Synthetic Communications: An International Journal for Rapid Communication of Synthetic Organic Chemistry
Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/lsyc20

Comparative Study of Selected Coupling Reagents in Dipeptide Synthesis

Joseph Dudash Jr. [a b], Jianjun Jiang [a], Scott C. Mayer [a] & Madeleine M. Joullié [a]

[a] Department of Chemistry, University of Pennsylvania Philadelphia, PA 19104-6323

[b] Department of Chemistry, Stanford University, Stanford, CA, 94305

Version of record first published: 23 Sep 2006.

To cite this article: Joseph Dudash Jr., Jianjun Jiang, Scott C. Mayer & Madeleine M. Joullié (1993): Comparative Study of Selected Coupling Reagents in Dipeptide Synthesis, Synthetic Communications: An International Journal for Rapid Communication of Synthetic Organic Chemistry, 23:3, 349-356

To link to this article: http://dx.doi.org/10.1080/00397919308009787

SYNTHETIC COMMUNICATIONS, 23(3), 349-356 (1993)

COMPARATIVE STUDY OF SELECTED COUPLING REAGENTS IN DIPEPTIDE SYNTHESIS

Joseph Dudash, Jr.†, Jianjun Jiang, Scott C. Mayer, and Madeleine M. Joullié*
Department of Chemistry, University of Pennsylvania
Philadelphia, PA 19104-6323

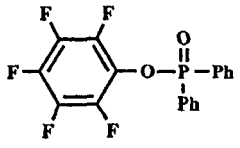
ABSTRACT: *A comparative study of the effectiveness of selected coupling reagents in dipeptide synthesis was conducted. Included in the study were a new coupling reagent, pentafluorophenyl diphenylphosphinate (FDPP, 1), benzotriazol-1-yl-oxytris(dimethylamino)phosphonium hexafluorophosphate (BOP, 2), 2-(1H-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HBTU, 3), and isobutyl chloroformate (IBCF, 4). We found that BOP (2) afforded the highest yields and lowest limits of racemization.*

The syntheses of biologically important peptides and cyclopeptides require the coupling of amino acids. A major problem in such couplings is the racemization of the amino acids. A new coupling reagent, pentafluorophenyl diphenyl phosphinate (FDPP, **1**), was recently reported to effect dipeptide coupling in high yield and without racemization.[1]

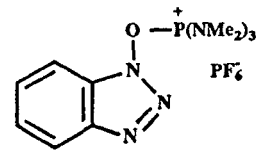
Our goal was to test this new coupling reagent in the synthesis of a series of dipeptides, including one of the reported examples. As a method of comparison, the same dipeptides were synthesized using other coupling reagents. Of particular interest in our study was the preparation of dipeptides with the N-(9-fluorenylmethoxycarbonyl) (Fmoc) and benzyloxycarbonyl (Cbz) amine protecting groups and the application of these reagents to the coupling of secondary amines. FDPP (**1**) was also utilized in the coupling of a macrocycle. Product yields, HPLC diastereomeric yields [2], and optical rotations were obtained and then compared (**Table**).

† Current address: Department of Chemistry, Stanford University, Stanford, CA 94305.

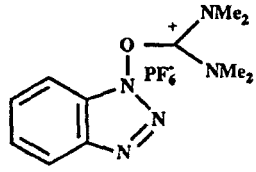
* To whom correspondence should be addressed.



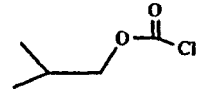
FDPP, 1



BOP, 2



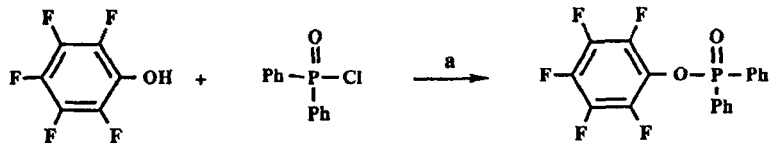
HBTU, 3



IBCF, 4

The following reagents were investigated: pentafluorophenyl diphenylphosphinate (FDPP, **1**)[1], benzotriazol-1-yl-oxytris(dimethylamino)phosphonium hexafluorophosphate (BOP, **2**)[3], 2-(1H-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HBTU, **3**)[4], and isobutyl chloroformate (IBCF, **4**)[5]

Pentafluorophenyl diphenylphosphinate (FDPP, **1**)[1] was synthesized as shown in **Scheme 1**.



[a] imidazole, CH_2Cl_2

FDPP, 1

Scheme 1

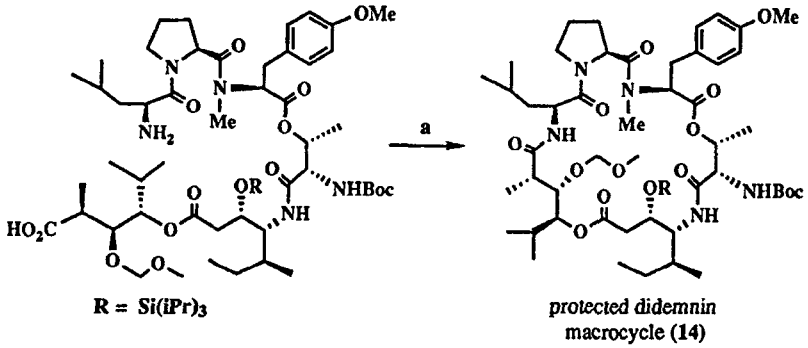
RESULTS AND CONCLUSIONS:

1. Our study shows that FDPP provides both high yields of product and good optical purity.

2. In almost all cases the other methods afforded better percentage yields than FDPP. When Fmoc was used as the protecting group (**6-9**), the yields were significantly lower than those obtained for the Boc compounds. In all cases, BOP proved to be more efficient than FDPP where Fmoc was the protecting group.

3. FDPP gave good results with proline methyl ester dipeptides (**12, 13**), while attempts to couple a carboxyl protected N-methyl phenylalanine with Cbz-leucylproline failed.

4. In the coupling of the amino acid components of a macrocycle (**14**) [6], a 68% yield of highly pure product was obtained with FDPP. These results were the best achieved thus far for this reaction (**Scheme 2**).



Scheme 2

5. Overall BOP proved to be the most successful coupling reagent for dipeptide syntheses, giving better yields and better optical purity without the use of racemization inhibitors.

EXPERIMENTAL

General. All reagents were obtained commercially and were used without further purification. N,N-Dimethylformamide (DMF) was distilled from phosphorous pentoxide. Methylene chloride was distilled from calcium chloride. Acetonitrile was HPLC grade. ^1H spectra were recorded on a Brucker/IBM AC-250 (250 MHz) or a Brucker AMX-500 (500 MHz). Optical rotations (in degrees) were measured with a Perkin-Elmer-Model 241 polarimeter at the sodium D line. Column chromatography was carried out on E. Merck silica gel 60 (230-400 mesh) using the solvent system listed under the individual experiments. HPLC analysis was performed on an ISCO Model 2350 with a Whatman column partisil 10 ODS-325 using acetonitrile to water (75/25 to 100/0) and UV detection at 254

nm wavelength. Yields reported were those percentages of separated diastereomers obtained from a Hewlett Packard 3390A Integrator.

Pentafluorophenyl diphenyl phosphinate (FDPP, 1)[1]. FDPP was synthesized by mixing equimolar amounts of diphenylphosphinic chloride and imidazole in methylene chloride at room temperature and then dropwise addition of an equimolar amount of pentafluorophenol dropwise (**Scheme 1**). The product was purified by short column chromatography on silica gel using a 20% ethyl acetate in petroleum ether solvent system and dried under reduced pressure for two days over phosphorous pentoxide, to yield 92% of the desired product. IR ($CHCl_3$) 3075 (w), 3015 (m), 2685 (w), 2475 (w), 1600 (m), 1515 (s), 1480 (m), 1440 (s), 1320 (m), 1245 (s), 1155 (m), 1135 (s), 1110 (m), 1070 (w), 1010 (s), 1000 (s), 985 (m), 805 (w) cm^{-1}. (^1H NMR (250 MHz, Me_2CO-d6) δ 7.58–7.81 (m, 6H, *p*- and *m*–H), 7.88–8.08 (m, 4H, *o*–H).

Cyclo-[N-(tert-butoxycarbonyl)-O-[[N-[(2S,3S,4S)-4-[(3S,4R,5S)-4 -amino-3-[(triisopropylsilyl)oxy]-5-methylheptanoyl]oxy-3-(methoxymethoxy)-2,5-dimethylhexanoyl]-L-leucyl]-L-prolyl-N,O-dimethyl-L-tyrosyl]-L-threonyl] (14). The acid precursor (40 mg, 35.2 μmol) was first dissolved in DMF (4 ml). To this solution was added diisopropylethylamine (18.3 μl, 105 μmol) followed by the coupling reagent FDPP (16 mg, 41.6 μmol). The reaction was stirred at room temperature for 4 h. After removal of DMF by distillation, the reaction mixture was diluted with diethyl ether (30 ml). This solution was washed with 10% HCl solution (4 ml), 5% $NaHCO_3$ solution (4 ml), and brine (4 ml), dried (Na_2SO_4), and concentrated in vacuo. The crude product was purified by silica gel flash chromatography using 30% to 40% ethyl acetate/petroleum ether as eluents . The product (**14**) was obtained as a white foam (26.8 mg, 68 % yield); ^1H NMR (250 MHz, $CDCl_3$) δ 0.82-1.07 (m, 36H), 1.11-1.37 (m, 11H), 1.44 (s, 9H), 1.48-1.67 (m, 3H), 1.70-1.89 (m, 1H), 1.93-2.21 (m, 5H), 2.50 (s, 3H), 2.57 (dd, 2H), 2.89 (d, 1H), 3.15-3.25 (m, 1H), 3.34 (d, 1H), 3.42 (s, 3H), 3.54 (dd, 1H), 3.58-3.67 (m, 1H), 3.69-3.77 (m, 1H), 3.80 (s, 3H), 3.93 (d, 1H), 4.09 (t, 1H), 4.33 (t, 2H), 4.55-4.67 (m, 1H), 4.68-4.81 (m, 3H), 4.82-5.06 (m, 3H), 6.87 (d, 2H), 7.01 (d, 2H), 7.25-7.34 (m, 1H), 7.52 (d, 1H); IR ($CHCl_3$) 3450 (m), 3400 (m), 1755-1715 (s), 1670-1650 (s), 1510 (m), 1470 (m), 1380 (w), 1260 (w), 1170 (s), 1120 (w), 1050 (m), 930 (w), 890 (w) cm^{-1}; HRMS calcd for $C_{58}H_{100}N_5O_{14}Si$ (M + H) 1118.704. Found 1118.700; R_f 0.68 (80:20, $CHCl_3$:acetone); $[\alpha]_D^{20}$ -66.9° (c=0.72, $CHCl_3$).

Table

DIPEPTIDE*	METHOD	YIELD	% L-L DIASTEREOMER	OPTICAL ROTATION (in $CHCl_3$)
BocLeu-	FDPP	78%	92%	+31.04° (c=0.60)
TrpOMe (5)[1]	BOP	87%	98%	+46.12° (c=0.60)
FmocIle-	FDPP	57%	88%	+22.97° (c=0.80)
TrpOMe (6)	BOP	77%	96%	+30.00° (c=0.70)
FmocMet-	FDPP	54%	86%	+10.82° (c=0.70)
PheOMe (7)	BOP	66%	95%	+17.76° (c=0.60)
FmocSer(O-*t* Bu)-	FDPP	62%	89%	+24.47° (c=1.1)
PheOMe (8)	BOP	72%	96%	+36.50° (c=1.1)
FmocTrp-	FDPP	64%	87%	-4.18° (c=0.60)
IleOMe (9)	BOP	71%	93%	-7.08° (c=1.1)
CbzAla-	FDPP	59%	100%	-14.68° (c=1.1)
LeuOMe (10)[7]	BOP	81%	98%	-14.40° (c=1.3)
CbzAla-	FDPP	90%	99%	-30.83° (c=0.60)
ValOMe (11)[8]	BOP	55%	91%	-25.23° (c=0.80)
	IBCF	95%	72%	-7.43° (c=1.4)
CbzGly-	FDPP	100%	82%	-33.91° (c=1.4)
ProOMe (12)[9]	BOP	90%	87%	-49.46° (c=0.90)
	IBCF	73%	99%	-60.81° (c=0.80)
CbzLeu-	FDPP	79%	91%	-62.40° (c=1.0)
ProOMe (13)[6]	HBTU	53%	100%	-74.11° (c=0.90)

* All amino acids are in the L-configuration

The procedures used to synthesize the dipeptides shown in the **Table** are described. The coupling reagents used are shown in parentheses.

PROCEDURE A (FDPP). Equimolar amounts (0.5 or 1 mmol scale) of the carboxylic acid and amine components and three equivalents of diisopropylethyl amine were stirred in dimethyl formamide (5 ml) as the solvent. Then 1.2 equivalents of FDPP were added, and the reaction was stirred at room temperature under argon for 2 h. The solution was then diluted with 15 ml of 1N potassium sulfate and 25 ml of ethyl acetate. The organic layer was separated and washed twice with 20 ml portions of saturated sodium bicarbonate and with 20 ml of 10% citric acid. The organic layer was dried over sodium sulfate and concentrated. The product was purified by column chromatography using a solvent system of 2% methanol in methylene chloride.

PROCEDURE B (BOP). Equimolar amounts (0.5 or 1 mmol) of the carboxylic acid and amine components and BOP were dissolved in acetonitrile (7 ml), and the coupling reaction was initiated by the addition of 2.3 equivalents of diisopropylethylamine. The reaction was stirred at room temperature for 2 h. The solution was diluted with 25 ml of ethyl acetate, and the organic layer was washed with 20 ml portions each of 3M hydrochloric acid, saturated sodium bicarbonate, and saturated sodium chloride. The organic layer was dried over sodium sulfate and concentrated. The product was purified by column chromatography using a 5% methanol in methylene chloride solvent system.

PROCEDURE C (HBTU). The amine (0.01 mmol) and 1.3 equivalents of the carboxylic acid were dissolved in methylene chloride (0.58 ml) at 0 °C under nitrogen. HBTU (1.3 equivalents) and N-methyl morpholine (0.025 ml) were added. The reaction was stirred for 30 minutes at 0 °C and then at room temperature for 2h. The residue was diluted with 15 ml of ethyl acetate and washed with 1.5 ml each of 10% hydrochloric acid, 5% sodium bicarbonate, and brine. The organic layer was dried over sodium sulfate and concentrated. The product was purified by column chromatography using a solvent system of 20% ethyl acetate in petroleum ether.

PROCEDURE D (IBCF). Equimolar amounts (0.5 or 1 mmol) of the carboxylic acid and isobutyl chloroformate with N-methyl morpholine (0.5 ml) were stirred for 15 minutes in methylene chloride (5 ml) at 0 °C. The amine component (equimolar amount) was then added, and the reaction was stirred for 45 minutes. The reaction mixture was then extracted three times with 5 ml portions of sodium

bicarbonate, followed by 5 ml of 10% citric acid, and 5 ml of water. The organic layer was dried over sodium sulfate and concentrated. The product was purified by column chromatography using a 2% methanol in methylene chloride solvent system.

OPTICAL ROTATION VALUES

N-t-Butyloxycarbonyl-L-leucyl-L-tryptophan Methyl Ester (5) Lit[1] $[\alpha]_D^{25}$ -14.4 (c=1.0, MeOH); Found $[\alpha]_D^{25}$ +31.05 (c=0.6, $CHCl_3$)

N-Carbobenzoxy-L-alanyl-L-leucine Methyl Ester (10) Lit[7] $[\alpha]_D^{23}$ -18.2 (c=0.318, EtOH); Found $[\alpha]_D^{25}$ -14.68 (c=1.1, $CHCl_3$)

N-Carbobenzoxy-L-alanyl-L-valine Methyl Ester (11) Lit[8] not reported; Found $[\alpha]_D^{25}$ -30.83 (c=0.6, $CHCl_3$)

N-Carbobenzoxy-L-glycyl-L-proline Methyl Ester (12) Lit[9] $[\alpha]_D^{20}$ -82.2 (c=1.0, MeOH); Found $[\alpha]_D^{25}$ -60.81 (c=0.8, $CHCl_3$)

N-Carbobenzoxy-L-leucyl-L-proline Methyl Ester (13) Lit[6] $[\alpha]_D^{21}$ -61.1 (c=0.585, $CHCl_3$); Found $[\alpha]_D^{25}$ -74.11 (c=0.9, $CHCl_3$)

SPECTRAL DATA

N-9-Fluorenylmethoxycarbonyl-L-isoleucyl-L-tryptophan Methyl Ester (6). ^1H NMR (250 MHz, $CDCl_3$) δ 0.75-0.94 (m, 6H), 0.98-1.14 (m, 1H), 1.34-1.51 (m, 1H), 1.71-1.87 (m, 1H), 3.23 (qd, 2H), 3.53 (s, 3H), 3.89 (t, 1H), 4.04 (t, 1H), 4.24 (t, 1H), 4.37 (t, 1H), 4.97 (q, 1H), 5.78 (d, 1H), 6.83 (s, 1H), 6.97-7.38 (m, 9H), 7.46 (d, 1H), 7.55 (d, 1H), 7.71 (dd, 2H), 8.47 (s, 1H); IR ($CHCl_3$) 3620 (w), 3490 (m), 3440 (m), 2980 (s), 1720 (s), 1680 (s), 1500 (m), 1450 (m), 1360 (m), 1345 (m), 1240 (m), 1040 (s), 875 (w) cm^{-1}.

HRMS calcd for $C_{33}H_{36}N_3O_5$ (M + H) 554.2655. Found 554.2649.; R_f 0.38 (5:95, MeOH:CH_2Cl_2).

N-9-Fluorenylmethoxycarbonyl-L-methionyl-L-phenylalanine Methyl Ester (7). ^1H NMR (250 MHz, $CDCl_3$) δ 1.88-2.00 (m, 2H), 2.02 (s, 3H), 2.52 (t, 2H), 3.00-3.15 (m, 2H), 3.65 (s, 3H), 4.05-4.30 (m, 2H), 4.32-4.50 (m, 2H), 4.78 (q, 1H), 5.83 (d, 1H), 6.90-7.40 (m, 10H), 7.54 (d, 2H), 7.73 (d, 2H); IR ($CHCl_3$) 3620 (w), 3430 (m), 2970 (s), 2930 (s), 1730 (s), 1680 (s), 1490 (s), 1450 (s), 1370 (m), 1250 (s), 1045 (s), 880 (m) cm^{-1}.

HRMS calcd for $C_{30}H_{33}N_2O_5S$ (M + H) 533.2032. Found 533.2092; R_f 0.48 (5:95, MeOH:CH_2Cl_2).

N-9-Fluorenylmethoxycarbonyl-L-t-butylseryl-L-phenylalanine Methyl Ester (8). ^1H NMR (250 MHz, $CDCl_3$) δ 1.17 (s, 9H), 3.10-3.16 (m, 2H), 3.39 (t, 1H),

3.72 (s, 3H), 3.77-3.87 (m, 1H), 4.25 (d, 2H), 4.40 (d, 2H), 4.85-4.94 (m, 1H), 5.70 (d, 1H), 7.08-7.42 (m, 9H), 7.60 (d, 2H), 7.75 (d, 2H); IR ($CHCl_3$) 3420 (w), 2950 (m), 1725 (s), 1675 (s), 1490 (s), 1450 (m), 1370 (m), 1250 (m), 1180 (m), 1070 (m), 1030 (m), 880 (w) cm^{-1}.

HRMS calcd for $C_{32}H_{37}N_2O_6$ (M + H) 545.2651. Found 545.2616; R_f 0.47 (5:95, MeOH:CH_2Cl_2).

N-9-Fluorenylmethoxycarbonyl-L-tryptophyl-L-isoleucine Methyl Ester(9). ^1H NMR (250 MHz, $CDCl_3$) δ 0.64-0.86 (m, 6H), 0.92-1.08 (m, 1H), 1.19-1.36 (m, 1H), 1.65-1.82 (m, 1H), 3.09-3.35 (m, 2H), 3.52 (s, 3H), 4.12 (t, 1H), 4.33 (d, 2H), 4.47 (dd, 1H), 4.61 (d, 1H), 5.77 (d, 1H), 6.52 (d, 1H), 6.95 (s, 1H), 7.01-7.42 (m, 7H), 7.52 (t, 2H), 7.63 (d, 1H), 7.74 (d, 2H), 8.39 (s, 1H); IR ($CHCl_3$) 3620 (w), 3490 (m), 3430 (m), 2980 (s), 2940 (s), 1730 (s), 1680 (s), 1495 (s), 1450 (s), 1340 (m), 1245 (s), 1145 (m), 1045 (s), 875 (w) cm^{-1}.

HRMS calcd for $C_{33}H_{36}N_3O_5$ (M + H) 554.2655. Found 554.2618; R_f 0.36 (5:95, MeOH:CH_2Cl_2).

Acknowledgment. Support from NIH (CA-40081) is gratefully acknowledged.

REFERENCES

1. Chen, S.; Xu, J. *Tetrahedron Lett.* **1991**, *32*, 6711.
2. Van der Auwera, C.; Van Damme, S.; Anteunis, M.J.O. *Int. J. Peptide Protein Res.* **1987**, *29*, 464.
3. Le Nguyen, D.; Seyer, R.; Heitz, A.; Castro, B. *J. Chem. Soc. Perkin Trans. I* **1985**, 1025.
4. Knorr, R.; Trzeciak, A.; Bannwarth, W.; Gillessen, D. *Tetrahedron Lett.* **1989**, *30*, 1927.
5. Rodriguez, M.; Goodman, M. *J. Med. Chem.* **1984**, *27*, 1668.
6. Li, W.-R.; Ewing, W.R.; Harris, B.D.; Joullié, M.M. *J. Am. Chem. Soc.* **1990**, *112*, 7659.
7. Naithani, V.K. *Hoppe Seyler's Z. Physiol. Chem.* **1972**, *353* 1806.
8. Nutt, R.F.; Chen, K.-M.; Joullié, M.M. *J. Org. Chem.* **1984**, *49*, 1013.
9. Acher, F.; Wakselman, M. *J. Org. Chem.* **1984**, *49*, 4133.

(Received in USA 18 August, 1992)