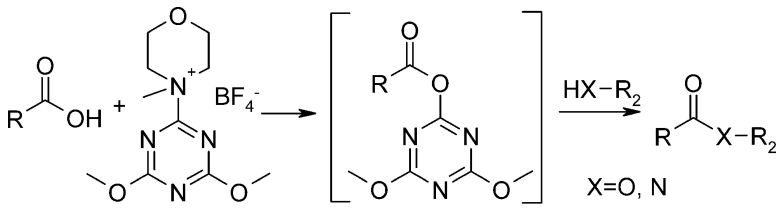
Article

N-Triazinylammonium Tetrafluoroborates. A New Generation of Efficient Coupling Reagents Useful for Peptide Synthesis

Zbigniew J. Kamiski, Beata Kolesiska, Justyna Kolesiska, Giuseppina Sabatino, Mario Chelli, Paolo Rovero, Micha Baszczyk, Marek L. Gwka, and Anna Maria Papini

J. Am. Chem. Soc., **2005**, 127 (48), 16912-16920• DOI: 10.1021/ja054260y • Publication Date (Web): 10 November 2005



More About This Article

Additional resources and features associated with this article are available within the HTML version:

- Supporting Information
- Links to the 7 articles that cite this article, as of the time of this article download
- Access to high resolution figures
- Links to articles and content related to this article
- Copyright permission to reproduce figures and/or text from this article

View the Full Text HTML

J|A|C|S
ARTICLES
Published on Web 11/10/2005

N-Triazinylammonium Tetrafluoroborates. A New Generation of Efficient Coupling Reagents Useful for Peptide Synthesis

Zbigniew J. Kamiński,*,† Beata Kolesińska,† Justyna Kolesińska,† Giuseppina Sabatino,‡,§ Mario Chelli,‡,§ Paolo Rovero,‡,|| Michał Błaszczyk,⊥ Marek L. Główka,⊥ and Anna Maria Papini*,‡,§

Contribution from the Institute of Organic Chemistry, Łódź University of Technology, 90-924 Łódź, Poland, Laboratory of Peptide and Protein Chemistry and Biology, Polo Scientifico, Università di Firenze, I-50019 Sesto Fiorentino, Florence, Italy, Dipartimento di Chimica Organica "Ugo Schiff" and CNR-ICCOM, Polo Scientifico, Università di Firenze, I-50019 Sesto Fiorentino, Florence, Italy, Dipartimento di Scienze Farmaceutiche, Università di Firenze, I-50019 Sesto Fiorentino, Florence, Italy, and Institute of General and Ecological Chemistry, Łódź University of Technology, 90-924 Łódź, Poland

Received June 28, 2005; E-mail: kaminsz@mail.p.lodz.pl; annnamaria.papini@unifi.it

Abstract: A new generation of triazine-based coupling reagents (TBCRs), designed according to the concept of "superactive esters", was obtained by treatment of 4-(4,6-dimethoxy-1,3,5-triazin-2-yl)-4-methylmorpholinium (DMTMM) chloride with lithium or silver tetrafluoroborate. The structure of 4-(4,6-dimethoxy-1,3,5-triazin-2-yl)-4-methylmorpholinium tetrafluoroborate was confirmed by X-ray diffraction. Activation of carboxylic acids by using this reagent proceeds via triazine "superactive ester". The coupling reagent was successfully used for the synthesis of Z-, Boc-, and Fmoc-protected dipeptides derived from natural and unnatural sterically hindered amino acids and for fragment condensation, in 80−100% yield and with high enantiomeric purity. The manual SPPS of the ACP(65−74) peptide fragment (H-Val-Gln-Ala-Ala-Ile-Asp-Tyr-Ile-Asn-Gly-OH) proceeded significantly faster than with TBTU or HATU, as well as the automated SPPS of the same fragment gave a purer product than by using TBTU or PyBOP. The reagent was also demonstrated to be efficient in on-resin head-to-tail cyclization of constrained cyclopeptides, in SPPS synthesis of Aib peptides, and in the synthesis of esters from appropriate acids, alcohols, and phenols. The high efficiency and versatility of this new generation of TBCRs confirm, for the first time, the usefulness of the concept of "superactive esters" in rational design of the structure of coupling reagents.

Introduction

Activation of carboxylic acids for the formation of amide or ester bonds is a key step in the synthesis of a large number of bioorganic molecules, in particular during peptide synthesis. Following the growing interest in peptides, numerous coupling reagents have been developed and become commercially available, including carbodiimides,[1] alone or plus additives (HOBt, HOAt),[2] and phosphonium[3] and uronium (iminium) salts.[4] Most of them engage the benzotriazole[5] or azabenzotriazole[6] ring system as a crucial fragment of their structure, which was subsequently transformed into a good leaving group at a more advanced stage of reaction. Two decades of domination of benzotriazole-based chemistry stimulated the progress in peptide synthesis to a high level of effectiveness. However, the growing need for new and more complex peptide structures, particularly for biomedical studies and, very recently, for the large-scale production of peptides as drugs, required manufacturing peptide products by efficient synthetic strategies, at reasonably low prices. Therefore, the search for new, more versatile, and low-cost reagents becomes again a great challenge. Several comprehensive review articles[7] summarized the great effort undertaken, but up to now, no versatile coupling reagent useful for both amide and ester bond formation, as well as for solution and solid-phase peptide synthesis, has been yet developed.

† Institute of Organic Chemistry, Łódź University of Technology.
‡ Laboratory of Peptide and Protein Chemistry and Biology, Polo Scientifico, Università di Firenze.
§ Dipartimento di Chimica Organica "Ugo Schiff" and CNR-ICCOM, Polo Scientifico, Università di Firenze.
|| Dipartimento di Scienze Farmaceutiche, Università di Firenze.
⊥ Institute of General and Ecological Chemistry, Łódź University of Technology.

(1) Sheehan, J. C.; Hess, G. P. *J. Am. Chem. Soc.* **1955**, *77*, 1067−1068.
(2) a) König, W.; Geiger, R.; *Chem. Ber.* **1970**, *103*, 788−798. (b) Carpino, L. A. *J. Am. Chem. Soc.* **1993**, *115*, 4397−4398.
(3) a) Castro, B.; Dormoy, J. R.; Evin, G.; Selve, C. *Tetrahedron Lett.* **1975**, *16* (14), 1219−1222. (b) Coste, J.; Le-Nguyen, B.; Castro, B. *Tetrahedron Lett.* **1990**, *31*, 205−208.
(4) (a) Carpino, L. A.; Imazumi, H.; El-Faham, A.; Ferrer, F. J.; Zhang, C.; Lee, Y.; Foxman, B. M.; Henklein, P.; Hanay, C.; Mugge, C.; Wenschuh, H.; Klose, J.; Beyermann, M.; Bienert, M. *Angew. Chem., Int. Ed.* **2002**, *41*, 441−445. (b) Li, P.; Xu, J. C. *J. Chem. Soc., Perkin Trans. 2* **2001**, 113−120.
(5) (a) Ferrer, F. J.; Carpino, L. A. Proceeding of the 17-th American Peptide Symposium, San Diego, CA, June 9−14; Lebl, M., Houghten, R. A., Eds.; American Peptide Society: San Diego, CA, 2001; pp 95−96. (b) Tantry, S. J.; Babu, V. V. S. *Lett. Peptide Sci.* **2002**, *9*, 35−41. (c) Carpino, L. A.; Henklein, P.; Foxman, B. M.; Abdelmoty, I.; Costisella, B.; Wray, V.; Domke, T.; El-Faham, A.; Muegge, C. *J. Org. Chem.* **2001**, *66*, 5245−5247.
(6) (a) Carpino, L. A.; Xia, J.; Zhang, C.; El-Faham, A. *J. Org. Chem.* **2004**, *69*, 62−71. (b) Carpino, L. A.; Ferrer, F. J. *Org. Lett.* **2001**, *3*, 2793−2795.

10.1021/ja054260y CCC: $30.25

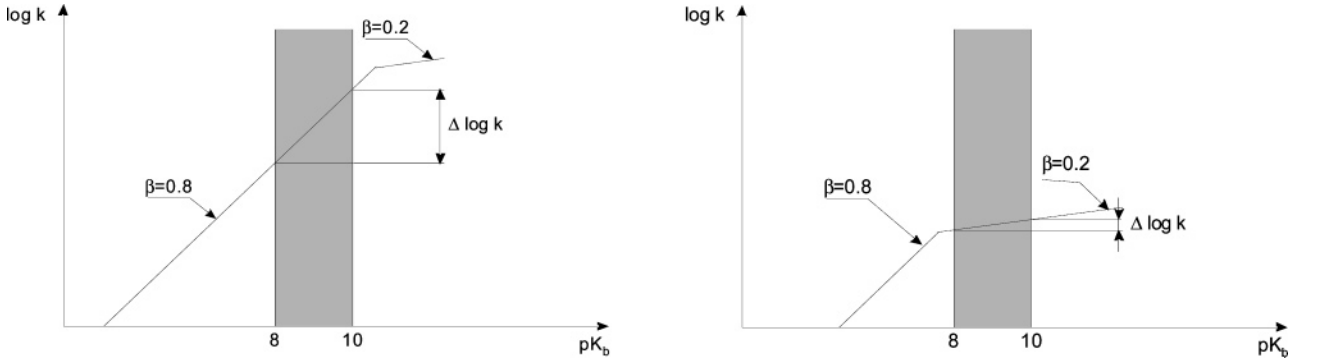


Figure 1. Nonlinear Brønsted relation for acylation of amino component typical for peptide synthesis (shadowed area) with active ester (on the left side; $\beta = 0.8$; large value of Δ log k) and "superactive ester" (right side; $\beta = 0.2$; small value of Δ log k).

The basic postulation in the design of the reagent structure was the dominance of its reactivity over all other coupling methods, a broad range of possible applications, a modular constitution, enabling extensive modification of its properties, and a reasonably low cost of native form as well as of its congeners. With this aim, we pointed our attention toward Triazine-Based Coupling Reagents (TBCRs)[8] due to the structural features postulated in the concept of "superactive esters",[9] disposing the reagent for attractive modification of the mechanism of the peptide bond formation and the outstanding coupling results obtained in the model experiments.

Previous studies proved the participation of 2-acyloxy-1,3,5-triazines **1**[10] as powerful acylating intermediates in condensations involving TBCRs. The mechanism of amine acylation with 2-acyloxy-1,3,5-triazines **1** studied by the kinetic isotope effects method was found substantially different than that postulated for typical active esters. Thus, the magnitude of kinetic isotope effects of ^{2}H and ^{15}N for acylation of aniline with 2-trimethylacetyloxy-4,6-dimethoxy-1,3,5-triazine has been found indicative of the stepwise reaction, with the formation of tetrahedral intermediate (TI) and its decomposition being nearly equally rate-limiting.[11] These studies excluded the one-step mechanism and have shown the dramatic shift of the breaking point characteristic for the change of the rate-determining step (A_N*$D_N^{\ddagger}$ versus $A_N^{\ddagger}$*D_N mechanism)[12] toward substrates about 3 pK_a units less basic than typical amino components used in the peptide synthesis (Figure 1, shadowed area). This is in contrast to the most of classic active esters used as acylating agents in the peptide synthesis for which the change of the mechanism was observed only for substrates more basic than typical amino components. The kinetic relationship observed for ester **1** should be very advantageous, because of the expectations of a more uniform rate of peptide bond formation (Figure 1, on the right side, small Δ log k) than that in acylation involving classic active esters (Figure 1, on the left side, large Δ log k). For any acylating reagent, the rate of acylation[13] depends on the basicity of the acylated amine, and the Brønsted parameter β_{nuc} (defined as the slope in plots of log k against pK_b) gives a measure of this relationship.

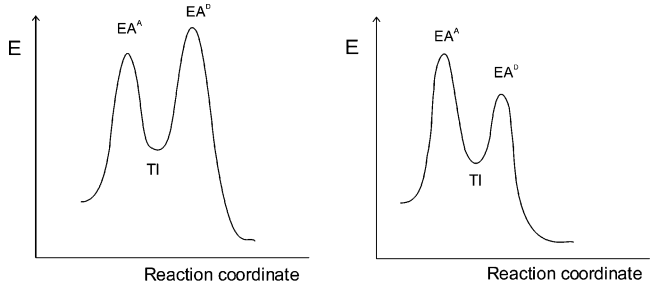


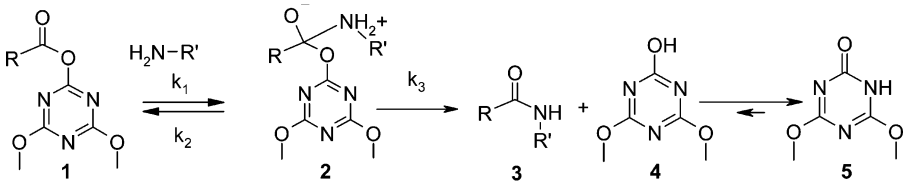
Figure 2. Energetic profile of amine acylation by active ester (left, rate-limiting TI breakdown) and "superactive ester" (right, fast TI breakdown, rate-limiting attack of amine on acylating reagent).

Thus, the change of the rate-limiting step[14] in the multistage reaction corresponds to a biphasic dependence on amine basicity, with the slope $\beta_{nuc} = 0.9 \div 1.2$ breaking off sharply to a much lower value of $\beta_{nuc} = 0.2 \div 0.1$ for strongly basic amines. For a large number of acylating reagents,[15] $\beta_{nuc} = 0.9 \div 1.2$ has been assigned to the rate-limiting breakdown of TI. The lower value of β_{nuc} (0.2−0.1) has been assigned to rate-limiting attack of the amine on the acylating reagent[16] ($A_N^{\ddagger}$*D_N mechanism according to IUPAC[12] recommendations). An equal contribution of the both mechanisms has been assigned to the breaking point.

According to the concept of "superactive esters",[9] lowering the energy of the transition state EA^D (leading TI to collapse to

(7) (a) Marder, O.; Albericio, F. *Chimica Oggi* **2003**, *21*, 35−40. (b) Han, S.-Y.; Kim, Y-. *Tetrahedron* **2004**, *60*, 2447−2467. (c) Bray, B. L. *Nat. Rev. Drug Discovery* **2003**, *2*, 587−593. (d) Kent, S. *J. Pept. Sci*. **2003**, *9*, 574−593. (e) Basso, A.; Wrubl, F. *Spec. Chem. Mag.* **2003**, *23*, 28−30. (f) Pittman, C. U. *Polym. News* **2003**, *28*, 183−184. (g) Stachelhaus, T. *Bioforum* **2003**, *26*, 626−628. (h) Keller, U.; Schauwecker, F. *Comb. Chem. High Throughput Screening* **2003**, *6*, 527−540. (i) Eggen, I. F. *Spec. Chem. Mag.* **2003**, *23*, 42−44. (j) Patchornik, A. *Polym, Adv. Technol.* **2002**, *13*, 1078−1090. (k) Zhang, W. *Tetrahedron*, **2003**, *59*, 4475−4489. (l) Najera, C. *Synlett* **2002**, *9*, 1388−1403. (m) Mizhiritskii, M.; Shpernat, Y. *Chimica Oggi* **2002**, *20*, 43−45, Chem. Abstr., 138:137537. (n) Albericio, F.; Chinchilla, R.; Dodsworth, D. J.; Najera, C. *Org. Prep. Proced. Int.* **2001**, *33*, 203−303.
(8) (a) Kamiński, Z. J. *Tetrahedron Lett.* **1985**, *26*, 2901−2904. (b) Kamiński, Z. J. *Synthesis* **1987**, 917−921. (c) Kamiński, Z. J.; Papini, A. M.; Kolesińska, B.; Kolesińska, J.; Jastrząbek, K. PL Pat. Appl. P-367852, from 10.05.2004. (d) For review, see: Kamiński, Z. J. *Biopolymers* **2000**, *55*, 140−165.
(9) Kamiński, Z. J. *Int. J. Pept. Protein Res.* **1994**, *43*, 312−319.
(10) (a) Kamiński Z. J. *J. Prakt. Chem*. **1990**, *332*, 579−583. (b) Sochacki, M.; Kamiński, Z. *J. Org. Mass Spectrom.* **1994**, *29*, 102−105.
(11) (a) Kamiński, Z. J.; Paneth, P.; O'Leary, M. H. *J. Org. Chem*. **1991**, *56*, 5716−5718. For other papers dealing with kinetic isotope effects in acyl transfer reactions, see: (b) Limbach, H. H.; Klein, O.; Del Amo, J. M. L.; Elguero, J. *Z. Phys. Chem*. **2004**, *218*, 17−49. (c) Iftimie, R.; Schofield, J. *J. Chem. Phys*. **2001**, *115*, 5891−5902. (d) Marlier, J. F. *Acc. Chem. Res*. **2001**, *34*, 283−290.
(12) IUPAC Commision for Physical Organic Chemistry, *Pure Appl. Chem*. **1989**, *61*, 23−56.
(13) For review, see: Williams, A. *Acc. Chem. Res*. **1989**, *22*, 387−392. For theoretical aspects of acyl group transfer, see: (a) Lee, I.; Kim, C. K.; Li, H. G.; Sohn, C. K.; Kim, C. K.; Lee, H. W.; Lee, B. S. *J. Am. Chem. Soc*. **2000**, *122*, 11162−11172.
(14) (a) Jencks, W. P.; Gilchrist, M. *J. Am. Chem. Soc*. **1968**, *90*, 2622−2637. (b) Castro, E. A.; Aliaga, M.; Campodonico, P.; Santos, J. G. *J. Org. Chem*. **2002**, *67*, 8911−8916.
(15) (a) Bond, P. M.; Castro, A. E.; Moodie, R. B. *J. Chem. Soc., Perkin Trans. 2* **1976**, 68−72. (b) Palling, D. J.; Jencks, W. P. *J. Am. Chem. Soc*. **1984**, *106*, 4869−4876.
(16) Satterthwait, A. C.; Jencks, W. P. *J. Am. Chem. Soc*. **1974**, *96*, 7018−7044.

Scheme 1. Postulated Mechanism of Coupling with TBCRs



final product) below the energy of the transition state formation EA^A (Figure 2, right) required leaving groups capable of an additional energetically favored process associated with their departure. It has been postulated that the energetically favored process of prototropic rearrangement of the enol **4** to the significantly more stable keto form[9] **5** provides sufficient driving force to change the energetic profile of the coupling reaction involving TBCRs (Scheme 1).

In fact, triazine esters **1** have already been found more reactive than any other acylating reagent, including *N*-hydroxybenzotriazole esters.[17] Moreover, many authors used successfully triazine reagents for the synthesis of peptides,[18] carboxylic acid esters,[19] amides,[20] pholic acid antagonists,[21] β-lactames,[22] carotenoids,[23] fullerenes,[24] and others compounds.[25,26]

Sporadic failures in some synthetic applications raised severe doubts concerning the correctness of the entire concept of "superactive esters". Moreover, in the literature there are some ambiguities concerning the energetic effects of keto−enol isomeric,[27] synchronization of both processes,[28] as well as other aspects of an acyl transfer mechanism.[29] Therefore, eradication of all these doubts was essential, and the "proof of concept" was undertaken demonstrating the efficacy of triazines not only in model studies but also in a very broad range of typical synthetic applications. These studies confirmed the predominance of the triazine-based "superactive esters" over the series of the well-known coupling reagents, considered up to now as the most efficient agents for ester and amide bond formation, in manual and automatic peptide synthesis, in solution and solid phase.

Results and Discussion

Development of New Improved TBCRs. A preliminary investigation of efficacy of the old generation (chloride salts) of 4-(4,6-dimethoxy-1,3,5-triazin-2-yl)-4-methylmorpholinium (DMTMM) chloride[30] evidenced the formation of methyl chloride in the demethylation side reaction,[31] which dramatically distorted stoichiometry, decreased the yield, and caused contamination of the final products. The degradation process was particularly favored in anhydrous aprotic solvents and during the storage of the salt.[31] The demethylation product of DMTMM was isolated and unequivocally identified as 4-(4,6-dimethoxy-1,3,5-triazin-2-yl)morpholine (**6**) by X-ray diffraction. The study showed very strong conjugation of the morpholine nitrogen with the triazine ring, tested by the shortening of C(2)−N(*morpholine)* bond to 1.350(2) Å, being equal to the two endocyclic C−N bond lengths of 1.353(2) Å and 1.347(2) Å (Figure 3), thus making a regular guanidine fragment.

Due to the conjugation, the triazine ring was found coplanar with the C−N−C fragment of the morpholine ring. The crystallographic data were fully consistent with the crystal structure determined by Kaftory et al.[32] and strongly suggested an attack of a nucleophilic chloride anion on a methyl group affording compound **6** with elimination of methyl chloride.

To prevent this degradation pathway, the nucleophilic chloride anion was changed with the less nucleophilic and larger tetrafluoroborate anion. The modular structure of the TBCRs (Figure 4) permitted the necessary modifications of the structure and a new generation of triazine coupling reagents as tetrafluoroborates **9a**−**d** (Scheme 2) has been prepared directly from the easily accessible precursor 2-chloro-4,6-dimethoxy-1,3,5-triazine (CDMT).

The synthetic procedure leading to the tetrafluoroborate salts of TBCRs **9a**−**d** involved in situ formation of *N*-triazinylammonium chlorides by treatment of the triazine **7** with appropriate tertiary amines **8a**−**d**, followed by replacement of the chloride anion with the tetrafluoroborate anion by treatment with silver or lithium tetrafluoroborate (Table 1). Two monocyclic **8a**,**b** and two bicyclic tertiary amines **8c**,**d** were used for the syntheses of TBCRs **9a**−**d**.

(17) Główka, M. L.; Iwanicka, I.; Kamiński, Z. J. *Acta Crystallogr.* **1990**, *C46*, 2211−2216.
(18) a) Kamiński, Z. J. *Synthesis* **1987**, 917−920. (b) Garrett, C. E.; Jiang, X.; Prasad, K.; Repic, O. *Tetrahedron Lett.* **2002**, *43*, 4161−4165.
(19) Kamińska, J. E.; Kamiński, Z. J.; Góra, J. *Synthesis* **1999**, 593−596.
(20) (a) Rayle, H. L.; Fellmeth, L. *Org. Process Res. Dev.* **1999**, *3*, 172−176. (b) Bandgar, B. P.; Pandit, S. S. *Tetrahedron Lett.* **2003**, *44*, 3855−3858. (c) Kunishima, M.; Yoshimura, K.; Morigaki, H.; Kawamata, R.; Terao, K.; Tani, S. *J. Am. Chem. Soc.* **2001**, *123*, 10760−10761.
(21) (a) Barnett, C.; Wilson, T. M.; Wendel, S. R.; Winningham, M. J.; Deeter, J. B. *J. Org. Chem.* **1994**, *59*, 7038−7045. (b) Read, M. W.; Miller, M. L.; Ray, P. S. *Tetrahedron* **1999**, *55*, 373−392. (c) Taylor, E. C.; Liu, B. *Heterocycles* **1997**, *45*, 241−254. (d) Taylor, E. C.; Liu, B. *Tetrahedron Lett.* **1999**, *40*, 4023−4026. (e) Barnett, C. J.; Wilson, T. M. *Tetrahedron Lett.* **1989**, *30*, 6291−6294. (f) Campbell, S. F.; Plews, R. M. *J. Med. Chem.* **1987**, *30*, 1794−1798. (g) Taylor, E. C.; Gillespi, P.; Patel, M. *J. Org. Chem.* **1992**, *57*, 3218−3225. (h) Taylor, E. C.; Patel, H. H.; Jun, J. G. *J. Org. Chem.* **1995**, *60*, 6684−6687. (i) Taylor, E. C.; Mao, Z. *J. Org. Chem.* **1996**, *61*, 7973−7974.
(22) Lee, H. W.; Kang, T. W.; Cha, K. H.; Kim, E. N.; Choi, N. H. *Synth. Commun.* **1998**, 28, 1339−1350.
(23) Tatman, D.; Liddell, P. A.; Moore, T. A.; Gust, D.; Moore, A. L. *Photochem. Photobiol.* **1998**, *68*, 459−466.
(24) (a) Bahr, J. L.; Kuciauskas, D.; Liddell, P. A.; Moore, A. L.; Moore, T. A.; Gust, D. *Photochem. Photobiol.* **2000**, *72*, 598−611. (b) Kodis, G.; Liddell, P. A.; Garza, L.; Moore, A. L.; Moore, T. A.; Gust, D. *J. Mater. Chem.* **2002**, *12*, 2100−2108. (c) Liddell, P. A.; Kodis, G.; De la Garza, L.; Bahr, J. L.; Moore, A. L.; Moore, T. A.; Gust, D. *Helv. Chim. Acta* **2001**, *84*, 2765−2783.
(25) Tucker, J. A.; Allwine, D. A.; Grega, K. C.; Barbachyn, M. R.; Klock, J. L. *J. Med. Chem.* **1998**, *41*, 3727−3735.
(26) (a) Kamiński, Z. J.; Kolesińska, B.; Kamińska, J. E.; Góra, J. *J. Org. Chem.* **2001**, *66*, 6276−6281. (b) Ono, S.; Inoue, Y.; Yoshida, T.; Ashimori, A.; Kosaka, K.; Imada, T.; Fukaya, C.; Nakamura, N. *Chem. Pharm. Bull.* **1999**, *47*, 1685−1693. (c) De Luca, L.; Giacomelli, G.; Porcheddu, A. *Org. Lett.* **2001**, *3*, 1519−1522. (d) De Luca, L.; Giacomelli, G.; Taddei, M. *J. Org. Chem.* **2001**, *66*, 2534−2537. (e) Bandgar, B. P.; Pandit, S. S. *Tetrahedron Lett.* **2003**, 2331−2333. (f) Nayyar, N. K.; Hutchinson, D. R.; Martinelli, M. J. *J. Org. Chem.* **1997**, *62*, 982−991.
(27) Bailey, J.; Kettle, L. J.; Cherryman, J. C.; Mitchell, J. B. O. *CrystEngComm* **2003**, *5*, 498−502.
(28) (a) Bernasconi, C. F.; Ragains, M. L.; Bhattacharya, S. *J. Am. Chem. Soc.* **2003**, *125*, 12328−12336. (b) Zhong, Z.; Snowden, T. S.; Best, M. D.; Anslyn, E. V. *J. Am. Chem. Soc.* **2004**, *126*, 3488−3495. (c) Yen, S. J.; Lin, C. Y.; Ho, J. J. *J. Phys. Chem. A* **2000**, *104*, 11771−11776.
(29) Ishida, T.; Kato, S. *J. Am. Chem. Soc.* **2003**, *125*, 12035−12048.
(30) Kamiński, Z. J.; Paneth, P.; Rudziński, J. *J. Org. Chem.* **1998**, *63*, 4248−4255.
(31) Kamiński, Z. J.; Darski, S. *XLII Conf. Pol. Chem Soc. Asoc. Chem. Eng., Rzeszów* **1999**, S 3, P-32.
(32) Fridman, N.; Kapon, M.; Kaftory, M. *Acta Crystallogr., Sect. C* **2003**, *C59*, 687−689.

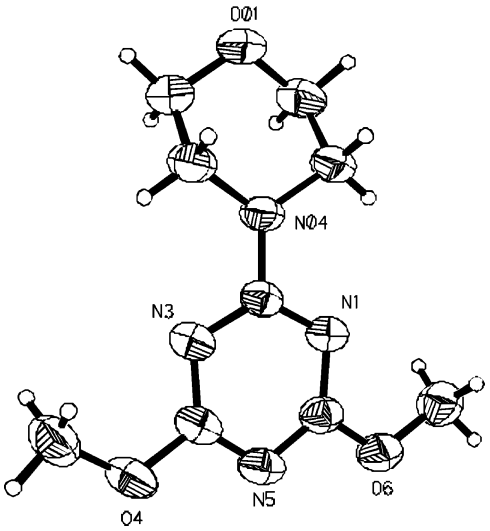


Figure 3. ORTEP representation of 4-(4,6-dimethoxy-1,3,5-triazin-2-yl)-morpholine (**6**) with thermal ellipsoid drawn at 50% probability level.

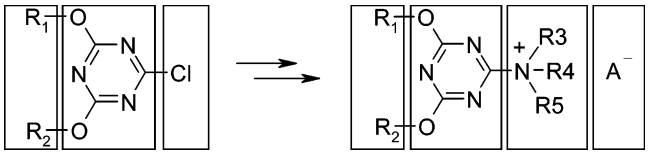


Figure 4. Modular structure of TBCRs.

Scheme 2. Synthesis of TBCRs **9a**−**d**

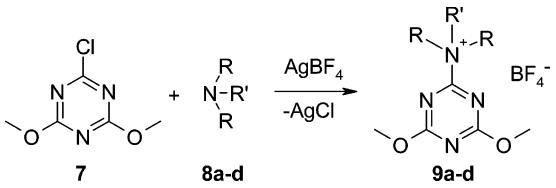


Table 1. Synthesis of Triazinylammonium Tetrafluoroborates **9a**−**d**

Entry	Amine	Triazinylammonium tetrafluoroborate	Yield [%]
1.1	 **8a**	**9a**	73
1.2	 **8b**	**9b**	62
1.3	 **8c**	**9c**	70
1.4	 **8d**	**9d**	77

The structures of the salts **9a**−**d** were confirmed by ^{1}H NMR, ^{13}C NMR, and X-ray diffraction studies on **9a**. Its crystal structure (Figure 5), being the very first known example of a s-triazine substituted with a quaternary ammonium group, has been determined from a monocrystal grown from acetonitrile.

X-ray structure determination of tetrafluoroborate **9a** showed that the triazinyl substituent occupies the axial position in the morpholine ring, being the methyl group in the equatorial position. The observation is in accordance with our previous suggestions based on the analysis of the NMR spectrum of the chloride salt.[30]

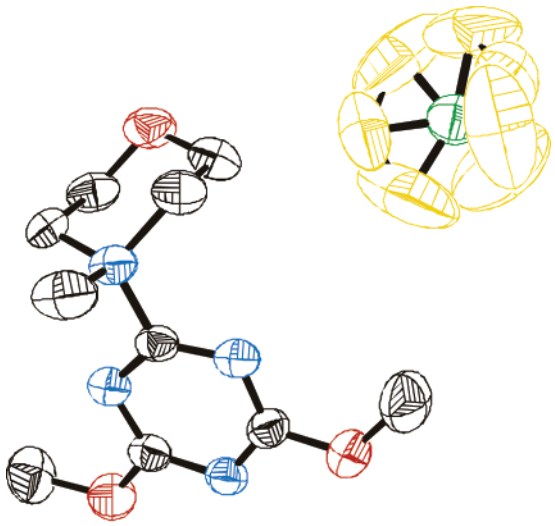


Figure 5. ORTEP representation of **9a** with thermal ellipsoid drawn at 50% probability level.

Applications of 4-(4,6-Dimethoxy-1,3,5-triazin-2-yl)-4-methylmorpholinium Tetrafluoroborate (9a). A. Amide and Ester Bond Formation in Liquid Phase. *N*-Methylmorpholine (NMM) has usually been used in a condensation reaction with classic CDMT or DMTMM; therefore in this paper we selected **9a** as the most representative new generation coupling reagent, anticipating its versatility as a common and unique low-cost reagent useful for ester and amide bond formation for both solution and solid-phase synthesis. We have proved that activation of carboxylic acids **10a**,**b** using **9a** yielded the same reactive intermediates **11a**,**b** (Scheme 3 and Table 2) as prepared by means of the classic triazine reagent **7**.

Moreover, we demonstrated that TBCR **9a** is very efficient in the synthesis of orthogonally protected dipeptides with Z, Boc, and Fmoc protecting groups, prepared from a broad range of natural and unnatural hindered amino acids (Table 3).

In solution, all protected dipeptides were obtained with very high purity, according to LC-SI-MS determination. Chromatographically pure products were obtained also in the case of the sterically hindered aminoisobutyric acid (Aib, Table 3, entry 3.5, 3.6) and derivatives of serine or threonine O-protected in the side chain with a bulky *t*-Bu group (Table 3, entry 3.21−3.23), activated with **9a** and then used as acylating components. Moreover, all couplings proceeded with exceedingly low racemization of the N-terminal amino acid. Only in the case of the C-terminal amino acid residue some racemization was observed, especially for the C-terminal phenylalanine residue, that could be caused by harsh conditions of acidolytic degradation of peptides to amino acids prior to the determination of their enantiomeric purity.

Reagent **9a** was demonstrated to be efficient in fragment coupling 2 + 3 and 2 + 5. Coupling of Z-Asp(OAla-OH)-OAl with human β-casein [57−59], H-Ile-Pro-Tyr-NH_2, and of Boc-Orn(Z)-Leu-OH with human β-casein [54−59], H-Glu(OBz)-Pro-Ile-Pro-Tyr-NH_2, by **9a** gave the peptides Z-Asp(Ala-Ile-Pro-Tyr-NH_2)-OAl and Boc-Orn(Z)-Leu-Glu(OBz)-Pro-Ile-Pro-Tyr-NH_2 in 92% and 89% yield, respectively (see Table 4). The

Scheme 3. Synthesis of Triazine Active Esters **11a,b** from **9a**

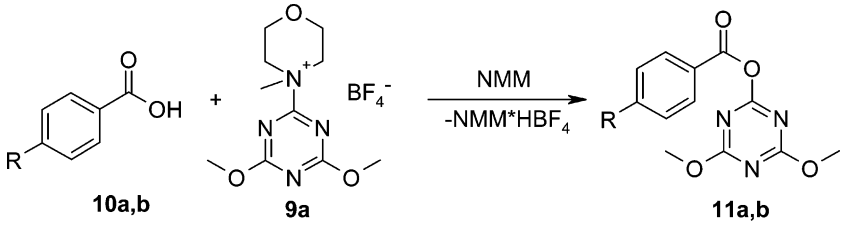


Table 2. Synthesis of 2-Acyloxy-4,6-dimethoxy-1,3,5-triazines **11a,b**

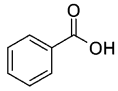
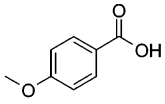
Entry	Carboxylic acid	2-Acyloxy-4,6-dimethoxy-1,3,5-triazine	Yield [%]
2.1	 **10a**	**11a**	88
2.2	 **10b**	**11b**	86

Table 3. Dipeptides Synthesized in Solution by Means of **9a**

entry	peptide[a]	yield [%]	purity [%]	[α]	%L/%D[b] N-prot. AA	%L/%D[b] C-prot. AA
3.1	Fmoc-Ala-Leu-OMe	85	96	−15.8 ($CHCl_3$, $c = 1.65$)	100/0	100/0
3.2	Fmoc-Ala-Ala-OMe	93	98	−25.8 ($CHCl_3$, $c = 1.08$)	100/0	100/0
3.3	Fmoc-Ala-Phe-OMe	89	98	+22.7 $CHCl_3$, $c = 1.03$)	100/0	100/0
3.4	Z-Aib-Ala-OMe	87	94	−2.0 ($CHCl_3$, $c = 0.7$)		100/0
3.5	Z-Aib-Phe-OMe	79	100	+35.4 $CHCl_3$, $c = 1.22$)		100/0
3.6	Z-Aib-Leu-OMe	88	100	−1.6 ($CHCl_3$, $c = 1.64$)		100/0
3.7	Boc-Orn(Z)-Leu-OMe	87	100	−21.1 ($CHCl_3$, $c = 1.08$)	100/0	100/0
3.8	Fmoc-Ala-Gly-OEt	81	100	−16.4 ($CHCl_3$, $c = 1.1$)	100/0	
3.9	Fmoc-Phe-Gly-OEt	85	97	−9.7 ($CHCl_3$, $c = 1.07$)	100/0	
3.10	Fmoc-Val-Ala-OMe	88	95	−18.1 ($CHCl_3$, $c = 1.05$)	100/0	100/0
3.11	Fmoc-Cys(Trt)-Ala-OMe	98	80	+9.5 ($CHCl_3$, $c = 1.0$)	100/0	100/0
3.12	Fmoc-Cys(Trt)-Phe-OMe	87	82	+14.8 ($CHCl_3$, $c = 1.04$)	100/0	100/0
3.13	Fmoc-Pro-Ala-OMe	98	100	−47.6 ($CHCl_3$, $c = 1.06$)	100/0	100/0
3.14	Fmoc-Pro-Phe-OMe	97	100	−35.3 ($CHCl_3$, $c = 1.0$)	100/0	96.4/3.6
3.15	Fmoc-Arg(Pbf)-Ala-OMe	88	87	−11.5 ($CHCl_3$, $c = 1.25$)	100/0	100/0
3.16	Fmoc-Arg(Pbf)-Phe-OMe	85	98	+6.2 ($CHCl_3$, $c = 1.05$)	100/0	99/1
3.17	Fmoc-Asp(OtBu)-Ala-OMe	72	93	−10.1 ($CHCl_3$, $c = 1.2$)	100/0	100/0
3.18	Fmoc-Asp(OtBu)-Phe-OMe	68	85	+31.0 ($CHCl_3$, $c = 0.92$)	100/0	97.5/2.5
3.19	Fmoc-Asn(Trt)-Ala-OMe	96	89	+5.7 ($CHCl_3$, $c = 1.0$)	100/0	98.6/1.4
3.20	Fmoc-Asn(Trt)-Phe-OMe	96	87	+5.4 ($CHCl_3$, $c = 1.22$)	100/0	97.3/2.7
3.21	Fmoc-Ser(*t*Bu)-Ala-OMe	85	100	+25.0 ($CHCl_3$, $c = 1.05$)	100/0	98.4/1.6
3.22	Fmoc-Ser(*t*Bu)-Phe-OMe	93	100	+49.1 ($CHCl_3$, $c = 1.02$)	100/0	100/0
3.23	Fmoc-Thr(*t*Bu)-Ala-OMe	70	100	+35.7 ($CHCl_3$, $c = 0.9$)	100/0	98.2/1.8
3.24	Fmoc-Thr(*t*Bu)-Phe-OMe	92	85	+19.2 ($CHCl_3$, $c = 1.03$)	100/0	98.1/1.9

[a] The dipeptides were synthesized in solution following the typical procedure for the formation of the amide bond (see Experimental Section and Supporting Information). [b] Enantiomeric purities were determined by GC analysis on a ChirasilVal column after degradation of peptides to amino acids and subsequent formation of volatile N-Tfa-AA-OMe.

syntheses of the amide fragments [57−59] and [54−59] of human β-casein, responsible for nitric oxide release from neutrophils of peripheral blood, were previously described.[33]

Very encouraging results were obtained when **9a** has been used in the ester synthesis. Usually, acylation of alcohols, which are less nucleophilic than amines, requires more vigorous reaction conditions and the use of more powerful acylating reagents. The triazine reagent **9a** gave pentafluorophenyl, allyl, or more sterically hindered methyl esters in satisfactory yield and exceedingly high purity (see Table 5).

The convenient and effective purification procedure was made by using the weakly basic properties of a triazine ring. Thus, simple washing of products with diluted acid solutions removed

(33) (a) Kamiński, Z. J.; Saleh, B.; Kolesińska, B.; Redliński, A.; Rudziński, J. *Acta Pol. Pharm.* **2005**, *62*, 53−57. (b) Rysz, J.; Redliński, A.; Mudyna, J.; Luciak, M.; Kamiński, Z. J. *Acta Polon. Pharm.* **2000**, *57*, 11−14.

Table 4. 2 + 3 and 2 + 5 Fragment Couplings by **9a**

entry	carboxy fragment	amino fragment	peptide[a]	yield [%]	purity [%]	%L/%D[b]
4.1	Z-Asp(Ala-OH)-OAl	H−Ile-Pro-Tyr-NH_2	Z-Asp(Ala-Ile-Pro-Tyr-NH_2)-OAl	92	89	98.8/1.2 (Ala)
4.2	Boc-Orn(Z)-Leu-OH	H-Glu(OBz)-Pro-Ile-Pro-Tyr-NH_2	Boc-Orn(Z)-Leu-Glu(OBz)-Pro-Ile-Pro-Tyr-NH_2	89	99	97/3 (Leu)

[a] The peptides were synthesized by fragment condensation in solution, following the typical procedure for the formation of the amide bond (see Experimental Section and Supporting Information). [b] Enantiomeric purities of activated amino acid were determined by GC analysis on a ChirasilVal column after degradation of peptides to amino acids and subsequent formation of volatile N-Tfa-AA-OMe.

Table 5. Synthesis of Esters of Carboxylic Acids by Means of **9a**

entry	esters	yield [%]	mp [°C]	[α]	purity [%]
4.1	Fmoc-Asp(O*t*Bu)-OPfp	88	55−57	−2.5 ($CHCl_3$, $c = 1.0$)	99
4.2	Fmoc-Asp(O*t*Bu)-OAl	86	80−82	−18.8 (DMF, $c = 1.0$)	100
4.3	Fmoc-Glu(O*t*Bu)-OAl	90	78−80	+1.5 ($CHCl_3$, c =1.0)	93
4.4	Fmoc-Ala-O-menthyl	86	92−94	−32.4 ($CHCl_3$, $c = 1.2$)	100
4.5	biotin-OSu	84	138−140	+31.0 (DMF, $c = 1.0$)	100

Table 6. Stability of TBCR **9a** in DMF[a]

entry	time	stability (%)
5.1	1 h	100
5.2	6 h	>99
5.3	12 h	>99
5.4	24 h	>99
5.5	48 h	98.3
5.6	1 month	98

[a] Stability studies were performed via HPLC analysis of aliquots from stock solutions (0.25 M) of the various coupling reagents in DMF at indicated times. Yields were calculated according to the integration of the peak area at 220 nm.

2-hydroxytriazine coproduct **4** and any other side products, as well as the excess of unreacted reagents.

B. Amide and Ester Bond Formation in Solid Phase. Manual SPPS. Solid-Phase Peptide Synthesis (SPPS) has become more and more strategic for peptide chemistry since Merrifield[34] proposed it in 1963. The success in the stepwise elongation of the peptide chain depends on the yield of the repetitive deprotection/coupling cycles. As the purification of the intermediates is impossible, the yield of each step must be the highest, to avoid many deletion peptide sequences as side products. This can be achieved only with a coupling reagent which is stable under SPPS conditions, operates in stoichiometric quantities, is soluble in most of the solvents recommended for the given solid support, minimizes racemization, and gives crude final peptides of the highest purity. As expected, the tetrafluoroborate **9a** was significantly more stable than the corresponding DMTMM chloride. Even after 1 month storage in anhydrous DMF, **9a** underwent no more than 2% degradation (Table 6).

The stability of coupling reagent **9a** was also examined in the presence of NMM (Table 7), because peptide bond formation is usually carried out in the presence of this base. Analysis performed by HPLC confirmed that tetrafluoroborate **9a** is stable in the presence of NMM and, what is particularly important in the case of slow coupling reactions, less than 1% degradation products was observed after 3 h.

To demonstrate the efficiency of triazine-based reagent **9a** in SPPS and to compare its performance to commonly used benzotriazole (TBTU) or azabenzotriazole-based coupling reagents (HATU), we synthesized the peptide fragment ACP(65−74) (ACP, Acyl Carrier Protein: H-Val-Gln-Ala-Ala-Ile-Asp-Tyr-Ile-Asn-Gly-OH). The development of internal secondary structures hampers the formation of the desired amide bond; therefore this peptide is a model of a difficult sequence used as a test peptide in several papers regarding the setup of SPPS strategies. In fact, the segment includes sterically hindered couplings, and it is prone to interchain aggregation with resulting reduction in amino function accessibility. In the case of the Fmoc/*t*Bu strategy, this phenomenon can cause both slow Fmoc-deprotection and poor couplings.[35]

Table 7. Stability of Tetrafluoroborate **9a** in the Presence of NMM (1 equiv)

entry	time	stability (%)
6.1	5 min	>99
6.2	30 min	>99
6.3	1 h	>99
6.4	2 h	>99
6.5	3 h	>99

Table 8. Comparative Study of the Synthesis of ACP (65−74) by TBTU, HATU, or TBCR **9a**

coupling reagent	purity of crude ACP (65–74) [%]	time of coupling [min]
TBTU	69	45
HATU	87	30
9a	84	15

ACP(65−74) was synthesized on a manual synthesizer starting from Fmoc-Gly-Wang resin. To emphasize the differences in terms of reactivity, we used a standard excess of the acylating mixture in a single coupling procedure. In all experiments the coupling progress was monitored by the Kaiser test.[36] The yield of the peptide was determined using LC-ESI-MS. The results revealed that coupling reactions of each amino acid by TBCR **9a** proceeded substantially faster (15 min) than with HATU (30 min) or TBTU (45 min) (Table 8).

Another approach for the evaluation of effectiveness of this new generation of coupling reagent involves its application in

(34) Merrifield, R. B. *J. Am. Chem. Soc.* **1963**, *85*, 2149−2154.

(35) Atherton, E.; Sheppard, R. C. *Solid-Phase Peptide Synthesis -A pratical approach.* Oxford University Press: 1989.

(36) Kaiser, E.; Colescott, R. L.; Bossinger, C. D.; Cook, P. I. *Anal. Biochem.* **1970**, *34*, 595−598.

the coupling of sterically hindered substrates. Usually, the most representative example used is the α,α-disubstituted amino acid α-aminoisobutyric acid (Aib). We already successfully incorporated Aib residue into the peptide chain in solution (stepwise, as well as by segment coupling approach) using the old generation of triazine reagents in the synthesis of [Aib]^2-enkephalins,[37] and Aib-Aib-Aib sequence in N-terminal hexapeptide emerimicin III.[38] Therefore, we used the new generation reagent **9a** to the SPPS synthesis of Aib peptides.

The SPPS synthesis of the [Aib^2,Aib^3]-enkephalin analogue has been carried out manually under standard conditions of the Fmoc/tBu strategy. The progress of the Aib-Aib fragment synthesis on the resin was monitored by the Kaiser test. The assembly of the first Fmoc-Aib-OH to the growing peptide chain on the resin proceeded under standard conditions (with no preactivation) and was completed within 2 h. Substantially slower coupling was observed for the incorporation of the second Fmoc-Aib-OH. However, increasing the excess of Fmoc-Aib-OH to 3 equiv, and the reaction time to 4 h, we overcome the synthetic difficulties and completed the synthesis without repeating the coupling procedure. It is evident that the active species of Fmoc-Aib-OH with **9a** is sufficiently stable to degradation under SPPS conditions. The subsequent acylation of the N-terminal Aib-Aib fragment with Fmoc-Tyr(O*t*Bu)-OH in the presence of **9a** was completed within 0.5 h, affording the [Aib^2,Aib^3]-enkephaline analogue (isolated after cleavage from the resin), as 95% pure product, according to HPLC.

Intramolecular peptide forming reaction, is the key step in the synthesis of constrained head-to-tail cyclopeptides due to the high tendency of the corresponding linear peptides to oligomerize. Classical approaches to the synthesis of a cyclic peptide generally involve preparation of the partially protected linear precursor (by solution or solid-phase approaches), followed by cyclization in solution under high dilution conditions. However, solution-phase methodologies, even in high dilution conditions, suffer from several drawbacks, such as cyclodimerization and cyclooligomerization side reactions. If the peptide remains anchored on a solid support, the cyclization takes advantage of the pseudodilution phenomenon, which favors intramolecular resin-bound reactions, minimizing interchain interactions. Therefore, homodetic head-to-tail cyclopeptides can be conveniently synthesized by SPPS anchoring a trifunctional amino acid to the resin by its side chain. The combination of this strategy with an orthogonal three-dimensional protection scheme, such as the Fmoc/tBu/OAl SPPS, results in a powerful head-to-tail cyclization methodology.[39]

We used **9a** in the preparation of head-to-tail constrained cyclopeptides, to prove the efficacy in the on-resin ciclopeptides synthesis. We started from Fmoc-Asp(O-Wang resin)-OAl, obtained by anchoring the side chain of aspartic acid residues to the resin[40] via reaction with DIPCDI. The linear peptide chain was elongated following a tridimensional Fmoc/*t*Bu/OAl protection strategy. After deprotection of the C-terminal carboxyl function of aspartic acid (anchored to the resin via its side chain), an intramolecular peptide bond was formed with **9a** yielding the desired cyclopeptide. As model peptides we chose three RGD containing sequences of different lengths: a cyclohexapeptide cyclo(YVFRGD), a cyclopentapeptide cyclo(VFRGD), and the most constrained cyclotetrapeptide cyclo(FRGD).

Table 9. On-Resin Cyclization of Head-to-Tail Cyclopeptides with TBCR **9a**

cyclopeptide	cyclo/linear (%)
cyclo(YVFRGD)	84
cyclo(VFRGD)	81
cyclo(FRGD)	76

It is already well-known that the cyclization with aminium (uronium) salts should be generally performed without excess of coupling reagent (1 equiv of coupling reagents in the presence of 2 equiv of tertiary base), to avoid the formation of guanidinium side products. We applied the same conditions (stoichiometric amounts of reagents), to compare the efficacy of all reagents under the same conditions. In all experiments, the cyclization yield (%) was determined as a ratio between the concentration of the cyclopeptide and the corresponding linear peptide isolated after the cleavage from the resin.

The data collected in Table 9 are evidence that fast and efficient formation of cyclo(YVFRGD) with TBCR **9a** proceeded after only 1 h of reaction time. Good cyclization yields were also obtained in the more difficult syntheses of the constrained cyclopeptides cyclo(FRGD) and cyclo(VFRGD).

C. SPPS by an Automatic Multiple Peptide Synthesizer. The protocols routinely used on an automatic multiple peptide solid-phase synthesizer take advantage of potent coupling reagents and a large excess of the acylating mixture. On the other hand, it is well-known that overactivation may lead to undesired side reactions. For example, aminium salts can react with an N-terminal amino component giving a guanidine derivative.[41] Moreover, the high cost of some protected amino acids (particularly the unnatural ones) and coupling reagents suggests maintaining their consumption to a reasonably low level, compatibly with the success of the synthesis.

In automatic multiple solid-phase syntheses, used to obtain simultaneously peptides not only of different sequences but also of different length, the coupling reagents should be efficient in terms of yield and preservation of optical purity of the final products. In particular, the coupling reagents should be applied repetitively under standard conditions to a wide range of substrates including less reactive and sterically hindered amino acids. In addition, using an automatic multiple peptide synthesizer, coupling reagents, preferably commercially available and easy-to-use, should have the following characteristics: fast reactions at room temperature, good solubility in the common solvents, and stability of their solutions for several days.

We compared the performance of **9a** with two other well-known coupling reagents (TBTU and PyBOP), using an automatic multiple peptide synthesizer, where the coupling reagents are stored in solution for a time depending on the length of the peptide to be synthesized. The SPPSs were performed on a multiple automatic batch synthesizer (a fully automated

(37) Rolka, K.; Kamiński, Z. J.; Słomczyńska, U.; Baran, L.; Przegaliński, E.; Leplawy, M. T.; Kupryszewski, G. *Pol. J. Chem.* **1984**, *58*, 1232−1235.
(38) Kamiński, Z. J.; Saleh, B.; Kolesińska, B.; Redliński, A.; Rudziński, J. *Acta Pol. Pharm.* **2005**, *62* (2) 117−120.
(39) Rovero, P. In *Solid-Phase Synthesis: A Practical Guide*; Kates, S. A., Albericio, F., Eds.; M. Dekker: New York, 2000.
(40) (a) Sabatino, G.; Chelli, M.; Mazzucco, S.; Ginanneschi, M.; Papini, A. M. *Tetrahedron Lett.* **1999**, *40*, 809−812. (b) Alcaro, M. C.; Sabatino, G.; Uziel, J.; Chelli, M.; Ginanneschi, M.; Rovero, P.; Papini, A. M. *J. Pept. Sci.* **2004**, *10* (4), 218−224.
(41) Albericio, F.; Bofill, J. M.; El-Faham, A.; Kates, S. A. *J. Org. Chem.* **1998**, *63*, 9678−9683.

Table 10. Solid-Phase Synthesis of ACP (65−74) by an Automatic Multiple Peptide Synthesizer Using Different Coupling Reagents

coupling reagent	yield [%]
TBCR **9a**	63
TBTU	63
PyBOP	60

robotic instrument capable of the simultaneous synthesis of up to 96 different peptides) using a Fmoc/*t*Bu protection scheme. We carried out simultaneously three syntheses of ACP(65−74), in the same conditions (solvents, temperature, reaction times), but using different coupling reagents.

Multiple synthesis of ACP(65−74) (Table 10) under identical conditions for all reagents gave expected product identified by LC-ESI-MS with a small trace of deletion peptide des-Gln[66]. The des-Gln[66] deletion peptide was previously identified as the main byproduct during the automatic synthesis by using the commercially available DMTMM.[42]

Summary

It has been proved that the concept of "superactive esters" is a tool useful for designing new coupling reagents. The idea of acceleration of the coupling process in the case of "superactive esters" is based on facile departure of the leaving group by its rearrangement in an energetically favored, consecutive process to a stable, chemically inert and neutral side product. This general approach for designing coupling reagents can be considered as a valuable alternative, which eliminates the overactivation of the acylating intermediate. The new generation of modified triazine reagents, with improved stability due to the use of non-nucleophilic tetrafluoroborate counterion, was introduced. The new coupling reagents are highly versatile in ester and peptide synthesis in solution as well as in the solid phase. Their application in the synthesis gave very regular coupling results for the whole range of amino acid substrates derived from natural and un-natural sterically hindered amino acids and substantially reduced amounts of side products under a broad range of reaction conditions.

Experimental Section

4-(4,6-Dimethoxy-1,3,5-triazin-2-yl)-4-methylmorpholinium Tetrafluoroborate (9a). NMM (1.10 mL, 10 mmol) was added dropwise to a vigorously stirred solution of 2-chloro-4,6-dimethoxy-1,3,5-triazine (1.76 g, 10 mmol) in dichloromethane (20 mL), cooled to 5 °C. The mixture was stirred at 5 °C for 0.5 h after which time a suspension of silver tetrafluoroborate (1.94 g, 10 mmol) in acetonitrile (20 mL) was added. The stirring was continued for an additional 2 h, the precipitate was filtered off, and the filtrate evaporated to dryness at a temperature not exceeding 20 °C. The solid residue was washed with THF and recrystallized from acetonitrile/diethyl ether affording **9a** (2.39 g, 73%), mp 199−200 °C. Anal. Calcd for $C_{10}H_{17}BF_4N_4O_3$: C, 36.61; H, 5.22. Found: C, 36.44; H, 5.46. ^{1}H NMR (250 MHz, CD_3CN): δ 3.39 (s, 3H, CH_3-N); 3.71 (t, 2H, J = 8.5 Hz, $N-CH_2-CH-O$); 3.75 (t, 2H, J = 10 Hz, $-N-CH-CH_2-O-$); 3.99 (m, 2H, $N-CH_2-CH_2-O-$); 4.11 (s, 6H, 2 × CH_3-O); 4.46 (dd, 2H, J_1= 10 Hz, J_2 = 2 Hz, $N-CH_e-C$). ^{13}C NMR (62.8 MHz, CD_3CN): δ 56.89 (CH_3-N); 57.82 (2 × CH_3-O); 61.10 (2 × CH_2); 62.77 (2 × CH_2); 171.23 (N−C−N); 175.01 (N−C−N). IR (KBr): 1636, 1540, 1488, 1392, 1072 (broad), 944, 864, 788, 712 cm^{-1}.

1-(4,6-Dimethoxy-1,3,5-triazin-2-yl)-1-methylpiperydinium Tetrafluoroborate (9b). *N*-Methylpiperidine (0.99 g, 10 mmol) was added to a vigorously stirred solution of 2-chloro-4,6-dimethoxy-1,3,5-triazine (1.76 g, 10 mmol) in dichloromethane (20 mL), cooled to 5 °C. The mixture was stirred at 5 °C for 0.5 h, after which time a suspension of silver tetrafluoroborate (1.94 g, 10 mmol) in acetonitrile (40 mL) was added. The stirring was continued for an additional 2 h, the precipitate was filtered off, and the filtrate evaporated to dryness at a temperature not exceeding 20 °C. The solid residue was washed with THF (2 × 20 mL), dried, and recrystallized from acetonitrile/diethyl ether affording **9b** (2.03 g, 62%), mp 111−113 °C. Anal. Calcd for $C_{11}H_{19}BF_4N_4O_2$: C, 40.52; H, 5.87. Found: C, 40.31; H, 5.64. ^{1}H NMR (250 MHz, CD_3CN): δ 4.41 (broad d, 2H, J = 12 Hz, N−CH−C), 4.10 (s, 6H, $O-CH_3$), 3.56 (dt, 2H, J_1 = 12 Hz, J_2 = 3 Hz, N−CH−C), 3.31 (s, 3H, $N-CH_3$), 1.50−2.00 (m, 6H, 3 × CH_2). ^{13}C NMR (62.8 MHz, CD_3CN): δ 21.3, 22.0 ($C-CH_2-C$), 55.5 (CH_3-N), 57.7 (CH_3-O), 62.5 ($N-CH_2$), 171.8 (N−C−N), 175.1 (N−C−N).

1-(4,6-Dimethoxy-1,3,5-triazin-2-yl)quinuclidinium Tetrafluoroborate (9c). A vigorously stirred solution of 2-chloro-4,6-dimethoxy-1,3,5-triazine (1.76 g, 10 mmol) and quinuclidine (1.11 g, 10 mmol) in dichloromethane (20 mL), cooled to 0 °C, was treated with a solution of lithium tetrafluoroborate (0.94 g, 10 mmol) in acetonitrile (40 mL). The stirring was continued for 2 h, the precipitate was filtered off, and the filtrate evaporated to dryness at a temperature not exceeding 20 °C. The solid residue was washed with THF (2 × 20 mL), dried, and recrystallized from acetonitrile/diethyl ether affording **9c** (2.36 g, 70%), mp 115−117 °C. Anal. Calcd for $C_{12}H_{19}BF_4N_4O_2$: C, 42.63; H, 5.66. Found: C, 42.38; H, 6.02. ^{1}H NMR (250 MHz, CD_3CN): δ 2.05, 2.11 (dd, AB system, 6H, J_1 = 11 Hz, J_2 = 8 Hz, J_3 = 3.3 Hz, $N-C-CH_2-$), 2.27 (hept., 1H, J = 3.3 Hz, C−*H*), 3.86, 3.91 (d, AB system, 6H, J_1 = 11 Hz, J_2 = 8 Hz, J_3 = 8 Hz, $N-CH_2-$), 4.09 (s, 6H, $O-CH_3$). ^{13}C NMR (62.8 MHz, CD_3CN): 24.1, 24.37 ($CH-CH_2-C$), 57.40 (CH_3-O), 57.45 ($N-CH_2$), 173.4 (N−C−N), 174.5 (N−C−N).

2-Acyloxy-4,6-dimethoxy-1,3,5-triazine (11a,b): Typical Procedure. The appropriate carboxylic acid (1 mmol) and NMM (0.11 mL, 1 mmol) were added to a vigorously stirred solution of **9a** (0.328 g, 1 mmol) in CH_3CN (2 mL), cooled to 0 °C. The stirring was continued for 1.5 h, after which time the solvent was evaporated under reduced pressure. The residue was dissolved in CH_2Cl_2 (10 mL), and the solution was washed successively with water, 0.5 M aqueous $KHSO_4$, water, 0.5 M aqueous $NaHCO_3$, and water again. The organic layer was dried with $MgSO_4$, filtered, and concentrated to dryness. The residue was dried under vacuum with P_2O_5 and KOH to constant weight affording the neutral products **11a** or **11b**.

Formation of the Peptide Bond: Typical Procedure. N-Protected amino acid (10 mmol) and NMM (1.10 mL, 10 mmol) were added to a vigorously stirred solution of **9a** (3.28 g, 10 mmol) in CH_3CN (20 mL), cooled to 0 °C. The stirring was continued for an additional 60 min, after which time the amino component (10 mmol) was added, and the mixture stirred for an additional 2 h at 0 °C and overnight at room temperature. The solvent was evaporated under reduced pressure, and the residue was dissolved in $CHCl_3$ (30 mL). The solution was washed successively with water, 0.5 M aqueous $KHSO_4$, water, 0.5 M aqueous $NaHCO_3$, and water again. The organic layer was dried with $MgSO_4$, filtered, and concentrated to dryness. The residue was dried under vacuum with P_2O_5 and KOH to constant weight affording the neutral peptide.

Formation of the Ester Bond: Typical Procedure. NMM (0.11 mL, 1 mmol) was added dropwise to a solution of **9a** (0.328 g, 1 mmol) and appropriate carboxylic acid (1 mmol) in CH_3CN (5 mL) at 0 °C. The solution was stirred at 0 °C for an additional 2 h, then a mixture of the appropriate alcohol (1.25 mmol) and a catalytic amount of DMAP were added. Stirring was continued at 0 °C for 0.5 h, and then the mixture was left at room temperature for 14 h. The solvent was evaporated, and the residue was suspended in $CHCl_3$ (10 mL). The suspension was washed with H_2O (5 mL), 0.5 M $KHSO_4$ (5 mL), H_2O

(42) Sabatino, G.; Mulinacci, B.; Alcaro, M. C.; Chelli, M.; Rovero, P.; Papini, A. M. *Lett. Pept. Sci*. **2002**, *9*, 119−123.

(5 mL), 0.5 M $NaHCO_3$ (5 mL), and H_2O (5 mL). The organic layer was dried (Na_2SO_4), the solvent evaporated, and the residue was treated with hexane (20 mL) in order to crystallize.

Automatic Synthesis of ACP(65−74) with TBCRs. The automatic SPPSs were performed on an automatic batch synthesizer equipped with a 40-wells reaction block using an Fmoc/*t*Bu protection scheme. The resin was swollen for 40 min in DMF. A robotic needlelike arm allows the distribution of reagents predissolved in DMF. Fmoc deprotections were performed with 25% piperidine in DMF and washed with DMF. The linear peptides were synthesized starting from Fmoc-Gly-Wang resin (0.63 mmol/g). The yield of the peptides was determined using analytical HPLC-ESI-MS (ESI Ion Trap LCQ Advantage ThermoFinnigan; using a Phenomenex Aqua C18 column (5 μm, 150 mm × 2.0 mm) (flow rate: 200 μL/min) with a gradient of 10−60% CH_3CN/H_2O in 20 min with 0.1% TFA.

Cyclopeptides Synthesis. A. Fmoc-Asp(O-Wang-resin)-OAl. DIPCDI (0.32 mL, 2 mmol) was added to a solution of Fmoc-Asp-OAl (1.5 g, 4 mmol) in dry DCM (15 mL). The solution was stirred at 0 °C under N_2. After 20 min the solution was concentrated, and the residue was dissolved in DMF (10 mL). This terminally protected amino acid solution and a solution of DMAP (12 mg, 0.1 mmol) in DMF (0.5 mL) were added to the Wang resin (1.0 g, 1.2 mmol/g), preswollen in DMF for 30 min, and vortexed for 1 h. The resin was washed with DMF (3 × 2 min) and DCM (2 × 2 min) and then endcapped with acetic anhydride (1.9 mL, 20 equiv) and NMM (2.2 mL, 20 equiv) in DCM (10 mL) for 1.5 h. The resin, washed with DCM (2 × 2 min), DMF (2 × 2 min), and DCM (2 × 2 min), was dried under vacuum, and the resin loading (0.25 mmol/g) was determined from the Fmoc release monitored by UV absorption at 301 nm.

General Procedure for the Solid-Phase Cyclopeptide Synthesis. The linear peptides anchored to the resin were synthesized on the automatic synthesizer following the standard Fmoc/tBu protocol as described above. To avoid DKP formation, Fmoc deprotection on the second residue was performed with a fast protocol (20% piperidine in DMF, 3 × 5 min).

To remove the allyl group, the peptide−resins were dried under vacuum and swollen in dry DCM (2 × 20 min) under Ar. The resins were shaken for 5 min with a solution of $PhSiH_3$ (24 equiv) in dry DCM under Ar, and then a solution of $Pd(PPh_3)_4$ (0.25 equiv) in dry DCM was added. After 40 min the resins were washed with dry DCM. The treatment with $PhSiH_3/Pd(PPh_3)_4$ was repeated once again. The resins were washed with DCM, a solution of 0.5% sodium diethyldithiocarbamate in DMF, DMF, and DCM. the Fmoc group was removed, and then the resins were treated with the different coupling in order to obtain the cyclization. We used 1 equiv of coupling reagents and 2 equiv of DIPEA for the cyclization reactions, and the resins were washed with DMF (5 × 2 min).

Peptide cleavage from the resin and contemporary deprotection of the amino acid side chains were carried out for 3 h at room temperature with TFA/TIS/H_2O (95:2.5:2.5). The yield of the peptides was determined using analytical HPLC-ESI-MS spectrometry using a Phenomenex Aqua C_{18} column (5 μm, 150 mm × 2.0 mm) (flow rate: 200 μL/min) with a gradient of 5−30% CH_3CN/H_2O in 20 min with 0.1% HCOOH.

Acknowledgment. We thank PRIN 2002, MIUR (Italy), and Fondazione Ente Cassa di Risparmio di Firenze (Italy), for financially supporting the Laboratory of Peptide and Protein Chemistry and Biology of the University of Florence, and the Polish State Committee for Scientific Research (KBN), the Project 4-T09A 189 25 for financially supporting the Institute of Organic Chemistry of the Łódź University of Technology, Łódź (Poland).

Supporting Information Available: Materials and Methods (PDF) and chemical data (CIF). This material is available free of charge via the Internet at http://pubs.acs.org.

JA054260Y