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1-Ethyl 2-Halopyridinium Salts, Highly Efficient Coupling Reagents for Hindered Peptide Synthesis both in Solution and the Solid-Phase

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Abstract—1-Ethyl-2-halopyridinium salts, BEP, FEP, BEPH and FEPH, were synthesized and proved to be very effective for the synthesis of hindered peptides containing *N*-methylated or C_α,C_α-dialkylated amino acid residues. HPLC monitoring of model reactions indicated that these pyridinium salts demonstrated higher reactivities, lower racemization than the commonly used halogenated uronium and phosphonium salts. The efficiency of these pyridinium type coupling reagents was further proved by the synthesis of a series of hindered oligopeptides and active esters with good yields and convenient workup. The 8–11 tetrapeptide fragment of Cyclosporin A (CsA) and the pentapeptide moiety of Dolastatin 15 were also successfully synthesized using these pyridinium salts. The efficiency of these pyridinium type coupling reagents for SPPS was also demonstrated by the solid-phase synthesis of the extremely hindered 8–11 peptide segment of CsA and the linear undecapeptide of CsO. The mechanism of the pyridinium salt mediated coupling reactions was also studied by ^{1}H NMR, IR and HPLC. It was proposed that the major reactive intermediates were the corresponding acyl halide and acyloxypyridinium salts of the *N*-protected amino acid or peptide.

Introduction

In the past decades, many new peptide coupling reagents have been designed, synthesized and commonly used in peptide synthesis. Among these reagents, HOBt- and HOAt-based uronium, phosphonium and immonium salts, such as BOP,[1] HBTU,[2] PyBOP,[3] HBPyU,[4] HATU,[5] HAPyU,[6] AOP,[7] PyAOP,[8] BOMI,[9] BDMP,[10] BDMP,[11] and AOMP have been proven to be very efficient (Fig. 1). The predominance of carbodiimide[12] and active ester techniques[13] have been gradually replaced with onium salts. These reagents can efficiently promote the formation of unhindered amide bonds, while the chain assembly of sterically hindered peptides containing *N*-methyl or C_α,C_α-dialkyl amino acid residues is inefficient using the above reagents except the HOAt-derived onium salts.[14] However, these HOAt-based reagents are expensive and unsuitable for large scale peptide synthesis, and may react with amino components to form the corresponding guanidinium derivatives, especially in peptide segment condensation and cyclization.[15] As an alternative approach, halogenated coupling reagents, such as PyBroP,[16] BroP,[17] CIP,[18] TFFH,[19] BTFFH,[20] PyCIU,[4b] CDTP,[21] CMMM, BOP-Cl,[22] and BEMT[23] shown in Fig. 2, were also found to be efficient

Keywords: 1-ethyl-2-halopyridinium; cyclosporin A; dolastatin 15.

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Abbreviations: Aib, α-aminoisobutyric acid; AOMP, 5-(1*H*-7-azabenzotriazol-l-yloxy)-3,4-dihydro-l-methyl 2*H*-pyrrolium hexachloroantimonate; AOP, (1*H*-7-azabenzotriazol-1-yloxy)tris(dimethylamino)phosphonium hexafluoroposphate; BDMP, 5-(1*H*-Benzotriazol-1-yl)-3,4-dihydro-1-methyl 2*H*-pyrrolium hexachloroantimonate: *N*-oxide; BEMT, 2-bromo-3-ethyl-4-methyl thiazolium tetrafluoroborate; BEP, 2-bromo-1-ethyl pyridinium tetrafluoroborate; BEPH, 2-bromo-1-ethyl pyridinium hexachloroantimonate; BOMI, *N*-(1*H*-benzotriazol-1-ylmethylene)-*N*-methylmethanaminium hexachloroantimonate *N*-oxide; BOP, (1*H*-benzotriazol-1-yloxy)tris(dimethylamino) phosphonium hexafluorophosphate; BOP-Cl, *N*,*N*′-bis(2-oxo-3-oxazolidinyl)phosphinic chloride; BPMP, 1-(1*H*-benzotriazol-1-yloxy)phenylmethylene pyrrolidinium hexachloroantimonate; BTFFH, 1,1,3,3-bis(tetramethylene) florouronium hexafluorophosphate; CMMM, chloro(4-morphoino)methylene morpholinium hexafluorophosphate; DCC, dicyclohexlcarbodiimide; DIEA, *N*,*N*(-diisopropylenthylamine; DMAP, 4-dimethylaminopyridine; FEP, 2-fluoro-1-ethyl pyridinium tetrafluoroborate; FEPH, 2-fluoro-1-ethyl pyridinium hexachloroantimonate; HAPyU, 1-(1-pyrrolidinyl-1*H*-1,2,3-triazolo[4,5-*b*]pyridin-1-ylmethylene)pyrrolidinium hexafluorophosphate *N*-oxide; HATU, *N*-[(dimethylamino)-1*H*-1,2,3-triazolo[4,5-*b*]pyridin-1-ylmethylene)-*N*-methylmethanaminium hexafluoro-phosphate *N*-oxide; HBPipU, *O*-(1*H*-benzotriazol-l-yl)-*N*,*N*,*N*′,*N*′-bis(pentamethylene)uronium hexafluoro-phosphate; HBPyU *O*-(1*H*-benzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-bis(tetramethylene)urinium hexafluorophosphate;; HBTU, *N*-[(1*H*-benzotriazol-1-yl)-(dimethylamino)methylene]-N-methylmethanaminium hexafluorophosphate *N*-oxide; HOAt, 1-hydroxy-7-azabenzotriazole; HOBt, 1-hydroxybenzotriazole; PE, petroleum; PyAOP, (1*H*-7-azabenzotriazol-1-yloxy)tris(pyrrolidino)phosphonium hexafluorophosphate; PyBOP, (1*H*-benzotriazol-1-yloxy)tris(pyrrolidino)phosphonium hexafluorophosphate; PyBrop, bromotrpyrrolidinophosphonium hexafluorophosphate; PyCloP, chlorotripyrrolidinophosphonium hexafluorophosphate; PyClU, 1,1,3,3-bis(tetramethylene) chlorouronium hexafluorophosphate; TFFH, tetramethylfluoromamidinium hexafluorophosphate. Nomenclature and symbols of amino acids and peptides generally follow the recommendations of the IUPAC-IUB Joint Commission of Biochemical Nomenclature in: *Pure Appl. Chem.* **1984**, *56*, 595–624.

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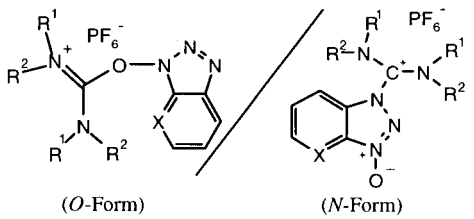
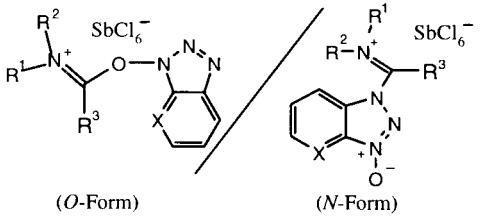


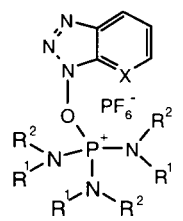
Uronium/aminium salts

HBTU: $R^1 = R^2 = CH_3$; X = CH (*N*-form structure)
HBPyU: R^1, $R^2 = (CH_2)_4$; X = CH (unknown)
HATU: $R^1 = R^2 = CH_3$; X = N (*N*-form structure)
HAPyU: R^1, $R^2 = (CH_2)_4$; X = N (*N*-form structure)



Immonium salts

BOMI: $R^1 = R^2 = CH_3$, R^3 = H; X = CH (*N*-form structure)
BDMP: R^1, $R^3 = (CH_2)_3$, $R^2 = CH_3$; X = CH (*N*-form structure)
BPMP: $R^1 = R^2 = (CH_2)_4$, R^3 = Ph; X = CH (unknown)
AOMP: R^1, $R^3 = (CH_2)_3$, $R^2 = CH_3$; X = N (unknown)



Phosphonium salts

BOP: $R^1 = R^2 = CH_3$; X = CH
PyBOP: R^1, $R^2 = (CH_2)_4$; X = CH
AOP: $R^1 = R^2 = CH_3$; X = N
PyAOP: R^1, $R^2 = (CH_2)_4$; X = N

Figure 1. HOBt- and HOAt-based uronium, phosphonium and immonium type peptide coupling reagents.

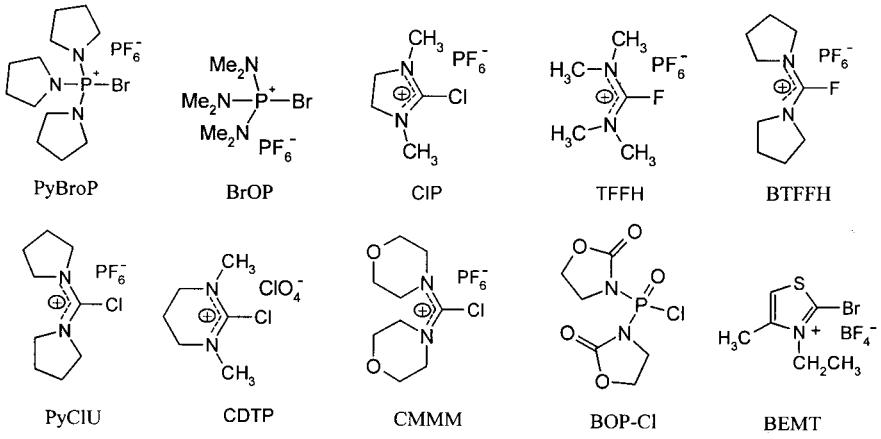
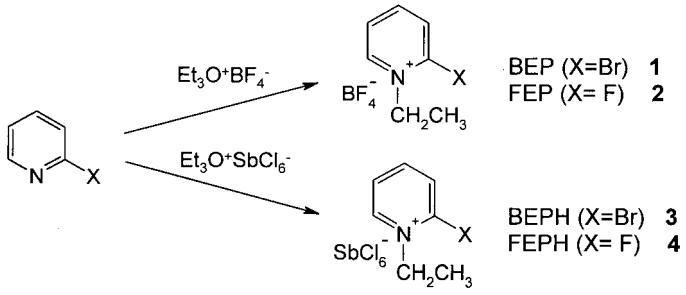


Figure 2. The structures of the halogenated peptide coupling reagents.



Scheme 1. Synthesis of 1-ethyl-2-halopyridinium salts.

for the synthesis of hindered peptides, especially for the scale-up of the preparation of peptides since they are inexpensive. Unfortunately, these halogenated reagents, except BOP-Cl and BEMT, usually result in high racemization during coupling, especially for segment condensation. Therefore, it is necessary and significant to develop more efficient and inexpensive coupling reagents to meet the needs of the synthesis of increasingly challenging peptides and peptidomimetics, as well as the establishment of peptide libraries.

Results and Discussion

Our molecular design was based upon the general structures of the halouronium salts since the efficiency of the uronium type coupling reagents seemed slightly better than the corresponding phosphonium analogues.[7] Analyzing the structures of these halouronium salts, we can see that the carbocation of each uronium salt is well stabilized by the two conjoint nitrogens and the lone electron pairs of the nitrogen atoms are delocalized within the N–C–N bonds, which causes the carbocation to share a relatively high electron density; consequently the uronium salt demonstrates relatively low reactivity in the nucleophilic reaction involved in peptide synthesis. Therefore, we tried to replace one nitrogen with other atoms without lone electron pairs or more electronegative atoms with lone electron pairs to enhance the reactivity of the reaction-mediated carbocations in uronium molecules. In previous studies, we replaced one nitrogen with sulfur to develop a 2-halothiazolium type reagent BEMT, and proved its high efficiency.[23] Herein, we attempted to replace one nitrogen with a carbon atom. Considering meantime considered the stability of generated molecules, it was obvious that the α-halopyridinium salts were the most suitable candidates. Mukaiyama et al., have studied 2-halo and 2-bromopyridinium iodides, and used them to synthesize esters,[24] lactones[25] and carboxamides.[26] However, the condensation reactions had to be carried out under the condition of refluxing in methylene chloride due to the poor solubility of these pyridinium iodides in conventional solvents. Such

Table 1. Comparison of racemization and reactivity of 2-halopyridinium salts with other coupling reagents (model reaction: *Z*-Gly-Phe-OH+Val-OCH_3·HCl→*Z*-Gly-Phe-Val-OCH_3; reaction conditions: solvent CH_2Cl_2 (0.1 M); temp. −10°C; base DIEA)

Coupling reagent	Yield % (t=2 min)	$t^{1/2}$ (min)	D-isomer content (%)
PyBroP	6.1	79	22.3
BOP-Cl	5.3	∽90[a]	4.1
PyClU	5.6	>120	33.2
BTFFH	9.4	49	25.9
CMMM	2.3	>120	31.4
BEMT	45.9	–[b]	2.7
BEMT[c]	84.3	<2[b]	1.3
FEP	75.6	<2[b]	2.3
FEPH	67.7	<2[b]	2.1
BEP	50.8	<2[b]	4.6
BEPH	52.3	<2[b]	4.6
FEP[c]	86.7	<2[b]	1.5
BEP[c]	75.9	<2[b]	1.4

[a] The $t^{1/2}$ value of BOP–Cl cannot be evaluated accurately due to its poor solubility in CH_2Cl_2.
[b] The coupling reactions were accomplished within 2 min.
[c] HOAt (1 equiv.) was added as an additive.

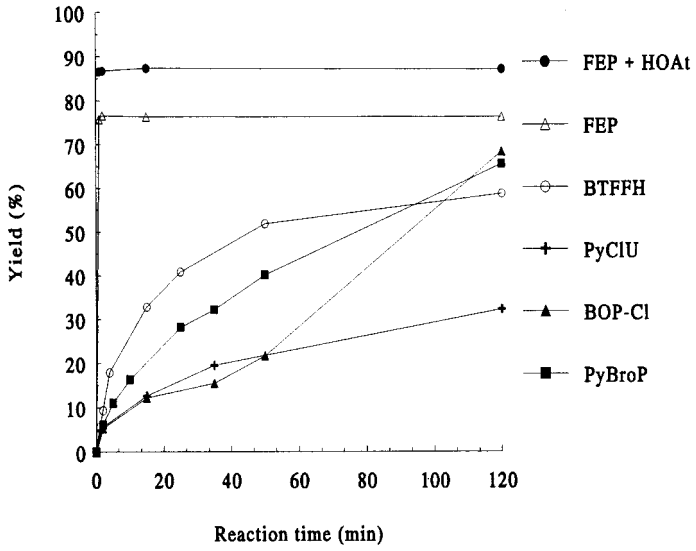


Figure 3. Comparison of reactivity of FEP with other coupling reagents (the reaction model and conditions were the same as in Table 1).

conditions were too rigorous and unsuitable for the synthesis of peptides, especially bio-active peptides. To improve the solubility of these pyridinium compounds, the tetrafluoroborate and hexachloroantimonate counterions were adopted. To further enhance the reactivity of these compounds, 2-fluoropyridinium salts were also selected as peptide coupling reagent candidates since the rate of hydrolysis of the 2-fluoropyridiniums were much faster than those of 2-bromo or 2-chloro substituted analogues.[27] Based upon the above considerations, we designed 2-halopyridinium salts BEP, FEP, BEPH, and FEPH for evaluation of their efficiency in peptide synthesis.

Compared to other halogenated coupling reagents, the 2-halopyridinium salts can be more readily synthesized by *N*-alkylation of 2-halopyridine using triethyloxonium tetrafluoroborate or hexachloroantimonate with nearly quantitative yield as colorless shelf-stable crystals (Scheme 1).

To evaluate the efficiency of these pyridinium salts in peptide synthesis, the model reaction of [2+1] segment condensation, *Z*-Gly-Phe-OH+Val-OCH_3·HCl→*Z*-Gly-Phe-Val-OCH_3, was selected and monitored by HPLC. The reactions were carried out in dilute solutions (0.1 mol/L) to ensure all substrates and products were well dissolved in the solution. Table 1 and Fig. 3 showed that these 2-halopyridinium salts were much more reactive than widely used halogenated reagents, such as PyBroP, PyClU, BTFFH and BOP-Cl. The coupling was accomplished within 2 min under the tested reaction conditions as shown in Fig. 3. The coupling yields were further improved if base was added slowly or dropwise to avoid the 2-halopyridinium salts reacting too violently. It is obvious that the racemization of product using 2-halopyridinium salts was much lower than those of other halogenated coupling reagents as shown in Table 1 except the reagent BOP-Cl and BEMT. If HOAt was added as an additive, the

Table 2. Preparation of oligopeptides using 2-halopyridinium type coupling reagents (the CO–NH bond formed in the peptide is indicated by *, all products were confirmed by ^{1}H NMR, EIMS and other characterization)

Peptide	Reagent	Yield (%)[a]	Mp (°C)	$[\alpha]_D$ (conc., solv., temp.)
Fmoc-Nva*-Sar-OBzl	BEP	98	38–39	−9.3 (0.3, $CHCl_3$, 23°C)
Z-MeVal*-MeVal-OMe	BEP	95	Oil	−206 (1, MeOH, 19°C)
Fmoc-Leu*-Ala-OBzl	FEP	91	124–126	−47.9 (1, MeOH, 20°C)
Fmoc-Val*-MeVal-OCH_3	BEP	88	Oil	−86.0 (0.3, $CHCl_3$, 23°C)
Fmoc-MeLeu*-MeVal-OCH_3	FEP	95	Oil	−135.6 (1, MeOH, 20°C)
Boc-Pro*-Pro-OBzl	BEP	90	Oil	−109.4 (1, $CHCl_3$, 23°C)
Z-Aib*-Aib-OCH_3	BEP	96	108–109	–
Fmoc-MeLeu*-MeVal-OBzl	BEP	91	Oil	−122 (0.2, $CHCl_3$, 22°C)
Z-Val*-Leu-Ala-OBu^t	FEP	97	131–132	−56.3 (1, MeOH, 20°C)
Fmoc-MeLeu*-MeLeu-MeVal-OBzl	BEP	48	Oil	−114.8 (1, MeOH, 20°C)
Z-Leu*-Val-Leu-Ala-OBu^t	BEP	92	144–146	−62.1 (1, MeOH, 20°C)
Fmoc-D-Ala*-MeLeu-MeLeu-MeVal-OBzl	BEP	94	36–37	−145.4 (0.5, $CHCl_3$, 25°C)
Boc-Val-Val-MeVal*-Pro-Pro-OBzl	BEP	88	89–90	−181 (0.3, $CHCl_3$, 23°C)
Boc-Val*-MeVal-OMe	FEP	92	Oil	−136.0 (1, MeOH, 20°C)
Boc-Pro*-Pro-OBzl	FEP	94	Oil	−127.4 (1, MeOH, 21°C)
Z-Leu*-Ala-OBu^t	FEP	89	Glassy solid	−38.2 (1, MeOH, 20°C)
Z-Aib*-Aib-OCH_3	FEP	95	108–110	–

[a] Isolated yield based upon N^{α}-protected amino acid or peptide.

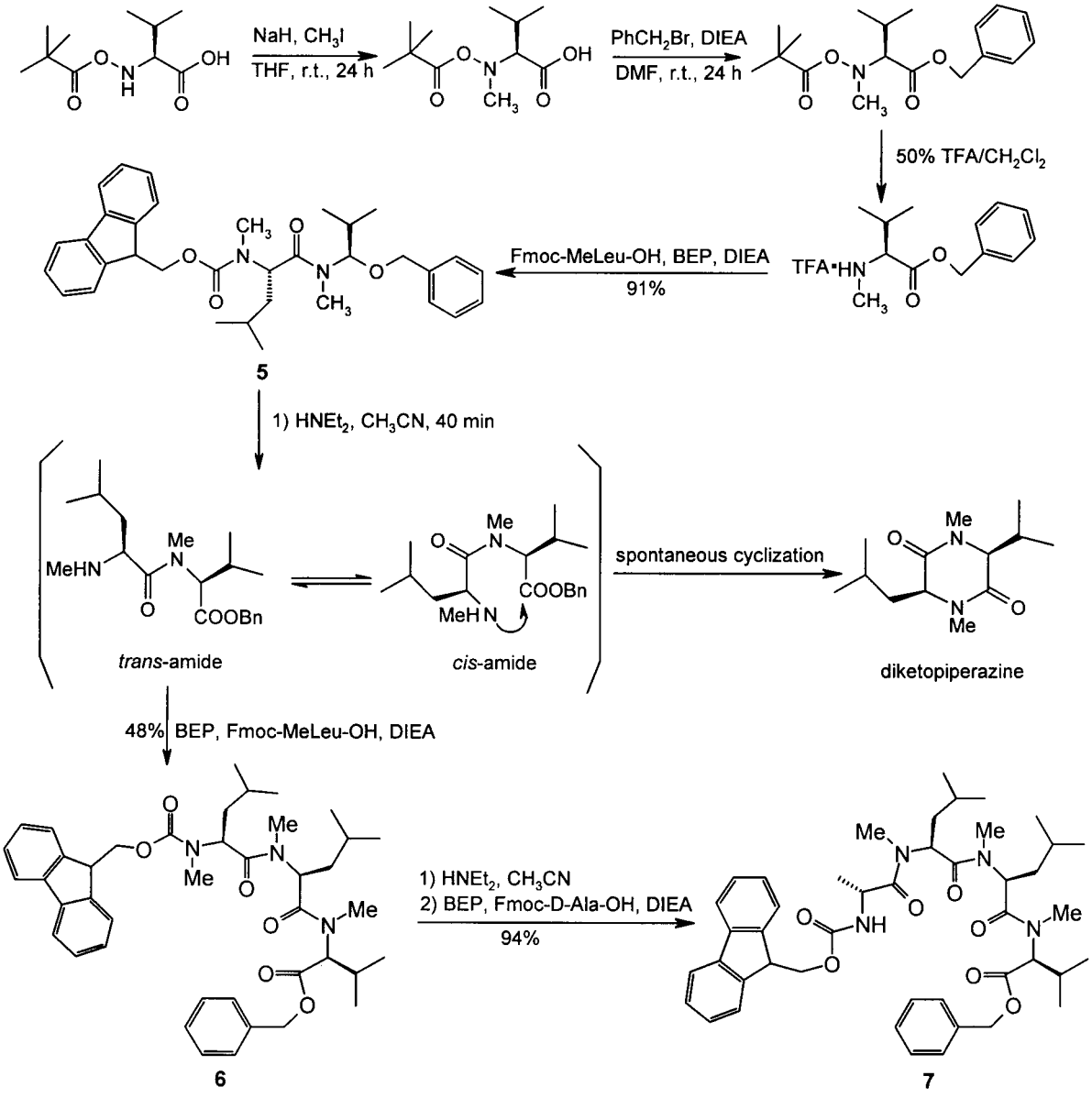
Table 3. Synthesis of active esters using 2-halopyridinium salts

Substrate		Reagent	Product[a]	Yield (%)[b]	Mp (°C)
Carboxylic acid	Alcohol				
Boc-Aib-OH	HOBt	FEP	Boc-Aib-OBt	82	Oil
Boc-Aib-OH	KOPfp	FEP	Boc-Aib-OPfp	76	105–106
Fmoc-Sar-OH	HOSu	FEP	Fmoc-Sar-OSu	81	Glassy solid
Fmoc-Sar-OH	HOOBt	BEP	Fmoc-Sar-OOBt	77	Glassy solid
Fmoc-Sar-OH	HOPcp	BEPH	Fmoc-Sar-OPcp	85	113–114
CBZ-Ala-OH	Bu^t-OH	FEP	CBZ-Ala-OBu^t	64	Oil

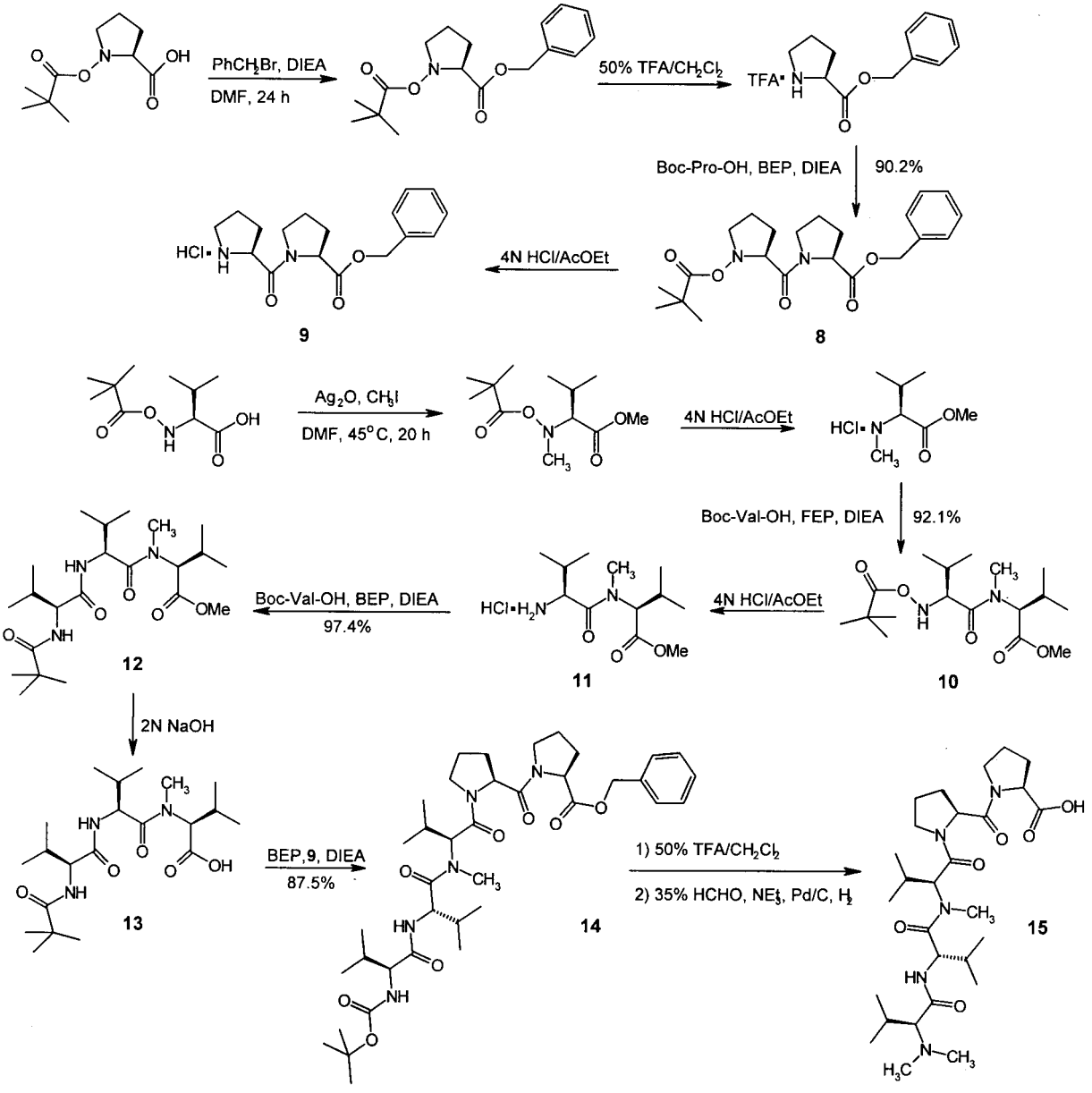
[a] All products were confirmed by ^1H NMR, EIMS and other characterization.
[b] Isolated yield based on carboxylic acid component.

racemization could be further suppressed due to its anchimeric assistance effect.[28]

In order to evaluate the efficiency of these pyridinium type coupling reagents for hindered peptide synthesis, a series of oligopeptides were prepared as shown in Table 2. In a typical experimental procedure, DIEA (3.2 equiv.) was added to a cooled mixture (−10°C) of N^{α}-protected amino acid (1 equiv.), amino acid ester hydrochloride (1.1 equiv.), and 2-halopyridinium salt (1.1 equiv.) in



Scheme 2. Synthesis of the 8–11 fragment of Cyclosporin A using reagent BEP.

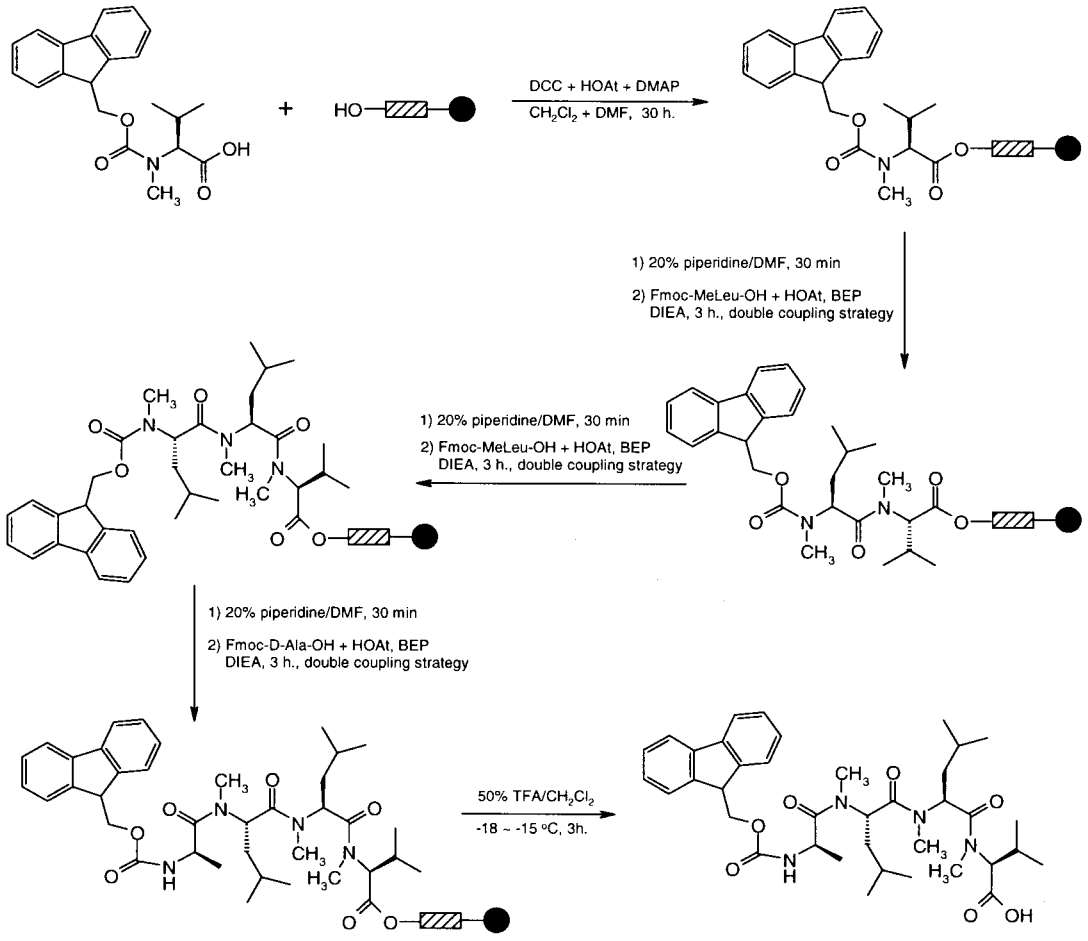


Scheme 3. Synthesis of pentapeptide moiety of Dolastatin 15.

CH_2Cl_2 (3–5 mL/mmol), stirred for 1 min cold and for 1 h at room temperature. The reaction time should be moderately prolonged for the coupling between *N*-methyl amino acids monitoring by TLC. The products can be easily purified by washing with 5% sodium bicarbonate, brine, 0.5 M citric acid and water successively to remove the byproducts *N*-ethyl-2-pyridine and other impurities.

These pyridinium type coupling reagents can also be used to prepare amides and esters, especially active esters and hindered esters, such as benzotriazolyl ester, pentafluorophenyl ester, succinimidyl ester, 2,3-dihydro-4-oxobenzotriazinyl ester and *tert*-butyl ester (Table 3). These active esters are commonly used in the synthesis of lactones and lactams.

To further demonstrate the efficiency of 2-halopyridinium salts in hindered peptide couplings, two extensively *N*-alkylated peptide segments were synthesized successfully using these reagents. During the synthesis of the 8–11 tetrapeptide fragment of Cyclosporin A, the C-terminus was intentionally protected as a benzyl ester to investigate the capability of these pyridinium type reagents, such as BEP, in preventing the spontaneous formation of diketopiperazine. Under this unfavorable circumstance, the tripeptide Fmoc-MeLeu-MeLeu-MeVal-OBzl **6** was still obtained in 48% yield by sequential deprotection and coupling of Fmoc-MeLeu-MeVal-OBzl **5** with Fmoc-MeLeu-OH using reagent BEP (Scheme 2). According to the report by Wenger during the synthesis of Cyclosporin A using modified mixing pivalic anhydride method, the same desired tripeptide was not obtained at all due to the spontaneous formation of diketopiperazine.[29] This encouraging result indicated that BEP was such a powerful coupling

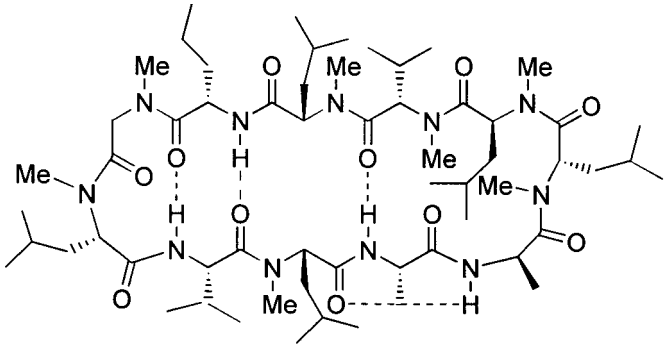


Scheme 4. Synthesis of the 8–11 segment of Cyclosporin A in the solid-phase using BEP.

reagent that could efficiently promote the formation of hindered peptide bonds, even preferentially to spontaneous diketopiperazine formation.

Using these α-halopyridinium type reagents, we also synthesized the hindered pentapeptide moiety of Dolastatin 15, which is a pseudopeptide bearing promising antineoplastic activity.[30] The [3+2] segment condensation strategy was adopted and the Boc group was used for the N^{α}-protection, thus a 58% overall yield of the protected pentapeptide **14** was obtained via seven coupling steps. After removal of the Boc protecting group with trifluoroacetic acid, the final product **15** was obtained by reductive alkylation in the presence of 35% formaldehyde and 10% Pd/C (Scheme 3). During elongation of the peptide chain, the yield of each step was almost all above 90%, no *N*-carboxyanhydride (NCA) was detected although such by-products would become dominant for halophosphonium type reagents, such as PyBroP and PyCloP, mediated coupling reactions in the case of Boc-protected amino acids as carboxylic components.[31]

The efficiency of these α-halopyridinium salts can be further proved by the successful total synthesis of the immunosuppressive cyclic undecapeptide Cyclosporin O in 18–23% overall yield with the rationally combined utilization of reagent BDMP and BEMT.[32]



Cyclosporin O (CsO)

These α-halopyridinium-type coupling reagents can also be used in solid-phase peptide synthesis, especially for the synthesis of peptides containing *N*-methyl amino acid residues. Using reagent BEP, the extremely hindered 8–11 tetrapeptide of Cyclosporin A was synthesized with the Wang resin. The purity of the obtained crude peptide was 95% without any purification after removal from the Wang resin with 50% trifluoroacetic acid (Scheme 4).

For further determination of the widespread usefulness of these 2-halopyridinium type reagents, we also synthesized

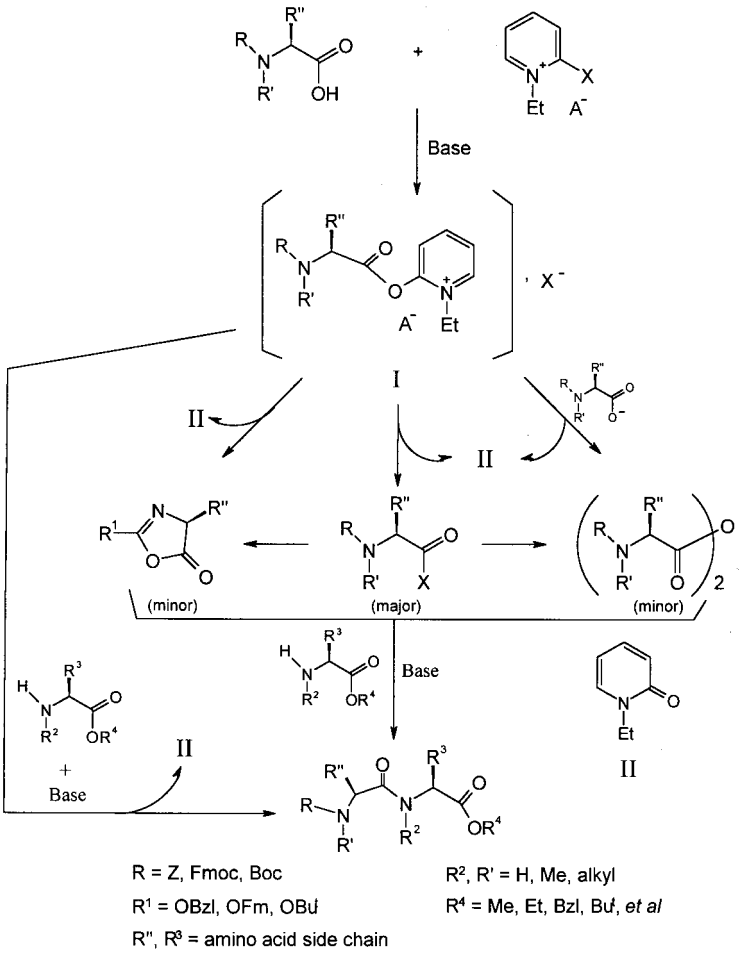


Figure 4. Proposed mechanism for α-halopyridinium salt mediated coupling reactions.

the linear undecapeptide CsO by the solid-phase method using BEP and FEP, respectively. The C-terminal residue Fmoc-Ala-OH was anchored onto the Wang resin using DCC/HOAt/DMAP in CH_2Cl_2. Because the application of the ninhydrin test for monitoring the coupling of *N*-methyl amino acids is impossible, the double coupling strategy (2×3 h) was adopted. To further reduce the racemization during coupling, HOAt was added as an additive. Thus, the reaction condition used in the coupling was: (3 equiv. of Fmoc-AA-OH/DMF+3 equiv. of coupling reagent+3 equiv. of HOAt+9 equiv. of DIEA)/1 equiv. of NH_2-AA′-Wang resin with a coupling time of 2×3 h. The cleavage of the peptide from the resin was carried out using 50% TFA in CH_2Cl_2 at low temperature (−18 to −15°C). Unfortunately, the purity of the obtained linear undecapeptide was no more than 5% shown by ESI-MS spectrum although the results of UV analysis[33] during the Fmoc deprotection indicated that the coupling yield of each step was above 90%. It is most likely that the amide bonds between hindered amino acids are prone to undergo hydrolysis in the strongly acidic medium.[34]

A tentative mechanism for 2-halopyridinium salt mediated coupling reactions was proposed and studied by ^{1}H NMR and IR. The model reaction was carried out in $CDCl_3$ by treating Boc-Val-OH with 2-halopyridinium salt in the present of NEt_3. It was indicated that the reaction mixture, analyzing by ^{1}H NMR and IR, consists of 2-*tert*-butoxy-4-isopropyl-L-5(4*H*)-oxazolone and a small amount of symmetric anhydride of Boc-Val-OH, besides Boc-Val-OH, $Et_3HN^+X^-$ and the byproduct *N*-ethyl-2-pyridone **II** released from 2-halopyridinium salts, compared to the spectra of authentic compounds prepared in advance. No NCA was detected during the reaction. However, it is reasonable to speculate that the oxazolones and symmetric anhydrides may not be the main active intermediates since the reactivity of the two species is not high enough to cause the coupling of *Z*-Gly-Phe-OH with Val-OCH_3·HCl to be accomplished within 2 min at −10°C in a dilute solution and promote the coupling reactions between *N*-methyl or C_α,C_α-dialkyl amino acids effectively with low racemization. Therefore, we speculated that other more reactive intermediates, such as (acyloxy)pyridinium salt and acyl halide, might participate in the coupling reaction, but they were very liable to decomposition and hardly detected. Because acyl fluorides are insensitive to the attack of neutral nucleophile, such as water and alcohol, than acyl bromides and acyl chlorides, we carried out another model reaction by treating the dichloromethane solution of the Fmoc-Val-OH with FEP in the presence of NEt_3 at −10°C, and monitored by IR. The appearance of the characteristic absorption of the carbonyl fluoride group was observed at 1845 cm^{-1}. This indicated the Fmoc-Val-F was generated immediately when the base NEt_3 was added. On exposing

the reaction mixture to the air for 1 h, the intensity of the absorption at 1845 cm^{-1} decreased to approximately 60% of the original value due to hydrolysis of Fmoc-Val-F.

Based upon the above results, we speculated that the first step of carboxylic acid activation by 2-halopyridinium salts involves the formation of an unstable acyloxypyridinium salt intermediate **I**, in turn the intermediate reactes directly with amino component to give the product or is competitively converted into the acid halide which is subsequently converted into the dipeptide by aminolysis. A small amount of 5(4*H*)-oxazolone and symmetric anhydride of the carboxylic component are generated as minor active intermediates during coupling, the former species is also very labile to cause racemization due to its tautomerization and enolization (Fig. 4). Comparing to halouronium and halophosphonium type coupling reagents, the high reactivity of α-halopyridinium slats may be attributed to the instability of the pyridinium salts themselves and the higher reactivity of the (acyloxy)pyridinium salts **I** than those of (acyloxy)uronium and (acyloxy)phosphonium salts due to the obvious electronic effect.

Conclusions

The 2-halopyridinium type coupling reagents, which can be readily prepared from 2-halopyridine in one step as shelf-stable crystals, demonstrated very high reactivity, low racemization and excellent yields in peptide synthesis both in solution and the solid-phase, especially the synthesis of hindered peptides containing *N*-methyl amino acid residues.

Experimental

Melting points were determined in capillary tubes and are uncorrected. IR spectra were measured with Schimadazu IR-440 spectrometer. NMR spectra were recorded on Bruker AM 300 or Bruker DRX 400 instruments. Chemical shifts are reported as ppm (δ units) downfield from a tetramethylsilane internal standard. Because in most cases the spectra demonstrated multiple conformations, generally with different coupling constants for the different rotamers, *J* values are for the most part omitted. Mass spectra were taken with HP5890A, and VG QUATTRO mass spectrometers. Elemental analyses for carbon, hydrogen and nitrogen were determined on a MOD-1106 elemental analyzer. Optical rotations were recorded with a Perkin–Elmer 241 MC polarimeter. HPLC analyses were carried out on a waters or Varian-SY-5000 instrument and using either a Kromasil RP-18 (5×250 mm) or a Waters μBondapak C18 (4.6×300 mm) column. Flash column chromatography was performed with 300–400 meshes silica gel, and analytical thin layer chromatography was performed on precoated silica gel plates (GF-254) with the systems (v/v) indicated. Solvents and reagents were purified by standard methods as necessary. The 60–90°C Petroleum (PE) ether was used.

DCC, PyBroP, HOAt, 2-bromo pyridine and 2-fluoro pyridine were purchased from Aldrich Chemical Co. of Milwaukee, WI, and used without purification. PyClU, BTFFH, BOP-Cl and amino acid derivatives were prepared according to literature methods.[4,20,22] Amino acids were all L-configuration if not otherwise stated. Cbz-*N*-methyl amino acids were synthesized by the procedure of McDermott and Benoiton.[35] Fmoc-*N*-methyl amino acids were synthesized by the procedure of Freidinger et al.[36]

General procedure for the synthesis of pyridinium type coupling reagents

To a solution of triethyloxonium tetrafluoroborate or hexachloroantimonate (10 mmol) in 10 mL $ClCH_2CH_2Cl$, 2-halogen pyridine (10 mmol) was added slowly under an argon atmosphere. After stirring at room temperature for 1 h, and heating to 50°C for a further 30 min. The reaction mixture was cooled, diluted with anhydrous ether, filtered and washed with ether. The crude product was crystallized from acetone/ether to give corresponding pyridinium type reagents as colorless crystals.

2-Bromo-1-ethyl pyridinium tetrafluoroborate (BEP, 1). BEP was synthesized from 2-bromopyridine and $Et_3O^+BF_4^-$ according to the general procedure. Yield 95%, mp 103–104°C; [Found: C, 30.58; H, 3.22; N, 4.96. $C_7H_9BBrF_4N$ requires C, 30.70; H, 3.31; N, 5.11%]; ν_{max}(KBr) 3106w, 1617s, 1571m, 1500m, 1467m, 1296m, 1050vs, 786s, 718w, 521w; ^{1}H NMR (300 MHz, d_6-acetone): δ=1.69 (t, *J*=7.3 Hz, 3H, CH_2CH_3), 5.01 (q, *J*=7.3 Hz, 2H, CH_2CH_3), 8.24 (m, 1H, 5-C*H*-Py), 8.53–8.57 (m, 2H, 3,4-C*H*-Py), 9.32 (d, *J*=6.9 Hz, 1H, 6-C*H*-Py); FAB-MS *m/z*: 186 $[M-BF_4^-]^+$, 188 $[M-BF_4^-+2]^+$.

2-Fluoro-1-ethyl pyridinium tetrafluoroborate (FEP, 2). FEP was synthesized from 2-fluoropyridine and $Et_3O^+BF_4^-$ according to the general procedure. Yield 95%; mp 51–52°C; [Found: C, 39.32; H, 4.17; N, 6.54. $C_7H_9BF_5N$ requires C, 39.48; H, 4.26; N, 6.58%]; ν_{max}(KBr) 3039w, 1643s, 1587m, 1519s, 1475m, 1307m, 1070vs, 847m, 786s, 725w, 522w; ^{1}H NMR (300 MHz, d_6-acetone): δ=1.69 (t, *J*=7.3 Hz, 3H, CH_2CH_3), 4.85 (q, *J*=7.3 Hz, 2H, CH_2CH_3), 8.02–9.11 (m, 4H, aryl); ^{19}F NMR (60 MHz, d_6-acetone, CF_3COOH): δ=70.2 (s, 1F); FAB-MS *m/z*: 126 $[M-BF_4^-]^+$.

2-Bromo-1-ethyl pyridinium hexachloroantimonate (BEPH, 3). BEPH was synthesized from 2-bromopyridine and $Et_3O^+SbCl_6^-$ according to the general procedure. Yield 90%, mp 240–242°C; [Found: C, 16.01; H, 1.80; N, 2.87. $C_7H_9BrCl_6NSb$ requires C, 16.12; H, 1.74; N, 2.69%]; ν_{max}(KBr) 3133w, 2987w, 1614m, 1546s, 1465m, 1407m, 1212s, 1130s, 1053m, 741s, 630m; ^{1}H NMR (300 MHz, d_6-acetone): δ=1.68 (t, *J*=7.2 Hz, 3H, CH_2CH_3), 4.99 (q, *J*=7 Hz, 2H, CH_2CH_3), 8.22 (m, 1H, 5-C*H*-Py), 8.51–8.58 (m, 2H, 3,4-C*H*-Py), 9.29 (d, *J*=6.5 Hz, 1H, 6-C*H*-Py).

2-Fluoro-1-ethyl pyridinium hexachloroantimonate (FEPH, 4) FEPH was synthesized from 2-fluoropyridine and $Et_3O^+SbCl_6^-$ according to the general procedure. Yield 91%, mp 262–246°C; [Found: C, 18.09; H, 1.80; N, 3.02. $C_7H_9Cl_6NFSb$ requires C, 18.25; H, 1.97; N, 3.04%]; ν_{max} (KBr) 3088w, 2995w, 1639vs, 1586s, 1516s, 1474m, 1310m, 1149m, 1091m, 847m, 776s, 728w; ^{1}H NMR

(300 MHz, d_6-acetone): δ=1.75 (t, J=7.2 Hz, 3H, CH_2CH_3), 4.93 (q, J=7.2 Hz, 2H, CH_2CH_3), 8.15–9.15 (m, 4H, aryl); ^{19}F NMR (300 MHz, d_6-acetone, CF_3COOH): δ=68.8 (s, 1F).

HPLC monitoring of model reactions for the evaluation of the efficiency of halogenated coupling reagents with the model reaction *Z*-Gly-Phe-OH+Val-OMe·HCl→ *Z*-Gly-D/L-Phe-Val-OMe

In the presence of DIEA (78 μL, 0.448 mmol), *Z*-Gly-Phe-OH (50 mg, 0.14 mmol) and Val-Ome·HCl (26 mg, 0.154 mmol) were coupled with the tested reagent (0.154 mmol) in CH_2Cl_2 (1.5 mL) at −10°C. Boc-Phe-Val-OMe (66 mg, 0.18 mmol) was added as the internal reference. Aliquots (10 μL) from the reaction mixture were quenched and dissolved in 100 μL buffer solution (CH_3OH/H_2O/TFA: 50/50/1). The resultant samples were analyzed by HPLC to give the following results: *Z*-Gly-Phe-OH (t_R= 4.04 min); *Z*-Gly-L-Phe-Val-OMe (t_R=9.24 min); *Z*-Gly-D-Phe-Val-OMe (t_R=10.28 min); Boc-Phe-Val-OMe (t_R= 15.82 min) by comparing to the prepared reference compounds. Peak areas were compared in order to obtain the chemical yields (yield (%)=[(LL/X_1+DL/X_2)/aS]×100%). The percentage of epimers was calculated according to the equation: D (%)=[DL/X_2/(LL/X_1+DL/X_2)]×100%; where LL refers to the area of *Z*-Gly-L-Phe-Val-OMe, DL refers to that of *Z*-Gly-D-Phe-Val-OMe, S refers to that of Boc-Phe-Val-OMe, a=0.778 which is the molar ratio between the *Z*-Gly-Phe-OH and Boc-Phe-Val-OMe, X_1=1.269 and X_2=1.254 which are the determined correction factors for absorption difference (220 nm) between the references.

HPLC conditions: Column: Kromasil KR 100–10 C18 (4.6×25 cm). Eluant: 48% CH_3CN (0.1% TFA). Flow rate: 1.5 mL/min. Detection: 220 nm (0.5 AUFS).

Illustrations of the preparation of oligopeptides and esters using 2-halopyridinium salts *N*-[(9*H*-Fluoren-9-ylmethoxy)carbonyl]-valyl-*N*-methyl-valine methyl ester (Fmoc-Val-MeVal-OCH$_3$). To a cold solution of Fmoc-Val-OH (0.187 g, 0.55 mmol), MeVal-OCH_3·HCl (91 mg, 0.50 mmol) and BEP (0.151 g, 0.55 mmol) in 2 mL CH_2Cl_2, DIEA (0.28 mL, 1.6 mmol) was added. The reaction mixture was stirred at room temperature for 1 h. Yield: 0.206 g (88%), $[\alpha]_D^{23}$=−86.0 (c 0.3, $CHCl_3$), R_f 0.65 (AcOEt/PE: 1/1); ^{1}H NMR (300 MHz, $CDCl_3$) two conformers: δ=0.58–1.04 (m, 12H, CH_3 of Val, MeVal), 2.02–2.38 (2m, 2H, β-C*H* of Val, MeVal), 2.91, 3.07 (2s 1: 7, 3H, *N*-CH_3-MeVal), 3.71 (s, 3H, OCH_3), 4.07–5.02 (m, 5H, α-C*H*-MeVal, 9-C*H*-Fluorenyl, CH_2-Fmoc, α-C*H*-Val), 5.55 (d, J=9.3 Hz, 1H, N*H*-Val), 7.32 (t, J=7.4 Hz, 2H, 2, 7-C*H*-Fluorenyl), 7.41 (t, J=7.4 Hz, 2H, 3, 6-C*H*-Fluorenyl), 7.60 (d, J=7.3 Hz, 2H, 1,8-C*H*-Fluorenly), 7.77 (d, J=7.4 Hz, 2H, 4,5-C*H*-Fluorenyl); EIMS m/z: 467 $[M+H]^+$, 294 $[M-MeVal\text{-}OCH_3\text{–}CO]^+$, 179 $[Fmoc\text{-}CO_2]^+$.

***N*-[(9*H*-Fluoren-9-ylmethoxy)carbonyl]-*N*-methyl-leucyl-*N*-methyl-valine methyl ester (Fmoc-MeLeu-MeVal-OCH$_3$).** To a cold solution of Fmoc-MeLeu-OH (0.184 g, 0.50 mmol), MeVal-OCH_3·HCl (0.100 g, 0.55 mmol) and FEP (0.117 g, 0.55 mmol) in 2 mL CH_2Cl_2, DIEA (0.28 mL, 1.60 mmol) was added. The reaction mixture was stirred at room temperature for 4 h. Yield: 0.234 g (95%), $[\alpha]_D^{20}$=−135.6 (c 1, MeOH), R_f 0.66 (AcOEt/PE: 1/3); ^{1}H NMR (300 MHz, $CDCl_3$) four conformers: δ=0.46–1.06 (m, 12H, CH_3 of MeLeu, MeVal), 1.10–1.79 (m, 3H, γ-C*H*-MeLeu, β-CH_2-MeLeu), 1.90–2.26 (m, 1H, β-C*H*-MeVal), 2.65–2.98 (m, 6H, *N*-CH_3 of MeLeu, MeVal), 3.41, 3.63, 3.65, 3.70 (4s, 3H, OCH_3), 4.12–5.18 (m, 5H, 9-C*H*-Fluorenyl, CH_2-Fmoc, α-C*H*-MeVal, α-C*H*-MeLeu), 7.28–7.47 (m, 4H, 2,3,6,7-C*H*-Fluorenyl), 7.50–7.69 (m, 2H, 1,8-C*H*-Fluorenyl), 7.70–7.85 (m, 2H, 4,5-C*H*-Fluorenyl); EIMS m/z: 495 $[M+H]^+$, 463 $[M-OCH_3]^+$, 322 $[(M-MeVal\text{-}OCH_3]^+$, 179 $[Fmoc\text{-}CO_2]^+$.

***N*-(*tert*-Butyloxycarbonyl)-α-aminoisobutyric acid pentafluorophenyl ester (Boc-Aib-OPfp).** To a cold solution of Boc-Aib-OH (0.305 g, 1.50 mmol), KOPfp (0.333 g, 1.50 mmol) and FEP (0.352 g, 1.65 mmol) in 3 mL CH_2Cl_2, DIEA (0.29 mL, 1.65 mmol) was added. The reaction mixture was stirred at room temperature for 1 h. Yield: 0.419 g (76%), mp 105–106°C, R_f 0.70 (AcOEt/PE: 1/12); ν_{max}(KBr) 3389s, 2995m, 1772s, 1705s, 1521s, 1454m, 1388m, 1292m, 1258m, 1169m, 1080s, 995s; ^{1}H NMR (90 MHz, $CDCl_3$): δ=1.51 (s, 9H, 3CH_3-Bu^t), 1.68 (s, 6H, 2β-CH_3-Aib), 5.06 (br, 1H, N*H*): ^{19}F NMR (60 MHz, $CDCl_3$, CF_3COOH): δ=−73.9 (d, J=20 Hz, 2F), −79.5 (t, J=20 Hz, 1F), −84.0 (t, J=20 Hz, 2F); EIMS m/z: 370 $[M+H]^+$, 57 $[Bu^t]^+$.

Synthesis of the 8–11 protected tetrapeptide fragment of Cyclosporin A using reagent BEP *N*-[(9*H*-Fluoren-9-ylmethoxy)carbonyl]-*N*-methylleucyl-*N*-methylvaline benzyl ester (Fmoc-MeLeu-MeVal-OBzl, 5). To a cold solution of Fmoc-MeLeu-OH (0.143 g, 0.389 mmol), MeVal-OBzl·TFA (0.137 g, 0.409 mmol) and BEP (0.117 g, 0.428 mmol) in 2 mL CH_2Cl_2, DIEA (0.21 mL, 1.23 mmol) was added. The reaction mixture was stirred at room temperature for 4 h. Yield: 0.203 g (91%), R_f 0.53 (AcOEt/PE: 1/4), $[\alpha]_D^{22}$=−122 (c 0.2, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) four conformers: δ=0.39–0.95 (m, 12H, CH_3 of MeLeu, MeVal), 1.16–1.63 (m, 3H, γ-C*H*-MeLeu, β-CH_2-MeLeu), 1.97–2.23 (m, 1H, β-C*H*-MeVal), 2.55–2.88 (m, 6H, *N*-CH_3 of MeLeu, MeVal), 3.94–5.23 (m, 7H, 9-C*H*-Fluorenyl, CH_2-Fmoc, α-C*H*-MeVal, α-C*H*-MeLeu, CH_2-OBzl), 7.01–7.74 (m, 13H, aryl); EIMS m/z: 571 $[M+H]^+$, 555 $[M-CH_3]^+$, 463 $[M-OBzl]^+$, 322 $[M-MeVal\text{-}OBzl\text{-}CO]^+$, 179 $[Fmoc\text{-}CO_2]^+$, 91 $[PhCH_2]^+$.

***N*-[(9*H*-Fluoren-9-ylmethoxy)carbonyl]-*N*-methylleucyl-*N*-methylleucyl-*N*-methyl valine benzyl ester (Fmoc-MeLeu-MeLeu-MeVal-OBzl, 6).** Fmoc-MeLeu-MeVal-OBzl (0.148 g, 0.259 mmol) was dissolved in CH_3CN (2 mL) and treated with an equal volume of diethylamine under nitrogen atmosphere until TLC analysis indicated that the starting material disappeared (ca. 40 min). The solution was concentrated in vacuo, the residue was dissolved in CH_3CN and concentrated again to give H-MeLeu-MeVal-OBzl, which was further dried in vacuo for 2 h and precipitated the following coupling reaction without purification. To a cold solution of Fmoc-MeLeu-OH (0.114 g,

0.311 mmol), BEP (85 mg, 0.311 mmol) and H-MeLeu-MeVal-OBzl (0.259 mmol) in 2 mL CH_2Cl_2, DIEA (108 μL, 0.622 mmol) was added. The reaction mixture was stirred at room temperature for 4 h. Yield: 87 mg (48%), R_f 0.41 (AcOEt/PE: 1/4), $[\alpha]_D^{20}=-114.8$ (*c* 1, CH_3OH); ^1H NMR (300 MHz, $CDCl_3$) more than two conformers: $\delta=0.51-1.01$ (m, 18H, C*H*$_3$ of 2MeLeu, MeVal), 1.25–1.78 (m, 6H, 2β-C*H*$_2$-MeLeu, 2γ-C*H*-MeLeu), 2.19 (m, 1H, β-C*H*-MeVal), 2.64–2.91 (m, 9H, *N*-C*H*$_3$ of 2MeLeu, MeVal), 4.17–5.49 (m, 8H, 9-C*H*-Fluorenyl, C*H*$_2$-Fmoc, α-C*H*-MeVal, C*H*$_2$-OBzl, 2α-C*H*-MeLeu), 7.26–7.48 (m, 9H, 2, 3, 6, 7-C*H*-Fluorenyl, Ph-OBzl), 7.50–7.63 (m, 2H, 1,8-C*H*-Fluorenyl), 7.65–7.84 (m, 2H, 4,5-C*H*-Fluorenyl); EIMS *m/z*: 697 M^+, 477 $[M-MeVal-OBzl]^+$, 322 $[(M-MeLeu-MeVal-OBzl-CO]^+$, 179 $[(Fmoc-CO_2]^+$, 91 $[PhCH_2]^+$.

***N*-[(9*H*-Fluoren-9-ylmethoxy)carbonyl]-D-alanyl-*N*-methylleucyl-*N*-methylleucyl-*N*-methylvaline benzyl ester (Fmoc-D-Ala-MeLeu-MeLeu-MeVal-OBzl, 7).** Fmoc-MeLeu-MeLeu-MeVal-OBzl (0.080 g, 0.115 mmol) was deprotected according to above experimental procedure to give H-MeLeu-MeLeu-MeVal-OBzl, which was further dried in vacuo for 2 h and precipitated the following coupling reaction without purification. To a cold solution of Fmoc-D-Ala-OH (54 mg, 0.172 mmol), BEP (47 mg, 0.172 mmol) and H-MeLeu-MeLeu-MeVal-OBzl (0.115 mmol) in 1 mL CH_2Cl_2, DIEA (60 μL, 0.344 mmol) was added. The reaction mixture was stirred at room temperature overnight. Yield: 83 mg (94%), R_f 0.53 (AcOEt/PE: 1/2), $[\alpha]_D^{25}=-145.4$ (*c* 0.5, $CHCl_3$); ^1H NMR (300 MHz, $CDCl_3$) two conformers: $\delta=0.78-1.96$ (m, 27H, C*H*$_3$ of D-Ala, 2MeLeu, MeVal, 2β-C*H*$_2$-MeLeu, 2γ-C*H*-MeLeu), 2.18 (m, 1H, β-C*H*-MeVal), 2.52–3.04 (m, 9H, *N*-C*H*$_3$ of 2MeLeu, MeVal), 4.19–4.44 (m, 3H, 9-C*H*-Fluorenyl, C*H*$_2$-Fmoc), 4.67 (m, 1H, α-C*H*-D-Ala), 4.88 (d, *J*=10.5 Hz, 1H, α-C*H*-MeVal), 5.14 (m, 2H, C*H*$_2$-OBzl), 5.37–5.54 (m, 2H, 2α-C*H*-MeLeu), 5.76 (m, 1H, N*H*-D-Ala), 7.24–7.45 (m, 9H, 2, 3, 6, 7-C*H*-Fluorenyl, Ph-OBzl), 7.56 (d, *J*=7.3 Hz, 2H, 1,8-C*H*-Fluorenyl), 7.76 (d, *J*=7.4 Hz, 2H, 4,5-C*H*-Fluorenyl); EI-MS *m/z*: 768 M^+, 548 $[M-MeVal-OBzl]^+$, 422 $[M-MeLeu-MeVal-OBzl]^+$, 91 $[PhCH_2]^+$.

Synthesis of the pentapeptide moiety of Dolastatin 15 using pyridinium type coupling reagents *N*-(*tert*-Butyloxycarbonyl)-prolyl-proline benzyl ester (Boc-Pro-Pro-OBzl, 8). To a cold solution of Boc-Pro-OH (0.431 g, 2.0 mmol), Pro-OBzl·TFA (0.702 g, 2.2 mmol) and BEP (0.603 g, 2.2 mmol) in 3 mL CH_2Cl_2, DIEA (1.1 mL, 6.4 mmol) was added. The reaction mixture was stirred at room temperature for 1 h. Yield: 0.726 g (91%), $[\alpha]_D^{23}=-109.4$ (*c* 1, $CHCl_3$), R_f 0.26 (AcOEt/PE: 1/2); ^1H NMR (90 MHz, $CDCl_3$) two conformers: $\delta=1.44$ (s, 9H, Bu^t), 1.69–2.33 (m, 8H, 2β-C*H*$_2$-Pro, 2γ-C*H*$_2$-Pro), 3.31–3.88 (m, 4H, 2δ-C*H*$_2$-Pro), 4.05–4.52 (2m, 2H, 2α-C*H*-Pro), 4.91–5.10 (m, 2H,C*H*$_2$-OBzl), 7.32 (s, 5H, Ph-OBzl); EI-MS *m/z*: 403 $[M+H]^+$, 402 M^+, 302 $[M+H-Boc]^+$, 91 $[PhCH_2]^+$, 57 $[Bu^t]^+$.

Prolyl-proline benzyl ester hydrochloride (HCl·Pro-Pro-OBzl, 9). Boc-Pro-Pro-OBzl (0.825 g, 2.05 mol) was deprotected with 4N HCl/AcOEt (8 mL) at 0°C. Yield: 0.658 g (94.7%), mp 158–160°C, R_f 0.31 (AcOEt/MeOH: 1/1); EIMS *m/z*: 303 $[M+H]^+$, 91 $[PhCH_2]^+$.

***N*-(*tert*-Butyloxycarbonyl)-valyl-methylvaline methyl ester (Boc-Val-MeVal-OCH$_3$, 10).** To a cold solution of Boc-Val-OH (0.782 g, 3.6 mmol), MeVal-OCH$_3$·HCl (0.545 g, 3.0 mmol) and FEP (0.767 g, 3.6 mmol) in 5 mL CH_2Cl_2, DIEA (1.78 mL, 10.2 mmol) was added. The reaction mixture was stirred at room temperature for 2 h. Yield: 0.951 g (92%), R_f 0.56 (AcOEt/PE: 1/4), $[\alpha]_D^{20}=-136.0$ (*c* 1, MeOH); ^1H NMR (90 MHz, $CDCl_3$) two conformers: $\delta=0.75-1.11$ (m, 12H, C*H*$_3$ of Val, MeVal), 1.44 (s, 9H, Bu^t), 1.86–2.35 (2m, 2H, β-C*H* of Val, MeVal), 2.87, 3.08 (2s, 3H, *N*-C*H*$_3$-MeVal), 3.70 (s, 3H, OC*H*$_3$), 4.08–4.64 (2m, 2H, α-C*H* of Val, MeVal), 4.93, 5.25 (2d, *J*=10 Hz, 1H, N*H*-Val); EI-MS *m/z*: 345 $[M+H]^+$, 344 M^+, 57$[Bu^t]^+$.

Valyl-*N*-methylvaline methyl ester hydrochloride (HCl·Val-MeVal-OCH$_3$, 11). Boc-Val-MeVal-OCH$_3$ (0.812 g, 2.36 mol) was deprotected with 4N HCl/AcOEt (8 mL). Yield: 0.605 g (91.4%), mp 181–182°C, R_f 0.48 (AcOEt/MeOH: 4/1).

***N*-(*tert*-Butyloxycarbonyl)-valyl-valyl-methylvaline methyl ester (Boc-Val^1-Val^2-MeVal^3-OCH$_3$, 12).** To a cold solution of Boc-Val-OH (83 mg, 0.38 mmol), Val-MeVal-OCH$_3$·HCl (97 mg, 0.35 mmol) and BEP (104 mg, 0.38 mmol) in 1 mL CH_2Cl_2, DIEA (0.19 mL, 1.11 mmol) was added. The reaction mixture was stirred at room temperature for 1 h. Yield: 0.15 g (97%), mp: 138–139°C, R_f 0.43 (AcOEt/PE: 1/4); ^1H NMR (300 MHz, d_6-acetone) one main conformers: $\delta=0.79-1.15$ (m, 18H, C*H*$_3$ of Val^1, Val^2, MeVal), 1.41 (s, 9H, Bu^t), 1.95–2.28 (3m, 3H, β-C*H* of Val^1, Val^2, MeVal), 3.11 (s, 3H, *N*-C*H*$_3$-MeVal), 3.68 (s, 3H, OC*H*$_3$), 4.03 (m, 1H, α-C*H*-MeVal), 4.70 (m, 1H, α-C*H*-Val^2), 4.86 (d, *J*=10.7 Hz, 1H, α-C*H*-Val^1), 6.08, 7.57 (2d, *J*=8.5 Hz, 2H, N*H* of Val^1, Val^2); EI-MS *m/z*: 444 $[M+H]^+$, 412 $[M-OCH_3]^+$, 57 $[Bu^t]^+$.

***N*-(*tert*-Butyloxycarbonyl)-valyl-valyl-methylvaline (Boc-Val^1-Val^2-MeVal^3-OH, 13).** Boc-Val-Val-MeVal-OCH$_3$ (0.855 g, 1.93 mmol) was saponified with 2N NaOH to give product. Yield: 0.812 g (98%), mp 72–74°C, R_f 0.76 (AcOEt/MeOH: 1/1); EI-MS *m/z*: 430 $[M+H]^+$, 412 $[M-H_2O]^+$, 329 $[M+H-Boc]^+$, 57$[Bu^t]^+$.

***N*-(*tert*-Butyloxycarbonyl)-valyl-valyl-methylvalyl-prolyl-proline benzyl ester (Boc-Val^1-Val^2-MeVal^3-Pro^4-Pro^5-OBzl, 14).** To a cold solution of Boc-Val-MeVal-OH (0.652 g, 1.52 mmol), Pro-Pro-OBzl·HCl (0.540 g, 1.59 mmol) and BEP (0.458 g, 1.67 mmol) in 7 mL CH_2Cl_2, DIEA (0.83 mL, 4.78 mmol) was added at 0°C. The reaction mixture was stirred at room temperature for 2 h. Yield: 0.949 g (88%), mp 94–96°C, $[\alpha]_D^{23}=-181$ (*c* 0.3, $CHCl_3$); [Found: C, 63.08; H, 8.38; N, 9.54. $C_{38}H_{59}N_5O_8\cdot0.5H_2O$ requires C, 63.13; H, 8.37; N, 9.69%]; ν_{max}(KBr) 3319s, 2967s, 2877sh, 1746sh, 1716sh, 1663sh, 1634vs, 1499s, 1442s, 1171s, 1094w, 699w; ^1H NMR (300 MHz, d_6-acetone): $\delta=0.73-1.01$ (m, 18H, C*H*$_3$ of Val^1, Val^2, MeVal^3), 1.42 (s, 9H, Bu^t), 1.72–2.31 (3m, 11H, β-C*H* of Val^1, Val^2, MeVal^3, 2β-C*H*$_2$-Pro, 2γ-C*H*$_2$-Pro), 3.13 (s, 3H, *N*-C*H*$_3$-MeVal^3), 3.56–3.69 (m, 2H, δ-C*H*$_2$-Pro), 3.75–3.87 (m, 2H, δ-C*H*$_2$-Pro), 4.05 (m, 1H,

α-C*H*-MeVal[3]), 4.48 (dd, J_1=4.2 Hz, J_2=8.7 Hz, 1H, α-C*H*-Pro), 4.63 (dd, J_1=4.6 Hz, J_2=8.9 Hz, 1H, α-C*H*-Pro), 4.75 (d, J=7.9 Hz, 1H, α-C*H*-Val[2]), 5.10 (d, J=10.9 Hz, 1H, α-C*H*-Val[1]), 5.07, 5.19 (AB, J=12.5 Hz, 2H, CH_2-OBzl), 6.20 (d, J=10 Hz, 1H, N*H*-Val[2]), 7.35–7.40 (m, 5H, Ph-OBzl), 7.48 (d, J=8 Hz, 1H, N*H*-Val[1]); ESI-MS *m/z*: 1450 $[M+M+Na]^+$, 753 $[M+K]^+$, 737 $[M+Na]^+$, 715 $[M+H]^+$, 546 $[(M+H+K)/2]^+$, 538 $[(M+H+Na)/2]^+$.

***N*,*N*-Dimethyl-valyl-valyl-methylvalyl-prolyl-proline (Me$_2$Val[1]-Val[2]-MeVal[3]-Pro[4]-Pro[5]-OH, 15).** Boc-Val-Val-MeVal-Pro-Pro-OBzl (35 mg, 0.049 mmol) was deprotected by 50% TFA in 2 mL CH_2Cl_2 to give TFA·H-Val-Val-MeVal-Pro-Pro-OBzl as white solid, which was dissolved in 2.5 mL methanol and reductively alkylated in the presence of 10% Pd/C (80 mg), 37% formaldehyde aqueous solution (0.32 mL, 3.92 mmol) and NEt_3 (7 μL, 0.049 mmol) at room temperature. Another part of 35% formaldehyde solution (0.15 mL) was added after 24 h and the reaction mixture was further hydrogenated for 48 h. Yield: 22 mg (82%), R_f 0.27 (MeOH); ν_{max}(KBr) 3400s, 2972s, 1730sh, 1673vs, 1639vs, 1473m, 1291w, 1202s, 1137sh, 799w, 721w; ^{1}H NMR (400 MHz, acetone-d$_6$): δ=0.76–1.12 (m, 18H, CH_3 of Me$_2$Val[1], Val[2], MeVal[3]), 1.81–2.37 (m, 11H, β-C*H* of Me$_2$Val[1], Val[2], MeVal[3], 2β-CH_2-Pro, 2γ-CH_2-Pro), 2.98 (s, 6H, 2*N*-CH_3-Me$_2$Val[1]), 3.19 (s, 3H, *N*-CH_3-MeVal[3]), 3.57–3.73 (m, 2H, δ-CH_2-Pro), 3.75–3.92 (m, 2H, δ-CH_2-Pro), 3.95 (d, J=8.5 Hz, 1H, α-C*H*-MeVal[3]), 3.61–4.05 (br, 1H, COO*H*), 4.45 (m, 1H, α-C*H*-Pro), 4.66 (dd, J_1=5.1 Hz, J_2=8.5 Hz, 1H, α-C*H*-Pro), 4.75 (d, J=8.0 Hz, 1H, α-C*H*-Val[2]), 5.10 (d, J=11.0 Hz, 1H, α-C*H*-Me$_2$Val[1]); ESI-MS *m/z*: 1104.6 $[M+M+H]^+$, 575.1 $[M+Na]^+$, 552.5 $[M+H]^+$.

Synthesis of the 8–11 fragment of CsA and the total synthesis of linear undecapeptide of CsO in the solid-phase using pyridinium type reagent

Anchoring of Fmoc-Ala-OH on Wang Resin (general procedure). To a solution of Fmoc-Ala-OH (1.33 g, 4.27 mmol), HOAt (0.174 g, 1.28 mmol), DMAP (0.104 g, 0.854 mmol) and DCC (0.881 g, 4.27 mmol) in CH_2Cl_2/DMF (4/1, 25 mL), Wang resin (2.0 g, 0.854 mmol) was added at 0°C. The mixture was gently stirred 16 h at room temperature. The resin was filtered, washed successively with DMF (3×), MeOH (3×), DMF/H_2O 9/1 (v/v, 3×), DMF (3×) and MeOH (3×), and dried under vacuum. UV and HPLC analysis indicated 84% substitution of the resin. The unreacted hydroxyl group was capped using acetic anhydride.

Anchoring of Fmoc-MeVal-OH on Wang Resin. Fmoc-MeVal-OH was anchored onto Wang resin according to above general procedure except the reaction time was prolonged to 30 h to afford 85% substitution of the resin.

General procedure for the solid-phase peptide synthesis using 2-halopyridinium salts

Starting from Fmoc-Ala-Wang resin (0.3 g, 0.107 mmol) or Fmoc-MeVal-Wang resin (0.4 g, 0.145 mmol) Fmoc deprotection was performed with 20% piperidine in DMF (20 mL/mmol) for 30 min. The resin was filtered and washed with DMF (10×, each with 3 mL for 1 min). Fmoc-protected amino acid (3 equiv.), 2-halopyridinium salt (3 equiv.) HOAt (3 equiv.) in DMF (2 mL) was added to the resin, then DIEA (9 equiv.) was added and the resin mixutre was shaken for 3 h. The coupling reaction was performed twice.

The peptide was cleaved from the resin using 50% TFA at low temperature. The flask containing the above resin was chilled under an argon atmosphere in an ethylene glycol/CO_2 bath held at −15 to −20°C. TFA (8 mL) which was precooled in the same bath, was added to the flask, the reaction mixutre was stirred at the low temperature for 3 h. The TFA was removed at −15°C by distillation into a CO_2/acetone trap under vacuum, and then the residue was treated with AcOEt (5 mL) and concentrated repeatedly in vacuo. The residue was washed with AcOEt (200 mL). The AcOEt solution was concentrated, dried and evaluated the purity of the crude peptide by HPLC and ESIMS. The crude linear undecapeptide of CsO synthesized using BEP and FEP was evaluated by ESI-MS and showed no more than 5% purity.

HPLC analysis indicated the purifty of Fmoc-D-Ala-MeLeu-MeLeu-MeVal-OH synthesized by BEP was 95%. The overall yield of the tetrapeptide was 85%. t_R=10.7 min (HPLC conditions: Column: Kromasil KR 100–10 C18 (4.6×25 cm). Eluant: 48% CH_3CN (0.1% TFA). Flow rate: 1.5 mL/min. Detection: 220 nm (0.5 AUFS)). After purification, the final product characterized and proved to be the desired product Fmoc-D-Ala-MeLeu-MeLeu-MeVal-OH. Mp 80–82°C, R_f 0.62 (AcOEt/CH_3OH=10/1), $[\alpha]_D^{20}$=−102.0 (*c* 0.25, $CHCl_3$); [Found: C, 65.66; H, 8.01; N, 7.96. $C_{38}H_{54}N_4O_7 \cdot H_2O$ requires C, 65.49; H, 8.10; N, 8.04%]; ν_{max}(KBr) 3316w, 2960s, 1726s, 1643s, 1451m, 1247m, 1063m, 741w; ^{1}H NMR (300 MHz, $CDCl_3$): δ=0.76–1.20 (m, 21H, CH_3 of D-Ala, Leu, Leu, Val), 1.24–1.91 (m, 6H, 2β-CH_2-Leu, 2γ-C*H*-Leu), 2.32 (m, 1H, β-C*H*-Val), 2.77–3.90 (m, 9H, *N*-CH_3 of 2Leu, Val), 4.06–4.49 (m, 4H, 9-C*H*-Fluorenyl, CH_2-Fmoc, α-C*H*-Val), 4.72 (m, 1H, α-C*H*-D-Ala), 5.44–5.56 (m, 2H, 2α-C*H*-Leu), 5.92, 6.60 (2d, J=8.0 Hz, 1H, N*H*-D-Ala), 7.31 (m, 2H, 2,7-C*H*-Fluorenyl), 7.40 (t, J=7.3 Hz, 2H, 3, 6-C*H*-Fluorenyl), 7.60 (m, 2H, 1,8-C*H*-Fluorenyl), 7.76 (d, J=7.6 Hz, 2*H*, 4,5-CH-Fluorenyl); EI-MS *m/z*: 661 $[M-OH]^+$, 421 [M−MeLeu-MeVal-OH]$^+$, 179 [Fmoc-CO_2]$^+$.

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References

1. Castro, B.; Dromoy, J. R.; Evin, G.; Selve, C. *Tetrahedron Lett.* **1975**, 1219.
2. Dourtoglou, V.; Ziegler, J. C.; Gross, B. *Tetrahedron Lett.* **1978**, 1269.
3. Coste, J.; Le-Nguyen, D.; Castro, B. *Tetrahedron Lett.* **1990**, *31*, 205.

4. (a) Chen, S. Q.; Xu, J. C. *Tetrahedron Lett.* **1992**, *33*, 647. (b) Coste, J.; Frerot, E.; Jouin, P. *Tetrahedron Lett.* **1991**, *32*, 1967.
5. Carpino, L. A.; El-Faham, A.; Albericio, F. *Tetrahedron Lett.* **1994**, *35*, 2279.
6. Ehrlich, A.; Rothemund, S.; Brudel, M.; Beyermann, M.; Carpino, L. A.; Bienert, M. *Tetrahedron Lett.* **1993**, *34*, 4781.
7. Carpino, L. A.; El-Faham, A.; Minor, C. A.; Albericio, F. *J. Chem., Soc. Chem. Commun.* **1994**, 201.
8. Alsina, J.; Giralt, E.; Albericio, F. *Tetrahedron Lett.* **1996**, *37*, 4195.
9. (a) Li, P.; Xu, J. C. *Tetrahedron Lett.* **1999**, *40*, 3605. (b) Li, P.; Xu, J. C. *J. Pept. Res.* **2000**, *55*, 110.
10. Li, P.; Xu, J. C. *Chem. Lett.* **1999**, 1163.
11. Li, P.; Xu, J. C. *Tetrahedron Lett.* **2000**, *41*, 721.
12. (a) Sheehan, J. C.; Hess, G. P. *J. Am. Chem. Soc.* **1955**, *77*, 1067. (b) Sarantakis, D.; Teichman, J.; Lien, E. L.; Fenichel, R. *Biochem. Biophys. Res. Commun.* **1976**, *73*, 336. (c) Shechan, J. C.; Cruickshank, P. A.; Boshart, G. L. *J. Org. Chem.* **1961**, *26*, 2525.
13. (a) Wieland, T.; Schaefer, W. *Angew. Chem.* **1951**, *63*, 146. (b) Bodanszky, M. *Nature* **1955**, *175*, 685.
14. Humphrey, J. M.; Chamberlin, A. R. *Chem. Rev.* **1997**, *97*, 2243.
15. (a) Albericio, F.; Bofill, J. M.; El-Faham, A.; Kates, S. A. *J. Org. Chem.* **1998**, *63*, 9678. (b) Story, S. C.; Aldrich, J. V. *Int. J. Pept. Protein Res.* **1994**, *43*, 292.
16. Frerot, E.; Coste, J.; Pantaloni, A.; Dufour, M.-H.; Jouin, P. *Tetrahedron* **1991**, *47*, 259.
17. Coste, J.; Dufour, M.-N.; Pantaloni, A.; Castro, B. *Tetrahedron Lett.* **1990**, *31*, 669.
18. Akaji, K.; Kuriyama, N.; Kiso, Y. *Tetrahedron Lett.* **1994**, *35*, 3315.
19. (a) Carpino, L. A.; El-Faham, A. *J. Am. Chem. Soc.* **1995**, *117*, 5401. (b) Vojkovsky, T.; Drake, B. *Org. Prep. Proced. Int.* **1997**, *29*, 497.
20. El-Faham, A. *Chem. Lett.* **1998**, 671.
21. Przybylski, J.; Wasilewski, K.; Legowska, A. Pol. PL 170331 B1, filed 10/30/92, published 11/29/96, assigned to Uniwersytet Gdanski, Pol., 6 pp (Poland).
22. (a) Van der Auwera, C.; Anteunis, M. J.O. *Int. J. Pept. Protein Res.* **1987**, *29*, 574. (b) Van der Auwera, C.; Anteunis, M. O. *Bull. Soc. Chim. Belg.* **1986**, *95*, 203.
23. Li, P.; Xu, J. C. *Tetrahedron Lett.* **1999**, *40*, 8301.
24. Mukaiyama, T.; Usui, M.; Shimada, E. *Chem. Lett.* **1975**, 1045.
25. Mukaiyama, T.; Usui, M.; Saigo, K. *Chem. Lett.* **1976**, 49.
26. Bald, E.; Saigo, K.; Mukaiyama, T. *Chem. Lett.* **1975**, 1163.
27. Barlin, G. B.; Benbow, J. A. *J. Chem. Soc., Perkin Trans. 2* **1974**, 790.
28. Carpino, L. A. *J. Am. Chem. Soc.* **1993**, *115*, 4397.
29. Wenger, R. M. *Helv. Chim. Acta.* **1983**, *66*, 2672.
30. Pettit, G. R.; Kamano, Y.; Dufresne, C.; Cerny, R. L.; Herald, C. L.; Schmidt, J. M. *J. Org. Chem.* **1989**, *54*, 6005.
31. Frerot, E.; Coste, J.; Poncet, J.; Jouin, P. *Tetrahedron Lett.* **1992**, *33*, 2815.
32. Li, P.; Xu, J. C. *J. Org. Chem.* **2000**, *65*, 2951.
33. (a) For quantitation of Fmoc protected petide segment on resin, UV absorbance of the fulvene-piperidine adduct at 301 nm was measured after cleavage of the Fmoc group with piperidine. (b) Meienhofer, J.; Waki, M.; Heimer, E.; Lambros, T. J.; Makofske, R.; Chang, C.-D. *Int. J. Pept. Protein Res.* **1979**, *13*, 35.
34. Albericio, F.; Cases, M.; Alsina, J.; Triolo, S. A.; Carpino, L. A.; Kates, S. A. *Tetrahedron Lett.* **1997**, *38*, 4853 (and references cited therein).
35. McDermott, J. R.; Benoiton, N. L. *Can. J. Chem.* **1973**, *51*, 1915–1919.
36. Freidinger, R. M.; Hinkle, J. S.; Perlow, D. S.; Arison, B. H. *J. Org. Chem.* **1983**, *48*, 77–81.