Novabiochem®

Innovations 3/10

Critical evaluation of in situ coupling reagents for SPPS

With the plethora of coupling reagents available for mediating amide bond formation in peptide synthesis, making a rational decision as to the optimal reagent for a given application can be difficult. In this Novabiochem® Innovations, we compare a wide range of novel and commercially available coupling reagents (Figure 1) for efficiency, solution stability and solubility, with a view to simplifying the selection of coupling reagents.

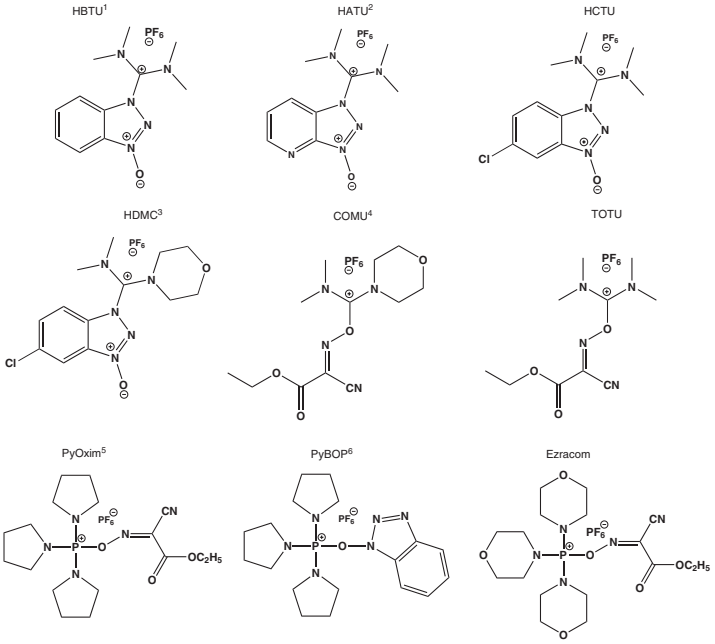

Fig. 1: Coupling reagents examined in this study.

Solubility

Solubility of coupling reagents was tested by placing 2.5 mmol of coupling reagent in a measuring cylinder. DMF was added in 10 ml aliquots and the mixture stirred for 5 minutes between additions of DMF (Table 1). Solubilities above 1.5 M were not tested.

Table 1: Solubilities of coupling reagents.

Coupling reagent	Molarity (lit)	Molarity [7]
HATU	0.43[3]	0.45
HBTU	0.46[3]	0.5
HCTU	0.50[3]	0.8
HDMC	1.00[3]	0.75
COMU	1.44[4]	1.5
TOTU		0.8
PyBOP	1.5[5]	1.5
PyOxim	2.5[5]	1.5
Ezracom		0.4

Stability

Open- and closed-vial stability of 0.25 M solutions of coupling reagents in DMF was determined by ^{1}H nmr time course studies in d7-DMF at room temperature (Tables 2 & 3) [7]. These results indicate that solutions of standard uronium coupling reagents have excellent long term stability in open vials, whereas those of phosphonium coupling reagents should not be stored in open vials for longer than 2 days. Uronium coupling reagents appear to be a good choice for use with synthesizers based on an open XY platform.

Closed vial stability closely mimics the conditions employed on synthesizers such as the ABI 433 or PTI Symphony. As expected solutions of uronium coupling reagents are extremely stable under those conditions. Phosphonium reagent solutions can be safely used up to 7 days if stored under these conditions. Surprisingly, and in contradiction of published results, COMU appeared to be rather unstable in solution.

Table 2: Open-vial stabilities of coupling reagents [7].

Coupling reagent	0 days	1 day	2 days
COMU	96	47	14
COMU (dried)	98	58	16
HDMC	99	98	93
HATU	99	98	97
HBTU	99	99	99
HCTU	99	99	98
TOTU	98	72	42
PyBOP	99	73	34
PyOxim	99	82	47
Ezracom	98	86	59

Table 3: Closed-vial stabilities of coupling reagents [7].

Coupling reagent	2 days	7 days	14 days
COMU	67	46	36
HDMC	99	99	98
HATU	99	99	98
HBTU	100	98	98
HCTU	99	99	98
TOTU	89	85	74
PyBOP	88	85	81
PyOxim	90	88	86
Ezracom	90	85	79

The colors of the solutions were also determined by measuring the OD of the solutions at 400 nm (Figure 2). In general the color of the solution correlates with stability.

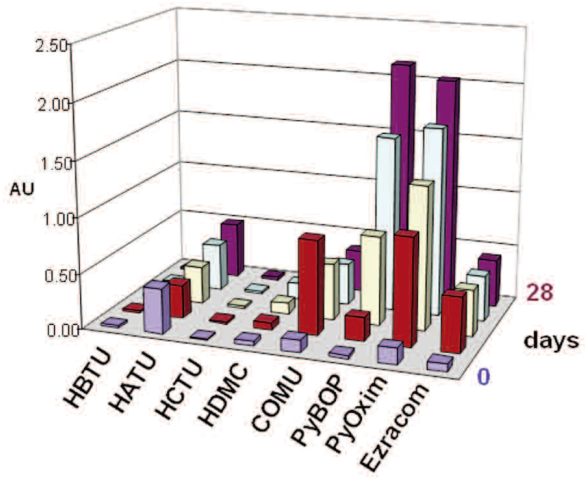

Figure 2: Solution stability of coupling reagents determined by solution OD at 400 nm [7].

Coupling efficiency

Three peptides selected from the literature [2, 3] (Table 4) were used as models to evaluate the efficiency of the coupling reagents.

	Peptide sequence
1	H-Tyr-Sar-Sar-Phe-Leu-NH_2
2	H-Tyr-Aib-Aib-Phe-Leu-NH_2
3	H-Tyr-MeLeu-MeLeu-Phe-Leu-NH_2

Table 4: Peptides prepared in this study.

Peptide synthesis conditions

The peptides were assembled under the conditions shown in Table 5. In every case the C-terminal three residues were coupled for 1 h. In the case of peptides 1 and 3 the final two residues were coupled for 5 mins to exaggerate the differences in efficiencies between the coupling reagents under test. With peptide 2, a 1 h coupling time was used for all residues.

Table 5: Conditions used for synthesis of test peptides.

	Conditions
Resin	Rink amide AM resin
Instrument	ABI 433A
Coupling	Fmoc-Aaa-OH:Coupling reagent:DIPEA (4:4:8)
Deblock	20% Piperidine (3 or 4 times 3 min)
Cleavage	TFA/water/TIS (95:2.5:2.5) for 3 h

Results

In our hands, only peptide 3 proved useful as a tool for evaluating these coupling reagents: peptide 1 was efficiently assembled by all reagents tested; with peptide 2, the des-Aib peptide could not be baseline resolved from the desired pentapeptide using three different HPLC columns; whereas with peptide 3, all the by-products from synthesis are fully resolved and the peptide appears to be highly discriminating between coupling reagents (Figure 3).

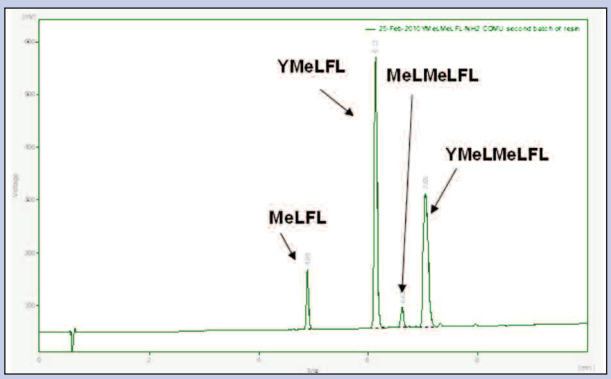

Fig. 3: HPLC profile of H-Tyr-MeLeu-MeLeu-Phe-Leu-NH_2 using COMU.

Table 6 summarizes the results obtained using peptide 3 as the model. These data reveal remarkedly consistent trends. The nature of the coupling reagent leaving group appears to be the most important factor influencing efficiency. The activating moiety appears to have little influence. HATU was the most efficient reagent for amide bond formation. All four Oxyma-based reagents, COMU, TOTU, PyOxim and Ezracom, gave very similar results and were more effective than Cl-HOBt and HOBt-based reagents. HOBt-based reagents were the least effective.

Table 6: Composition of products obtained using various coupling reagents in the synthesis of peptide 3 [7].

% Compostion of products by HPLC				
Coupling reagents	MeLFL	YMeLFL	MeLMeLFL	YMeLMeLFL
PyBOP	60	31	5	4
PyOxim	10	45	5	40
HBTU	57	31	8	4
HCTU	31	35	9	25
HATU	0	16	7	77
TOTU	5	58	2	35
COMU	9	47	4	40
Ezracom	9	50	5	36
HDMC	24	46	7	21

Conclusions

PyOxim appears to combine high reactivity and solubility with moderate stability, making it an excellent choice of coupling reagent for synthesizers employing closed-bottle reagent storage. Furthermore, unlike uronium-based coupling reagents, it will not give rise to guanidinylated by-products, making it ideal for coupling of slow-to-activate amino acids, cyclizations and fragment condensations. HATU was the most effective coupling reagent of those tested, but its high cost limits its use to special applications. COMU should be dissolved and used immediately. For synthesizers employed open-vials, uronium-based reagents should be employed owing to their high stability.

Ordering information

Novabiochem®'s coupling reagents

851004	BOP	5 g 25 g 100 g
851085	COMU	5 g 25 g 100 g
851091	DEPBT	5 g 25 g 100 g
851013	HATU	5 g 25 g
851006	HBTU	5 g 25 g 100 g
851012	HCTU	5 g 25 g 100 g
851011	MSNT	1 g 5 g 25 g
851009	PyBOP®	5 g 25 g 100 g
851010	PyBrOP®	5 g 25 g 100 g
851087	PyClocK	5 g 25 g 100 g
851095 *NEW*	PyOxim	5 g 25 g 100 g
851008	TBTU	5 g 25 g 100 g
851090	TFFH	1 g 5 g 25 g
851088	TOTU	5 g 25 g 100 g
851007	WSC•HCl	5 g 25 g

References

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