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# Highly Efficient Synthesis of Sterically Hindered Peptides Containing *N*-Methylated Amino Acid Residues using a Novel 1*H*-Benzimidazolium Salt

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**Abstract**—Novel 1*H*-benzimidazolium type peptide coupling reagent, CMBI, was designed, synthesized, and shown to be efficient in the promotion of the formation of sterically hindered amide and ester bonds. Its high efficiency was proved by model reaction tests and the successful synthesis of various hindered oligopeptides and peptide segments containing *N*-methyl amino acid residues with fast reaction speeds, low racemization and excellent yields. A mechanism for amide bond formation mediated by the reagent was proposed. © 2000 Elsevier Science Ltd. All rights reserved.

## Introduction

The development of novel and highly efficient peptide coupling reagents has long been one of the most active and attractive topics in the peptide field. During the past decades, many coupling reagents have been designed, synthesized and commonly used in peptide synthesis,[1] even in the whole field of organic synthesis.[2] The useful and creative exploitation of phosphonium,[3] uronium,[4] and immonium[5] type reagents has made the synthesis of unhindered peptides easy and routine. However, the peptides, which are isolated from fungi, bacteria, marine sponges and other lower animal forms, are usually extensively *N*-methylated and exhibit a variety of biological activities. The results of the chain elongations of these sterically hindered peptides are not satisfied using the above reagents except the HOAt-derived onium salts and halogenerated onium salts, such as HATU,[6] HAPyU,[7] PyBroP,[8] PyClU,[4c] CIP,[9] TFFH[10] and BTFFH.[11] To tackle this problem and meet the needs of the efficient synthesis of increasingly structural challenging peptides and peptidomimetics, we have developed the greatly applicable

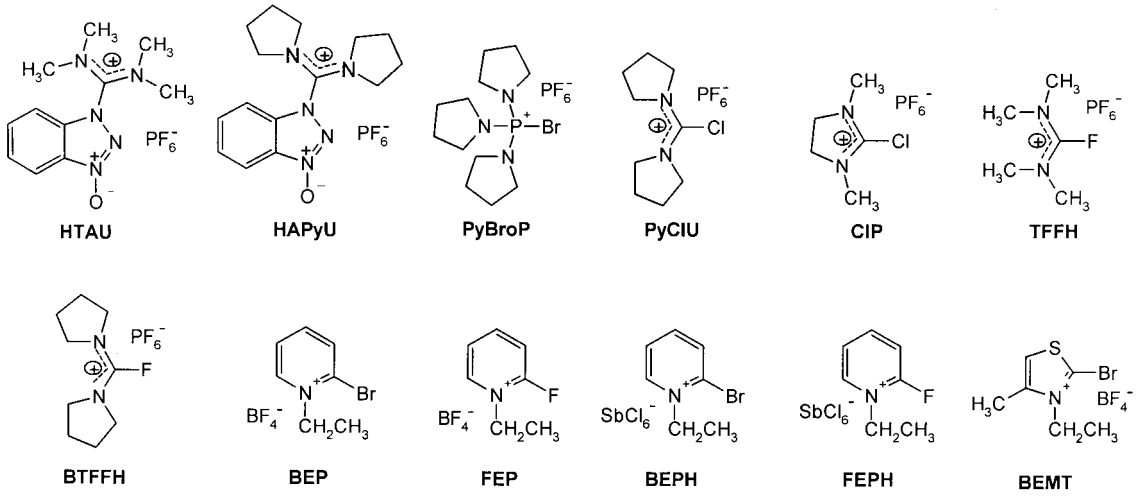


**Figure 1.** The structures of the HOAt-based and halogenated onium type peptide coupling reagents.

*Keywords*: coupling reagent; CMBI; actinomycin D.

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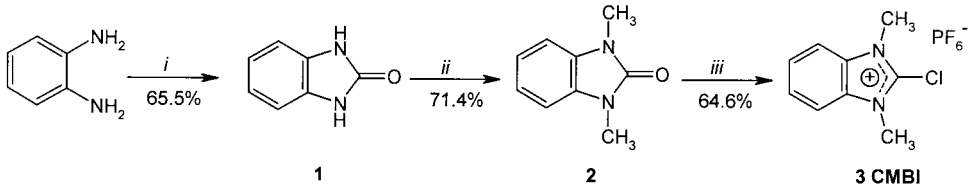



**Scheme 1.** Synthesis of 1*H*-benzimidazolium type coupling reagent CMBI. Reagents and conditions: (i) $H_2NCONH_2$, neat, 140–145°C, 1 h; (ii) $CH_3I$, KOH, $CH_3COCH_3$, reflux 30 min; (iii) (a) $PCl_5$, $POCl_3$, reflux, 40–55 min; (b) $KPF_6/H_2O$, 5–10°C, 30 min.

thiazolium and pyridinium type reagents, BEMT, BEP, FEP, BEPH and FEPH (Fig. 1).[12] Herein, we will further report a novel 1*H*-benzimidazolium type peptide coupling reagent, 2-chloro-1,3-dimethyl 1*H*-benzimidazolium hexafluorophosphate (CMBI), and demonstrate its high efficiency in hindered peptide synthesis.

## Results and Discussion

The design of reagent CMBI was based upon the molecular structure of the uronium salt CIP by fusing a benzene ring to the carbocation matrix of CIP to delocalize the lone electronic pairs of the two nitrogen atoms in the molecule. Thus, the reaction-mediating carbocation atom in CMBI would share relatively low electron density and consequently exhibit higher reactivity in nucleophilic substitution reactions involved in peptide synthesis.[13]

The synthesis of the compound CMBI has been well studied, and we exploited a concise and efficient synthetic route (Scheme 1). Starting from cheap and easy available material, 1,2-benzenediamine, we can easily synthesize CMBI via three steps. The product is a crystalline, non-hygroscopic, and shelf-stable solid.

To evaluate the efficiency of reagent CBMI in peptide synthesis, two model reactions were selected and monitored by HPLC. Since the major drawback of halogenated onium type reagents is the high racemization during segment condensations, we intentionally selected the epimerization-prone [2+1] segment condensation, *Z*-Gly-L-Phe-OH+L-Val-$OCH_3$·HCl+coupling reagent→Z-Gly-D/L-Phe-L-Val-$OCH_3$, as a model reaction. The test results showed that the DL-isomer content of product is 16.8% for CMBI, 33.2% for PyClU, 22.3% for PyBroP, and 25.9% for BTFFH, which indicated CMBI bearing relatively higher racemization-suppressing capability. This reagent also exhibited high reactivity, which was reflected by the evaluation of the coupling yield of the above model reaction. For example, the yield is 19.7% for CMBI, meantime only 5.6% for PyClU, 6.1% for PyBroP, and 9.4% for BTFFH after two minutes reaction at −10°C in $CH_2Cl_2$, with DIEA as a base. To appraise the capability of CBMI in the promotion of the formation of sterically hindered amide bonds, the model reaction, *Z*-MeVal-OH+MeVal-$OCH_3$·HCl+coupling reagent→*Z*-MeVal-MeVal-$OCH_3$, was adopted and monitored by HPLC. It was shown that the coupling yield was 22.6% for CMBI, 10.6% for PyBroP, 14.3% for BOP-Cl, and 22.1% for BEP after 35 min reaction at 25°C in dilute $CH_2Cl_2$ solution, with DIEA as a base. These results suggested that reagent CMBI might exhibit high efficiency in the synthesis of hindered peptides containing *N*-methyl amino acid residues.

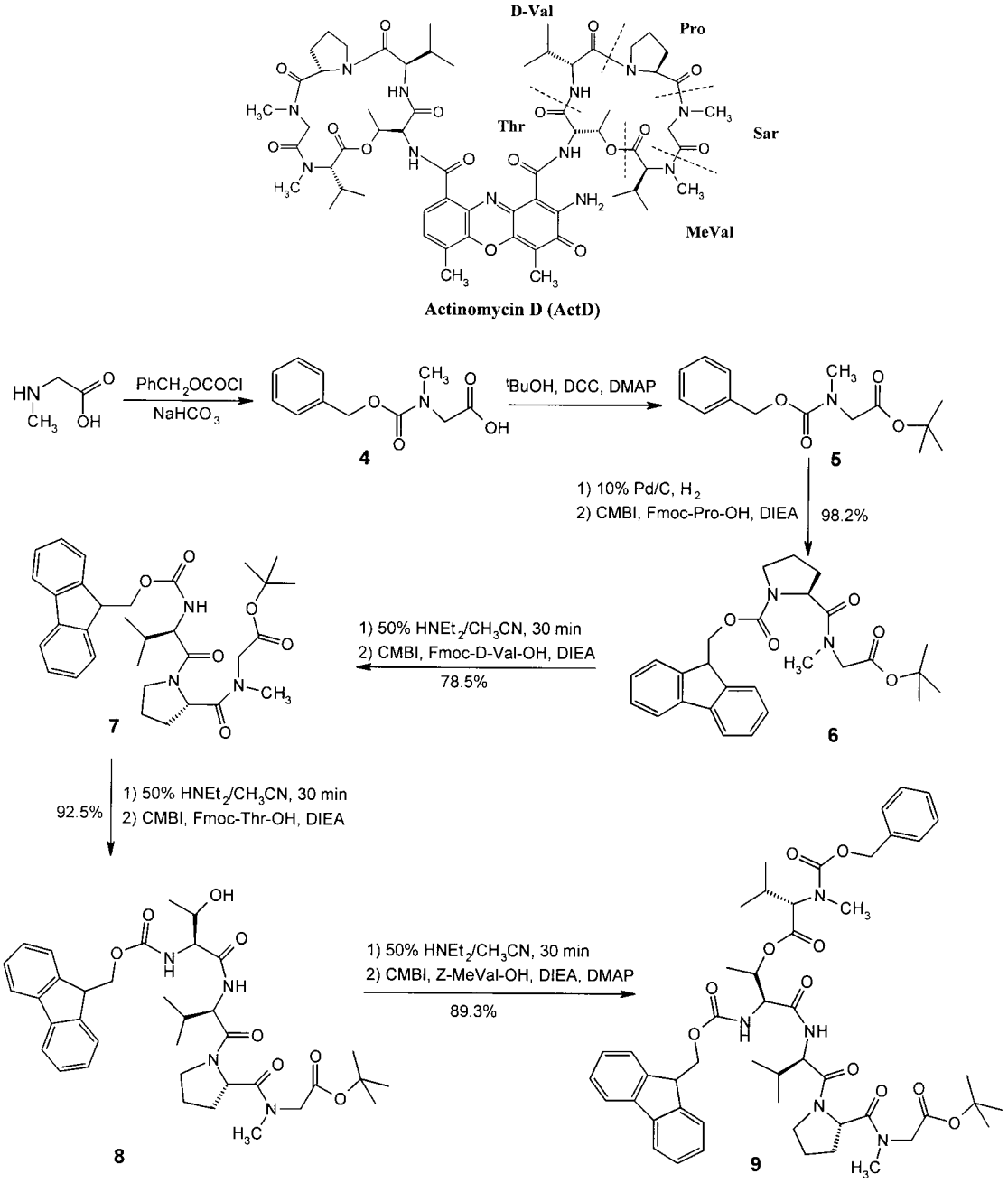
The high applicability of this 1*H*-benzimidazolium type reagent was further verified by the successful synthesis of a series of hindered oligopeptides shown in Table 1. In most cases, the yields of products were almost quantitative. The coupling reactions can be carried out by the 'one pot' experimental procedure, and the carboxylic components need not be pre-activated. In a typical experimental procedure, DIEA (3.2 equiv.) was added to a cooled mixture (−10°C) of $N^{\alpha}$-protected amino acid (1 equiv.), amino acid ester hydrochloride (1.1 equiv.), and CMBI (1.1 equiv.) in $CH_2Cl_2$ (3–5 mL/mmol), the reaction solution was stirred at room temperature and monitored by TLC. After the completion of the coupling, the reaction mixture was concentrated and purified directly by flash column chromatography on silica gel.

Encouraged by the above promising results, we synthesized the protected pentapeptide moiety of the anticancer drug, Actinomycin D, using this reagent. This depsipeptide was extensively *N*-alkylated, and was the major obstacle of the total synthesis of ActD.[14] To avoid the spontaneous formation of diketopiperazine during the $N^{\alpha}$-deprotection of dipeptide **6**, the C-terminal was protected as a *tert*-butyl ester. In the synthesis of compound **8**, the hydroxyl group in the side chain of Fmoc-Thr-OH was not protected, and no side-reaction was observed during coupling. The final esterification step was also accomplished with CMBI to

**Table 1.** Preparation of peptides using CBMI as a coupling reagent

| Peptide[a] | Yield (%)[b] | Appearance | $[\alpha]_D$ (conc., solv., temp) |
|---|---|---|---|
| Fmoc-Sar$^{\Phi}$-Aib-$OCH_3$ | 99.0 | Foamy solid | – |
| Fmoc-Pro$^{\Phi}$-Sar-Obzl | 99.4 | Glassy solid | −33.6 (1, $CHCl_3$, 21°C) |
| Fmoc-MeVal$^{\Phi}$-Sar-Obzl | 98.1 | Viscous oil | −98.1 (1, $CHCl_3$, 22°C) |
| *Z*-MeVal$^{\Phi}$-MeVal-$OCH_3$ | 99.5 | Oil | −206 (1, $CH_3OH$, 23°C) |
| Fmoc-Pro$^{\Phi}$-Sar-OBu$^t$ | 98.2 | Glassy solid | −36.2 (1, $CHCl_3$, 23°C) |
| Boc-Leu-Gly$^{\Phi}$-Leu-Gly-Val-Obzl | 94.2 | Solid (Mp 146–147°C) | −19.7 (*c* 1, $CHCl_3$, 22°C) |

[a] The amide bond formed in the peptide is indicated by a Φ. All products were confirmed by $^1$H NMR, EIMS and other characterization.
[b] Isolated yield based upon $N^{\alpha}$-protected amino acid or peptide.




**Scheme 2.** Synthesis of the protected depsipeptide of ActD using reagent CMBI.

give the desired pentapeptide **9** in 89.3% yield after 4 h reaction. The successful synthesis of this hindered depsipeptide further proved the high efficiency of reagent CMBI in peptide synthesis (Scheme 2).

With regards to the mechanism of CMBI mediated coupling reaction, we propose that the carboxylate anion initially attack the carbocation in the CMBI molecule to form an unstable acyloxybenzimidazolium intermediate **10**, which in turn reacts with the amino component to give product with the release of the by-product, 1,3-dimethyl-2-benzimidazolinone **11**. Alternatively, the intermediate **10** might be converted competitively into the corresponding acid chloride **12**, which could also be transformed into the desired peptide by aminolysis. A small amount of the symmetric anhydride **13** and 5(4*H*)-oxazolone **14** of the carboxylic component may be generated as minor and relatively less reactive intermediates during coupling. The latter was also very liable to epimerization due to its spontaneous tautomerization or enolization under basic reaction condition (Fig. 2).

## Conclusions

A novel 1*H*-benzimidazolium type coupling reagent, CMBI, was designed, synthesized, and found to be very efficient for the synthesis of hindered peptides containing *N*-methyl amino acid residues. Its applicability was demonstrated by the successful synthesis of a series of hindered peptides with

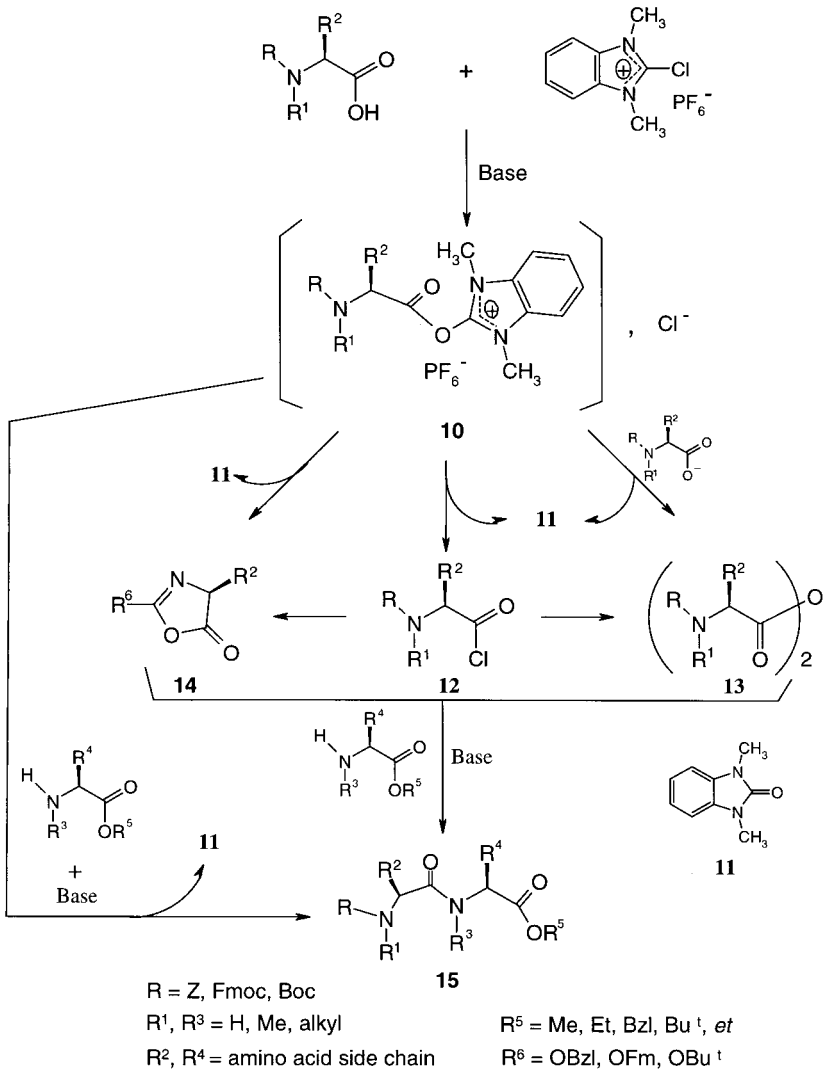



**Figure 2.** Proposed mechanism for reagent CMBI mediated coupling reactions.

fast reaction speeds, low racemization and excellent yields. The major reaction intermediates of CMBI mediated coupling were proposed to be the acyloxybenzimidazolium and acid chloride of the carboxylic component.

## Experimental

Melting points were taken on digital melting point apparatus. Infrared spectra were recorded on an Schimadazu IR-440 spectrometer. Mass spectra were recorded on HP 5989A and VG QUATTRO mass spectrometers. $^1$H NMR spectra were recorded on Bruker AM 300 (300 MHz) and Bruker DRX-400 (400 MHz) using TMS as internal standard. Combustion analysis for elemental composition was carried out on an Italy MOD 1106 analyzer. Optical rotations were determined using 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. Amino acids were L-configuration if not otherwise stated.

PyBroP and DMAP were purchased from Aldrich Chemical Co. of Milwaukee, WI, and used without purification. PyClU, BTFFH and amino acid derivatives were prepared according to literature methods.[4c,11] Cbz-*N*-methyl amino acids were synthesized by the procedure of McDermott and Benoiton.[15] Fmoc-*N*-methyl amino acids were synthesized by the procedure of Freidinger et al.[16] 2-Benzimidazolinone **1** and 1,3-dimethyl-2-benzimidazolinone **2** were synthesized according to literature methods.[17]

**2-Chloro-1,3-dimethyl 1*H*-benzimidazolium hexafluorophosphate (CMBI, 3).** Phosphorus pentachloride (8.34 g, 40 mmol) was dissolved in phosphorus oxychloride (50 mL) at 50°C, 1,3-dimethyl benzimidazolin-2-one (3.25 g, 20 mmol) was added batch-wise under nitrogen atmosphere. After the reaction mixture was refluxed for 40–55 min, when the evolution of hydrochloride was ceased, it was cooled and $CH_2Cl_2$ (30 mL) was added, then the resultant

suspension was filtered under nitrogen atmosphere, washed with cold $CH_2Cl_2$ and dried in vacuo. The solid was dissolved in cold water, then saturated aqueous solution of $KPF_6$ (3.68 g, 20 mmol) was added gradually. After stirring 30 min the reaction mixture was filtered, the filter cake was washed with ether, dried in vacuo and recrystallized from acetone/$Et_2O$ to give product as white crystalline solid. Yield: 4.22 g (64.6%), mp 155–156°C; [Found: C, 32.86; H, 3.00; N, 8.37. $C_9H_{10}ClF_6N_4P$ requires C, 33.10; H, 3.09; N, 8.58%]; $\nu_{max}$(KBr) 3060w, 1610w, 1538s, 1471m, 1413m, 1191w, 1014w, 836vs, 762sh, 557s, 478w $cm^{-1}$; $^1H$ NMR (300 MHz, acetone-$d_6$): $\delta$=4.26 (s, 6H, *N*-C$H_3$), 7.79 (m, 2H, 5,6-C*H*-aryl), 8.07 (m, 2H, 4,7-*CH*-aryl); FAB-MS *m/z*: 181 $[M-PF_6]^+$, 183 $[M+2-PF_6]^+$.

**The evaluation of the efficiency of various peptide 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 (%)=$[(LL/X_1+DL/X_2)/a\cdot S]\times$ 100%). The percentage of epimers was calculated according to the equation: D%=$[DL/X_2/(LL/X_1+DL/X_2)]\times100\%$; 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).

**The evaluation of the efficiency of various peptide coupling reagents with the model reaction *Z*-MeVal-OH+MeVal-OMe·HCl→*Z*-MeVal-MeVal-OMe.** In the presence of DIEA (84 μL, 0.48 mmol), *Z*-MeVal-OH (40 mg, 0.15 mmol) and MeVal-OMe·HCl (30 mg, 0.165 mmol) were coupled with the tested reagent (0.165 mmol) in $CH_2Cl_2$ (1.5 mL) at 25°C. Boc-Phe-Phe-OMe (64 mg, 0.15 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*-MeVal-OH ($t_R$=6.39 min); *Z*-MeVal-MeVal-OMe ($t_R$= 15.51 min); Boc-Phe-Phe-OMe ($t_R$=9.99 min) by comparing to the preformed reference compounds. Peak areas were compared in order to obtain the chemical yields (yield (%)=$[(P/x)/S]\times100\%$), where *P* refers to the area of *Z*-MeVal-MeVal-OMe, *S* refers to that of Boc-Phe-Phe-OMe, $X_1$=1.004 which is 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: 74% $CH_3CN$ (0.1% TFA). Flow rate: 0.6 mL/min. Detection: 220 nm (0.5 AUFS).

***N*-{*N*-[(9*H*-Fluoren-9-ylmethoxy)carbonyl]-*N*-methylglycyl}-α-aminoisobutyric acid methyl ester (Fmoc-Sar-Aib-OCH$_3$).** To a cold suspension of Fmoc-Sar-OH (0.311 g, 1.0 mmol), Aib-$OCH_3$·HCl (0.154 g, 1.10 mmol) and CMBI (0.327 g, 1.10 mmol) in $CH_2Cl_2$ (4 mL), DIEA (0.56 mL, 3.2 mmol) was added at −10°C. After the reaction mixture was stirred at room temperature for 1 h, it was concentrated. The residue was purified by flash chromatography on silica gel to give products as white foamy solid Yield: 0.406 g (99.0%), mp 104–105°C, $R_f$ 0.32 (AcOEt/PE: 1/1); $^1H$ NMR (300 MHz, $CDCl_3$): $\delta$=1.55 (s, 6H, 2β-C$H_3$-Aib), 3.03 (s, 3H, *N*-C$H_3$-Sar), 3.71 (s, 3H, OC$H_3$), 3.89–4.55 (m, 5H, 9-C*H*-Fluorenyl, C$H_2$-Fmoc, α-C$H_2$-Sar), 6.59 (br, 1H, N*H*-Aib), 7.32 (t, *J*=7.3 Hz, 2H, 2, 7-C*H*-Fluorenyl), 7.41 (t, *J*=7.3 Hz, 2H, 3, 6-C*H*-Fluorenyl), 7.60 (m, 2H, 1,8-C*H*-Fluorenyl), 7.78 (d, *J*= 7.5 Hz, 2H, 4,5-C*H*-Fluorenyl); EIMS *m/z*: 411 $[M+H]^+$, 379 $[M-OCH_3]^+$, 294 $[M-Aib\text{-}OCH_3]^+$, 179 $[Fmoc\text{-}CO_2]^+$.

***N*-[(9*H*-Fluoren-9-ylmethoxy)carbonyl]-prolyl-sacrosine benzyl ester (Fmoc-Pro-Sar-OBzl).** To a cooled solution of Fmoc-Pro-OH (0.337 g, 1.0 mmol), Sar-OBzl·HCl (0.237 g, 1.10 mmol) and CMBI (0.359 g, 1.10 mmol) in 4 mL $CH_2Cl_2$, DIEA (0.56 mL, 3.2 mmol) was added at −10°C. The reaction mixture was stirred at room temperature for 1 h. Yield: 0.495 g (99.4%), $R_f$ 0.35 (AcOEt/PE=1/1), $[\alpha]_D^{21}$=−33.6 (*c* 1, $CHCl_3$); [Found: C, 72.01; H, 6.02; N, 5.47. $C_{30}H_{30}N_2O_5$ requires C, 72.27; H, 6.06; N, 5.62%]; $^1H$ NMR (300 MHz, $CDCl_3$) four conformers $\delta$ 1.72–2.31 (m, 4H, γ-C$H_2$-Pro, β-C$H_2$-Pro), 2.84, 3.01, 3.15, 3.36 (4s, 3H, *N*-C$H_3$-Sar), 3.45–3.94 (2m, 2H, δ-C$H_2$-Pro), 4.10–4.86 (m, 6H, 9-C*H*-Fluorenyl, C$H_2$-Fmoc, α-C$H_2$-Sar, α-C*H*-Pro), 5.15 (m, 2H, C$H_2$-OBzl), 7.28–7.46 (m, 9H, 2,3,6,7-C*H*-Fmoc, Ph-OBzl), 7.52–7.81 (2m, 4H, 1,4,5,8-C*H*-Fluorenyl); EIMS *m/z*: 275 $[M-Fmoc]^+$, 179 $[Fmoc\text{-}CO_2]^+$, 91 $[PhCH_2]^+$.

***N*-[(9*H*-Fluoren-9-ylmethoxy)carbonyl]-*N*-methyl valyl-sarcosine benzyl ester (Fmoc-MeVal-Sar-OBzl).** Fmoc-MeVal-OH (0.212 g, 0.60 mmol), Sar-OBzl·TFA (0.193 g, 0.66 mmol) and CMBI (0.216 g, 0.66 mmol) was dissolved in 4 mL $CH_2Cl_2$, then DIEA (0.33 mL, 1.92 mmol) was added at −10°C. The reaction mixture was stirred at room temperature for 2 h. Yield: 0.303 g (98.1%), $[\alpha]_D^{22}$=−98.1 (*c* 1, $CHCl_3$), $R_f$ 0.75 (AcOEt/Pe=1/1); [Found: C, 72.13; H, 6.58; N, 5.29. $C_{31}H_{34}N_2O_5$ requires C, 72.35; H, 6.66; N, 5.44%]; $^1H$ NMR (300 MHz, $CDCl_3$) more than two conformers: $\delta$ 0.50–1.03 (m, 6H, 2γ-C$H_3$-Val), 2.01–2.42 (m, 1H, β-C*H*-Val), 2.62–3.32 (multi s, 6H, *N*-C$H_3$-Val, *N*-C$H_3$-Sar), 4.03 (m, 6H, 9-C*H*-Fluorenyl, C$H_2$-Fmoc, α-C$H_2$-Sar, α-C*H*-Val), 5.19 (m, 2H, C$H_2$-OBzl), 7.18–7.88 (m, 13H, aryl-Fmoc, Ph-OBzl); EIMS *m/z*: 515 $[M+H]^+$, 336 $[M-Sar\text{-}OBzl]^+$, 179 $[Fmoc\text{-}CO_2]^+$, 91 $[PhCH_2]^+$.

***N*-(*tert*-Butyloxycarbonyl)-leucyl-glycyl-leucyl-glycyl-valine benzyl ester (Boc-Leu-Gly-Leu-Gly-Val-OBzl).** To a cooled suspension of Boc-Leu-Gly-OH (0.254 g, 0.88 mmol), HCl·Leu-Gly-Val-OBzl (0.331 g, 0.80 mmol) and CMBI (0.287 g, 0.88 mmol) in 50 mL $CH_2Cl_2$, DIEA (0.45 mL, 2.56 mmol) was added at −10°C. The reaction mixture was stirred at room temperature for 1 h. Yield: 0.488 g (94.2%), mp 146–147°C, $R_f$ 0.71 (AcOEt), $[\alpha]_D^{22}=-19.7$ (*c* 1 $CHCl_3$); [Found: C, 60.31; H, 8.35; N, 10.66. $C_{33}H_{53}N_5O_8\cdot0.5H_2O$ requires C, 60.35; H, 8.29; N, 10.66%]; $\nu_{max}$(KBr) 3299vs, 2960s, 1730sh, 1633vs, 1533vs, 1368m, 1248s, 1168s, 1047w, 698w $cm^{-1}$; $^1H$ NMR (400 MHz, $CDCl_3$): δ=0.72–1.06 (m, 18H, 4δ-C*H*$_3$-Leu, 2γ-C*H*$_3$-Val), 1.42 (s, 9H, Bu$^t$), 1.14–1.98 (m, 6H, 2β-C*H*$_2$-Leu, 2γ-C*H*-Leu), 2.19 (m, 1H, β-C*H*-Val), 3.85–4.28 (m, 5H, 2α-C*H*$_2$-Gly, α-C*H*-Leu), 4.59 (dd, *J*=8.6 Hz, *J*′=5.2 Hz, 1H, α-C*H*-Val), 4.72 (m, 1H, α-C*H*-Leu), 5.10, 5.21 (AB, *J*=12.2 Hz, 2H, C*H*$_2$-OBzl), 5.55 (br, 1H, N*H*-Gly), 7.34 (s, 5H, Ph-OBzl), 7.48 (br, 1H, N*H*-Leu), 7.62 (br, 1H, N*H*-Val), 7.67 (br, 1H, N*H*-Gly), 7.81 (br, 1H, N*H*-Leu); ESIMS *m/z*: 1965 $[M+M+M+Na]^+$, 1319 $[M+M+Na]^+$, 984 $[(M+M+M+Na+H)/2]^+$, 671 $[M+Na]^+$, 648 $[M+H]^+$.

**Synthesis of the hindered depsipeptide moiety of Actinomycin D using reagent CMBI *N*-[(9*H*-Fluoren-9-ylmethoxy)carbonyl]-prolyl-sarcosine *tert*-butyl ester (Fmoc-Pro-Sar-OBu$^t$, 6).** To a cooled solution of Sar-OBu$^t$ (0.798 g, 5.5 mmol), Fmoc-Pro-OH (1.69 g, 5.0 mmol) and CMBI (1.80 g, 5.5 mmol) in 5 mL $CH_2Cl_2$, DIEA (1.83 mL, 10.5 mmol) was added at −10°C. The reaction mixture was stirred at room temperature for 1 h. Yield: 2.28 g (98.2%), $R_f$ 0.28 (AcOEt/PE: 1/2), $[\alpha]_D^{23}=-36.2$ (*c* 1, $CHCl_3$); $^1H$ NMR (300 MHz, $CDCl_3$) four conformers δ=1.43, 1.46, 1.49 (3s, 9H, Bu$^t$), 1.70–2.35 (m, 4H, γ-C*H*$_2$-Pro, β-C*H*$_2$-Pro), 2.86, 3.01, 3.15, 3.38 (4S, 3H, *N*-C*H*$_3$-Sar), 3.45–3.88 (2m, 2H, δ-C*H*$_2$-Pro), 4.10–4.92 (m, 6H, 9-C*H*-Fluorenyl, C*H*$_2$-Fmoc, α-C*H*$_2$-Sar, α-C*H*-Pro), 7.31 (t, *J*=7.3 Hz, 2H, 2, 7-C*H*-Fluorenyl), 7.40 (t, *J*=7.3 Hz, 2H, 3, 6-C*H*-Fluorenyl), 7.63 (m, 2H, 1,8-C*H*-Fluorenyl), 7.73 (d, *J*=7.3 Hz, 2H, 4,5-C*H*-Fluorenyl); EIMS *m/z*: 391 [M−OBu$^t$]+, 179 $[Fmoc\text{-}CO_2]^+$, 57 $[Bu^t]^+$.

***N*-[(9*H*-Fluoren-9-ylmethoxy)carbonyl]-D-valyl-prolyl-sarcosine *tert*-butyl ester (Fmoc-D-Val-Pro-Sar-OBu$^t$, 7).** Compound **6** (2.14 g, 4.61 mmol) was dissolved in 15 mL $CH_3CN$, and treated with diethylamine (5 mL) under nitrogen atmosphere until TLC analysis indicated 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 *N*-Fmoc-deprotected peptide, which was further dried in vacuo for 2 h and utilized for the following coupling reaction without further purification. CMBI (1.66 g, 5.07 mmol), Fmoc-D-Val-OH (1.72 g, 5.07 mmol) and the above residue were dissolved in 6 mL $CH_2Cl_2$, then DIEA (2.57 mL, 14.8 mmol) was added at −10°C. The reaction mixture was stirred at room temperature for 1 h. Yield: 2.04 g (78.5%), mp 72–73°C, $R_f$ 0.57 ($CHCl_3$/$CH_3OH$: 20/1), $[\alpha]_D^{21}=-19.0$ (*c* 0.5, $CHCl_3$); [Found: C, 67.24; H, 7.25; N, 7.03. $C_{32}H_{41}N_3O_6\cdot0.5H_2O$ requires C, 67.11; H, 7.39; N, 7.33%]; $\nu_{max}$(KBr) 3285m, 2975s, 1740vs, 1720sh, 1647vs, 1450s, 1232s, 1159s, 1116m, 1029m, 742s $cm^{-1}$; $^1H$ NMR (300 MHz, $CDCl_3$) more than two conformers δ=0.82–1.10 (m, 6H, 2γ-C*H*$_3$-Val), 1.45, 1.46, 1.49 (3s, 9H, Bu$^t$), 1.71–2.45 (m, 5H, γ-C*H*$_2$-Pro, β-C*H*-Val, β-C*H*$_2$-Pro), 2.99–3.41 (multi s, 3H, *N*-C*H*$_3$-Sar), 3.52–3.89 (2m, 2H, δ-C*H*$_2$-Pro), 4.10–4.92 (m, 7H, 9-C*H*-Fluorenyl, C*H*$_2$-Fmoc, α-C*H*$_2$-Sar, α-C*H*-Pro, α-C*H*-Val), 5.31, 5.56 (2d, *J*=9.3 Hz, 1H, N*H*-Val), 7.27 (t, *J*=7.3 Hz, 2H, 2,7-C*H*-Fluorenyl), 7.37 (t, *J*=7.3 Hz, 2H, 3,6-C*H*-Fluorenyl), 7.63 (m, 2H, 1,8-C*H*-Fluorenyl), 7.76 (d, *J*=7.4 Hz, 2H, 4,5-C*H*-Fluorenyl); EIMS *m/z*: 564 $[M+H]^+$, 419 $[M-Sar\text{-}OBu^t]^+$, 322 $[M-Pro\text{-}Sar\text{-}OBu^t]^+$, 179 $[Fmoc\text{-}CO_2]^+$, 57 $[Bu^t]^+$.

***N*-[(9*H*-Fluoren-9-ylmethoxy)carbonyl]-threonyl-D-valyl-prolyl-sarcosine *tert*-butyl ester (Fmoc-Thr-D-Val-Pro-Sar-OBu$^t$, 8).** Compound **7** (0.409 g, 0.726 mmol) was deprotected according to the above experimental procedure to give H-D-Val-Pro-Sar-OBu$^t$, which was further dried in vacuo for 2 h and participated the following coupling reaction without purification. To a cooled solution of Fmoc-Thr-OH (0.297 g, 0.871 mmol), CMBI (0.284 g, 0.871 mmol) and the above H-D-Val-Pro-Sar-OBu$^t$ in 3 mL $CH_2Cl_2$, DIEA (0.43 mL, 2.47 mmol) was added. The reaction mixture was stirred at room temperature for 1 h. Yield: 0.446 g (92.5%), mp 96–97°C, $R_f$ 0.34 ($CHCl_3$/$CH_3OH$: 20/1), $[\alpha]_D^{23}=-24.6$ (*c* 1, $CHCl_3$); [Found: C, 64.06; H, 7.53; N, 8.61. $C_{36}H_{48}N_4O_8\cdot0.5H_2O$ requires C, 64.17; H, 7.33; N, 8.32%]; $\nu_{max}$(KBr) 3318s, 2975m, 2877sh, 1739vs, 1668sh, 1644vs, 1450s, 1232s, 1159m, 761sh, 742m $cm^{-1}$; $^1H$ NMR (300 MHz, $CDCl_3$) two conformers δ=0.85–1.04 (m, 6H, 2γ-C*H*$_3$-Val), 1.23 (d, *J*=7.8 Hz, 3H, γ-C*H*$_3$-Thr), 1.46, 1.49 (2s, 9H, Bu$^t$), 1.76–2.49 (m, 5H, γ-C*H*$_2$-Pro, β-C*H*-Val, β-C*H*$_2$-Pro), 2.95, 3.13 (2s 1: 4.6, 3H, *N*-C*H*$_3$-Sar), 3.34–4.66 (m, 11H, δ-C*H*$_2$-Pro, 9-C*H*-Fluorenyl, C*H*$_2$-Fmoc, α-C*H*$_2$-Sar, α-C*H*-Pro, α-C*H*-Val, α-C*H*-Thr, β-C*H*-Thr), 6.02 (br, 1H, O*H*-Thr), 4.86, 6.85 (2m, 2H, N*H*-Val, N*H*-Thr), 7.34 (t,, *J*=7.3 Hz, 2H, 2,7-C*H*-Fluorenyl), 7.41 (t,, *J*=7.3 Hz, 2H, 3,6-C*H*-Fluorenyl), 7.64 (d, *J*=7.3 Hz, 2H, 1,8-C*H*-Fluorenyl), 7.78 (d, *J*=7.3 Hz, 2H, 4,5-C*H*-Fluorenyl); EIMS *m/z*: 664 $M^{+\cdot}$, 324 $[M\text{-}D\text{-}Val\text{-}Pro\text{-}Sar\text{-}OBu^t]^+$, 179 $[Fmoc\text{-}CO_2]^+$, 57 $[Bu^t]^+$.

***O*-[*N*-Benzyloxycarbonyl-*N*-methylvalyl]-*N*-[(9*H*-Fluoren-9-ylmethoxy)carbonyl]-threonyl-D-valyl-prolyl-sarcosine *tert*-butyl ester (Fmoc-Thr(OMeVal-*Z*)-D-Val-Pro-Sar-OBu$^t$, 9).** Compound **8** (0.532 g, 0.80 mmol), *Z*-MeVal-OH(0.318 g, 1.20 mmol), DMAP (98 mg, 0.8 mmol) and CMBI (0.392 g, 1.20 mmol) was dissolved in 10 mL $CH_2Cl_2$, then DIEA (0.28 mL, 1.6 mmol) was added at −10°C. The reaction mixture was stirred at room temperature for 4 h. Yield: 0.652g (89.3%), mp 92–92.5°C, $R_f$ 0.44 ($CHCl_3$/$CH_3OH$: 20/1), $[\alpha]_D^{23}=-22.6$ (*c* 1, $CHCl_3$); [Found: C, 64.78; H, 7.19; N, 7.36. $C_{50}H_{65}N_5O_{11}\cdot H_2O$ requires C, 64.57; H, 7.26; N, 7.53%]; $\nu_{max}$(KBr) 3298s, 2972s, 1740vs, 1705sh, 1673sh, 1624sh, 1500m, 1451s, 1231m, 1157s, 1054m, 760sh, 741m $cm^{-1}$; $^1H$ NMR (400 MHz, $CDCl_3$) more than three conformers δ=0.77–1.08 (m, 12H, 4γ-C*H*$_3$ of D-Val, MeVal), 1.24 (m, 3H, γ-C*H*$_3$-Thr), 1.41, 1.45, 1.47 (3s, 9H, Bu$^t$), 1.76–2.47 (m, 6H, γ-C*H*$_2$-Pro, β-C*H*-D-Val, β-C*H*-MeVal, β-C*H*$_2$-Pro), 2.81–3.13 (m, 6H, *N*-C*H*$_3$-Sar, *N*-C*H*$_3$-MeVal), 3.23, 4.59 (2m, 2H, α-C*H*$_2$-Sar), 3.52–3.73 (m, 2H, δ-C*H*$_2$-Pro), 3.76–4.92 (m, 7H, α-C*H*-Thr, 9-C*H*-Fluorenyl, C*H*$_2$-Fmoc, α-C*H*-Pro,

α-C*H*-D-Val, α-C*H*-MeVal), 5.20 (m, 2H, C$H_2$-*Z*), 5.38 (m, 1H, β-C*H*-Thr), 6.03, 7.05 (2m, 2H, N*H*-D-Val, N*H*-Thr), 7.22–7.48 (m, 9H, 2, 3, 6, 7-C*H*-Fluorenyl, Ph-*Z*), 7.63 (m, 2H, 1,8-C*H*-Fluorenyl), 7.75 (d, $J$= 7.1 Hz, 2H, 4,5-C*H*-Fluorenyl); ESIMS *m/z*: 1846 $[M+ M+Na]^+$, 1824 $[M+M+H]^+$, 1387 $[(M+M+M+K+H)/2]^+$, 935 $[M+Na]^+$, 913 $[M+H]^+$.

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## References

1. Humphrey, J. M.; Chamberlin, A. R. *Chem. Rev.* **1997**, *97*, 2243–2266.
2. Nicolaou, K. C.; Vourloumis, D.; Winssinger, N.; Baran, P. S. *Angew. Chem., Int. Ed. Engl.* **2000**, *39*, 44–122.
3. (a) Castro, B.; Dromoy, J. R.; Evin, G.; Selve, C. *Tetrahedron Lett.* **1975**, 1219–1222. (b) Coste, J.; Le-Nguyen, D.; Castro, B. *Tetrahedron Lett.* **1990**, *31*, 205–208. (c) Carpino, L. A.; El-Faham, A.; Minor, C. A.; Albericio, F. *J. Chem. Soc., Chem. Commun.* **1994**, 201–203. (d) Alsina, J.; Giralt, E.; Albericio, F. *Tetrahedron Lett.* **1996**, *37*, 4195–4198.
4. (a) Dourtoglou, V.; Ziegler, J. C.; Gross, B. *Tetrahedron Lett.* **1978**, 1269–1272. (b) Chen, S. Q.; Xu, J. C. *Tetrahedron Lett.* **1992**, *33*, 647–650. (c) Coste, J.; Frerot, E.; Jouin, P. *Tetrahedron Lett.* **1991**, *32*, 1967–1970. (d) Kiso, Y.; Fujiwara, Y.; Kimura, T.; Nishitani, A.; Akaji, K. *Int. J. Pept. Protein Res.* **1992**, *40*, 308–314.
5. (a) Li, P.; Xu, J. C. *Tetrahedron Lett.* **1999**, *40*, 3605–3608. (b) Li, P.; Xu, J. C. *Tetrahedron Lett.* **2000**, *41*, 721–724. (c) Li, P.; Xu, J. C. *J. Pept. Res.* **2000**, *55*, 110–119. (d) Li, P.; Xu, J. C. *Chin. J. Chem.* **2000**, *18*, 85–93.
6. Carpino, L. A.; El-Faham, A.; Albericio, F. *Tetrahedron Lett.* **1994**, *35*, 2279–2282.
7. Ehrlich, A.; Rothemund, S.; Brudel, M.; Beyermann, M.; Carpino, L. A.; Bienert, M. *Tetrahedron Lett.* **1993**, *34*, 4781–4784.
8. Frerot, E.; Coste, J.; Pantaloni, A.; Dufour, M.-N.; Jouin, P. *Tetrahedron* **1991**, *47*, 259–270.
9. Akaji, K.; Kuriyama, N.; Kiso, Y. *Tetrahedron Lett.* **1994**, *35*, 3315–3318.
10. (a) Carpino, L. A.; El-Faham, A. *J. Am. Chem. Soc.* **1995**, *117*, 5401–5402. (b) Vojkovsky, T.; Drake, B. *Org. Prep. Proced. Int.* **1997**, *29*, 497–498.
11. El-Faham, A. *Chem. Lett.* **1998**, 671–672.
12. (a) Li, P.; Xu, J. C. *Tetrahedron Lett.* **1999**, *40*, 8301–8304. (b) Li, P.; Xu, J. C. *J. Org. Chem.* **2000**, in press.
13. Semiempirical PM3 calculations showed the net charge of the central carbocation of reagents CIP and CMBI is −0.995 and −0.498, respectively, which also reflects that the carbocation of CBMI would share relatively low electron density.
14. Abbreviations: ActD, Actinomycin D; BEMT, 2-bromo-3-ethyl-4-methyl thiazolium tetrafluoroborate; BEP, 2-bromo-1-ethyl pyridinium tetrafluoroborate; BEPH, 2-bromo-1-ethyl pyridinium hexachloroantimonate; BOP-Cl, *N*,*N*′-bis(2-oxo-3-oxazolidinyl)phosphinic chloride; BTFFH, 1,1,3,3-bis(tetramethylene) florouronium hexafluorophosphate; CMBI, 2-chloro-1,3-dimethyl 1*H*-benzimidazolium hexafluorophosphate; DIEA, *N*,*N*′-diisopropylethylamine; 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 hexafluorophosphate *N*-oxide; HOAt, 1-hydroxy-7-azabenzotriazole; HOBt, 1-hydroxybenzotriazole; PyBroP, bromotripyrrolidinophosphonium hexafluorophosphate; PyClU, 1,1,3,3-bis(tetramethylene) chlorouronium hexafluorophosphate; TFFH, tetramethylfluoromamidinium hexafluorophosphate.
15. McDermott, J. R.; Benoiton, N. L. *Can. J. Chem.* **1973**, *51*, 1915–1919.
16. Freidinger, R. M.; Hinkle, J. S.; Perlow, D. S.; Arison, B. H. *J. Org. Chem.* **1983**, *48*, 77–81.
17. Efros, L. S.; Porai-Koshits, B. A.; Farbenshtein, S. G. *Zh. Obshch. Khim.* **1953**, *23*, 1691–1696.