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Assessment of 6Cl-HOBt-based coupling reagents in solid-phase cyclopeptide synthesis

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Introduction

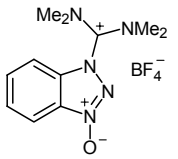
We recently described a comparative study [1,2] on the efficiency of the 6Cl-HOBt-based coupling reagents TCTU, and HCTU (Luxembourg Industries Ltd, Tel Aviv, Israel) synthesizing a difficult peptide sequence, ACP(65-74), and a longer peptide, rMOG(35-55). We demonstrated, using a multiple peptide synthesizer (Advanced ChemTech APEX 396), that these coupling reagents were efficient in both cases.

Due to the great interest in SAR studies of cyclic peptides, presenting a restricted conformation, we compared TCTU and HCTU with other aminium coupling reagents (TBTU, in a first instance) in the solid-phase on-resin cyclization of head-to-tail cyclopeptides [3], by anchoring the side chain of Fmoc-Asp-OAl to the Wang resin [4,5].

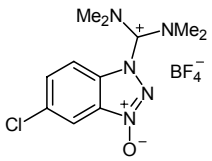
Results and Discussion

In order to undertake a comparative study between the efficiency of different coupling reagents in the solid-phase cyclization reaction, as model peptides we chose three RGD containing sequences (biologically interesting) [5]. In particular, we synthesized the cyclotetrapeptide cyclo(FRGD), the cyclopentapeptide cyclo(VFRGD) and the cyclohexapeptide cyclo(SVFRGD), in which hindered amino acids are present (Phe, Val). Moreover, the cyclohexapeptide contains also a Ser residue, a well-known amino acid to induce extensive racemization during the coupling reaction [2].

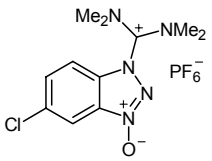
Starting from Fmoc-Asp(Wang resin)-OAl (0.24 mmol/g), we synthesized the linear peptides Fmoc-FRGD(Wang resin)-OAl, Fmoc-VFRGD(Wang resin)-OAl and Fmoc-SVFRGD(Wang resin)-OAl by the standard SPPS protocol. After deprotection of the C-terminal carboxyl function of Asp (anchored to the resin via its side chain) with a solution of $PhSiH_3/Pd(PPh_3)_4$ (20 equiv./0.25 equiv. in dry DCM, under Ar), the Fmoc group was removed with piperidine (25% in DMF). The comparative study was undertaken performing the solid-phase on-resin head-to-tail cyclizations in parallel, on a multiple peptide synthesizer, using 1 equiv. of one of the three different aminium coupling reagents, TBTU, TCTU or HCTU, in the presence of DIPEA (2 equiv.).



TBTU



TCTU



HCTU

During the synthesis of the cyclohexapeptide cyclo(SVFRGD), we carried out microcleavages at different cyclization times (45 min, 90 min, 3 h, overnight), in order to evaluate the effectiveness of the different coupling reagents for the on-resin cyclization reaction. The synthesis of cyclo(VFRGD) and cyclo(FRGD) was performed using HCTU or TCTU as coupling reagents and 2 h of cyclization time. The cleavage from the resins (and contemporary deprotection of the amino-acids side chains) were carried out in 2 h, at room temperature, with TFA/TIS/H_2O (95 : 2.5 : 2.5). The crude products were precipitated with diethyl ether, centrifuged, re-dissolved in H_2O and lyophilized. The cyclization yield (%) on the crude products was determined by RP-HPLC on a ThermoFinnigan Surveyor system (equipped with a diode array detector) coupled to the ESI-MS (ESI Ion Trap LCQ Advantage ThermoFinnigan), using a Phenomenex Aqua C18 column (5 μm, 150 × 2.0 mm) (flow rate: 200 μL/min) with a gradient of 5–30% CH_3CN/H_2O with 0.1% TFA for cyclo(SVFRGD) and 5–30% CH_3CN/H_2O with 0.1% HCOOH for cyclo(FRGD), and cyclo(VFRGD).

Preliminary data (Fig. 1) concerning the cyclization step to obtain cyclo(SVFRGD) showed that both HCTU and TCTU are very efficient after only 45 min of reaction time, while a lower cyclization percentage with TBTU was reached only after 3 hours. The cyclization yields (%) were determined as a ratio between the cyclopeptide and the corresponding linear peptide concentrations.

We obtained a good cyclization yields also in the more difficult synthesis of the constrained cyclopeptides cyclo(FRGD) and cyclo(VFRGD), both with HCTU and TCTU (Table 1).

Interestingly, in the synthesis of the cyclopentapeptide cyclo(VFRGD) no dimer was detected. The evaluation of racemization and other side reactions [6] is under investigation.

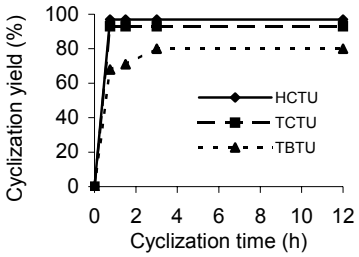


Fig. 1 Comparison of three coupling reagents (HCTU, TCTU, and TBTU) at different cyclization times in the synthesis of cyclo(SVFRGD).

Table 1. Cyclization yields after 2 h.

Cyclopeptide	HCTU	TCTU
cyclo(VFRGD)	90%	90%
cyclo(FRGD)	75%	87%

Acknowledgments

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References

1. Sabatino, G., Mulinacci, B., Alcaro, M.C., Chelli, M., Rovero, P., Papini, A.M., *Lett. Pept. Sci*. **9**, 119–123 (2002).
2. Di Fenza, A., Rovero, P., *Lett. Pept. Sci*. **9**, 125–129 (2002).
3. Rovero, P., In: Kates, S.A. and Albericio, F. (Eds.), *Solid-Phase Synthesis: A Practical Guide*, M. Dekker, New York, 2000, p. 331.
4. Sabatino, G., Chelli, M., Alcaro, M.C., Ginanneschi, M., Papini, A.M., *Tetrahedron Lett.* **40**, 809–812 (1999).
5. Alcaro, M.C., Sabatino, G., Uziel, J., Chelli, M., Ginanneschi, M., Rovero, P., Papini, A.M., *J. Pept. Sci.* **9**, in press.
6. Arttamangkul, S., Arbogast, B., Barofsky, D., Aldrich, J.V. Aldrich, *Lett. Pept. Sci*. **3**, 357–370 (2002).