



TBDCs: ADVANCED COUPLING REAGENTS FOR LIQUID PHASE PEPTIDE SYNTHESIS (LPPS)

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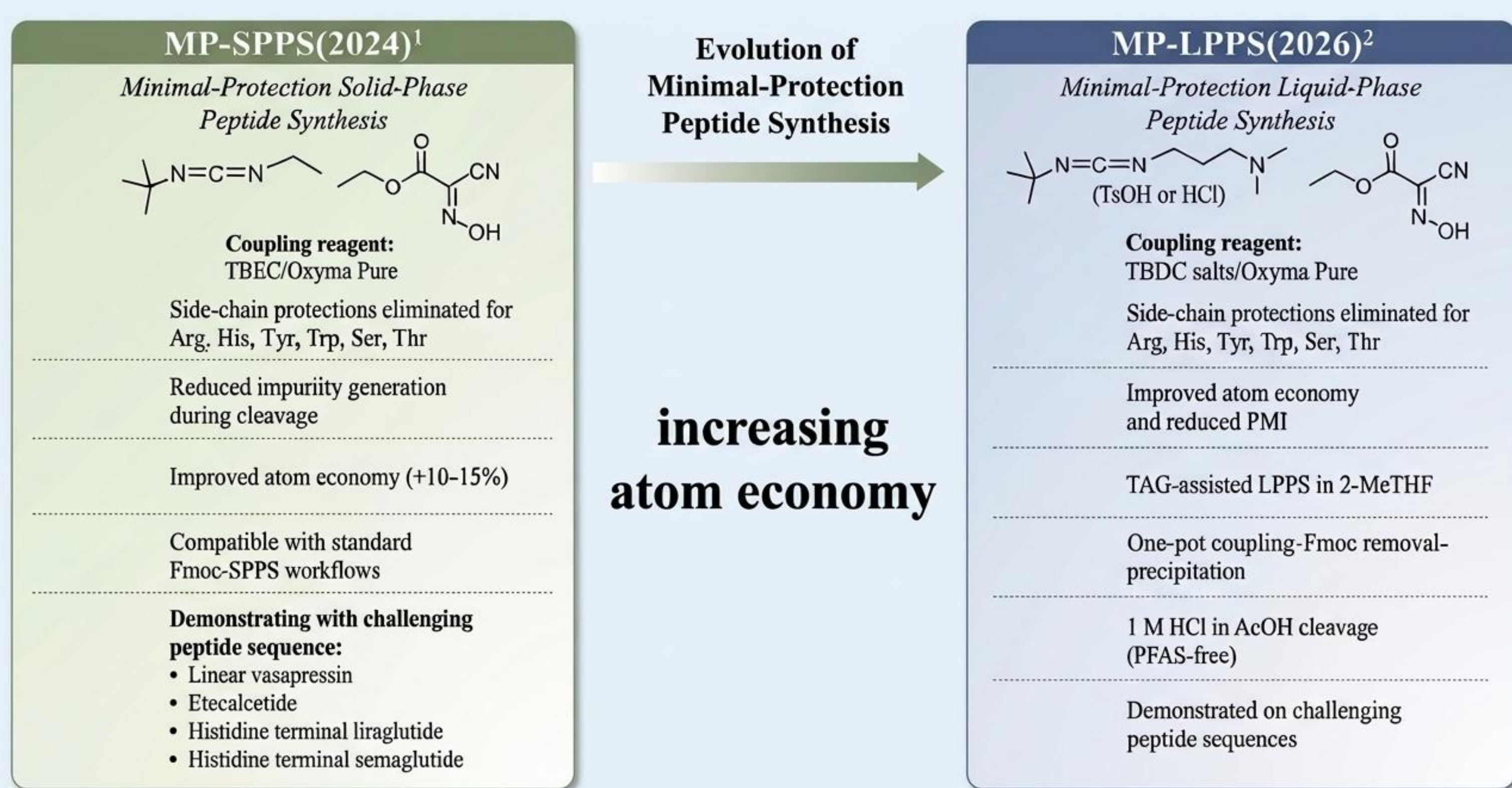
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Abstract

The growing market demand for oligopeptides has led to increased efforts toward the development of sustainable and more efficient synthetic processes for these significant bioactive molecules. In 2024, our group demonstrated that amino acids without side-chain protecting groups could be employed in Solid Phase Peptide Synthesis (SPPS), increasing the Atom Economy (AE) of the process.¹ In this work, we introduce a novel carbodiimide, N-(3-dimethylaminopropyl)-N'-tert butylcarbodiimide (TBDC), as the p-toluenesulfonic acid salt, for Liquid Phase Peptide Synthesis (LPPS). We successfully applied it to the synthesis of peptides containing arginine, histidine, tyrosine, and tryptophan without side-chain protecting groups, using a green solvent, thereby increasing both AE and Process Mass Intensity (PMI) for LPPS. In line with a PFAS-free approach, we also removed trifluoroacetic acid (TFA) from the final cleavage step, which was performed using a commercially available 1 M HCl solution in acetic acid. Overall, these results further improve the sustainability of TAG-assisted peptide chemistry.



Minimal Protection Peptide Synthesis : From SPPS to LPPS

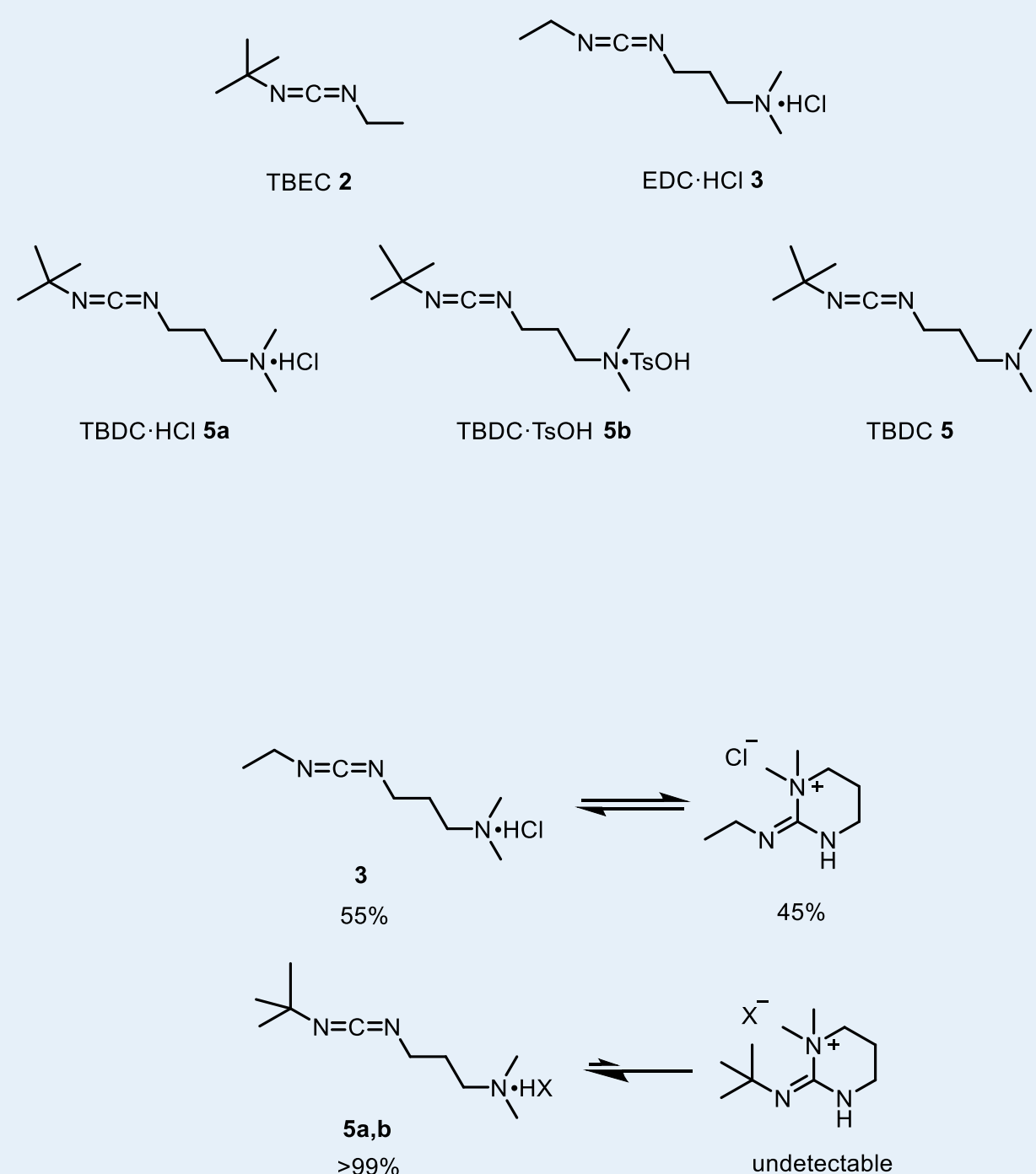


The coupling reagent

Green = Complete solubility; Yellow = Partial solubility ; Red = Complete insolubility

Solvent	TBEC 2	EDC-HCl 3	TBDC-HCl 5a	TBDC-TsOH 5b
DMF	Green	Red	Red	Green
DCM	Green	Red	Red	Green
Anisole	Green	Red	Yellow	Green
DMC	Green	Red	Red	Green
DEC	Green	Red	Red	Green
NOP	Green	Red	Red	Green
NBP	Green	Red	Red	Green
NBP:DMC 8:2	Green	Red	Red	Green
EtOAc	Green	Red	Red	Green
DMSO	Green	Red	Red	Green
EtOAc:DMSO 7:3	Green	Red	Red	Green
ACN	Green	Red	Red	Green
ACN:DMSO 7:3	Green	Red	Red	Green
PrOAc	Green	Red	Red	Green
ⁱ PrOAc	Green	Red	Red	Green
^t BuOAc	Green	Red	Red	Green
CPME	Green	Red	Red	Yellow
2-MeTHF	Green	Red	Red	Green
2-MeTHF:DMSO 9:1	Green	Red	Red	Green
GVL	Green	Yellow	Red	Green
DOS	Green	Red	Red	Green
TEP	Green	Red	Red	Green
Water	Red	Red	Red	Green

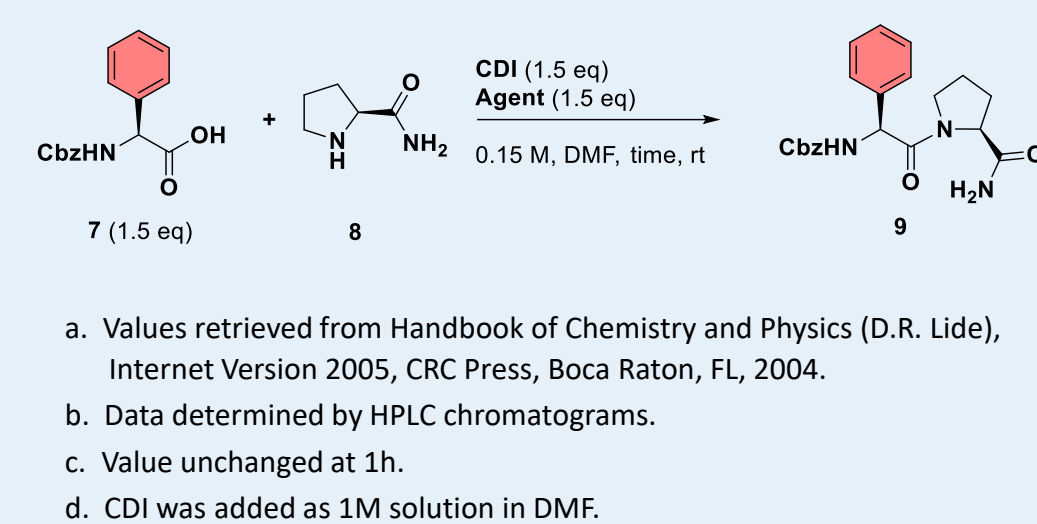
DMF: dimethyl formamide; **DCM:** dichloromethane; **DEC:** diethyl carbonate; **NOP:** N-octyl pyrrolidone; **NBP:** N-butyl pyrrolidone, **DMC:** dimethyl carbonate; **EtOAc:** ethyl acetate; **DMSO:** dimethylsulphoxide; **PrOAc:** propyl acetate; **ⁱPrOAc:** isopropyl acetate; **^tBuOAc:** tertbutyl acetate; **CPME:** cyclopentyl methyl ether; **2-MeTHF:** 2-methyl tetrahydrofuran; **GVL:** gamma Valero lactone; **DOS:** 1,3-dioxolane; **TEP:** triethyl phosphate.



TBDC is less prone to intramolecular cyclization than EDC !

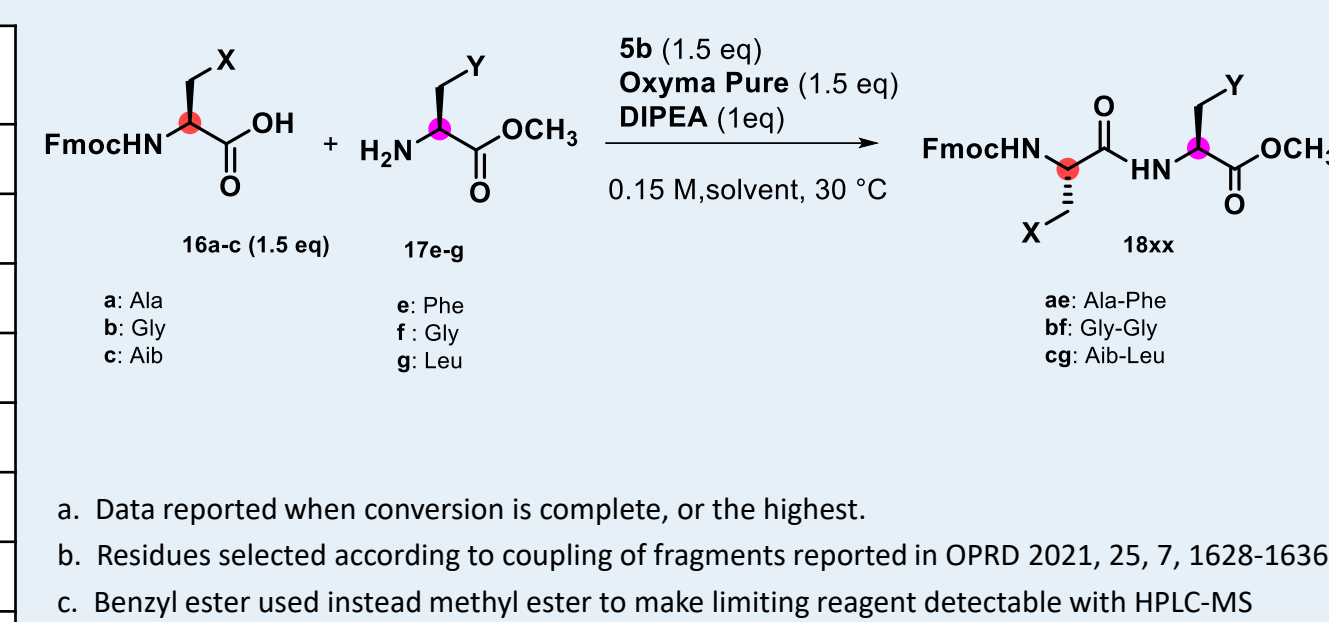
Evaluation of ability to avoid racemization

Entry	CDI	Acid	Acid pKa ^a	Agent (eq)	C _{max} % ^b	Time (min)	LL/DL ^b
1	2	-	-	Oxyma Pure	95.7	15 ^c	98:2
2	5	CH ₃ SO ₃ H	-1.9	Oxyma Pure	77.6	60	96:4
3	5	p-TolSO ₃ H	-2.8	Oxyma Pure	86.8	60	96:4
4	5	TCA	0.6	Oxyma Pure	91.0	60	69:31
5	5	TFA	0.2	Oxyma Pure	89.1	60	91:9
6	3	-	-	OxymaPure	100	15	93:7
7	5a ^d	-	-	OxymaPure	100	30	97:3
8	5b ^d	-	-	OxymaPure	100	30	97:3
9	3	-	-	HOBt	100	15	75:25
10	5a ^d	-	-	HOBt	100	15	81:19
11	5a ^d	-	-	HOAt	100	15	87:13
12	5a ^d	-	-	Oxyma B	100	60	96:4

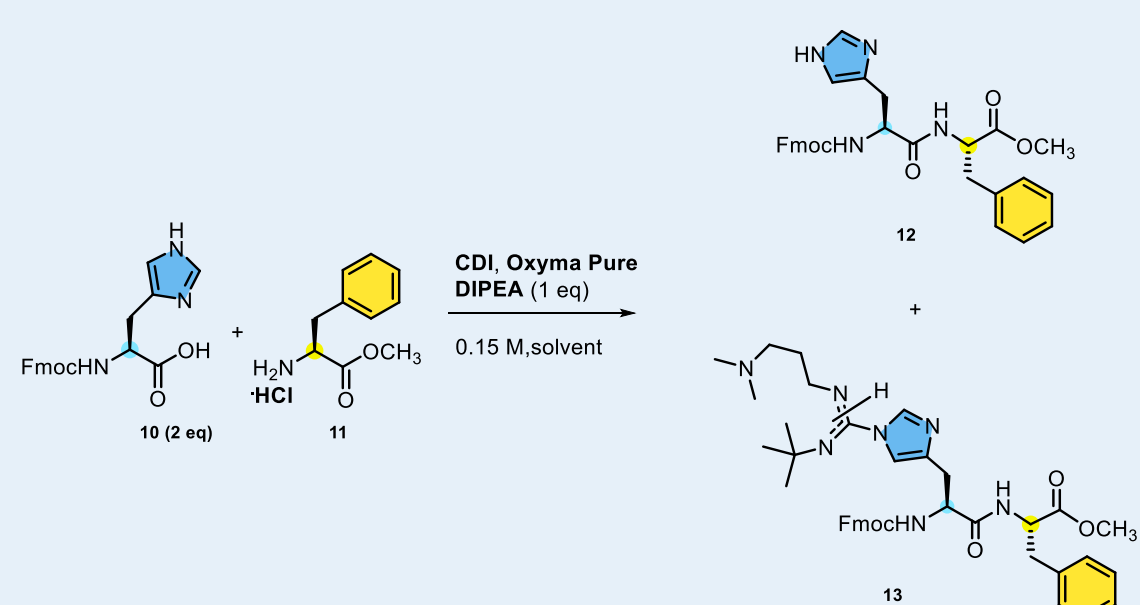
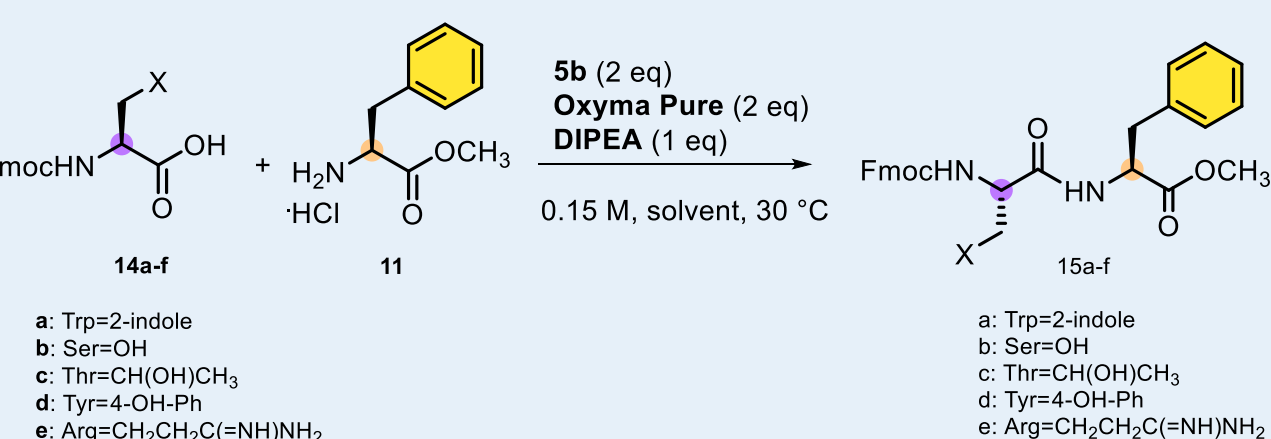


Coupling conditions for lyphophilic residues in green solvents (Tirzepatide)

Entry	AA ^b	Solvent	C _{max} % ^a	Time (h)
1		DMF	97.9 (91.3)	1.5
2	18ae	ACN:DMSO 7:3	86.6 (84.6)	3.0
3		MeTHF:DMSO 9:1	93.1 (91.2)	1.5
4		DMF	100 (92.4)	3.0
5	18bf ^c	ACN:DMSO 7:3	80.2 (69.6)	5.0
6		MeTHF:DMSO 9:1	76.9 (75.6)	6.0
7		DMF	100	0.5
8	18cg ^c	ACN:DMSO 7:3	100 (95.2)	2.0
9		MeTHF:DMSO 9:1	100 (99.3)	2.0



Coupling conditions for sidechain unprotected residues in green solvents



Entry	AA	Solvent	C _{max} % ^a	Time (h)
1		DMF	100	1h
2	14a	MeTHF	100	1h
3		MeTHF:DMSO 9:1	100	1h
4		DMF	100	1h
5	14b	MeTHF	100	1h
6		MeTHF:DMSO 9:1	100	1.5h
7		DMF	100	1h
8	14c	MeTHF	100	0.5h
9		MeTHF:DMSO 9:1	100	1h
10		DMF	100	0.5h
11	14d	MeTHF	100	0.5h
12		MeTHF:DMSO 9:1	100	1h
13		DMF	100	0.5h
14		MeTHF	100	0.5h
15	14e ^b	MeTHF:DMSO 9:1	100	0.5h

a. Data reported when conversion is complete, or the highest.

b. OxymaPure 6 was used in 5 eq, according to Green Chem. 2024, 26, 10929-10939.

Entry	CDI	CDI (eq)	Oxyma Pure (eq)	Solvent	C _{max} % ^b	Time (h)	T (°C)	LL/DL ^b	12/13 ^b
1	3	2	2	DMF	90.8 ^a	2.0	30	97:3	85.6:14.4
2	5a	2	2	DMF	100	2.0	30	97:3	99.7:0.2
3	5b	2	2	DMF	100	1.5	30	98:2	99.4:0.6
4	5a	2	4	DMF	100	1.5	30	98:2	98.8:1.2
5	5b	2	4	DMF	100	1.5	30	99:1	100:0
6	5a	2	4	MeTHF:DMSO 9:1	100	1.0	30	97:3	99:1
7	5b	2	4	MeTHF:DMSO 9:1	100	1.0	30	97:3	98.8:1.2
8 ^a	5a ^c	1+1	3	DMF	100 (65.0)	3.0	30	97:3 (98:2)	100:0
9 ^a	5b	1+1	3	DMF	100 (72.1)	3.0	30	98:2 (98:2)	100:0
10 ^a	5a ^c	1+1	3	MeTHF:DMSO 9:1	100 (82.8)	1.5	30	97:3 (98:2)	100:0
11 ^a	5b	1+1	3	MeTHF:DMSO 9:1	100 ^d (64.6)	4.0	30	96:4 (98:2)	100:0
12 ^a	5a ^c	1+1	4	MeTHF:DMSO 9:1	100 (78.1)	1.5	30	97:3 (98:2)	100:0
13 ^a	5b	1+1	4	MeTHF:DMSO 9:1	100 (81.6)	1.5	30	97:3 (98:2)	100:0

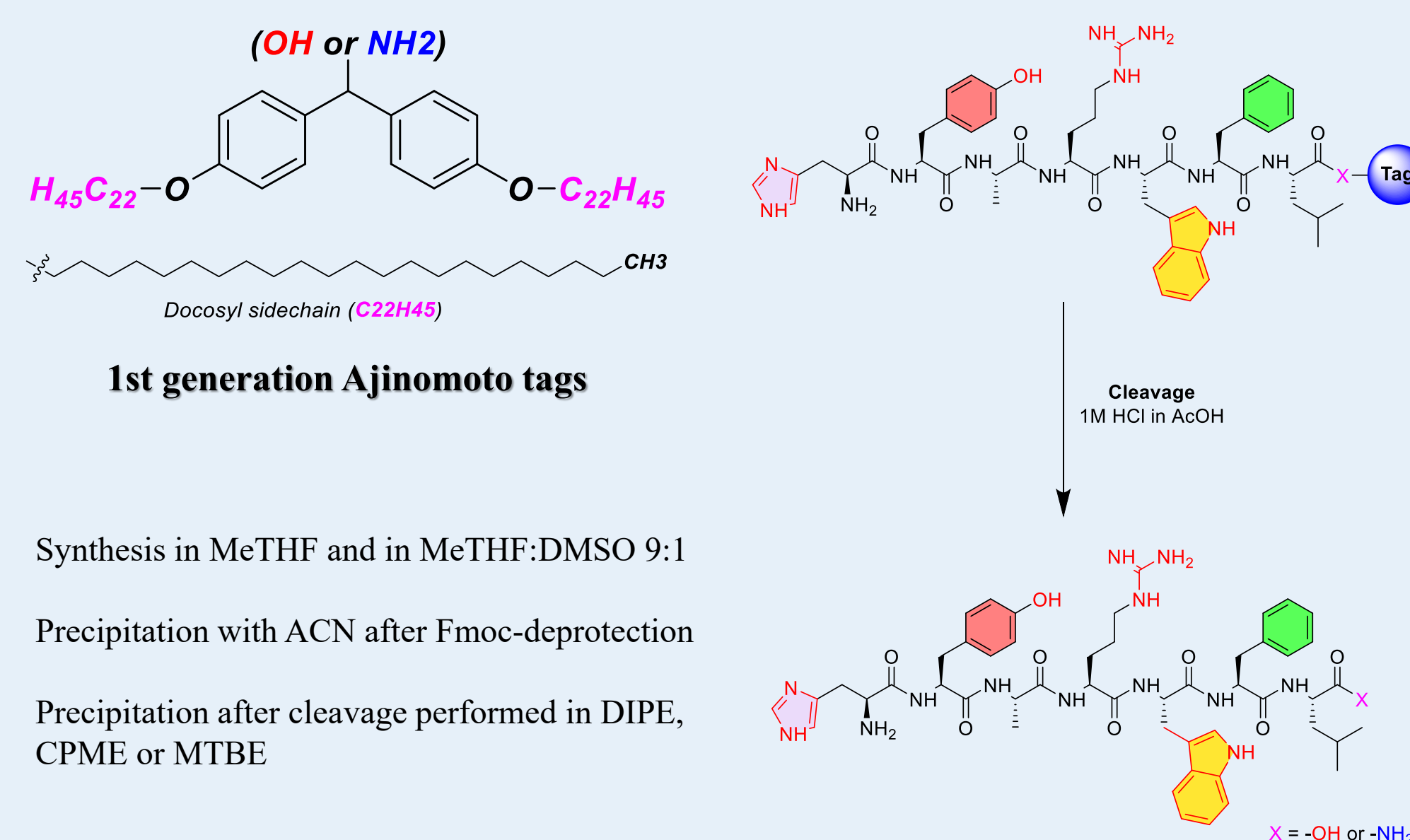
a. Portionwise addition of CDI, second portion of CDI (1eq) added after 1h.

b. Data reported when conversion is complete, or the highest. In brackets, the values after 1h.

c. Addition of 5a was performed by direct addition of solid carbodiimide to ensure complete solubilization in solvent mixture, while in all the other experiments a preformed 1M solution of carbodiimide was preferred.

d. Conversion reached 99.2% at 3h (previous point in the kinetic study).

LPPS of a challenging sequence



Entry	Sequence	Purity ^a (%)	Yield (%)
1	H-HYARWFL-OH	100	81
2	H-HYARWFL-NH ₂	>98	80

^a Determined via HPLC on crude product obtained after precipitation

References: ¹ T. Fantoni et al., Green Chem, **2024**, 26, 10929-10939; ² D. Corbisiero, N. Patrian et al., ACS Sustainable Chem. Eng., **2026**, 14, 26, 11707-11721

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