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Synthesis of sterically-hindered peptidomimetics using 4-(4,6-dimethoxy-1,3,5-triazine-2-yl)-4-methyl-morpholinium chloride

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This Letter is dedicated to Professor E. J. Corey on the occasion of his 80th birthday

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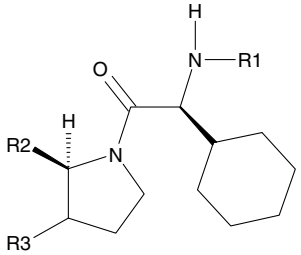
ABSTRACT

Our study demonstrated that 4-(4,6-dimethoxy-1,3,5-triazine-2-yl)-4-methyl-morpholinium chloride (DMTMM) is a versatile coupling reagent for the synthesis of sterically-hindered peptidomimetics. It is superior to HBTU/HOBt and CDMT in controlling racemization and N-arylation, respectively.

To support our drug discovery program, we had to synthesize several peptidomimetics that exhibit good in vitro activity in multiple cell lines. As shown in generic structure **1**, these peptidomimetics contain two sterically-hindered motifs, namely the pyrrolidine and cyclohexyl rings. Syntheses of sterically-hindered peptides or chiral amides have been challenging since racemization and side reactions are common competing pathways. Although coupling reagents such as thiazolium[1] or benzimidazolium[2] salts have been reported for the synthesis of sterically-hindered peptides, they were not considered by us because of their unavailability on large scale. To assemble the peptidomimetics of our interest, we decided to start with a method employing *O*-(benzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate (HBTU)[3] and hydroxy-benzotriazole (HOBt), a combination that is commonly used for peptide syntheses involving sterically-hindered amino acids as reported by Knorr[4] and Giralt.[5] Our initial efforts to couple sterically-hindered amine **9** and acid **5** using HBTU/HOBt were unsatisfactory. We observed 25% of the undesired diastereomer **7**, which was caused by a racemization pathway that occurred during the amide bond formation (Table 1,



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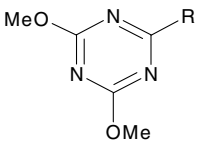

2a, R = OH
2b, R = Cl

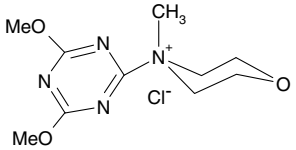

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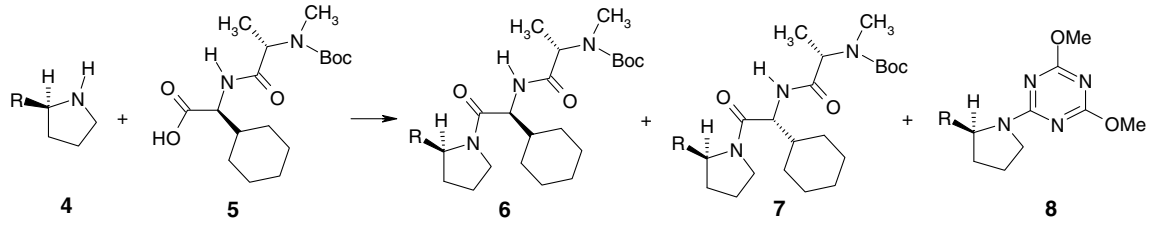
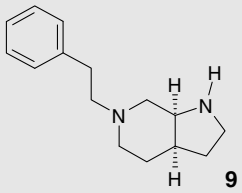
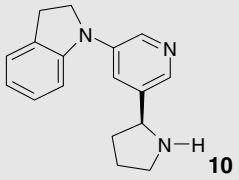
Table 1
Coupling of sterically-hindered amine **4** and acid **5**



Entry	Amine	Coupling method	% **6**[a]	% **7**[b] (racemization)	% **8**[a] (N-arylation)
1	 **9**	HBTU/HOBt	75	25	NA
2	**9**	CDMT	25	0	75
3	**9**	DMTMM	97.5	0.5	2
4	**9**	BOP-Cl	94.5	1.5	NA[b]
5	 **10**	HBTU/HOBt	53	47	NA
6	**10**	DMTMM	90	0	10

[a] Product distribution was determined by HPLC (UV absorption, area normalization) analysis of the reaction mixture or of the subsequent 'Boc-free' mixture after TFA treatment.
[b] A different, un-identified by-product (4%) was observed.

entry 1). Recently, we reported a peptide synthesis protocol that can significantly reduce racemization using 2-chloro-4,6-dimethoxy-1,3,5-triazine (CDMT, **2b**) in a one-pot, one-step procedure.[6] This methodology was applied to the amide formation between **9** and **5** and yielded racemization-free **6**. However, a serious side reaction involving N-arylation of amine **4** and CDMT contributed to the formation of **8** as the major product in 75% HPLC yield (Table 1, entry 2).

To suppress formation of **8**, we decided to probe the same coupling reaction using 4-(4,6-dimethoxy-1,3,5-triazine-2-yl)-4-methyl-morpholinium chloride (DMTMM, **3**), an analogue of CDMT. Since the discovery of DMTMM by Kaminski a decade ago,[7] several laboratories demonstrated its utility for the synthesis of esters,[8] amides,[9] and simple peptides.[10] DMTMM is a white solid, commercially available, and stable under certain conditions.[11] From a 'green' chemistry point of view, DMTMM is attractive. First, the coupling reactions can be performed in the presence of water.[12] Secondly, the byproduct 2-hydroxy-4,6-dimethoxy-1,3,5-triazine (**2a**), generated during the reaction, can be easily separated and recycled for DMTMM manufacturing.[11] Since DMTMM can tolerate water or alcohol,[12] peptide synthesis involving serine does not require the protection of its hydroxyl functionality.[10] In a case study, Kunishima reported the suppression of racemization for a tripeptide synthesis involving Z(OMe)-Gly-L-Ala-OH and H-L-Phe-Obzl.[13] Despite all the advantages mentioned above, DMTMM has not been used extensively for peptide syntheses. Here, we disclose another attractive utility of DMTMM, especially for the synthesis of sterically-hindered peptidomimetics.

Gratifyingly, employing DMTMM (instead of CDMT) did afford the desired peptide **6** in 97.5% yield, along with 0.5% of diastereo-

Table 2
Coupling of acid **5** with various sterically-hindered amine amines

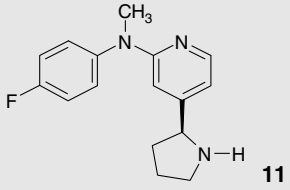
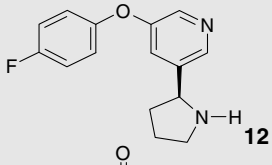
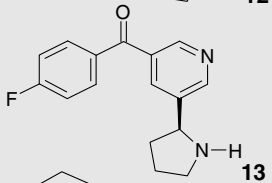
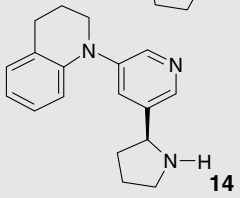
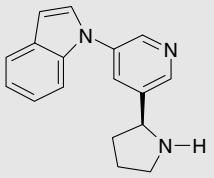
Entry	Amines	% **6**[a] (isolated)	% **7**[a] (racemization)	% **8**[a] (N-arylation)
1	 **11**	96 (95)	3	1
2	 **12**	>97 (82)	<1.0	2
3	 **13**	91 (77)	5	4
4	 **14**	92 (79)	0	8

Table 2 (*continued*)

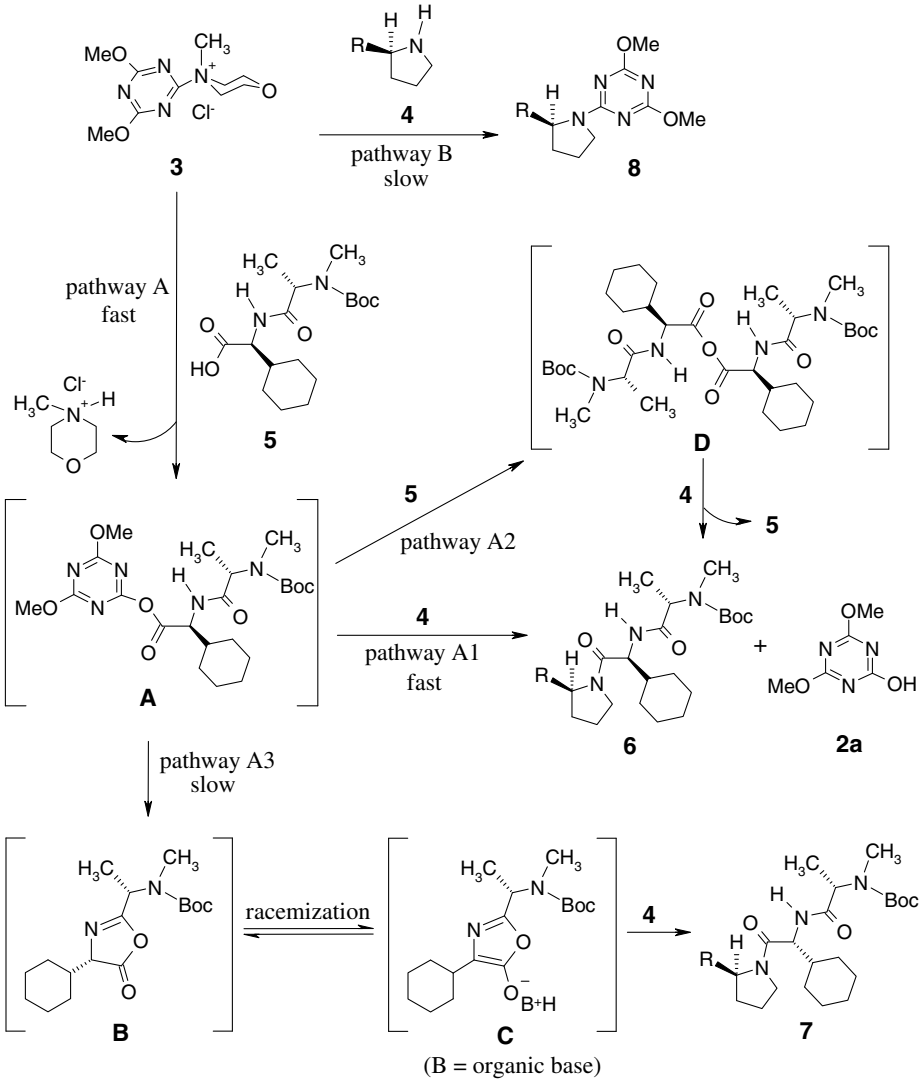
Entry	Amines	% **6**[a] (isolated)	% **7**[a] (racemization)	% **8**[a] (N-arylation)
5	 **15**	88 (73)	0	12

[a] Product distribution was determined by HPLC (UV absorption, area normalization) analysis of the reaction mixture or of the subsequent 'Boc-free' mixture after TFA treatment.

mer **7** and 2% of **8** (N-arylation) (Table 1, entry 3). A comparison study was performed using bis(2-oxo-3-oxazolidinyl)phosphinic chloride (BOP-Cl) as the coupling reagent, which is known for suppressing racemization on peptide synthesis involving sterically-hindered amines.[14] Coupling amine **9** with acid **5** using BOP-Cl, we observed 1.5% racemization (Table 1, entry 4), which is comparable to that of DMTMM. In another racemization comparison study for the coupling of hindered amine **10** with acid **5**, DMTMM was significantly advantageous over HBTU/HOBt: the former contributed an undetectable amount and the latter 47% of racemization (entries 4 and 5). Encouraged by these good results, we applied DMTMM to the syntheses of several other sterically-hindered analogues to explore the generality. The results are summarized in Table 2. Among five other amines examined, racemization was determined to be in a range of 0–5%. For the competing N-arylation pathway, adduct **8** was minimized to single-digit percentages (entries 1–4). In one instance, crude product contained 12% of **8**, which was removed from **6** by chromatography without difficulty due to their intrinsic difference in Rf values, and afforded the desired amide **6** in 73% yield after purification (entry 5).

A typical experimental procedure follows. In a 100-mL, 4-necked flask equipped with a mechanical stirrer, a thermometer, and an addition funnel were charged amine **11** (1.4 g, 5 mmol), acid **5** (1.7 g, 5 mmol), and ethyl acetate (20 mL). The resulting mixture was cooled to −20 °C. DMTMM (1.5 g, 5.3 mmol) was added, and the mixture was allowed to warm to ambient temperature and stirred for an additional 45 min. Any precipitate was removed by filtration and rinsed with ethyl acetate (10 mL). The combined filtrate was washed with 0.5 M aqueous citric acid (20 mL). The organic layer was separated, concentrated under vacuum at 25 °C, and purified by chromatography (silica gel) to obtain the corresponding amide **6** as a foamy solid (2.8 g, 95% yield).[15]

A plausible pathway proposed in Scheme 1 could be used to explain the versatility of DMTMM in controlling both racemization



Scheme 1. Plausible pathways for the amide bond formation.

and N-arylation for the synthesis of sterically-hindered peptidomimetics. As described in the experimental procedure above, DMTMM (**3**), amine **4**, and acid **5** were mixed at low temperature (−20 °C) and allowed to warm to ambient temperature for the completion of reaction. Amide **6** formation presumably went through an activated-ester **A** (pathway A), which could react either with amine **4** (pathway A1) affording **6** or with acid **5** (pathway A2) furnishing symmetrical anhydride **D**.[16] Intermediate **D** could react with **4** to afford 1 equiv each of **6** and **5**, which can be recycled to generate **A** and subsequently more **6**. This 'one-stage' procedure, forming activated-ester **A** and immediately reacting with amine **4** or acid **5,** can shorten the lifetime of **A** in the solution and minimize the probability of transforming it into oxazolones **B** and **C** (pathway A3), which are responsible for the racemization of the stereogenic center.[17] Steric effect presumably is accountable for the ability of DMTMM to suppress N-arylation. Interaction of bulky DMTMM with hindered amine **4** leading to **8** (pathway B) was unfavored (relative to pathway A) due to the pair's severe steric hindrance.

In conclusion, our study demonstrated that 4-(4,6-dimethoxy-1,3,5-triazine-2-yl)-4-methyl-morpholinium chloride (DMTMM) is a versatile coupling reagent for the synthesis of sterically-hindered peptidomimetics. It is superior to HBTU/HOBt and CDMT in controlling racemization and N-arylation, respectively.

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15. Representative analytical data of amides: (a) Amide from **9** and **5**: ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.15–7.31 (m, 5H), 6.60 (br s, 1H), 4.64 (t, J = 8.6 Hz, 0.5H), 4.50 (t, J = 8.5 Hz, 0.5H), 4.30 (m, 0.5H), 4.06 (m, 0.5H), 3.72 (q, 9.5 Hz, 0.5H), 3.55–3.65 (m, 1H), 3.45 (m, 0.5H), 3.30 (m, 0.5H), 3.13 (m, 0.5H), 2.75–2.90 (m, 6H), 2.55–2.70 (m, 3H), 2.42 (m, 0.5H), 2.00–2.35 (m, 3.5H), 1.60–1.90 (m, 9H), 1.50 (s, 9H), 1.32 (m, 3H), 0.95–1.25 (m, 6H). Racemization assessment was performed as follows. The Boc group of crude amide (**9** + **5**) was removed by treating with TFA and analyzed with HPLC: Zorbax SB-C18, 3 × 150 mm, 3.5-μm, 40 °C, flow rate = 0.5 mL/min, mobile phase A: CH_3CN, mobile phase B: buffer (0.05 M NaH_2PO_4, pH 2.5 with H_3PO_4), equilibrated with A:B = 10:90 for 5 min then gradient: $t_{0\,min}$ A:B = 10:90, $t_{12\,min}$ A:B = 40:60, $t_{15\,min}$ A:B = 90:10. Retention time: (*R*,*S*)-diastereomer = 8.7 min, (*S*,*S*)-diastereomer = 9.2 min. (b) Amide from **11** and **5**: ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.1 (d, J = 5.0, 1H), 7.23 (dd, J = 8.9, 5.0 Hz, 2H), 7.1 (t, J = 8.9 Hz, 2H), 6.40 (dd, J = 5.4, 1.3 Hz, 1H), 6.30 (s, 1H), 4.91 (dd, J = 8.2, 4.1 Hz, 1H), 4.60 (dd, J = 8.4, 7.2 Hz, 1H), 3.91 (m, 1H), 3.66 (m, 1H), 3.42 (s, 3H), 2.79 (s, 3H), 2.21 (m, 1H), 1.97 (m, 2H), 1.55–1.80 (m, 7H), 1.48 (s, 9H), 1.32 (d, J = 7.3 Hz, 3H), 1.06–1.26 (m, 4H), 0.90–1.00 (m, 2H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 171.3, 170.4, 161.3, 159.3, 159.0, 147.9, 142.7, 128.4, 128.3, 128.2, 116.6, 116.5, 110.3, 105.6, 60.7, 60.6, 55.0, 48.1, 41.0, 38.9, 35.5, 30.1, 30.0, 28.0, 26.1, 26.0, 25.8, 24.2. MS (ES+ & AP+): m/z = 596 (M+H). (c) Amide from **13** and **5**: ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.78 (d, J = 1.9 Hz, 1H), 8.67 (d, J = 2.2 Hz, 1H), 7.92 (t, J = 2.2 Hz, 1H), 7.85 (dd, J = 5.4, 3.5 Hz, 2H), 7.20 (t, J = 8.5 Hz, 2H), 5.22 (dd, J = 4.7, 3.5 Hz, 1H), 4.64 (t, J = 7.3 Hz, 1H), 4.03 (dd, J = 10.1, 7.3 Hz, 1H), 3.78–3.87 (m, 1H), 2.78 (s, 3H), 2.36–2.45 (m, 1H), 2.03–2.13 (m, 2H), 1.87–1.96 (m, 1H), 1.50–1.74 (m, 6H), 1.46 (s, 9H), 1.32 (d, J = 6.9 Hz, 3H), 0.87–1.21 (m, 6H).
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17. (a) For the discussion of racemization of peptide synthesis via oxazolone intermediate, see: *The Peptides*; Gross, E., Meienhofer, J., Eds.; Academic Press: New York, 1979; Vol. 1, Chapter 7; (b) Bailey, P. D. *An Introduction to Peptide Chemistry*; John Wiley & Sons: Chichester, 1992; pp 125–129.