-Leu-Asp(OtBu)-TAG (3ea)

Following the general procedure, 2,6-lutidine (69.9 μ L, 0.60 mmol), Bu_4NClO_4 (136.8 mg, 0.40 mmol), Ph_3P (131.1 mg, 0.50 mmol), Fmoc-Val-OH (106.0 mg, 0.30 mmol), and H_2N -Asp(OtBu)-Tag (185.7 mg, 0.20 mmol) were added to Ar-purged MeCN (8.0 mL)-*c*-hex (4.0 mL). The solution was electrolyzed at constant current (0.67 mA/cm²) under Ar at 50 °C. After 4.8 F/mol of electricity based on H_2N -Asp(OtBu)-Tag was passed, the reaction mixture was extracted with *c*-hex (20 mL x 2), and then the solvent was replaced with MeCN. The resulting precipitants were collected by filtration and washed with MeCN to give the product in 96% yield (242.7 mg) as white solid.

¹H NMR (CDCl₃, 600 MHz) δ 7.76 (2H, d, $J = 7.6$ Hz), 7.59 (2H, t, $J = 6.9$ Hz), 7.40 (2H, td, $J = 7.6$ Hz, 2.8 Hz), 7.31 (2H, t, $J = 7.6$ Hz), 7.15 (1H, d, $J = 8.3$ Hz), 6.77 (1H, d, $J = 8.3$ Hz), 6.44-6.33 (2H, m), 5.24 (1H, d, $J = 8.3$ Hz), 5.18 (1H, d, $J = 11.7$ Hz), 5.15 (1H, d, $J = 11.7$ Hz), 4.86-4.75 (1H, m), 4.44-4.30 (2H, m), 4.25-4.17 (2H, t, $J = 6.9$ Hz), 3.91 (4H, m), 2.96 (1H, dd, $J = 16.5$ Hz, 4.1 Hz), 2.71 (1H, dd, $J = 16.8$ Hz, 4.1 Hz), 1.80-1.71 (4H, m), 1.71-1.58 (2H, m), 1.54-1.47 (1H, m), 1.45-1.19 (85H, m), 0.91 (6H, t, $J = 6.2$ Hz), 0.88 (6H, t, $J = 6.9$ Hz); ¹³C NMR (CDCl₃, 150 MHz) δ 171.8, 170.5, 170.0, 160.8, 158.3, 156.0, 143.9, 143.8, 141.3, 131.1, 127.7, 127.1, 125.1, 120.0, 119.9, 115.9, 104.5, 99.6, 81.8, 68.1, 68.1, 67.0, 63.0, 53.4, 48.7, 47.2, 42.1, 37.3, 31.9, 29.7, 29.7, 29.6, 29.4, 29.4, 29.3, 29.1, 28.0, 26.1, 26.0, 24.6, 22.9, 22.7, 22.0, 14.1; HRMS (ESI-MS) $[M+Na]^+$ calcd for $C_{80}H_{130}N_2O_9$ 1285.9669, found 1285.9691.

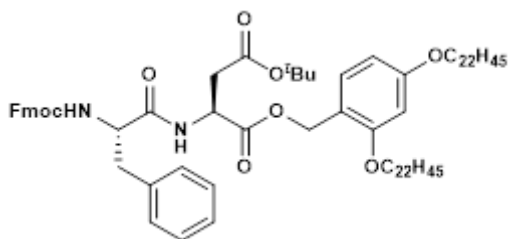


Fmoc-Ile-Asp(OtBu)-TAG (3fa)

Following the general procedure, 2,6-lutidine (69.9 μ L, 0.60 mmol), Bu_4NClO_4 (136.8 mg, 0.40 mmol), Ph_3P (131.1 mg, 0.50 mmol), Fmoc-Ile-OH (106.0 mg, 0.30 mmol),

and H₂N-Asp(O^tBu)-Tag (185.7 mg, 0.20 mmol) were added to Ar-purged MeCN (8.0 mL)-*c*-hex (4.0 mL). The solution was electrolyzed at constant current (0.67 mA/cm²) under Ar at 50 °C. After 4.8 F/mol of electricity based on H₂N-Asp(O^tBu)-Tag was passed, the reaction mixture was extracted with *c*-hex (20 mL x 2), and then the solvent was replaced with MeCN. The resulting precipitants were collected by filtration and washed with MeCN to give the product in 92% yield (233.6 mg) as white solid.

¹H NMR (CDCl₃, 600 MHz) δ 7.76 (2H, d, *J* = 7.6 Hz), 7.60 (2H, d, *J* = 7.6 Hz), 7.40 (2H, m), 7.31 (2H, t, *J* = 7.6 Hz), 7.15 (1H, d, *J* = 8.3 Hz), 6.73 (1H, d, *J* = 8.3 Hz), 6.45-6.33 (2H, m), 5.50 (1H, d, *J* = 8.9 Hz), 5.18 (1H, d, *J* = 11.7 Hz), 5.14 (1H, d, *J* = 11.7 Hz), 4.86-4.75 (1H, m), 4.39 (1H, dd, *J* = 10.7 Hz, 7.6 Hz), 4.35 (1H, dd, *J* = 10.7 Hz, 7.6 Hz), 4.21 (2H, t, 7.6 Hz), 4.14-4.03 (1H, m), 3.91 (4H, t, *J* = 6.9 Hz), 2.95 (2H, dd, *J* = 16.5 Hz, 4.1 Hz), 2.71 (1H, dd, *J* = 17.1 Hz, 4.1 Hz), 1.89-1.81 (1H, m), 1.81-1.70 (4H, m), 1.71-1.58 (2H, m), 1.51-1.22 (85H, m), 1.22 (2H, m), 0.97-0.83 (12H, m); ¹³C NMR(CDCl₃, 150 MHz) δ 170.7, 170.6, 170.2, 160.9, 158.5, 156.1, 144.0, 143.9, 141.4, 131.2, 127.8, 127.2, 125.2, 120.0, 115.9, 104.6, 99.7, 81.9, 68.2, 68.2, 63.1, 59.5, 48.7, 47.3, 38.2, 37.3, 32.0, 29.8, 29.8, 29.7, 29.5, 29.5, 29.3, 29.2, 28.1, 26.1, 25.0, 22.8, 15.2, 14.2, 11.6; HRMS (ESI-MS) [M+Na]⁺ calcd for C₈₀H₁₃₀N₂O₉ 1285.9669, found 1285.9680.

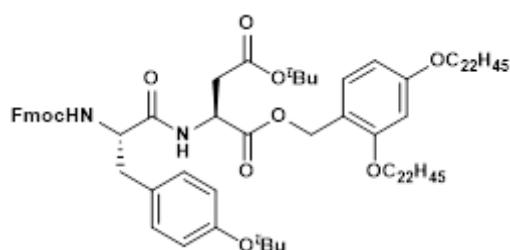


Fmoc-Phe-Asp(O^tBu)-TAG (3ga)

Following the general procedure, 2,6-lutidine (69.9 μl, 0.60 mmol), Bu₄NClO₄ (136.8 mg, 0.40 mmol), Ph₃P (104.9 mg, 0.40 mmol), Fmoc-Phe-OH (116.2 mg, 0.30 mmol), and H₂N-Asp(O^tBu)-Tag (185.7 mg, 0.20 mmol) were added to Ar-purged MeCN (8.0 mL)-*c*-hex (4.0 mL). The solution was electrolyzed at constant current (0.67 mA/cm²) under Ar at 40 °C. After 4.8 F/mol of electricity based on H₂N-Asp(O^tBu)-Tag was passed, the reaction mixture was extracted with *c*-hex (20 mL x 2), and then the solvent was replaced with MeCN. The resulting precipitants were collected by filtration and washed with MeCN to give the product in 97% yield (251.0 mg) as white solid.

¹H NMR (CDCl₃, 600 MHz) δ 7.76 (2H, d, *J* = 7.6 Hz), 7.53 (1H, d, *J* = 7.6 Hz), 7.51 (1H, d, *J* = 7.6 Hz), 7.39 (2H, t, *J* = 7.6 Hz), 7.30 (2H, t, *J* = 7.6 Hz), 7.26-7.19 (4H, m),

7.16 (2H, d, $J = 6.9$ Hz), 6.84 (1H, d, $J = 6.9$ Hz), 6.42-6.35 (2H, m), 5.33 (1H, d, $J = 7.6$ Hz), 5.19 (1H, d, $J = 11.7$ Hz), 5.15 (1H, d, $J = 11.7$ Hz), 4.82-4.72 (1H, m), 4.52-4.43 (1H, m), 4.38 (1H, dd, $J = 10.9$ Hz, 7.6 Hz), 4.30-4.22 (1H, m), 4.17 (1H, t, $J = 6.9$ Hz), 3.96-3.81 (4H, m), 3.24-2.98 (2H, m), 2.90 (1H, dd, $J = 16.5$ Hz, 3.4 Hz), 2.70 (1H, dd, $J = 16.5$ Hz, 4.1 Hz), 1.80-1.68 (4H, m), 1.45-1.20 (85H, m), 0.88 (6H, t, $J = 6.9$ Hz); ^{13}C NMR(CDCl_3 , 150 MHz) δ 170.4, 170.4, 169.9, 160.9, 158.3, 155.7, 143.8, 143.8, 141.3, 131.1, 129.4, 128.6, 127.7, 127.1, 125.2, 125.1, 120.0, 115.9, 104.6, 99.6, 81.7, 68.2, 68.1, 67.1, 63.0, 55.7, 48.9, 47.1, 38.5, 37.3, 31.9, 29.7, 29.7, 29.6, 29.4, 29.4, 29.3, 29.2, 28.0, 26.0, 22.7, 14.1; HRMS (ESI-MS) $[\text{M}+\text{Na}]^+$ calcd for $\text{C}_{83}\text{H}_{128}\text{N}_2\text{O}_9$ 1319.9512, found 1319.9533.

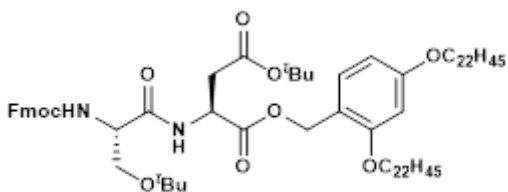


Fmoc-Tyr(O^tBu)-Asp(O^tBu)-TAG (3ha)

Following the general procedure, 2,6-lutidine (69.9 μl , 0.60 mmol), Bu_4NClO_4 (136.8 mg, 0.40 mmol), Ph_3P (104.9 mg, 0.40 mmol), Fmoc-Tyr(O^tBu)-OH (137.9 mg, 0.30 mmol), and $\text{H}_2\text{N-Asp(O}^t\text{Bu)-Tag}$ (185.7 mg, 0.20 mmol) were added to Ar-purged MeCN (8.0 mL)-*c*-hex (4.0 mL). The solution was electrolyzed at constant current (0.67 mA/cm²) under Ar at room temperature. After 4.8 F/mol of electricity based on $\text{H}_2\text{N-Asp(O}^t\text{Bu)-Tag}$ was passed, the reaction mixture was extracted with *c*-hex (20 mL x 2), and then the solvent was replaced with MeCN. The resulting precipitants were collected by filtration and washed with MeCN to give the product in 95% yield (261.0 mg) as white solid.

^1H NMR (CDCl_3 , 600 MHz) δ 7.75 (2H, d, $J = 7.6$ Hz), 7.58-7.49 (2H, m), 7.39 (2H, t, $J = 7.6$ Hz), 7.33-7.27 (2H, m), 7.16 (1H, d, $J = 7.6$ Hz), 7.06 (2H, d, $J = 7.6$ Hz), 6.87 (2H, d, $J = 7.6$ Hz), 6.80 (1H, d, $J = 7.6$ Hz), 6.44-6.34 (2H, m), 5.33 (1H, d, $J = 7.6$ Hz), 5.18 (1H, d, $J = 11.7$ Hz), 5.15 (1H, d, $J = 11.7$ Hz), 4.82-4.71 (1H, m), 4.48-4.40 (1H, m), 4.39-4.32 (1H, m), 4.31-4.24 (1H, m), 4.17 (1H, t, $J = 6.9$ Hz), 3.94-3.83 (4H, m), 3.12-3.02 (1H, m), 3.02-2.94 (1H, m), 2.90 (1H, dd, $J = 16.5$, 4.1 Hz), 2.70 (1H, dd, $J = 16.5$, 4.1 Hz), 1.80-1.68 (4H, m), 1.45-1.21 (94H, m), 0.88 (6H, t, $J = 6.9$ Hz); ^{13}C NMR(CDCl_3 , 150 MHz) δ 170.5, 170.4, 169.8, 160.8, 158.3, 155.7, 154.4, 143.83, 143.78, 141.3, 131.1, 129.8, 127.7, 127.1, 125.1, 125.1, 124.3, 120.0, 115.9, 104.6,

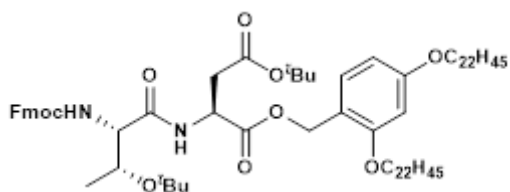
99.6, 81.7, 78.3, 68.2, 68.1, 67.1, 63.0, 55.9, 48.9, 47.1, 38.0, 37.3, 31.9, 29.72, 29.67, 29.6, 29.4, 29.4, 29.3, 29.2, 28.8, 27.9, 26.1, 26.0, 22.7, 14.1; HRMS (ESI-MS) $[M+Na]^+$ calcd for $C_{87}H_{136}N_2O_{10}$ 1392.0087, found 1392.0077.



Fmoc-Ser(OtBu)-Asp(OtBu)-TAG (3ia)

Following the general procedure, 2,6-lutidine (69.9 μ l, 0.60 mmol), Bu_4NClO_4 (136.8 mg, 0.40 mmol), Ph_3P (131.1 mg, 0.50 mmol), Fmoc-Ser(OtBu)-OH (153.4 mg, 0.40 mmol), and H_2N -Asp(OtBu)-Tag (185.7 mg, 0.20 mmol) were added to Ar-purged MeCN (8.0 mL)-*c*-hex (4.0 mL). The solution was electrolyzed at constant current (0.67 mA/cm²) under Ar at 40 °C. After 4.8 F/mol of electricity based on H_2N -Asp(OtBu)-Tag was passed, the reaction mixture was extracted with *c*-hex (20 mL x 2), and then the solvent was replaced with MeCN. The resulting precipitants were collected by filtration and washed with MeCN to give the product in 98% yield (253.1 mg) as white solid.

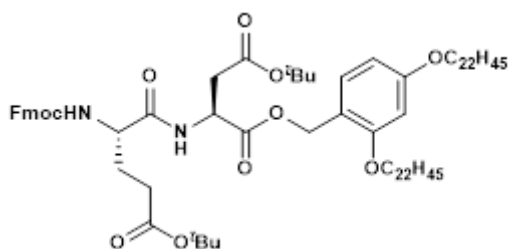
¹H NMR ($CDCl_3$, 600 MHz) δ 7.81 (1H, d, $J = 6.2$ Hz), 7.76 (2H, d, $J = 7.6$ Hz), 7.64-7.53 (2H, m), 7.40 (2H, t, $J = 7.6$ Hz), 7.31 (2H, t, $J = 7.6$ Hz), 7.16 (1H, d, $J = 7.6$ Hz), 6.44-6.35 (2H, m), 5.79 (1H, d, $J = 5.5$ Hz), 5.20 (1H, d, $J = 11.7$ Hz), 5.14 (1H, d, $J = 11.7$ Hz), 4.85-4.79 (1H, m), 4.41-4.33 (2H, m), 4.29-4.18 (2H, m), 3.95-3.86 (4H, m), 3.84-3.76 (1H, m), 3.41 (1H, t, $J = 8.6$ Hz), 2.91 (1H, dd, $J = 16.5$ Hz, 4.8 Hz), 2.74 (1H, dd, $J = 17.2$ Hz, 4.8 Hz), 1.80-1.71 (4H, m), 1.46-1.18 (94H, m), 0.88 (6H, t, $J = 6.9$ Hz); ¹³C NMR ($CDCl_3$, 150 MHz) δ 170.4, 170.2, 169.6, 160.8, 158.3, 156.0, 144.0, 143.8, 141.3, 130.9, 127.7, 127.1, 125.2, 125.2, 120.0, 116.0, 104.6, 99.6, 81.5, 74.4, 68.1, 68.1, 67.2, 62.8, 61.7, 54.2, 49.1, 47.1, 37.5, 31.9, 29.72, 29.67, 29.6, 29.42, 29.38, 29.3, 29.2, 28.0, 27.3, 26.1, 22.7, 14.1; HRMS (ESI-MS) $[M+Na]^+$ calcd for $C_{81}H_{132}N_2O_{10}$ 1315.9774, found 1315.9796.



Fmoc-Thr(O^tBu)-Asp(O^tBu)-TAG (3ja)

Following the general procedure, 2,6-lutidine (69.9 μ l, 0.60 mmol), Bu_4NClO_4 (136.8 mg, 0.40 mmol), Ph_3P (131.1 mg, 0.50 mmol), Fmoc-Thr(O^tBu)-OH (159.0 mg, 0.40 mmol), and $\text{H}_2\text{N-Asp(O}^t\text{Bu)-Tag}$ (185.7 mg, 0.20 mmol) were added to Ar-purged MeCN (8.0 mL)-*c*-hex (4.0 mL). The solution was electrolyzed at constant current (0.67 mA/cm²) under Ar at 40 °C. After 4.8 F/mol of electricity based on $\text{H}_2\text{N-Asp(O}^t\text{Bu)-Tag}$ was passed, the reaction mixture was extracted with *c*-hex (20 mL x 2), and then the solvent was replaced with MeCN. The resulting precipitants were collected by filtration and washed with MeCN to give the product in 93% yield (243.3 mg) as white solid.

¹H NMR (CDCl_3 , 600 MHz) δ 8.12 (1H, d, $J = 7.6$ Hz), 7.76 (1H, d, $J = 7.6$ Hz), 7.61 (1H, d, $J = 7.6$ Hz), 7.43-7.36 (2H, m), 7.31 (2H, t, $J = 7.6$ Hz), 7.19 (1H, d, $J = 8.9$ Hz), 6.44-6.37 (2H, m), 6.01 (1H, d, $J = 5.5$ Hz), 5.21 (1H, d, $J = 12.4$ Hz), 5.17 (1H, d, $J = 12.4$ Hz), 4.88-4.80 (1H, m), 4.42-4.32 (2H, m), 4.25-4.11 (3H, m), 3.95-3.87 (4H, m), 2.92 (1H, dd, $J = 17.2$ Hz, 4.8 Hz), 2.74 (1H, dd, $J = 17.2$ Hz, 4.1 Hz), 1.80-1.72 (4H, m), 1.47-1.20 (94H, m), 1.07 (3H, d, $J = 6.2$ Hz), 0.88 (6H, t, $J = 6.9$ Hz); ¹³C NMR (CDCl_3 , 150 MHz) δ 170.5, 169.6, 169.3, 160.7, 158.3, 156.0, 144.0, 143.7, 143.7, 141.3, 141.3, 131.0, 127.7, 127.1, 125.2, 120.0, 120.0, 116.1, 104.6, 99.5, 81.4, 75.5, 68.1, 67.0, 66.6, 62.9, 58.5, 49.0, 47.2, 37.6, 31.9, 29.7, 29.6, 29.6, 29.4, 29.4, 29.4, 29.3, 29.2, 28.1, 28.0, 26.1, 22.7, 16.5, 14.1; HRMS (ESI-MS) $[\text{M}+\text{Na}]^+$ calcd for $\text{C}_{82}\text{H}_{134}\text{N}_2\text{O}_{10}$ 1329.9931, found 1329.9909.

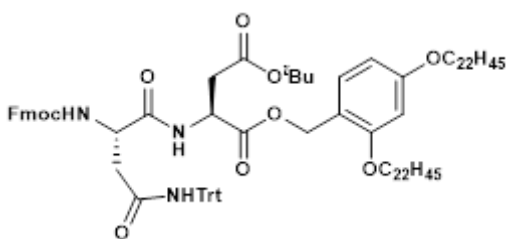


Fmoc-Glu(O^tBu)-Asp(O^tBu)-TAG (3ka)

Following the general procedure, 2,6-lutidine (69.9 μ l, 0.60 mmol), Bu_4NClO_4 (136.8 mg, 0.40 mmol), Ph_3P (104.9 mg, 0.40 mmol), Fmoc-Glu(O^tBu)-OH (133.1 mg, 0.30

mmol), and H₂N-Asp(O^tBu)-Tag (185.7 mg, 0.20 mmol) were added to Ar-purged MeCN (8.0 mL)-*c*-hex (4.0 mL). The solution was electrolyzed at constant current (0.67 mA/cm²) under Ar at room temperature. After 4.8 F/mol of electricity based on H₂N-Asp(O^tBu)-Tag was passed, the reaction mixture was extracted with *c*-hex (20 mL x 2), and then the solvent was replaced with MeCN. The resulting precipitants were collected by filtration and washed with MeCN to give the product in 92% yield (245.3 mg) as white solid.

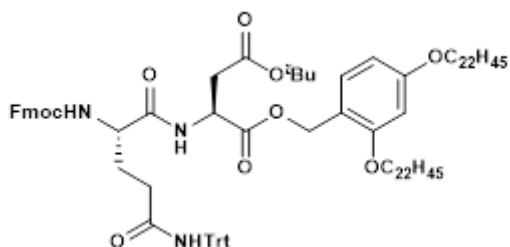
¹H NMR (CDCl₃, 600 MHz) δ 7.76 (2H, d, *J* = 7.6 Hz), 7.60 (2H, m), 7.39 (2H, t, *J* = 7.6 Hz), 7.31 (2H, t, *J* = 7.6 Hz), 7.19-7.09 (2H, m), 6.44-6.35 (2H, m), 5.72 (1H, d, *J* = 7.6 Hz), 5.18 (1H, d, *J* = 12.4 Hz), 5.15 (1H, d, *J* = 12.4 Hz), 4.86-4.80 (1H, m), 4.39-4.31 (2H, m), 4.28 (1H, dd, *J* = 13.1 Hz, 7.6 Hz), 4.21 (1H, t, *J* = 7.6 Hz), 3.95-3.86 (4H, m), 2.94 (1H, dd, *J* = 17.2 Hz, 4.8 Hz), 2.72 (1H, dd, *J* = 17.2 Hz, 4.8 Hz), 2.44-2.33 (2H, m), 2.13-2.05 (1H, m), 1.96-1.86 (1H, m), 1.80-1.71 (4H, m), 1.49-1.21 (94H, m), 0.88 (6H, t, *J* = 6.9 Hz); ¹³C NMR (CDCl₃, 150 MHz) δ 172.7, 171.0, 170.5, 169.7, 160.8, 158.3, 156.0, 144.0, 143.8, 141.3, 131.0, 127.7, 127.1, 125.2, 125.2, 120.0, 115.9, 104.6, 99.6, 81.8, 80.9, 68.2, 68.1, 67.1, 63.0, 54.1, 48.8, 47.1, 37.3, 31.9, 31.5, 29.7, 29.7, 29.6, 29.4, 29.4, 29.3, 29.1, 28.6, 28.1, 28.0, 26.1, 26.0, 22.7, 14.1; HRMS (ESI-MS) [M+Na]⁺ calcd for C₈₃H₁₃₄N₂O₁₁ 1357.9880, found 1357.9876.



Fmoc-Asn(Trt)-Asp(O^tBu)-TAG (3la)

Following the general procedure, 2,6-lutidine (69.9 μl, 0.60 mmol), Bu₄NClO₄ (136.8 mg, 0.40 mmol), Ph₃P (131.1 mg, 0.50 mmol), Fmoc-Asn(Trt)-OH (179.0 mg, 0.30 mmol), and H₂N-Asp(O^tBu)-Tag (185.7 mg, 0.20 mmol) were added to Ar-purged MeCN (8.0 mL)-*c*-hex (4.0 mL). The solution was electrolyzed at constant current (0.67 mA/cm²) under Ar at room temperature. After 4.8 F/mol of electricity based on H₂N-Asp(O^tBu)-Tag was passed, the reaction mixture was extracted with *c*-hex (20 mL x 2), and then the solvent was replaced with MeCN. The resulting precipitants were collected by filtration and washed with MeCN to give the product in 99% yield (300.5 mg) as white solid.

^1H NMR (CDCl_3 , 600 MHz) δ 7.75 (2H, d, $J = 6.9$ Hz), 7.60-7.50 (3H, m), 7.38 (2H, t, $J = 7.6$ Hz), 7.32-7.26 (7H, m), 7.26-7.11 (11H, m), 6.89 (1H, s), 6.43-6.34 (3H, m), 5.13 (2H, s), 4.85-4.78 (1H, m), 4.60 (1H, s), 4.38-4.27 (2H, m), 4.18 (1H, t, $J = 7.2$ Hz), 3.93-3.84 (4H, m), 3.09 (1H, d, $J = 14.8$ Hz), 2.81 (1H, dd, $J = 16.5$ Hz, 4.8 Hz), 2.64 (1H, dd, $J = 15.8$ Hz, 4.8 Hz), 2.55 (1H, dd, $J = 17.2$ Hz, 4.8 Hz), 1.78-1.71 (4H, m), 1.45-1.22 (85H, m), 0.88 (6H, t, $J = 6.9$ Hz); ^{13}C NMR(CDCl_3 , 150 MHz) δ 170.7, 170.5, 170.4, 169.5, 160.7, 158.2, 156.3, 144.3, 143.9, 143.7, 141.2, 130.8, 128.7, 128.0, 127.7, 127.1, 125.3, 119.9, 116.0, 104.5, 99.6, 81.7, 70.9, 68.1, 68.1, 67.4, 62.8, 51.6, 49.0, 47.1, 38.1, 37.3, 31.9, 29.7, 29.7, 29.6, 29.6, 29.4, 29.4, 29.3, 29.1, 27.9, 26.1, 26.0, 22.7, 14.1; HRMS (ESI-MS) $[\text{M}+\text{Na}]^+$ calcd for $\text{C}_{97}\text{H}_{139}\text{N}_3\text{O}_{10}$ 1529.0353, found 1529.0349.

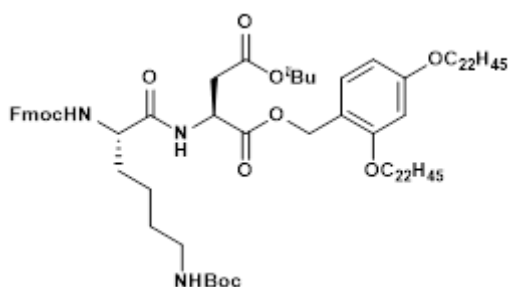


Fmoc-Gln(Trt)-Asp(O^tBu)-TAG (3ma)

Following the general procedure, 2,6-lutidine (69.9 μL , 0.60 mmol), Bu_4NClO_4 (136.8 mg, 0.40 mmol), Ph_3P (131.1 mg, 0.50 mmol), Fmoc-Gln(Trt)-OH (183.2 mg, 0.30 mmol), and $\text{H}_2\text{N-Asp(O}^t\text{Bu)-Tag}$ (185.7 mg, 0.20 mmol) were added to Ar-purged MeCN (8.0 mL)-*c*-hex (4.0 mL). The solution was electrolyzed at constant current (0.67 mA/cm^2) under Ar at room temperature. After 4.8 F/mol of electricity based on $\text{H}_2\text{N-Asp(O}^t\text{Bu)-Tag}$ was passed, the reaction mixture was extracted with *c*-hex (20 mL x 2), and then the solvent was replaced with MeCN. The resulting precipitants were collected by filtration and washed with MeCN to give the product in 99% yield (301.2mg) as white solid.

^1H NMR (CDCl_3 , 600 MHz) δ 7.75 (2H, d, $J = 7.6$ Hz), 7.58 (2H, d, $J = 6.9$ Hz), 7.41-7.34 (2H, m), 7.31-7.26 (8H, m), 7.26-7.19 (9H, m), 7.17 (1H, d, $J = 7.6$ Hz), 7.08 (1H, d, $J = 8.3$ Hz), 7.02 (1H, s), 6.40 (1H, d, $J = 1.4$ Hz), 6.35 (1H, d, $J = 8.3$ Hz), 5.83 (1H, d, $J = 6.9$ Hz), 5.10 (1H, d, $J = 11.7$ Hz), 5.07 (1H, d, $J = 11.7$ Hz), 4.85-4.78 (1H, m), 4.38-4.28 (2H, m), 4.20 (1H, t, $J = 7.2$ Hz), 4.18-4.111 (1H, m), 3.94-3.86 (4H, m), 2.82 (1H, dd, $J = 17.2$ Hz, 4.8 Hz), 2.68 (1H, dd, $J = 17.2$ Hz, 4.8 Hz), 2.50-2.38 (2H, m), 2.13-1.97 (2H, m), 1.80-1.69 (4H, m), 1.46-1.22 (85H, m), 0.88 (6H, t, $J = 6.9$ Hz); ^{13}C NMR(CDCl_3 , 150 MHz) δ 171.7, 171.1, 170.6, 169.7, 160.8, 158.3, 156.0, 144.6, 143.9,

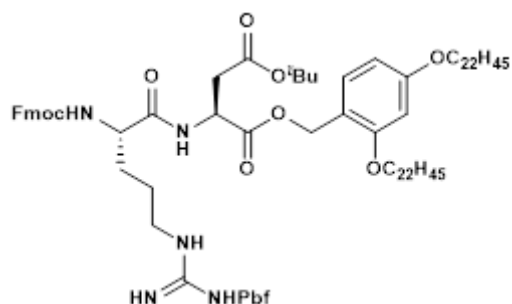
143.8, 141.3, 141.3, 131.1, 128.8, 127.9, 127.7, 127.1, 127.0, 125.2, 119.9, 115.8, 104.5, 99.6, 81.8, 70.7, 68.2, 68.1, 67.1, 63.0, 54.0, 48.9, 47.2, 37.3, 33.0, 31.9, 29.7, 29.6, 29.4, 29.4, 29.3, 29.1, 27.9, 26.1, 26.0, 22.7, 14.1; HRMS (ESI-MS) $[M+Na]^+$ calcd for $C_{98}H_{141}N_3O_{10}$ 1543.0509, found 1543.0519.



Fmoc-Lys(Boc)-Asp(O'Bu)-TAG (3na)

Following the general procedure, 2,6-lutidine (69.9 μ l, 0.60 mmol), Bu_4NClO_4 (136.8 mg, 0.40 mmol), Ph_3P (131.1 mg, 0.50 mmol), Fmoc-Lys(Boc)-OH (140.6 mg, 0.30 mmol), and H_2N -Asp(O'Bu)-Tag (185.7 mg, 0.20 mmol) were added to Ar-purged MeCN (8.0 mL)-*c*-hex (4.0 mL). The solution was electrolyzed at constant current (0.67 mA/cm²) under Ar at 60 °C. After 4.8 F/mol of electricity based on H_2N -Asp(O'Bu)-Tag was passed, the reaction mixture was extracted with *c*-hex (20 mL x 2), and then the solvent was replaced with MeCN. The resulting precipitants were collected by filtration and washed with MeCN to give the product in 91% yield (252.0 mg) as white solid.

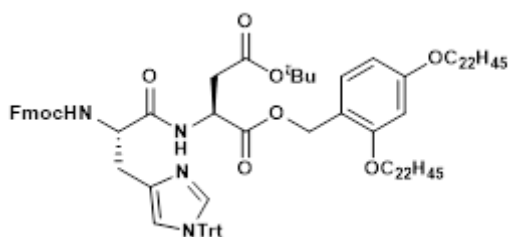
¹H NMR ($CDCl_3$, 600 MHz) δ 7.76 (2H, d, $J = 7.6$ Hz), 7.63-7.51 (2H, m), 7.40 (2H, t, $J = 7.6$ Hz), 7.31 (2H, t, $J = 7.6$ Hz), 7.16 (1H, d, $J = 8.3$ Hz), 6.81 (1H, d, $J = 8.3$ Hz), 6.45-6.34 (2H, m), 5.51 (1H, d, $J = 6.9$ Hz), 5.16 (2H, s), 4.86-4.79 (1H, m), 4.72 (1H, s), 4.37 (2H, d, $J = 6.9$ Hz), 4.26-4.14 (2H, m), 3.97-3.85 (4H, m), 3.18-3.00 (2H, m), 2.95 (1H, dd, $J = 17.2$ Hz, 4.8 Hz), 2.70 (1H, dd, $J = 17.2$ Hz, 4.1 Hz), 1.89-1.60 (6H, m), 1.51-1.21 (98H, m), 0.88 (6H, t, $J = 6.9$ Hz); ¹³C NMR($CDCl_3$, 150 MHz) δ 171.3, 171.3, 170.6, 170.0, 160.8, 158.4, 156.1, 156.0, 143.9, 143.8, 141.3, 131.2, 127.7, 127.1, 125.2, 119.9, 115.9, 104.6, 99.6, 81.9, 79.0, 68.2, 68.1, 67.1, 63.1, 54.7, 48.7, 47.2, 39.9, 37.2, 32.6, 31.9, 29.7, 29.7, 29.6, 29.6, 29.5, 29.4, 29.4, 29.3, 29.1, 28.5, 28.0, 26.1, 26.0, 22.7, 22.2, 14.1; HRMS (ESI-MS) $[M+Na]^+$ calcd for $C_{85}H_{139}N_3O_{11}$ 1401.0302, found 1401.0320.



Fmoc-Arg(Pbf)-Asp(O^tBu)-TAG (30a)

Following the general procedure, 2,6-lutidine (69.9 μ l, 0.60 mmol), Bu₄NClO₄ (136.8 mg, 0.40 mmol), Ph₃P (157.4 mg, 0.60 mmol), Fmoc-Arg(Pbf)-OH (222.1 mg, 0.30 mmol), and H₂N-Asp(O^tBu)-Tag (185.7 mg, 0.20 mmol) were added to Ar-purged MeCN (8.0 mL)-*c*-hex (4.0 mL). The solution was electrolyzed at constant current (0.67 mA/cm²) under Ar at 50 °C. After 4.8 F/mol of electricity based on H₂N-Asp(O^tBu)-Tag was passed, the reaction mixture was extracted with *c*-hex (20 mL x 2), and then the solvent was replaced with MeCN. The resulting precipitants were collected by filtration and washed with MeCN to give the product in 93% yield (290.1 mg) as white solid.

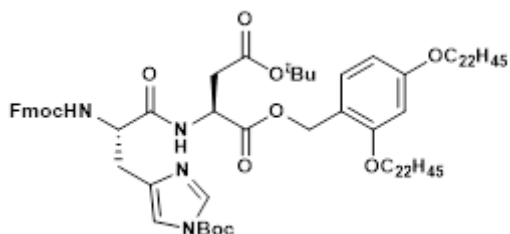
¹H NMR (CDCl₃, 600 MHz) δ 7.75 (2H, d, J = 7.6 Hz), 7.57 (2H, d, J = 7.6 Hz), 7.41-7.34 (2H, m), 7.32-7.26 (2H, m), 7.12 (1H, d, J = 8.3 Hz), 7.01 (1H, s), 6.42-6.34 (2H, m), 6.13-5.94 (2H, m), 5.72 (1H, s), 5.13 (2H, s), 4.91-4.84 (1H, m), 4.42-4.24 (3H, m), 4.18 (1H, t, J = 6.9 Hz), 3.91 (4H, t, J = 6.9 Hz), 3.27-3.10 (2H, m), 2.99-2.88 (3H, m), 2.69 (1H, dd, J = 17.2 Hz, 4.1 Hz), 2.60 (2.5H, s, major rotamer), 2.59 (0.5H, s, minor rotamer), 2.53 (2.5H, s, major rotamer), 2.52 (0.5H, minor rotamer), 2.09 (2.5H, s, major rotamer), 2.08 (0.5H, s, minor rotamer), 1.78-1.71 (4H, m), 1.70-1.53 (3H, m), 1.46-1.19 (93H, m), 0.88 (6H, t, J = 6.9 Hz); ¹³C NMR(CDCl₃, 150 MHz) δ 171.4, 171.3, 170.1, 160.9, 158.7, 158.3, 156.2, 143.8, 143.7, 141.3, 141.3, 138.4, 133.1, 132.3, 131.1, 127.7, 127.1, 125.1, 124.5, 120.0, 119.9, 117.4, 115.6, 104.6, 99.6, 86.3, 82.0, 68.2, 68.2, 67.1, 63.3, 53.9, 48.8, 47.1, 43.2, 40.5, 40.5, 40.4, 37.1, 31.9, 30.2, 29.7, 29.7, 29.6, 29.5, 29.4, 29.3, 29.1, 28.6, 27.9, 26.1, 26.0, 22.7, 19.3, 18.0, 14.1, 12.5; HRMS (ESI-MS) [M+Na]⁺ calcd for C₉₃H₁₄₇N₅O₁₂S 1581.0659, found 1581.0668.



Fmoc-His(Trt)-Asp(O'Bu)-TAG (3pa)

Following the general procedure, 2,6-lutidine (69.9 μ l, 0.60 mmol), Bu_4NClO_4 (136.8 mg, 0.40 mmol), Ph_3P (157.4 mg, 0.60 mmol), Fmoc-His(Trt)-OH (185.9 mg, 0.30 mmol), and $\text{H}_2\text{N-Asp(O'Bu)-Tag}$ (185.7 mg, 0.20 mmol) were added to Ar-purged MeCN (8.0 mL)-*c*-hex (4.0 mL). The solution was electrolyzed at constant current (0.67 mA/cm²) under Ar at room temperature. After 4.8 F/mol of electricity based on $\text{H}_2\text{N-Asp(O'Bu)-Tag}$ was passed, the reaction mixture was extracted with *c*-hex (20 mL x 2), and then the solvent was replaced with MeCN. The resulting precipitants were collected by filtration and washed with MeCN to give the product in 96% yield (dr =75:25, 294.4 mg) as white solid.

¹H NMR (CDCl_3 , 600 MHz) δ 7.82 (1H, d, J = 7.6 Hz), 7.75 (2H, d, J = 7.6 Hz), 7.65-7.53 (2H, m), 7.41-7.34 (3H, m), 7.34-7.26 (11H, m), 7.16-7.05 (7H, m), 6.98 (0.25H, d, J = 5.5 Hz), 6.74 (0.75H, d, J = 6.2 Hz), 6.69-6.58 (1H, m), 6.45-6.27 (2H, m), 5.16-5.04 (2H, m), 4.83-4.78 (0.75H, m), 4.78-4.74 (0.25H, m), 4.54 (0.75H, dd, J = 12.7 Hz, 5.5 Hz), 4.50 (0.25H, dd, J = 11.7 Hz, 5.5 Hz), 4.35-4.27 (2H, m), 4.24-4.12 (1H, m), 3.94-3.81 (4H, m), 3.12-2.94 (2H, m), 2.92-2.81 (1H, m), 2.76-2.69 (0.25H, m), 2.64 (0.75H, dd, J = 15.8 Hz, 3.4 Hz), 1.80-1.68 (4H, m), 1.45-1.21 (85H, m), 0.88 (6H, t, J = 6.9 Hz); ¹³C NMR (CDCl_3 , 150 MHz) δ 171.0, 170.6, 169.5, 160.6, 158.1, 156.3, 144.0, 144.0, 144.0, 143.9, 142.4, 141.2, 138.6, 138.4, 136.9, 136.8, 132.1, 132.1, 130.7, 129.8, 128.0, 127.6, 127.1, 127.1, 125.4, 125.3, 119.9, 119.6, 119.5, 116.0, 104.5, 99.6, 99.6, 81.5, 81.4, 75.3, 68.1, 68.0, 67.2, 62.7, 55.5, 55.0, 48.9, 47.1, 37.6, 37.5, 31.9, 30.6, 29.7, 29.7, 29.6, 29.6, 29.4, 29.4, 29.3, 29.1, 27.9, 27.9, 26.1, 26.0, 22.7, 14.1; HRMS (ESI-MS) $[\text{M}+\text{Na}]^+$ calcd for $\text{C}_{99}\text{H}_{140}\text{N}_4\text{O}_9$ 1552.0513, found 1552.0528.

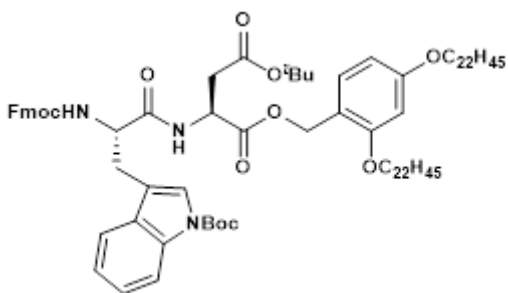


Fmoc-His(Boc)-Asp(O'Bu)-TAG (3qa)

Following the general procedure, 2,6-lutidine (69.9 μ l, 0.60 mmol), Bu_4NClO_4 (136.8 mg, 0.40 mmol), Ph_3P (157.4 mg, 0.60 mmol), Fmoc-His(Boc)-OH (143.2 mg, 0.30

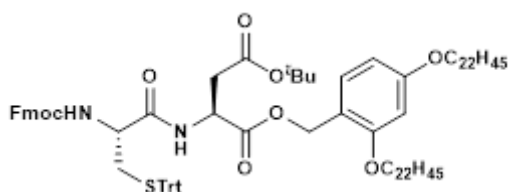
mmol), and H₂N-Asp(O^tBu)-Tag (185.7 mg, 0.20 mmol) were added to Ar-purged MeCN (8.0 mL)-*c*-hex (4.0 mL). The solution was electrolyzed at constant current (0.67 mA/cm²) under Ar at room temperature. After 4.8 F/mol of electricity based on H₂N-Asp(O^tBu)-Tag was passed, the reaction mixture was extracted with *c*-hex (20 mL x 2), and then the solvent was replaced with MeCN. The resulting precipitants were collected by filtration and washed with MeCN to give the product in 99% yield (274.8 mg) as white solid.

¹H NMR (CDCl₃, 600 MHz) δ 8.00 (1H, s), 7.75 (2H, d, *J* = 7.6 Hz), 7.64-7.50 (3H, m), 7.39 (2H, t, *J* = 7.2 Hz), 7.30 (2H, t, *J* = 6.9 Hz), 7.20 (1H, s), 7.12 (1H, d, *J* = 8.3 Hz), 6.47 (1H, d, *J* = 6.9 Hz), 6.43-6.29 (2H, m), 5.14 (1H, d, *J* = 11.7 Hz), 5.12 (1H, d, *J* = 11.7 Hz), 4.82-4.77 (1H, m), 4.63-4.48 (1H, m), 4.34 (2H, d, *J* = 7.6 Hz), 4.28-4.18 (1H, m), 3.94-3.82 (4H, m), 3.12 (1H, dd, *J* = 15.0 Hz, 4.1 Hz), 2.99 (1H, dd, *J* = 14.4 Hz, 5.5 Hz), 2.87 (1H, dd, *J* = 16.5 Hz, 4.1 Hz), 2.63 (1H, dd, *J* = 16.5 Hz, 3.4 Hz), 1.78-1.69 (4H, m), 1.59 (9H, s), 1.48-1.23 (85H, m), 0.88 (6H, t, *J* = 6.9 Hz); ¹³C NMR (CDCl₃, 150 MHz) δ 170.7, 170.4, 169.4, 160.7, 158.2, 156.1, 146.9, 144.0, 143.9, 141.3, 138.9, 136.8, 130.8, 127.6, 127.1, 127.1, 125.3, 125.3, 119.9, 116.0, 114.8, 104.5, 99.6, 85.5, 81.5, 68.1, 68.1, 67.3, 62.8, 54.6, 49.0, 47.1, 37.5, 31.9, 30.3, 29.7, 29.7, 29.6, 29.6, 29.4, 29.4, 29.3, 29.1, 27.9, 27.9, 26.1, 26.0, 22.7, 14.1; HRMS (ESI-MS) [M+Na]⁺ calcd for C₈₅H₁₃₄N₄O₁₁ 1409.9941, found 1409.9961.



Fmoc-Trp(Boc)-Asp(O^tBu)-TAG (3ra) Following the general procedure, 2,6-lutidine (69.9 μl, 0.60 mmol), Bu₄NClO₄ (136.8 mg, 0.40 mmol), Ph₃P (131.1 mg, 0.50 mmol), Fmoc-Trp(Boc)-OH (158.0 mg, 0.30 mmol), and H₂N-Asp(O^tBu)-Tag (185.7 mg, 0.20 mmol) were added to Ar-purged MeCN (8.0 mL)-*c*-hex (4.0 mL). The solution was electrolyzed at constant current (0.67 mA/cm²) under Ar at room temperature. After 4.8 F/mol of electricity based on H₂N-Asp(O^tBu)-Tag was passed, the reaction mixture was extracted with *c*-hex (20 mL x 2), and then the solvent was replaced with MeCN. The resulting precipitants were collected by filtration and washed with MeCN to give the product in 94% yield (271.1 mg) as white solid.

^1H NMR (CDCl_3 , 600 MHz) δ 8.13 (1H, s), 7.75 (2H, d, $J = 7.6$ Hz), 7.66 (1H, d, $J = 7.6$ Hz), 7.59-7.45 (3H, m), 7.42-7.34 (2H, m), 7.33-7.26 (3H, m), 7.22 (1H, t, $J = 7.6$ Hz), 7.14 (1H, d, $J = 8.3$ Hz), 6.74 (1H, d, $J = 7.6$ Hz), 6.44-6.31 (2H, m), 5.55 (1H, d, $J = 6.9$ Hz), 5.17 (1H, d, $J = 11.7$ Hz), 5.12 (1H, d, $J = 11.7$ Hz), 4.82-4.69 (1H, m), 4.63-4.50 (1H, m), 4.40-4.26 (2H, m), 4.25-4.13 (1H, m), 3.95-3.80 (4H, m), 3.33-3.09 (2H, m), 2.98-2.76 (1H, m), 2.71-2.54 (1H, m), 1.80-1.70 (4H, m), 1.64 (9H, s), 1.45-1.22 (85H, m), 0.88 (6H, t, $J = 6.9$ Hz); ^{13}C NMR (CDCl_3 , 150 MHz) δ 170.5, 170.2, 169.6, 160.8, 158.3, 155.8, 149.5, 143.8, 143.8, 141.2, 135.6, 130.9, 130.2, 127.7, 127.1, 125.1, 124.9, 124.5, 122.7, 119.9, 119.0, 115.9, 115.4, 114.9, 104.5, 99.6, 83.4, 81.7, 68.1, 68.1, 67.3, 62.9, 54.7, 48.8, 47.1, 37.2, 31.9, 29.7, 29.7, 29.6, 29.4, 29.4, 29.3, 29.1, 28.5, 28.2, 27.8, 26.0, 26.02, 22.7, 14.1; HRMS (ESI-MS) $[\text{M}+\text{Na}]^+$ calcd for $\text{C}_{90}\text{H}_{137}\text{N}_3\text{O}_{11}$ 1459.0145, found 1459.0140.

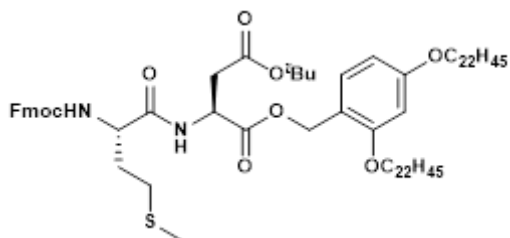


Fmoc-Cys(Trt)-Asp(O^tBu)-TAG (3sa)

Following the general procedure, 2,6-lutidine (69.9 μL , 0.60 mmol), Bu_4NClO_4 (136.8 mg, 0.40 mmol), Ph_3P (157.4 mg, 0.60 mmol), Fmoc-Cys(Trt)-OH (175.7 mg, 0.30 mmol), and $\text{H}_2\text{N-Asp(O}^t\text{Bu)-Tag}$ (185.7 mg, 0.20 mmol) were added to Ar-purged MeCN (8.0 mL)-*c*-hex (4.0 mL). The solution was electrolyzed at constant current (0.67 mA/cm^2) under Ar at 40 $^\circ\text{C}$. After 4.8 F/mol of electricity based on $\text{H}_2\text{N-Asp(O}^t\text{Bu)-Tag}$ was passed, the reaction mixture was extracted with *c*-hex (20 mL x 2), and then the solvent was replaced with MeCN. The resulting precipitants were collected by filtration and washed with MeCN to give the product in 95% yield (278.2 mg) as white solid.

^1H NMR (CDCl_3 , 600 MHz) δ 7.78-7.71 (2H, m), 7.62-7.49 (2H, m), 7.45-7.35 (8H, m), 7.30-7.23 (8H, m), 7.22-7.17 (3H, m), 7.11 (1H, d, $J = 8.3$ Hz), 6.85 (1H, d, $J = 6.9$ Hz), 6.43-6.30 (2H, m), 5.13 (2H, s), 5.01 (1H, d, $J = 7.6$ Hz), 4.72-4.66 (1H, m), 4.40-4.26 (2H, m), 4.19 (1H, t, $J = 6.2$ Hz), 3.94-3.84 (4H, m), 3.79 (1H, dd, $J = 13.1$ Hz, 7.6 Hz), 2.85 (1H, dd, $J = 17.1$ Hz, 4.1 Hz), 2.75-2.67 (2H, m), 2.63 (1H, dd, $J = 13.1$ Hz, 4.8 Hz), 1.74 (4H, tt, $J = 6.9$ Hz), 1.45-1.21 (85H, m), 0.88 (6H, t, $J = 6.9$ Hz); ^{13}C NMR (CDCl_3 , 150 MHz) δ 170.3, 169.8, 169.6, 160.7, 158.2, 155.8, 144.4, 141.3, 130.8, 129.6, 128.1, 127.7, 127.1, 126.9, 125.1, 119.9, 115.9, 104.5, 99.6, 81.6, 68.1, 68.1, 67.3, 62.9, 53.9, 49.0, 47.1, 37.3, 33.9, 31.9, 29.7, 29.7, 29.6, 29.6, 29.4, 29.4,

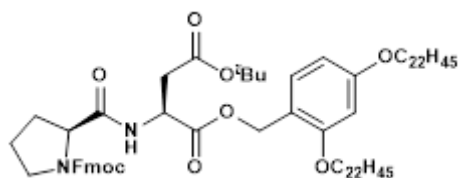
29.3, 29.1, 27.9, 26.1, 26.0, 22.7, 14.1; HRMS (ESI-MS) $[M+Na]^+$ calcd for $C_{96}H_{138}N_2O_9S$ 1518.0015, found 1518.0006.



Fmoc-Met-Asp(OtBu)-TAG (3ta)

Following the general procedure, 2,6-lutidine (69.9 μ L, 0.60 mmol), Bu_4NClO_4 (136.8 mg, 0.40 mmol), Ph_3P (104.9 mg, 0.40 mmol), Fmoc-Met-OH (111.4 mg, 0.30 mmol), and H_2N -Asp(OtBu)-Tag (185.7 mg, 0.20 mmol) were added to Ar-purged MeCN (8.0 mL)-*c*-hex (4.0 mL). The solution was electrolyzed at constant current (0.67 mA/cm²) under Ar at 50 °C. After 4.8 F/mol of electricity based on H_2N -Asp(OtBu)-Tag was passed, the reaction mixture was extracted with *c*-hex (20 mL x 2), and then the solvent was replaced with MeCN. The resulting precipitants were collected by filtration and washed with MeCN to give the product in 95% yield (242.7 mg) as white solid.

¹H NMR ($CDCl_3$, 600 MHz) δ 7.76 (2H, d, $J = 7.6$ Hz), 7.62-7.56 (2H, m), 7.40 (2H, t, $J = 7.2$ Hz), 7.34-7.28 (2H, m), 7.16 (1H, d, $J = 7.6$ Hz), 7.00 (1H, d, $J = 8.9$ Hz), 6.44-6.35 (2H, m), 5.59 (1H, d, $J = 8.3$ Hz), 5.18 (1H, d, $J = 11.7$ Hz), 5.14 (1H, d, $J = 11.7$ Hz), 4.88-4.80 (1H, m), 4.47-4.30 (3H, m), 4.21 (1H, t, $J = 6.9$ Hz), 3.96-3.87 (4H, m), 2.96 (1H, dd, $J = 17.1$ Hz, 4.8 Hz), 2.70 (1H, dd, $J = 17.2$ Hz, 4.8 Hz), 2.63-2.53 (2H, m), 2.09 (3H, s), 2.08-2.02 (1H, m), 2.00-1.93 (1H, m), 1.80-1.71 (4H, m), 1.46-1.20 (85H, m), 0.88 (6H, t, $J = 7.2$ Hz); ¹³C NMR ($CDCl_3$, 150 MHz) δ 170.7, 170.4, 169.9, 160.9, 158.4, 155.8, 143.9, 143.7, 141.3, 131.1, 127.7, 127.1, 125.1, 120.0, 120.0, 115.8, 104.6, 99.6, 81.9, 68.2, 68.1, 67.1, 63.1, 53.5, 48.7, 47.2, 37.3, 32.1, 31.9, 29.7, 29.7, 29.6, 29.6, 29.4, 29.4, 29.3, 29.2, 28.0, 26.1, 26.0, 22.7, 15.0, 14.1; HRMS (ESI-MS) $[M+Na]^+$ calcd for $C_{79}H_{128}N_2O_9S$ 1303.9233, found 1303.9230.

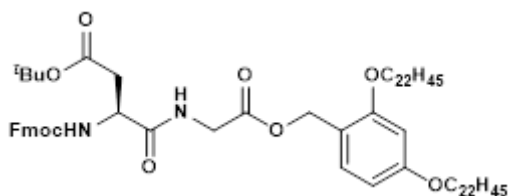


Fmoc-Pro-Asp(OtBu)-TAG (3ua)

Following the general procedure, 2,6-lutidine (69.9 μ L, 0.60 mmol), $NaClO_4$ (49.0 mg, 0.40 mmol), Ph_3P (209.8 mg, 0.80 mmol), Fmoc-Pro-OH (135.0 mg, 0.40 mmol), and

H₂N-Asp(O^tBu)-Tag (185.7 mg, 0.20 mmol) were added to Ar-purged MeCN (8.0 mL)-*c*-hex (4.0 mL). The solution was electrolyzed at constant current (0.67 mA/cm²) under Ar at 60 °C. After 4.8 F/mol of electricity based on H₂N-Asp(O^tBu)-Tag was passed, the reaction mixture was extracted with *c*-hex (20 mL x 2), and then the solvent was replaced with MeCN. The resulting precipitants were collected by filtration and washed with MeCN to give the product in 98% yield (247.0 mg) as white solid.

¹H NMR (CDCl₃, 600 MHz) δ 7.73 (2H, d, *J* = 7.6 Hz), 7.63-7.48 (2H, m), 7.42-7.33 (2H, m), 7.32-7.25 (3H, m), 7.10 (1H, brs), 6.45-6.29 (2H, m), 5.21-5.04 (2H, m), 4.82-4.70 (1H, m), 4.44-4.15 (4H, m), 3.96-3.83 (4H, m), 3.59-3.40 (2H, m), 2.85 (1H, dd, *J* = 16.5 Hz, 4.8 Hz), 2.73 (1H, dd, *J* = 16.5 Hz, 4.1 Hz), 2.22 (1H, brs), 2.12-1.99 (1H, brs), 1.96-1.81 (2H, m), 1.79-1.69 (4H, m), 1.49-1.22 (85H, m), 0.88 (6H, t, *J* = 7.6 Hz); ¹³C NMR (CDCl₃, 150 MHz) δ 171.8, 171.5, 170.7, 170.4, 169.8, 169.8, 160.7, 158.3, 155.7, 155.1, 144.3, 144.1, 143.9, 143.8, 141.3, 131.0, 127.7, 127.0, 125.2, 119.9, 116.3, 116.1, 115.8, 104.5, 104.5, 99.6, 81.6, 81.5, 68.1, 68.1, 67.9, 67.7, 63.0, 62.9, 60.9, 60.6, 48.9, 47.5, 47.2, 47.1, 47.0, 37.5, 37.4, 31.9, 31.3, 29.7, 29.7, 29.6, 29.6, 29.4, 29.4, 29.3, 29.1, 29.0, 27.9, 26.9, 26.1, 26.0, 24.5, 23.4, 22.7, 14.1; HRMS (ESI-MS) [M+Na]⁺ calcd for C₇₉H₁₂₆N₂O₉ 1269.9356, found 1296.9367.

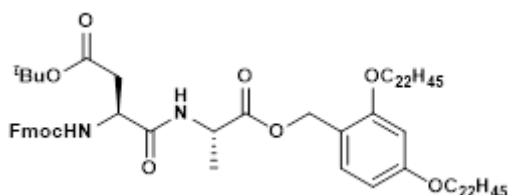


Fmoc-Asp(O^tBu)-Gly-TAG (3ab)

Following the general procedure, 2,6-lutidine (69.9 μl, 0.60 mmol), Bu₄NClO₄ (136.8 mg, 0.40 mmol), Ph₃P (131.1 mg, 0.50 mmol), Fmoc-Asp(O^tBu)-OH (123.4 mg, 0.30 mmol), and H₂N-Gly-Tag (162.9 mg, 0.20 mmol) were added to Ar-purged MeCN(8.0 mL)-*c*-hex (4.0 mL). The solution was electrolyzed at constant current (0.67 mA/cm²) under Ar at room temperature. After 4.8 F/mol of electricity based on H₂N-Gly-Tag was passed, the reaction mixture was extracted with *c*-hex (20 mL x 2), and then the solvent was replaced with MeCN. The resulting precipitants were collected by filtration and washed with MeCN to give the product in 97% yield (235.4 mg) as white solid.

¹H NMR (CDCl₃, 400 MHz) δ 7.76 (2H, d, *J* = 7.3 Hz), 7.59 (2H, d, *J* = 7.3 Hz), 7.40 (2H, t, *J* = 7.3 Hz), 7.35-7.27 (2H, m), 7.18 (1H, d, *J* = 8.2 Hz), 6.99 (1H, brs), 6.44-6.37 (2H, m), 5.96 (1H, d, *J* = 8.2 Hz), 5.16 (2H, s), 4.67-4.33 (3H, m), 4.23 (1H, t, *J* = 6.9 Hz), 4.10-3.87 (6H, m), 2.91 (1H, dd, *J* = 17.9 Hz, 4.1 Hz), 2.64 (1H, dd, *J* = 16.9

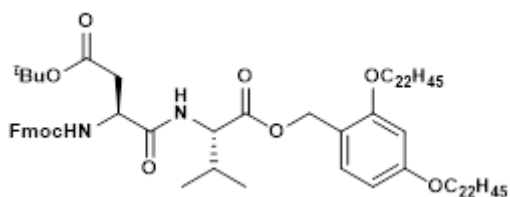
Hz, 6.4 Hz), 1.80-1.70 (4H, m), 1.48-1.20 (85H, m), 0.88 (6H, t, $J = 6.9$ Hz); ^{13}C NMR(CDCl_3 , 150 MHz) δ 170.5, 169.9, 168.4, 160.9, 158.4, 156.4, 143.8, 143.8, 141.3, 131.1, 127.7, 127.1, 125.1, 120.0, 115.9, 104.6, 99.6, 81.8, 68.2, 68.1, 67.3, 63.1, 48.8, 47.1, 44.3, 37.3, 31.9, 29.7, 29.7, 29.6, 29.6, 29.4, 29.4, 29.3, 29.2, 27.9, 26.1, 22.7, 14.1; HRMS (ESI-MS) $[\text{M}+\text{Na}]^+$ calcd for $\text{C}_{76}\text{H}_{122}\text{N}_2\text{O}_9$ 1229.9043, found 1229.9046.



Fmoc-Asp(O'Bu)-Ala-TAG (3ac)

Following the general procedure, 2,6-lutidine (69.9 μl , 0.60 mmol), Bu_4NClO_4 (136.8 mg, 0.40 mmol), Ph_3P (131.1 mg, 0.50 mmol), Fmoc-Asp(O'Bu)-OH (164.6 mg, 0.40 mmol), and $\text{H}_2\text{N-Ala-Tag}$ (165.7 mg, 0.20 mmol) were added to Ar-purged MeCN(8.0 mL)-*c*-hex (4.0 mL). The solution was electrolyzed at constant current (0.67 mA/cm^2) under Ar at 50 $^\circ\text{C}$. After 4.8 F/mol of electricity based on $\text{H}_2\text{N-Ala-Tag}$ was passed, the reaction mixture was extracted with *c*-hex (20 mL x 2), and then the solvent was replaced with MeCN. The resulting precipitants were collected by filtration and washed with MeCN to give the product in 94% yield (230.4 mg) as white solid.

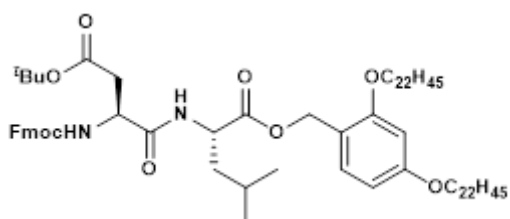
^1H NMR (CDCl_3 , 600 MHz) δ 7.76 (2H, d, $J = 7.6$ Hz), 7.59 (2H, d, $J = 6.9$ Hz), 7.40 (2H, t, $J = 7.6$ Hz), 7.31 (2H, t, $J = 7.6$ Hz), 7.16 (1H, d, $J = 8.3$ Hz), 7.12 (1H, d, $J = 6.9$ Hz), 6.45-6.37 (2H, m), 5.97 (1H, d, $J = 7.6$ Hz), 5.18 (1H, d, $J = 11.7$ Hz), 5.09 (1H, d, $J = 11.7$ Hz), 4.60-4.49 (2H, m), 4.45-4.36 (2H, m), 4.23 (1H, t, $J = 6.9$ Hz), 3.96-3.88 (4H, m), 2.92 (1H, dd, $J = 17.2$ Hz, 4.1 Hz), 2.60 (1H, dd, $J = 17.2$ Hz, 6.9 Hz), 1.79-1.71 (4H, m), 1.53-1.22 (88H, m), 0.88 (6H, t, $J = 6.9$ Hz); ^{13}C NMR(CDCl_3 , 150 MHz) δ 172.5, 171.3, 170.0, 160.9, 158.4, 155.9, 143.8, 143.7, 141.3, 131.1, 127.8, 127.1, 125.1, 120.0, 115.9, 104.5, 99.7, 81.9, 68.1, 67.3, 62.9, 50.9, 48.5, 47.1, 37.7, 31.9, 29.7, 29.7, 29.6, 29.6, 29.4, 29.4, 29.3, 29.2, 28.0, 26.0, 26.0, 22.7, 18.3, 14.1; HRMS (ESI-MS) $[\text{M}+\text{Na}]^+$ calcd for $\text{C}_{77}\text{H}_{124}\text{N}_2\text{O}_9$ 1243.9199, found 1243.9189.



Fmoc-Asp(O^tBu)-Val-TAG (3ad)

Following the general procedure, 2,6-lutidine (69.9 μ l, 0.60 mmol), Bu₄NClO₄ (136.8 mg, 0.40 mmol), Ph₃P (131.1 mg, 0.50 mmol), Fmoc-Asp(O^tBu)-OH (123.4 mg, 0.30 mmol), and H₂N-Val-Tag (171.3 mg, 0.20 mmol) were added to Ar-purged MeCN(8.0 mL)-*c*-hex (4.0 mL). The solution was electrolyzed at constant current (0.67 mA/cm²) under Ar at 60 °C. After 4.8 F/mol of electricity based on H₂N-Val-Tag was passed, the reaction mixture was extracted with *c*-hex (20 mL x 2), and then the solvent was replaced with MeCN. The resulting precipitants were collected by filtration and washed with MeCN to give the product in 98% yield (246.9 mg) as white solid.

¹H NMR (CDCl₃, 400 MHz) δ 7.76 (2H, d, J = 7.3 Hz), 7.59 (2H, d, J = 7.3 Hz), 7.40 (2H, t, J = 7.3 Hz), 7.31 (2H, t, J = 7.3 Hz), 7.22-7.05 (2H, m), 6.47-6.34 (2H, m), 6.05 (1H, d, J = 8.2 Hz), 5.18 (1H, d, J = 11.9 Hz), 5.06 (1H, d, J = 11.9 Hz), 4.64-4.54 (1H, m), 4.50 (1H, dd, J = 8.7 Hz, 4.6 Hz), 4.45-4.33 (2H, m), 4.23 (1H, t, J = 6.9 Hz), 3.98-3.85 (4H, m), 2.92 (1H, dd, J = 17.4 Hz, 4.1 Hz), 2.61 (1H, dd, J = 17.4 Hz, 7.3 Hz), 2.26-2.12 (1H, m), 1.81-1.68 (4H, m), 1.49-1.19 (85H, m), 0.95-0.81 (12H, m); ¹³C NMR(CDCl₃, 100 MHz) δ 171.6, 171.4, 170.5, 160.9, 158.5, 155.9, 143.9, 143.7, 141.3, 131.4, 127.7, 127.1, 125.1, 120.0, 116.0, 104.5, 99.7, 81.9, 68.1, 67.3, 62.7, 57.5, 50.9, 47.1, 37.7, 31.9, 31.1, 29.7, 29.6, 29.4, 29.4, 29.3, 29.2, 28.0, 26.1, 26.0, 22.7, 19.0, 17.5, 14.1; HRMS (ESI-MS) [M+Na]⁺ calcd for C₇₉H₁₂₈N₂O₉ 1271.9512, found 1271.9505.

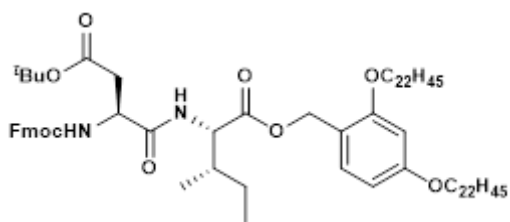


Fmoc-Asp(O^tBu)-Leu-TAG (3ae)

Following the general procedure, 2,6-lutidine (69.9 μ l, 0.60 mmol), Bu₄NClO₄ (136.8 mg, 0.40 mmol), Ph₃P (131.1 mg, 0.50 mmol), Fmoc-Asp(O^tBu)-OH (123.4 mg, 0.30 mmol), and H₂N-Leu-Tag (174.1 mg, 0.20 mmol) were added to Ar-purged MeCN(8.0 mL)-*c*-hex (4.0 mL). The solution was electrolyzed at constant current (0.67 mA/cm²)

under Ar at 50 °C. After 4.8 F/mol of electricity based on H₂N-Leu-Tag was passed, the reaction mixture was extracted with *c*-hex (20 mL x 2), and then the solvent was replaced with MeCN. The resulting precipitants were collected by filtration and washed with MeCN to give the product in 96% yield (241.9 mg) as white solid.

¹H NMR (CDCl₃, 400 MHz) δ 7.76 (2H, d, *J* = 7.3 Hz), 7.59 (2H, t, *J* = 7.3 Hz), 7.40 (2H, t, *J* = 7.3 Hz), 7.35-7.27 (2H, m), 7.16 (1H, d, *J* = 7.8 Hz), 6.99 (1H, d, *J* = 7.8 Hz), 6.46-6.35 (2H, m), 6.01 (1H, d, *J* = 8.2 Hz), 5.16 (1H, d, *J* = 11.9 Hz), 5.07 (1H, d, *J* = 11.9 Hz), 4.65-4.50 (2H, m), 4.40 (2H, d, *J* = 7.3 Hz), 4.22 (1H, t, *J* = 7.1 Hz), 3.92 (4H, t, *J* = 6.4 Hz), 2.92 (1H, dd, *J* = 17.4 Hz, 4.1 Hz), 2.59 (1H, dd, *J* = 16.9 Hz, 6.9 Hz), 1.81-1.51 (7H, m), 1.50-1.19 (85H, m), 0.92-0.82 (12H, m); ¹³C NMR (CDCl₃, 100 MHz) δ 172.5, 171.5, 170.2, 160.8, 158.4, 155.9, 143.8, 143.7, 141.3, 131.1, 127.8, 127.1, 125.1, 120.0, 116.0, 104.5, 99.6, 81.9, 68.1, 67.3, 62.7, 51.2, 50.9, 47.1, 41.5, 37.7, 31.9, 30.0, 29.7, 29.6, 29.6, 29.4, 29.4, 29.3, 29.2, 28.0, 26.1, 26.0, 24.7, 22.8, 22.7, 21.9, 14.1; HRMS (ESI-MS) [M+Na]⁺ calcd for C₈₀H₁₃₀N₂O₉ 1285.9669, found 1285.9652.

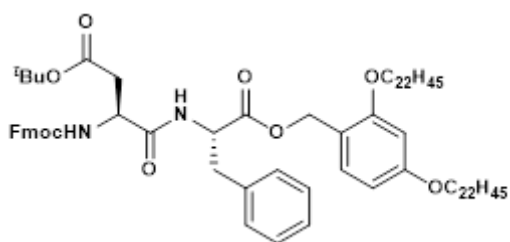


Fmoc-Asp(O^tBu)-Ile-TAG (3fa)

Following the general procedure, 2,6-lutidine (69.9 μl, 0.60 mmol), Bu₄NClO₄ (136.8 mg, 0.40 mmol), Ph₃P (131.1 mg, 0.50 mmol), Fmoc-Asp(O^tBu)-OH (123.4 mg, 0.30 mmol), and H₂N-Ile-Tag (188.0 mg, 0.20 mmol) were added to Ar-purged MeCN (8.0 mL)-*c*-hex (4.0 mL). The solution was electrolyzed at constant current (0.67 mA/cm²) under Ar at 60 °C. After 4.8 F/mol of electricity based on H₂N-Ile-Tag was passed, the reaction mixture was extracted with *c*-hex (20 mL x 2), and then the solvent was replaced with MeCN. The resulting precipitants were collected by filtration and washed with MeCN to give the product in 93% yield (234.4 mg) as white solid.

¹H NMR (CDCl₃, 400 MHz) δ 7.76 (2H, d, *J* = 7.3 Hz), 7.59 (2H, d, *J* = 7.3 Hz), 7.40 (2H, t, *J* = 7.3 Hz), 7.35-7.27 (2H, m), 7.22-7.07 (2H, m), 6.46-6.31 (2H, m), 6.05 (1H, d, *J* = 7.8 Hz), 5.17 (1H, d, *J* = 11.9 Hz), 5.06 (1H, d, *J* = 11.9 Hz), 4.63-4.48 (2H, m), 4.46-4.32 (2H, m), 4.23 (1H, t, *J* = 6.9 Hz), 3.91 (4H, t, *J* = 6.4 Hz), 2.91 (1H, dd, *J* = 17.4 Hz, 4.1 Hz), 2.61 (1H, dd, *J* = 17.4 Hz, 6.9 Hz), 1.98-1.82 (1H, m), 1.82-1.66 (4H, m), 1.49-1.20 (86H, m), 1.2-1.07 (1H, m), 0.94-0.79 (12H, m); ¹³C NMR (CDCl₃, 100

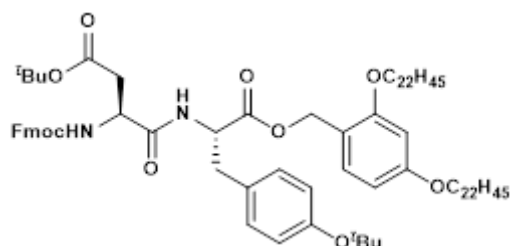
MHz) δ 171.6, 171.4, 170.3, 160.9, 158.4, 155.9, 143.9, 143.7, 141.3, 131.4, 127.7, 127.1, 125.1, 120.0, 116.0, 104.4, 99.6, 81.9, 68.1, 68.1, 67.3, 62.6, 56.9, 50.9, 47.1, 37.7, 31.9, 30.0, 29.7, 29.6, 29.4, 29.4, 29.3, 29.2, 28.0, 26.1, 24.9, 22.7, 15.5, 14.1, 11.7; HRMS (ESI-MS) $[M+Na]^+$ calcd for $C_{80}H_{130}N_2O_9$ 1285.9669, found 1285.9656.



Fmoc-Asp(O'Bu)-Phe-TAG (3ag)

Following the general procedure, 2,6-lutidine (69.9 μ l, 0.60 mmol), Bu_4NClO_4 (136.8 mg, 0.40 mmol), Ph_3P (131.1 mg, 0.50 mmol), Fmoc-Asp(O'Bu)-OH (164.6 mg, 0.40 mmol), and H_2N -Phe-Tag (180.9 mg, 0.20 mmol) were added to Ar-purged MeCN(8.0 mL)-*c*-hex (4.0 mL). The solution was electrolyzed at constant current (0.67 mA/cm²) under Ar at 40 °C. After 4.8 F/mol of electricity based on H_2N -Phe-Tag was passed, the reaction mixture was extracted with *c*-hex (20 mL x 2), and then the solvent was replaced with MeCN. The resulting precipitants were collected by filtration and washed with MeCN to give the product in 95% yield (245.9 mg) as white solid.

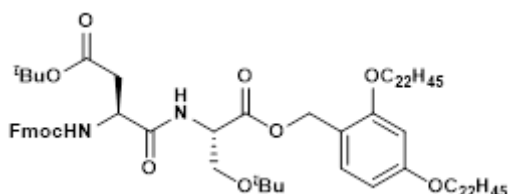
¹H NMR (CDCl₃, 400 MHz) δ 7.76 (2H, d, J = 7.3 Hz), 7.57 (2H, d, J = 7.3 Hz), 7.40 (2H, t, J = 7.3 Hz), 7.30 (2H, t, J = 7.3 Hz), 7.23-7.15 (3H, m), 7.12 (1H, d, J = 8.7 Hz), 7.09-7.03 (2H, m), 6.99 (1H, d, J = 7.3 Hz), 6.48-6.35 (2H, m), 5.92 (1H, d, J = 8.2 Hz), 5.17 (1H, d, J = 11.9 Hz), 5.09 (1H, d, J = 11.9 Hz), 4.88-4.76 (1H, m), 4.63-4.44 (1H, m), 4.43-4.28 (2H, m), 4.20 (1H, t, J = 6.9 Hz), 4.01-3.87 (4H, m), 3.22-3.00 (2H, m), 2.87 (1H, dd, J = 16.9 Hz, 4.1 Hz), 2.59 (1H, dd, J = 16.9 Hz, 6.4 Hz), 1.87-1.66 (4H, m), 1.50-1.20 (85H, m), 0.88 (6H, t, J = 6.9 Hz); ¹³C NMR(CDCl₃, 100 MHz) δ 171.2, 170.9, 170.1, 161.0, 158.5, 155.9, 143.8, 143.7, 141.3, 135.7, 131.6, 129.4, 128.5, 127.7, 127.1, 127.0, 125.1, 120.0, 115.8, 104.5, 99.7, 81.8, 68.2, 68.1, 67.3, 62.9, 53.5, 50.9, 47.1, 37.6, 37.4, 31.9, 30.0, 29.7, 29.6, 29.4, 29.4, 29.3, 29.2, 28.0 26.1, 22.7, 14.1; HRMS (ESI-MS) $[M+Na]^+$ calcd for $C_{83}H_{128}N_2O_9$ 1319.9512, found 1319.9483.



Fmoc-Asp(O^tBu)-Tyr(O^tBu)-TAG (3ah)

Following the general procedure, 2,6-lutidine (69.9 μ l, 0.60 mmol), Bu₄NClO₄ (136.8 mg, 0.40 mmol), Ph₃P (131.1 mg, 0.50 mmol), Fmoc-Asp(O^tBu)-OH (164.6 mg, 0.40 mmol), and H₂N- Tyr(O^tBu)-Tag (195.3 mg, 0.20 mmol) were added to Ar-purged MeCN(8.0 mL)-*c*-hex (4.0 mL). The solution was electrolyzed at constant current (0.67 mA/cm²) under Ar at room temperature. After 4.8 F/mol of electricity based on H₂N- Tyr(O^tBu)-Tag was passed, the reaction mixture was extracted with *c*-hex (20 mL x 2), and then the solvent was replaced with MeCN. The resulting precipitants were collected by filtration and washed with MeCN to give the product in 95% yield (261.4 mg) as white solid.

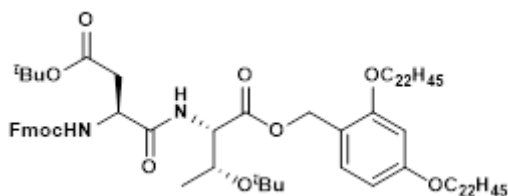
¹H NMR (CDCl₃, 400 MHz) δ 7.76 (2H, d, J = 7.3 Hz), 7.58 (2H, d, J = 7.3 Hz), 7.40 (2H, t, J = 7.3 Hz), 7.31 (2H, t, J = 7.3 Hz), 7.13 (1H, d, J = 8.2 Hz), 7.00 (1H, d, J = 7.8 Hz), 6.95 (2H, d, J = 8.2 Hz), 6.82 (2H, d, J = 8.2 Hz), 6.47-6.36 (2H, m), 5.92 (1H, d, J = 7.8 Hz), 5.16 (1H, d, J = 11.9 Hz), 5.09 (1H, d, J = 11.9 Hz), 4.85-4.75 (1H, m), 4.59-4.45 (1H, m), 4.42-4.29 (2H, m), 4.22 (1H, t, J = 7.3 Hz), 3.99-3.88 (4H, m), 3.13-2.97 (2H, m), 2.87 (1H, dd, J = 17.4 Hz, 3.7 Hz), 2.59 (1H, dd, J = 17.4 Hz, 6.9 Hz), 1.84-1.71 (4H, m), 1.53-1.20 (94H, m), 0.88 (6H, t, J = 6.9 Hz); ¹³C NMR(CDCl₃, 100 MHz) δ 171.2, 171.0, 170.0, 161.0, 158.5, 155.9, 154.4, 143.9, 143.7, 141.3, 131.6, 130.4, 129.9, 127.7, 127.1, 125.2, 124.1, 120.0, 115.9, 104.5, 99.6, 81.8, 78.2, 68.2, 68.1, 67.3, 62.8, 53.6, 50.9, 47.1, 37.5, 37.0, 31.9, 30.0, 29.7, 29.6, 29.4, 29.4, 29.3, 29.2, 28.8, 28.0, 26.1, 22.7, 14.1; HRMS (ESI-MS) [M+Na]⁺ calcd for C₈₇H₁₃₆N₂O₁₀ 1392.0087, found 1392.0067



Fmoc-Asp(O^tBu)-Ser(O^tBu)-TAG (3ai)

Following the general procedure, 2,6-lutidine (69.9 μ l, 0.60 mmol), Bu₄NClO₄ (136.8 mg, 0.40 mmol), Ph₃P (131.1 mg, 0.50 mmol), Fmoc-Asp(O^tBu)-OH (123.4 mg, 0.30

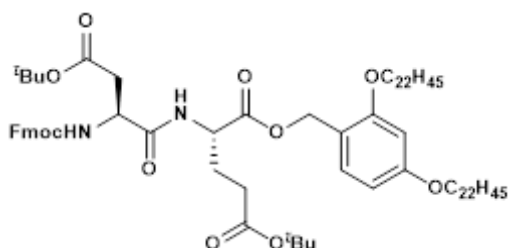
mmol), and H₂N-Ser(O^tBu)-Tag (180.1 mg, 0.20 mmol) were added to Ar-purged MeCN(8.0 mL)-*c*-hex (4.0 mL). The solution was electrolyzed at constant current (0.67 mA/cm²) under Ar at 40 °C. After 4.8 F/mol of electricity based on H₂N-Ser(O^tBu)-Tag was passed, the reaction mixture was extracted with *c*-hex (20 mL x 2), and then the solvent was replaced with MeCN. The resulting precipitants were collected by filtration and washed with MeCN to give the product in 98% yield (254.6 mg) as white solid. ¹H NMR (CDCl₃, 400 MHz) δ 7.76 (2H, d, *J* = 7.3 Hz), 7.59 (2H, d, *J* = 7.3 Hz), 7.43-7.34 (3H, m), 7.31 (2H, td, *J* = 7.3 Hz, 0.9 Hz), 7.18 (1H, d, *J* = 8.2 Hz), 6.44-6.34 (2H, m), 5.99 (1H, d, *J* = 7.8 Hz), 5.16 (2H, s), 4.71-4.55 (2H, m), 4.38 (2H, d, *J* = 7.3 Hz), 4.23 (1H, t, *J* = 7.3 Hz), 3.98-3.86 (4H, m), 3.83 (1H, dd, *J* = 8.7 Hz, 2.8 Hz), 3.54 (1H, dd, *J* = 8.7 Hz, 3.2 Hz), 2.87 (1H, dd, *J* = 17.4 Hz, 4.1 Hz), 2.66 (1H, dd, *J* = 16.9 Hz, 7.3 Hz), 1.82-1.70 (4H, m), 1.48-.20 (85H, m), 1.10 (9H, m), 0.88 (6H, t, *J* = 6.9 Hz); ¹³C NMR(CDCl₃, 100 MHz) δ 171.1, 170.5, 167.0, 160.6, 158.2, 155.8, 143.9, 143.8, 141.3, 130.8, 127.7, 127.1, 125.1, 120.0, 116.3, 104.6, 99.6, 81.8, 73.4, 68.1, 68.1, 67.3, 62.4, 61.6, 53.2, 50.7, 47.1, 38.0, 31.9, 29.7, 29.6, 29.4, 29.4, 29.3, 29.2, 28.1, 27.2, 26.1, 22.7, 14.1; HRMS (ESI-MS) [M+Na]⁺ calcd for C₈₁H₁₃₂N₂O₁₀ 1315.9774, found 1315.9763.



Fmoc-Asp(O^tBu)-Thr(O^tBu)-TAG (3aj)

Following the general procedure, 2,6-lutidine (69.9 μl, 0.60 mmol), Bu₄NClO₄ (136.8 mg, 0.40 mmol), Ph₃P (131.1 mg, 0.50 mmol), Fmoc-Asp(O^tBu)-OH (123.4 mg, 0.30 mmol), and H₂N-Thr(O^tBu)-Tag (182.9 mg, 0.20 mmol) were added to Ar-purged MeCN(8.0 mL)-*c*-hex (4.0 mL). The solution was electrolyzed at constant current (0.67 mA/cm²) under Ar at 40 °C. After 4.8 F/mol of electricity based on H₂N-Thr(O^tBu)-Tag was passed, the reaction mixture was extracted with *c*-hex (20 mL x 2), and then the solvent was replaced with MeCN. The resulting precipitants were collected by filtration and washed with MeCN to give the product in 95% yield (249.5 mg) as white solid. ¹H NMR (CDCl₃, 400 MHz) δ 7.76 (2H, d, *J* = 7.3 Hz), 7.60 (2H, d, *J* = 7.3 Hz), 7.39 (2H, t, *J* = 7.3 Hz), 7.35-7.27 (3H, m), 7.18 (1H, d, *J* = 8.7 Hz), 4.46-6.37 (2H, m), 6.03 (1H, d, *J* = 7.8 Hz), 5.15 (1H, d, *J* = 11.9 Hz), 5.03 (1H, d, *J* = 11.9 Hz), 4.75-4.57 (1H, m), 4.47 (1H, dd, *J* = 9.2 Hz, 1.4 Hz), 4.43-4.30 (2H, m), 4.29-4.17 (2H, m), 3.99-3.85

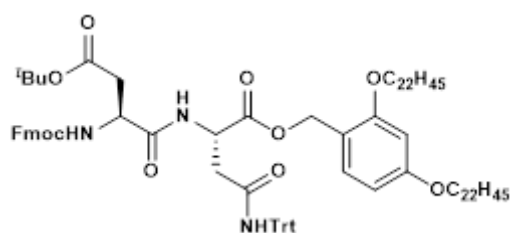
(4H, m), 2.88 (1H, dd, $J = 17.4$ Hz, 4.1 Hz), 2.66 (1H, dd, $J = 17.4$ Hz, 6.9 Hz), 1.81-1.70 (4H, m), 1.49-1.21 (85H, m), 1.16 (3H, d, $J = 6.4$ Hz), 1.08 (9H, s), 0.88 (6H, t, $J = 6.9$ Hz); ^{13}C NMR(CDCl_3 , 100 MHz) δ 171.2, 171.0, 170.4, 160.8, 158.4, 155.8, 143.9, 143.8, 141.3, 131.4, 127.7, 127.1, 125.2, 120.0, 115.9, 104.6, 99.6, 81.6, 73.9, 68.1, 68.1, 67.3, 67.1, 62.6, 58.2, 50.9, 47.2, 38.1, 31.9, 29.7, 29.6, 29.4, 29.4, 29.3, 29.1, 28.3, 28.1, 26.1, 22.7, 21.1, 20.0, 14.1; HRMS (ESI-MS) $[\text{M}+\text{Na}]^+$ calcd for $\text{C}_{82}\text{H}_{134}\text{N}_2\text{O}_{10}$ 1329.9931, found 1329.9918.



Fmoc-Asp(OtBu)-Glu(OtBu)-TAG (3ak)

Following the general procedure, 2,6-lutidine (69.9 μl , 0.60 mmol), Bu_4NClO_4 (136.8 mg, 0.40 mmol), Ph_3P (131.1 mg, 0.50 mmol), Fmoc-Asp(OtBu)-OH (164.6 mg, 0.40 mmol), and $\text{H}_2\text{N-Glu(OtBu)-Tag}$ (188.6 mg, 0.20 mmol) were added to Ar-purged MeCN(8.0 mL)-*c*-hex (4.0 mL). The solution was electrolyzed at constant current (0.67 mA/cm^2) under Ar at room temperature. After 4.8 F/mol of electricity based on $\text{H}_2\text{N-Glu(OtBu)-Tag}$ was passed, the reaction mixture was extracted with *c*-hex (20 mL x 2), and then the solvent was replaced with MeCN. The resulting precipitants were collected by filtration and washed with MeCN to give the product in 95% yield (254.3 mg) as white solid.

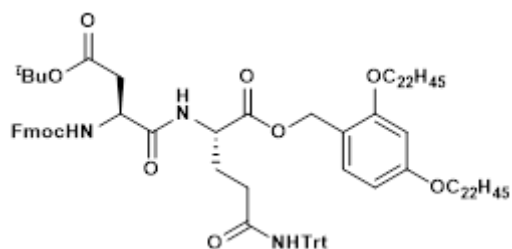
^1H NMR (CDCl_3 , 600 MHz) δ 7.76 (2H, d, $J = 7.6$ Hz), 7.66-7.51 (2H, m), 7.40 (2H, t, $J = 7.6$ Hz), 7.31 (2H, t, $J = 7.6$ Hz), 7.25-7.11 (2H, m), 6.46-6.34 (2H, m), 5.99 (1H, d, $J = 8.3$ Hz), 5.19 (1H, d, $J = 11.7$ Hz), 5.09 (1H, d, $J = 11.7$ Hz), 4.65-4.52 (2H, m), 4.41 (2H, d, $J = 6.9$ Hz), 4.23 (1H, t, $J = 6.9$ Hz), 3.97-3.85 (4H, m), 2.96 (1H, dd, $J = 17.3$ Hz, 3.4 Hz), 2.59 (1H, dd, $J = 17.3$ Hz, 5.5 Hz), 2.35-2.11 (3H, m), 2.01-1.88 (1H, m), 1.80-1.71 (4H, m), 1.53-1.20 (94H, m), 0.88 (6H, t, $J = 6.9$ Hz); ^{13}C NMR(CDCl_3 , 150 MHz) δ 172.0, 171.4, 170.4, 160.9, 158.4, 155.9, 143.9, 143.7, 141.3, 131.2, 127.8, 127.1, 125.1, 120.0, 115.9, 104.5, 99.7, 81.9, 80.5, 68.1, 68.1, 67.3, 63.0, 52.1, 51.0, 47.1, 37.6, 31.9, 31.2, 29.7, 29.7, 29.6, 29.4, 29.4, 29.3, 29.1, 28.1, 27.5, 26.1, 26.0, 22.7, 14.1; HRMS (ESI-MS) $[\text{M}+\text{Na}]^+$ calcd for $\text{C}_{83}\text{H}_{134}\text{N}_2\text{O}_{11}$ 1357.9880, found 1357.9891.



Fmoc-Asp(O^tBu)-Asn(Trt)-TAG (3a)

Following the general procedure, 2,6-lutidine (69.9 μ l, 0.60 mmol), Bu₄NClO₄ (136.8 mg, 0.40 mmol), Ph₃P (131.1 mg, 0.50 mmol), Fmoc-Asp(O^tBu)-OH (123.4 mg, 0.30 mmol), and H₂N-Asn(Trt)-Tag (222.8 mg, 0.20 mmol) were added to Ar-purged MeCN(8.0 mL)-*c*-hex (4.0 mL). The solution was electrolyzed at constant current (0.67 mA/cm²) under Ar at room temperature. After 4.8 F/mol of electricity based on H₂N-Asn(Trt)-Tag was passed, the reaction mixture was extracted with *c*-hex (20 mL x 2), and then the solvent was replaced with MeCN. The resulting precipitants were collected by filtration and washed with MeCN to give the product in 96% yield (289.9 mg) as white solid.

¹H NMR (CDCl₃, 400 MHz) δ 7.75 (2H, d, J = 7.3 Hz), 7.63-7.43 (3H, m), 7.37 (2H, d, J = 7.3 Hz), 7.29-7.10 (17H, m), 7.04 (1H, d, J = 8.2 Hz), 6.70 (1H, s), 6.39 (1H, d, J = 1.8 Hz), 6.31 (1H, dd, J = 8.2 Hz, 1.8 Hz), 5.78 (1H, d, J = 8.2 Hz), 5.13 (1H, d, J = 11.9 Hz), 5.06 (1H, d, J = 11.9 Hz), 4.83-4.74 (1H, m), 4.60-4.46 (1H, m), 4.32 (2H, d, J = 7.3 Hz), 4.18 (1H, t, J = 7.3 Hz), 3.95-3.79 (4H, m), 3.03 (1H, dd, J = 15.6 Hz, 4.6 Hz), 2.89-2.70 (2H, m), 2.60 (1H, dd, J = 16.5 Hz, 5.0 Hz), 1.79-1.68 (4H, m), 1.46-1.20 (85H, m), 0.88 (6H, t, J = 6.9 Hz); ¹³C NMR(CDCl₃, 100 MHz) δ 171.5, 171.4, 170.9, 170.7, 160.9, 158.4, 156.0, 144.7, 143.8, 143.6, 141.3, 131.2, 128.7, 127.8, 127.8, 127.1, 126.9, 125.1, 120.0, 115.8, 104.6, 99.7, 81.9, 70.5, 68.2, 68.1, 67.3, 63.0, 52.0, 47.1, 37.4, 33.3, 31.9, 29.7, 29.4, 29.4, 29.3, 29.1, 28.0, 26.1, 26.0, 22.7, 14.1; HRMS (ESI-MS) [M+Na]⁺ calcd for C₉₇H₁₃₉N₃O₁₀ 1529.0353, found 1529.0337.

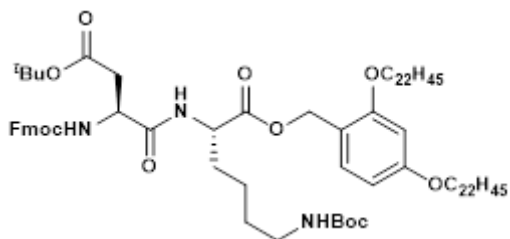


Fmoc-Asp(O^tBu)-Gln(Trt)-TAG (3am)

Following the general procedure, 2,6-lutidine (69.9 μ l, 0.60 mmol), Bu₄NClO₄ (136.8 mg, 0.40 mmol), Ph₃P (131.1 mg, 0.50 mmol), Fmoc-Asp(O^tBu)-OH (123.4 mg, 0.30

mmol), and H₂N-Asn(Trt)-Tag (225.6 mg, 0.20 mmol) were added to Ar-purged MeCN(8.0 mL)-*c*-hex (4.0 mL). The solution was electrolyzed at constant current (0.67 mA/cm²) under Ar at room temperature. After 4.8 F/mol of electricity based on H₂N-Gln(Trt)-Tag was passed, the reaction mixture was extracted with *c*-hex (20 mL x 2), and then the solvent was replaced with MeCN. The resulting precipitants were collected by filtration and washed with MeCN to give the product in 93% yield (282.0 mg) as white solid.

¹H NMR (CDCl₃, 400 MHz) δ 7.75 (2H, d, *J* = 7.3 Hz), 7.62-7.50 (2H, m), 7.39 (2H, t, *J* = 7.3 Hz), 7.33-7.26 (2H, m), 7.24-7.13 (18H, m), 6.43-6.33 (2H, m), 5.85 (1H, d, *J* = 8.2 Hz), 5.17 (1H, d, *J* = 11.9 Hz), 5.10 (1H, d, *J* = 11.9 Hz), 4.66-4.56 (1H, m), 4.56-4.46 (1H, m), 4.38 (2H, d, *J* = 6.0 Hz), 4.20 (1H, t, *J* = 6.9 Hz), 3.95-3.82 (4H, m), 2.94 (1H, dd, *J* = 17.5 Hz, 4.1 Hz), 2.59 (1H, dd, *J* = 17.5 Hz, 4.6 Hz), 2.41-2.16 (3H, m), 1.95-1.81 (1H, m), 1.80-1.67 (4H, m), 1.49-1.20 (85H, m), 0.88 (6H, t, *J* = 6.9 Hz); ¹³C NMR(CDCl₃, 100 MHz) δ 170.5, 170.4, 169.1, 160.6, 158.0, 155.9, 144.4, 144.0, 143.7, 141.2, 130.7, 128.7, 128.0, 127.7, 127.1, 125.2, 119.9, 116.0, 104.6, 99.6, 81.6, 70.9, 68.1, 68.1, 67.3, 62.9, 51.4, 49.5, 47.0, 38.3, 37.9, 31.9, 30.0, 29.7, 29.4, 29.4, 29.3, 29.1, 28.0, 26.1, 26.0, 22.7, 14.1; HRMS (ESI-MS) [M+Na]⁺ calcd for C₉₈H₁₄₁N₃O₁₀ 1543.0509, found 1543.0511.

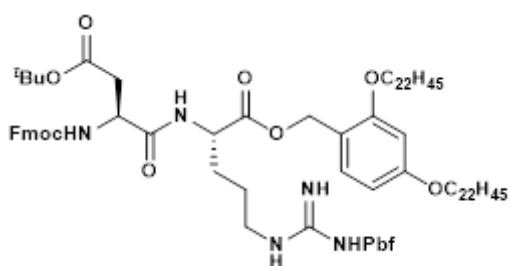


Fmoc-Asp(O^tBu)-Lys(Boc)-TAG (3an)

Following the general procedure, 2,6-lutidine (69.9 μL, 0.60 mmol), Bu₄NClO₄ (136.8 mg, 0.40 mmol), Ph₃P (131.1 mg, 0.50 mmol), Fmoc-Asp(O^tBu)-OH (123.4 mg, 0.30 mmol), and H₂N-Lys(Boc)-Tag (197.1 mg, 0.20 mmol) were added to Ar-purged MeCN(8.0 mL)-*c*-hex (4.0 mL). The solution was electrolyzed at constant current (0.67 mA/cm²) under Ar at 50 °C. After 4.8 F/mol of electricity based on H₂N-Lys(Boc)-Tag was passed, the reaction mixture was extracted with *c*-hex (20 mL x 2), and then the solvent was replaced with MeCN. The resulting precipitants were collected by filtration and washed with MeCN to give the product in 98% yield (255.6 mg) as white solid.

¹H NMR (CDCl₃, 400 MHz) δ 7.76 (2H, d, *J* = 7.3 Hz), 7.59 (2H, d, *J* = 7.3 Hz), 7.39 (2H, t, *J* = 7.3 Hz), 7.35-7.27 (2H, m), 7.16 (1H, d, *J* = 8.2 Hz), 7.08 (1H, d, *J* = 7.8 Hz), 6.45-6.36 (2H, m), 6.15-5.83 (1H, m), 5.17 (1H, d, *J* = 11.9 Hz), 5.09 (1H, d, *J* =

11.9 Hz), 4.68-4.49 (3H, m), 4.41 (2H, d, $J = 7.3$ Hz), 4.23 (1H, t, $J = 6.9$ Hz), 3.97-3.86 (4H, m), 3.09-2.97 (2H, m), 2.93 (1H, dd, $J = 17.4$ Hz, 4.6 Hz), 2.62 (1H, dd, $J = 17.4$ Hz), 1.91-1.62 (6H, m), 1.50-1.21 (98H, m), 0.88 (6H, t, $J = 6.9$ Hz); ^{13}C NMR(CDCl_3 , 100 MHz) δ 171.8, 171.3, 170.3, 160.9, 158.4, 156.0, 155.9, 143.9, 143.7, 141.3, 131.2, 127.8, 127.1, 125.1, 120.0, 115.9, 104.6, 99.7, 81.9, 79.0, 68.2, 68.1, 67.3, 62.8, 52.3, 51.0, 47.1, 40.23, 37.6, 31.9, 29.7, 29.6, 29.4, 29.4, 29.3, 29.2, 28.4, 28.0, 26.1, 26.0, 22.7, 22.2, 14.1; HRMS (ESI-MS) $[\text{M}+\text{Na}]^+$ calcd for $\text{C}_{85}\text{H}_{139}\text{N}_3\text{O}_{11}$ 1401.0302, found 1401.0276.

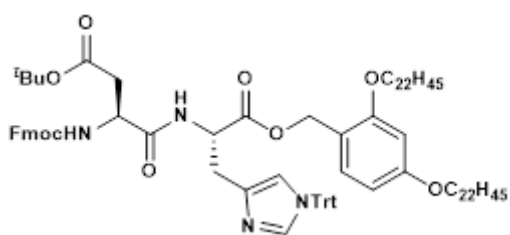


Fmoc-Asp(OtBu)-Arg(Pbf)-TAG (3ao)

Following the general procedure, 2,6-lutidine (69.9 μl , 0.60 mmol), Bu_4NClO_4 (136.8 mg, 0.40 mmol), Ph_3P (157.4 mg, 0.60 mmol), Fmoc-Asp(OtBu)-OH (123.4 mg, 0.30 mmol), and $\text{H}_2\text{N-Arg(Pbf)-Tag}$ (197.1 mg, 0.20 mmol) were added to Ar-purged MeCN(8.0 mL)-*c*-hex (4.0 mL). The solution was electrolyzed at constant current (0.67 mA/cm 2) under Ar at 50 $^\circ\text{C}$. After 4.8 F/mol of electricity based on $\text{H}_2\text{N-Arg(Pbf)-Tag}$ was passed, the reaction mixture was extracted with *c*-hex (20 mL x 2), and then the solvent was replaced with MeCN. The resulting precipitants were collected by filtration and washed with MeCN to give the product in 95% yield (296.7 mg) as white solid.

^1H NMR (CDCl_3 , 400 MHz) δ 7.74 (2H, d, $J = 7.8$ Hz), 7.61-7.52 (2H, m), 7.38 (2H, t, $J = 7.3$ Hz), 7.32-7.26 (2H, m), 7.13 (1H, d, $J = 8.2$ Hz), 6.44-6.36 (2H, m), 6.18-5.77 (4H, m), 5.16 (1H, d, $J = 11.9$ Hz), 5.08 (1H, d, $J = 11.9$ Hz), 4.64-4.46 (2H, m), 4.38 (2H, d, $J = 6.9$ Hz), 4.18 (1H, t, $J = 6.9$ Hz), 3.94-3.85 (4H, m), 3.26-3.04 (2H, m), 2.93-2.81 (3H, m), 2.64 (1H, dd, $J = 16.9$ Hz, 4.1 Hz), 2.56 (3H, s), 2.50 (3H, s), 2.06 (3H, s), 1.92-1.63 (7H, m), 1.60-1.21 (93H, m), 0.88 (6H, t, $J = 6.9$ Hz); ^{13}C

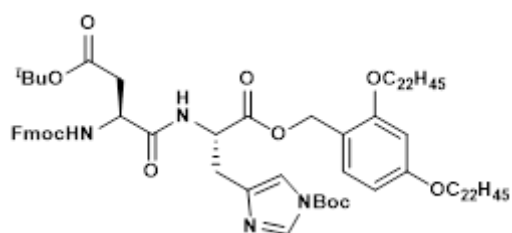
NMR(CDCl_3 , 100 MHz) δ 171.5, 171.0, 171.0, 161.0, 158.6, 158.4, 156.3, 156.0, 143.8, 143.5, 141.3, 138.4, 133.2, 132.3, 132.0, 131.3, 127.8, 127.1, 125.1, 124.5, 120.0, 117.4, 115.6, 104.6, 99.7, 86.2, 82.2, 68.2, 68.1, 67.4, 63.1, 51.8, 51.4, 47.0, 43.2, 40.6, 37.4, 31.9, 30.2, 29.7, 29.7, 29.5, 29.4, 29.3, 29.1, 28.6, 28.0, 26.1, 26.0, 24.6, 22.7, 19.2, 17.9, 14.1, 12.4; HRMS (ESI-MS) $[\text{M}+\text{Na}]^+$ calcd for $\text{C}_{93}\text{H}_{147}\text{N}_5\text{O}_{12}\text{S}$ 1581.0659, found 1581.0657.



Fmoc-Asp(O'Bu)-His(Trt)-TAG (3ap)

Following the general procedure, 2,6-lutidine (69.9 μL , 0.60 mmol), Bu_4NClO_4 (136.8 mg, 0.40 mmol), Ph_3P (157.4 mg, 0.60 mmol), Fmoc-Asp(O'Bu)-OH (123.4 mg, 0.30 mmol), and $\text{H}_2\text{N-His(Trt)-Tag}$ (227.3 mg, 0.20 mmol) were added to Ar-purged MeCN (8.0 mL)-*c*-hex (4.0 mL). The solution was electrolyzed at constant current (0.67 mA/cm^2) under Ar at room temperature. After 4.8 F/mol of electricity based on $\text{H}_2\text{N-His(Trt)-Tag}$ was passed, the reaction mixture was extracted with *c*-hex (20 mL x 2), and then the solvent was replaced with MeCN. The resulting precipitants were collected by filtration and washed with MeCN to give the product in 97% yield (297.9 mg) as white solid.

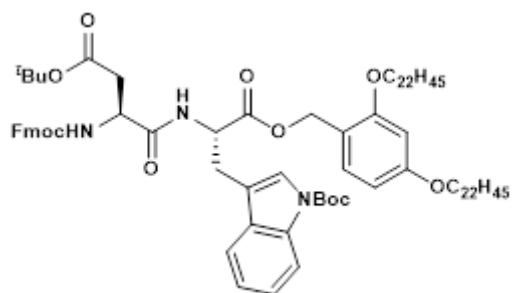
^1H NMR (CDCl_3 , 400 MHz) δ 8.12 (0.4H, d, $J = 7.3$ Hz, minor rotamer), 7.90 (0.6H, d, $J = 7.8$ Hz, major rotamer), 7.73 (2H, d, $J = 7.3$ Hz), 7.61-7.49 (2H, m), 7.36 (2H, t, $J = 7.3$ Hz), 7.31-7.25 (3H, m), 7.25-7.17 (9H, m), 7.08-6.93 (7H, m), 6.56-6.48 (1H, m), 6.48-6.41 (1H, m), 6.37 (1H, d, $J = 1.8$ Hz), 6.26 (1H, dd, $J = 8.2$ Hz, 1.8 Hz), 5.09 (1H, d, $J = 11.9$ Hz), 5.01-4.87 (1H, m), 4.86-4.75 (1H, m), 4.69-4.56 (1H, m), 4.37-4.21 (2H, m), 4.15 (0.4H, t, $J = 6.9$ Hz, minor rotamer), 4.07 (0.6H, t, $J = 6.9$ Hz, major rotamer), 3.90-3.79 (4H, m), 3.12-2.95 (2H, m), 2.94-2.58 (2H, m), 1.79-1.64 (4H, m), 1.46-1.21 (85H, m), 0.88 (6H, t, $J = 6.9$ Hz); ^{13}C NMR (CDCl_3 , 100 MHz) δ 170.9, 170.8, 170.7, 170.5, 170.4, 170.3, 170.2, 170.2, 170.1, 160.6, 160.5, 158.1, 158.1, 155.9, 155.9, 155.8, 144.0, 144.0, 143.9, 143.9, 143.8, 143.7, 143.7, 142.3, 142.2, 141.3, 138.7, 138.6, 136.5, 136.4, 130.6, 130.6, 129.7, 128.0, 127.7, 127.1, 127.1, 125.3, 125.3, 125.2, 119.9, 119.8, 119.5, 116.3, 116.2, 104.5, 99.6, 81.5, 81.3, 75.2, 75.2, 68.1, 68.0, 67.3, 67.1, 62.4, 62.4, 53.0, 52.9, 51.6, 51.4, 47.1, 37.7, 31.9, 30.0, 29.7, 29.6, 29.4, 29.4, 29.3, 29.1, 28.1, 28.0, 26.1, 26.0, 22.7, 14.1; HRMS (ESI-MS) $[\text{M}+\text{Na}]^+$ calcd for $\text{C}_{99}\text{H}_{140}\text{N}_4\text{O}_9$ 1552.0513, found 1552.0502.



Fmoc-Asp(O^tBu)-His(Boc)-TAG (3aq)

Following the general procedure, 2,6-lutidine (69.9 μ l, 0.60 mmol), Bu₄NClO₄ (136.8 mg, 0.40 mmol), Ph₃P (157.4 mg, 0.60 mmol), Fmoc-Asp(O^tBu)-OH (123.4 mg, 0.30 mmol), and H₂N-His(Boc)-Tag (198.9 mg, 0.20 mmol) were added to Ar-purged MeCN(8.0 mL)-*c*-hex (4.0 mL). The solution was electrolyzed at constant current (0.67 mA/cm²) under Ar at room temperature. After 4.8 F/mol of electricity based on H₂N-His(Boc)-Tag was passed, the reaction mixture was extracted with *c*-hex (20 mL x 2), and then the solvent was replaced with MeCN. The resulting precipitants were collected by filtration and washed with MeCN to give the product in 99% yield (274.8 mg) as white solid.

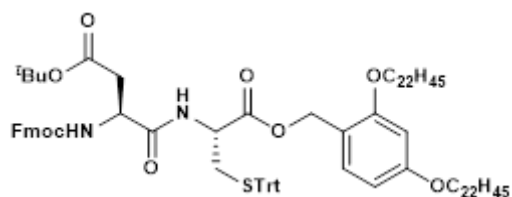
¹H NMR (CDCl₃, 600 MHz) δ 7.88 (1H, s), 7.76 (2H, d, J = 7.6 Hz), 7.67 (1H, d, J = 7.6 Hz), 7.61 (2H, t, J = 6.9 Hz), 7.39 (2H, t, J = 7.6 Hz), 7.30 (2H, t, J = 7.6 Hz), 7.13 (1H, d, J = 8.3 Hz), 7.06 (1H, s), 6.44-6.36 (2H, m), 6.13 (1H, d, J = 8.3 Hz), 5.14 (1H, d, J = 12.4 Hz), 5.11 (1H, d, J = 12.4 Hz), 4.86-4.81 (1H, m), 4.66-4.58 (1H, m), 4.38 (2H, d, J = 6.9 Hz), 4.24 (1H, t, J = 6.9 Hz), 3.96-3.88 (4H, m), 3.06 (2H, d, J = 5.5 Hz), 2.89 (1H, dd, J = 17.2 Hz, 4.8 Hz), 2.66 (1H, dd, J = 17.2 Hz, 6.2 Hz), 1.80-1.73 (4H, m), 1.55 (9H, s), 1.48-1.21 (85H, m), 0.88 (6H, t, J = 6.9 Hz); ¹³C NMR (CDCl₃, 150 MHz) δ 171.0, 170.7, 170.3, 160.7, 158.3, 155.9, 146.7, 143.9, 143.8, 141.3, 138.4, 136.8, 131.0, 127.7, 127.1, 125.2, 120.0, 116.1, 114.7, 104.5, 99.6, 85.4, 81.5, 68.2, 68.1, 67.3, 62.7, 52.4, 51.2, 47.2, 37.7, 31.9, 29.7, 29.7, 29.6, 29.4, 29.4, 29.3, 29.2, 28.0, 27.8, 26.1, 26.1, 22.7, 14.1; HRMS (ESI-MS) [M+Na]⁺ calcd for C₈₅H₁₃₄N₄O₁₁ 1409.9941, found 1409.9939.



Fmoc-Asp(O^tBu)-Trp(Boc)-TAG (**3ar**)

Following the general procedure, 2,6-lutidine (69.9 μ l, 0.60 mmol), Bu₄NClO₄ (136.8 mg, 0.40 mmol), Ph₃P (157.4 mg, 0.60 mmol), Fmoc-Asp(O^tBu)-OH (123.4 mg, 0.30 mmol), and H₂N-Trp(Boc)-Tag (208.7 mg, 0.20 mmol) were added to Ar-purged MeCN(8.0 mL)-*c*-hex (4.0 mL). The solution was electrolyzed at constant current (0.67 mA/cm²) under Ar at room temperature. After 4.8 F/mol of electricity based on H₂N-Trp(Boc)-Tag was passed, the reaction mixture was extracted with *c*-hex (20 mL x 2), and then the solvent was replaced with MeCN. The resulting precipitants were collected by filtration and washed with MeCN to give the product in 96% yield (275.8 mg) as white solid.

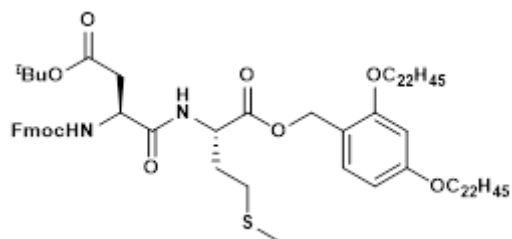
¹H NMR (CDCl₃, 400 MHz) δ 8.10 (1H, d, J = 6.4 Hz), 7.75 (2H, d, J = 7.3 Hz), 7.60-7.43 (4H, m), 7.38 (2H, t, J = 7.3 Hz), 7.32-7.25 (3H, m), 7.24-7.12 (2H, m), 7.02 (1H, d, J = 8.2 Hz), 6.45-6.32 (2H, m), 5.91 (1H, d, J = 8.2 Hz), 5.11 (1H, d, J = 11.9 Hz), 5.06 (1H, d, J = 11.9 Hz), 4.95-4.85 (1H, m), 4.59-4.45 (1H, m), 4.44-4.24 (2H, m), 4.18 (1H, t, J = 7.3 Hz), 3.97-3.85 (4H, m), 3.23 (2H, d, J = 5.5 Hz), 2.88 (1H, dd, J = 16.9 Hz, 3.2 Hz), 2.58 (1H, dd, J = 16.9 Hz, 6.9 Hz), 1.80-1.70 (4H, m), 1.63 (9H, s), 1.49-1.21 (85H, m), 0.88 (6H, t, J = 6.9 Hz); ¹³C NMR (CDCl₃, 100 MHz) δ 171.3, 171.1, 170.2, 160.9, 158.4, 155.9, 149.5, 143.9, 143.7, 141.3, 131.1, 130.5, 127.7, 127.1, 125.1, 124.5, 122.6, 120.0, 119.0, 115.7, 115.3, 114.8, 104.5, 99.7, 83.5, 81.8, 68.2, 68.1, 67.3, 63.1, 52.9, 51.0, 47.1, 37.5, 31.9, 30.0, 29.7, 29.6, 29.4, 29.4, 29.3, 29.1, 28.2, 28.0, 27.3, 26.1, 26.0, 22.7, 14.1; HRMS (ESI-MS) [M+Na]⁺ calcd for C₉₀H₁₃₇N₃O₁₁ 1459.0145, found 1459.0162.



Fmoc-Asp(O^tBu)-Cys(Trt)-TAG (3as)

Following the general procedure, 2,6-lutidine (69.9 μ l, 0.60 mmol), Bu₄NClO₄ (136.8 mg, 0.40 mmol), Ph₃P (157.4 mg, 0.60 mmol), Fmoc-Asp(O^tBu)-OH (123.4 mg, 0.30 mmol), and H₂N-Cys(Trt)-Tag (220.4 mg, 0.20 mmol) were added to Ar-purged MeCN(8.0 mL)-*c*-hex (4.0 mL). The solution was electrolyzed at constant current (0.67 mA/cm²) under Ar at 40 °C. After 4.8 F/mol of electricity based on H₂N-Cys(Trt)-Tag was passed, the reaction mixture was extracted with *c*-hex (20 mL x 2), and then the solvent was replaced with MeCN. The resulting precipitants were collected by filtration and washed with MeCN to give the product in 99% yield (297.8 mg) as white solid.

¹H NMR (CDCl₃, 400 MHz) δ 7.75 (2H, d, J = 7.8 Hz), 7.57 (2H, t, J = 7.8 Hz), 7.42-7.25 (10H, m), 7.25-7.07 (11H, m), 6.43-6.34 (2H, m), 5.93 (1H, d, J = 7.8 Hz), 5.21-5.01 (2H, m), 4.60-4.50 (1H, m), 4.50-4.43 (1H, m), 4.42-4.29 (2H, m), 4.20 (1H, t, J = 6.9 Hz), 3.92 (2H, t, J = 6.4 Hz), 3.86 (2H, t, J = 6.4 Hz), 2.80 (1H, dd, J = 17.4 Hz, 3.7 Hz), 2.71-2.47 (3H, m), 1.81-1.63 (4H, m), 1.50-1.20 (85H, m), 0.88 (6H, t, J = 6.9 Hz); ¹³C NMR (CDCl₃, 100 MHz) δ 171.0, 170.2, 169.8, 160.8, 158.3, 155.8, 144.3, 143.9, 143.7, 141.3, 141.3, 131.0, 129.5, 127.9, 127.7, 127.1, 126.8, 125.1, 120.0, 116.0, 104.6, 99.6, 81.8, 68.1, 68.1, 67.3, 66.8, 62.8, 51.7, 50.7, 47.1, 37.8, 33.7, 31.9, 29.7, 29.6, 29.4, 29.4, 29.3, 29.1, 28.0, 26.1, 26.0, 22.7, 14.1; HRMS (ESI-MS) [M+Na]⁺ calcd for C₉₆H₁₃₈N₂O₉S 1518.0015, found 1518.0044.

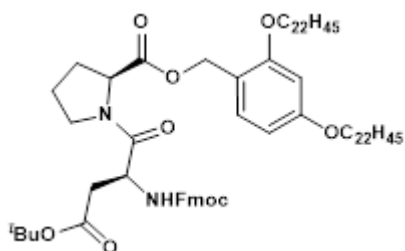


Fmoc-Asp(O^tBu)-Met-TAG (3at)

Following the general procedure, 2,6-lutidine (69.9 μ l, 0.60 mmol), Bu₄NClO₄ (136.8 mg, 0.40 mmol), Ph₃P (131.1 mg, 0.50 mmol), Fmoc-Asp(O^tBu)-OH (123.4 mg, 0.30 mmol), and H₂N-Met-Tag (177.7 mg, 0.20 mmol) were added to Ar-purged MeCN(8.0 mL)-*c*-hex (4.0 mL). The solution was electrolyzed at constant current (0.67 mA/cm²)

under Ar at 50 °C. After 4.8 F/mol of electricity based on H₂N-Met-Tag was passed, the reaction mixture was extracted with *c*-hex (20 mL x 2), and then the solvent was replaced with MeCN. The resulting precipitants were collected by filtration and washed with MeCN to give the product in 99% yield (253.2 mg) as white solid.

¹H NMR (CDCl₃, 600 MHz) δ 7.76 (2H, d, *J* = 7.6 Hz), 7.64-7.51 (2H, m), 7.40 (2H, t, *J* = 7.6 Hz), 7.32 (2H, t, *J* = 7.6 Hz), 7.24-7.11 (2H, m), 6.45-6.35 (2H, m), 5.98 (1H, d, *J* = 8.3 Hz), 5.20 (1H, d, *J* = 11.7 Hz), 5.09 (1H, d, *J* = 11.7 Hz), 4.67 (1H, dd, *J* = 12.4 Hz, 7.6 Hz), 4.61-4.50 (1H, m), 4.41 (2H, d, *J* = 6.9 Hz), 4.23 (1H, t, *J* = 6.9 Hz), 3.96-3.85 (4H, m), 2.95 (1H, dd, *J* = 17.2 Hz, 3.4 Hz), 2.60 (1H, dd, *J* = 17.5 Hz, 6.2 Hz), 2.52-2.38 (2H, m), 2.20-2.09 (1H, m), 2.05-1.91 (4H, m), 1.81-1.70 (4H, m), 1.52-1.21 (85H, m), 0.88 (6H, t, *J* = 6.9 Hz); ¹³C NMR (CDCl₃, 150 MHz) δ 171.4, 171.4, 170.3, 161.0, 158.5, 156.0, 143.8, 143.7, 141.3, 131.4, 127.8, 127.1, 125.1, 120.0, 115.8, 104.5, 99.7, 82.0, 68.2, 68.1, 67.3, 63.1, 52.0, 51.0, 47.1, 37.5, 31.9, 29.7, 29.7, 29.6, 29.6, 29.4, 29.4, 29.3, 29.2, 28.0, 26.1, 26.0, 22.7, 15.3, 14.1; HRMS (ESI-MS) [M+Na]⁺ calcd for C₇₉H₁₂₈N₂O₉S 1303.9233, found 1303.9235.



Fmoc-Pro-Asp(O^tBu)-TAG (3au)

Following the general procedure, 2,6-lutidine (69.9 μl, 0.60 mmol), Bu₄NClO₄ (136.8 mg, 0.40 mmol), Ph₃P (131.1 mg, 0.50 mmol), Fmoc-Asp(O^tBu)-OH (205.7 mg, 0.50 mmol), and H₂N-Pro-Tag (168.1 mg, 0.20 mmol) were added to Ar-purged MeCN(8.0 mL)-*c*-hex (4.0 mL). The solution was electrolyzed at constant current (0.67 mA/cm²) under Ar at room temperature. After 6.0 F/mol of electricity based on H₂N-Pro-Tag was passed, the reaction mixture was extracted with *c*-hex (20 mL x 2), and then the solvent was replaced with MeCN. The resulting precipitants were collected by filtration and washed with MeCN to give the product in 99% yield (249.5 mg) as white solid.

¹H NMR (CDCl₃, 400 MHz) δ 7.79-7.71 (2H, m), 7.62-7.51 (2H, m), 7.43-7.34 (2H, m), 7.34-7.26 (2H, m), 7.22-7.15 (0.83H, m, major rotamer), 7.15-7.08 (0.17H, m, minor rotamer), 6.46-6.33 (2H, m), 5.68 (0.83H, d, *J* = 8.9 Hz, major rotamer), 5.53 (0.17H, d, *J* = 9.2 Hz, minor rotamer), 5.21 (0.87H, d, *J* = 11.9 Hz, major rotamer), 5.15

(0.13H, d, $J = 11.9$ Hz, minor rotamer), 5.04 (0.87H, d, $J = 11.9$ Hz, major rotamer), 4.98 (0.13H, d, $J = 11.9$ Hz, minor rotamer), 4.94-4.85 (0.72H, m, major rotamer), 4.85-4.79 (0.12H, m, minor rotamer), 4.75-4.65 (0.13H, m, minor rotamer), 4.64-4.47 (1H, m), 4.44-4.25 (2H, m), 4.25-4.11 (1H, m), 3.98-3.83 (4H, m), 3.80-3.62 (2H, m), 2.77 (0.14H, dd, $J = 16.0$ Hz, 7.8 Hz, minor rotamer), 2.64 (0.86H, dd, $J = 15.6$ Hz, 4.6 Hz, major rotamer), 2.48 (1H, dd, $J = 15.6$ Hz, 8.2 Hz), 2.24-2.09 (1H, m), 2.08-1.92 (2H, m), 1.83-1.71 (4H, m), 1.49-1.21 (86H, m), 0.88 (6H, t, $J = 6.9$ Hz); ^{13}C NMR (CDCl_3 , 100 MHz) δ 171.6, 169.5, 169.5, 160.8, 158.3, 155.8, 143.8, 143.8, 141.3, 131.1, 127.7, 127.1, 125.2, 120.0, 116.3, 104.6, 99.7, 81.4, 68.1, 67.2, 62.5, 59.1, 49.8, 47.1, 46.9, 38.6, 31.9, 29.7, 29.6, 29.4, 29.4, 29.4, 29.3, 29.2, 29.0, 28.0, 26.1, 24.7, 22.7, 14.1; HRMS (ESI-MS) $[\text{M}+\text{Na}]^+$ calcd for $\text{C}_{79}\text{H}_{126}\text{N}_2\text{O}_9$ 1269.9356, found 1296.9345.

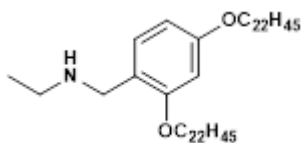
Synthesis of Leuprorelin

(1) Preparation of the Hydrophobic Benzyl Amine HCl salt

To a solution of the hydrophobic benzaldehyde (1.51 g, 2.0 mmol) in THF (45 mL) ethylamine hydrochloride (815.4 mg, 10.0 mmol) and DMF (14 mL), DIPEA 3.4 mL, MgSO_4 (4.00 g), acetic acid (1.50 mL), and $\text{NaBH}(\text{OAc})_3$ (211.9 mg x 4, 8.0 mmol) were added. The resulting reaction mixture was stirred at room temperature for 1.5 h followed by quench of remaining $\text{NaBH}(\text{OAc})_3$ by 0.1 N HCl aq. The resulting reaction mixture was filtered and extracted with c-hex, and organic phase was washed with 0.1 HCl aq. and brine, dried over MgSO_4 and concentrated in vacuo. To a crude product, MeCN was added to give precipitants. The precipitants were collected by filtration and purified by column chromatography (CHCl_3 : MeOH =50:1) to give the product as a white solid (1.29 g, 80% yield)

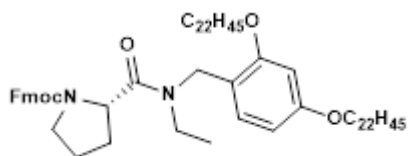
(2) Synthesis of Fmoc-Pro-NEtTag

To a solution of hydrophobic benzyl amine (328.3 mg, 0.40 mmol) in THF (8.0 mL), Fmoc-Pro-OH H_2O (202.4 mg, 0.60 mmol), DIPEA (224 μL , 1.32 mmol), and COMU (257.0 mg, 0.60 mmol) were added. The mixture was stirred for 20 min followed by solvent replacement with MeCN to give the product as a precipitate. Precipitate was collected by filtration and washed with MeCN to give the products quantitatively as light pink solid.



Hydrophobic benzyl amine

^1H NMR (CDCl_3 , 600 MHz) δ 7.09 (1H, d, $J = 7.6$ Hz), 6.44-6.38 (2H, m), 3.96-3.91 (4H, m), 3.72 (2H, s), 2.62 (2H, q, $J = 6.9$ Hz), 1.82-1.73 (4H, m), 1.64 (1H, broad s), 1.49-1.22 (76H, m), 1.11 (3H, t, $J = 6.9$ Hz), 0.88 (6H, t, $J = 6.9$ Hz); ^{13}C NMR (CDCl_3 , 150 MHz) δ 159.5, 158.1, 130.4, 120.9, 104.2, 99.7, 68.1, 67.8, 49.0, 43.2, 31.9, 29.7, 29.6, 29.6, 29.4, 29.4, 29.3, 29.3, 26.2, 26.1, 22.7, 15.3, 14.1.



Fmoc-Pro-NEtTag

^1H NMR (CDCl_3 , 600 MHz) δ 7.77-7.71 (2H, m), 7.68-7.50 (2H, m), 7.41-7.33 (2H, m), 7.33-7.23 (2H, m), 7.21-7.09 (0.8H, m), 6.89-6.84 (0.2H, m), 6.51-6.25 (1.8H, m), 5.91-5.87 (0.2H, m), 4.78-4.56 (2H, m), 4.49-4.23 (4H, m), 4.12-3.54 (7H, m), 3.51-3.17 (1H, m), 2.30-2.01 (2H, m), 1.99-1.83 (2H, m), 1.83-1.59 (4H, m), 1.47-1.21 (76H, m), 1.16-1.04 (3H, m), 0.88 (6H, t, $J = 6.9$ Hz); ^{13}C NMR (CDCl_3 , 150 MHz) δ 172.5, 172.4, 172.3, 172.0, 159.8, 159.7, 159.5, 159.5, 157.9, 157.3, 157.1, 154.9, 154.9, 154.6, 154.4, 144.6, 144.5, 144.4, 144.3, 144.0, 143.9, 141.3, 141.3, 141.2, 141.2, 141.1, 130.5, 130.0, 127.9, 127.7, 127.6, 127.6, 127.4, 127.1, 127.1, 127.0, 127.0, 125.7, 125.4, 125.2, 125.1, 119.9, 119.8, 119.7, 118.1, 117.0, 116.5, 104.9, 104.7, 104.4, 104.0, 99.6, 99.4, 99.3, 68.1, 68.0, 68.0, 67.9, 67.7, 67.4, 67.3, 67.1, 66.7, 57.2, 57.1, 56.9, 56.6, 54.8, 47.4, 47.4, 47.4, 47.3, 47.3, 47.2, 47.0, 46.9, 45.0, 44.7, 42.3, 41.9, 41.6, 41.5, 41.4, 41.2, 38.4, 31.9, 31.4, 31.3, 30.3, 30.3, 29.7, 29.7, 29.6, 29.6, 29.4, 29.4, 29.3, 29.2, 26.2, 26.1, 26.0, 26.0, 24.5, 24.4, 23.4, 23.3, 22.7, 14.1, 14.1, 14.1, 14.0, 12.7, 12.6.

(3) Fmoc-deprotection of Peptides

Following the **2.3**, Fmoc was deprotected and products were obtained.

(4) General Procedure for Electrochemical Peptide Elongation*

MeCN (8.0 mL) and *c*-Hex (4.0 mL) were purged with Ar gas for 2 min. To a purged solvents, 2,6-lutidine (69.9 μ L, 0.6 mmol), Bu₄NClO₄ (0.5 mmol), Ph₃P (157.4 mg, 0.6 mmol), Fmoc-protected amino acid (0.4 mmol), and *N*-deprotected tagged amino acid (0.2 mmol) were added. The solution was electrolyzed at constant current (0.67 mA/cm²) under Ar at 40 °C. After 4.8 F/mol of electricity based on *N*-deprotected tagged amino acid was passed, the reaction mixture was extracted with *c*-hex (20 mL x 2), and then the solvent was replaced with MeCN. The resulting precipitants were collected by filtration and washed with MeCN to give the products.

* When condensation with (1) Fmoc-Arg(Pbf)-OH, (2) Fmoc-Ser(OtBu)-OH, and (3) pGlu, following conditions were applied.

(1) Fmoc-Arg(Pbf)-OH 3.5 eq., Ph₃P 5.0 eq., 6.0 F/mol, 60 °C.

(2) Fmoc-Ser(OtBu)-OH 3.0 eq., Ph₃P 4.0 eq., 6.0 F/mol, 60 °C.

(3) pGlu 3.0 eq., Ph₃P 4.0 eq., 6.0 F/mol, 60 °C.

9. NMR スペクトル

