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Teaching old dogs (Fmoc-amine, azodicarboxylate, and phosphine) new tricks (triazolinones)

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ABSTRACT

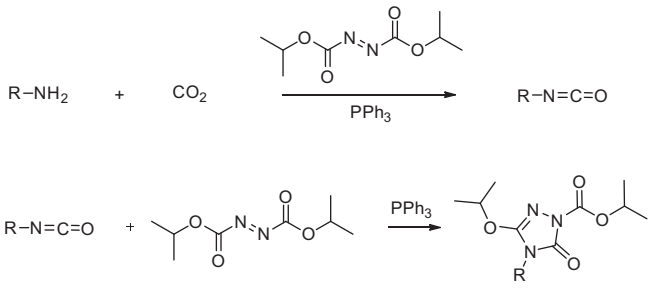
Exposure of Fmoc-amines to an excess of standard Mitsunobu reagents, azodicarboxylate and triphenylphosphine, at ambient temperature yielded triazolinone derivatives in high yield and purity. This transformation appeared to be general and the traditional Mitsunobu reaction can be carried out independently of triazolinone formation because the latter requires a high excess of reagents.

Introduction

The traditional outcome of a Mitsunobu reaction[1,2] is formation of a product from two components (unless intramolecular) via carbon–oxygen/nitrogen/carbon bond formation between an alcohol (source of the carbon) and acidic proton containing nucleophile (source of oxygen/nitrogen/carbon). Azodicarboxylates and phosphines serve as reagents without being included in the target product. However, numerous reactions involving components of either of these reagents in competitively formed products were reported, such as preparation of isocyanates (Scheme 1) from primary amines and carbon dioxide.[3,4] Syntheses of 1,2,4-triazole derivatives (Scheme 1) from isocyanates by Mitsunobu chemistry were reported by Alizadeh.[5] Herein, we report that the reaction of Fmoc protected amines with excess Mitsunobu reagents results in a clean formation of triazolinones, thus providing facile access to an interesting and useful class of heterocycles.

Results and discussion

Exposure of polymer-supported Fmoc-amines **1** to an excess of Mitsunobu reagents azodicarboxylates **2** and triphenylphosphine at ambient temperature led to the formation of an unexpected product (Scheme 2). The transformation was very clean and LC/MS analysis revealed that the product was substantially more hydrophilic than expected (shorter retention time on analytical reverse phase LC column) and lacked the Fmoc protection group (UV spectrum). We isolated and purified the product and determined that its structure is triazolinone derivative **3**. Both compounds **3a** and **3b**, prepared on Wang resin esterified with Fmoc-Ala-OH, exhibited similar ion patterns in their mass spectra (LC/MS analysis **3a**, rt 2.74 min, $[M+H]^+$ 274, $[M+NH_4]^+$ 291, $[2M+NH_4]^+$ 564, $[2M+Na]^+$ 569; LC/MS analysis **3b**, rt 3.37 min, $[M+H]^+$ 302, $[M+NH_4]^+$ 319, $[2M+NH_4]^+$ 620, $[2M+Na]^+$ 625). This pattern was found to be characteristic for all synthesized triazolinone derivatives. To access the scope and limitation of this simple and clean transformation under mild conditions we synthesized several model compounds using Fmoc-protected amino acids attached to Rink amide[6] **1a** and Wang[7] **1b** resins, respectively (Scheme 2). All reactions provided triazolinone derivatives (Table 1).



Scheme 1. Synthesis of isocyanates and triazoles using Mitsunobu reagents.

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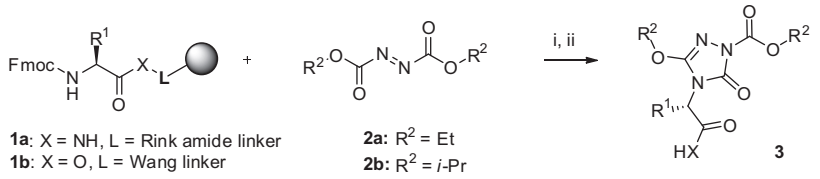


Scheme 2. Synthesis of triazolinones from polymer-supported Fmoc-amino acid, azodicarboxylate, and phosphine. Reagents and conditions: (i) PPh_3, anhydrous THF, rt, 1 h; (ii) 50% TFA in dichloromethane (DCM) (*v*/*v*), rt, 1 h.

Table 1
Synthesized triazolinones

Entry	Product	X	R^1	R^2	Purity[a] (%)	Yield[b] (%)
1	**3a**	O	$-CH_3$	$-CH_2CH_3$	87	77
2	**3b**	O	$-CH_3$	$-CH(CH_3)_2$	93	77
3	**3e**	NH	$-CH_3$	$-CH(CH_3)_2$	68	46
4	**4**	O	$-CH_3$	–Bn	61	47
5	**5**	O	$-CH_3$	$-C(CH_3)_3$	91	78

[a] Purity of the crude product before purification estimated from LC traces @ 230 nm.
[b] Total yield after purification of target compounds by semi-preparative reverse phase HPLC.

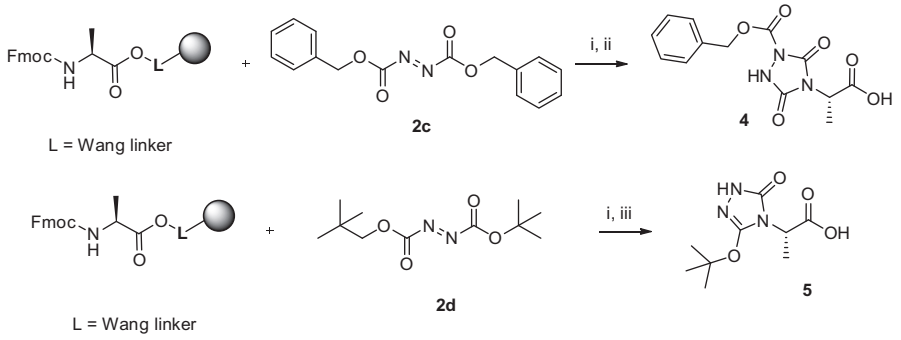
Next, we carried out the reaction with dibenzyl (**2c**) and di-*tert*-butyl (**2d**) azodicarboxylates. Whereas diethyl and di-*iso*-propyl azodicarboxylates yielded triazolinone derivatives **3**, dibenzyl azodicarboxylate formed, after acid-mediated cleavage from the resin, triazolinone derivative **4** (Scheme 3). The product prepared using di-*tert*-butyl azodicarboxylate **2d** decomposed in the cleavage cocktail (50% TFA in DCM) and it was released from the Wang resin by NaOH. The HRMS and ^1H and ^{13}C NMR spectra were consistent with triazolinone derivative **5** in which the Boc groups had been cleaved. To document that the transformations of triazolinone derivatives prepared from azodicarboxylates **2c** and **2d** to compounds **4** and **5** occurred during cleavage from the resin, we carried out experiments in solution.

Solid-phase synthesis is traditionally carried out with several-(typically three)-fold excess of reagents. Therefore, the triazolinone synthesis was advantageously carried out on solid phase since the excess of all components of the reaction mixture, except the resin-bound product, was simply removed by washing the resin beads. To address the effect of the use of excess Mitsunobu reagents and competitive traditional Mitsunobu reaction course (formation of an ester from acid and alcohol), we carried out experiments in solution. Fmoc-Ala-OH and an equimolar quantity of MeOH in anhydrous THF (both 0.1 M solution) were exposed to an excess of diisopropyl azodicarboxylate (DIAD) and PPh_3. Two molar excess of DIAD and PPh_3 (0.2 M solution) caused the expected standard Mitsunobu reaction with complete conversion of acid to an ester in 1 h, but did not exhibit the formation of any significant amount of triazolinone even after overnight reaction. Alternatively, the use of four fold excess (0.4 M solution) quantitatively converted the Fmoc-Ala-OMe to the triazolinone derivative in 1 h. This experiment confirmed that the traditional Mitsunobu reaction can be carried out independently and without competition from the formation of triazolinone by avoiding a large excess of reagents.

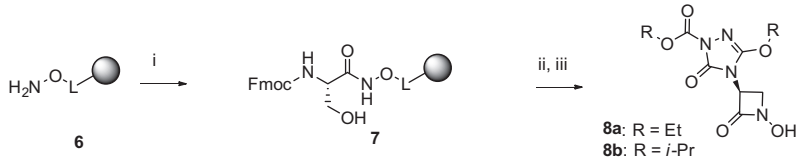
LC/MS analysis of the reaction of Fmoc-Ala-OH, MeOH, PPh_3, and azodicarboxylates **2c** and **2d** in solution revealed the presence of products **3c** and **3d** (XH=OCH_3), thus confirming that formation of **4** and **5** occurred in the cleavage cocktail used to release products from resin (LC/MS analysis **3c**, rt 7.93 min, $[M+H]^+$ 344, $[M+NH_4]^+$ 361, $[2M+NH_4]^+$ 704, $[2M+Na]^+$ 709; LC/MS analysis **3d**, rt 8.53 min, $[M+H]^+$ 412, $[M+NH_4]^+$ 429, $[2M+NH_4]^+$ 840, $[2M+Na]^+$ 845).

The high purity (61–91%) and high yield (47–78%) of the transformation to triazolinone derivatives with excess Mitsunobu reagents prompted us to explore the potential of combining the traditional course of Mitsunobu reaction with triazolinone formation on solid phase. As an example, we chose to use Miller's Mitsunobu-mediated β-lactam syntheses.[8] Thus, hydroxylamine resin **6**[9,10] was acylated with Fmoc-Ser-OH to yield resin **7** (Scheme 4). Using an excess of azodicarboxylates **2a** and **2b** and triphenylphosphine on resin **7** led to the formation of β-lactams[8] and at the same time transformation of the Fmoc-protecting group to triazolinone derivatives **8**. It is worth mentioning that the synthesis of β-lactams was previously reported on solid phase; however, the authors did not mention the formation of triazolinone.[11]

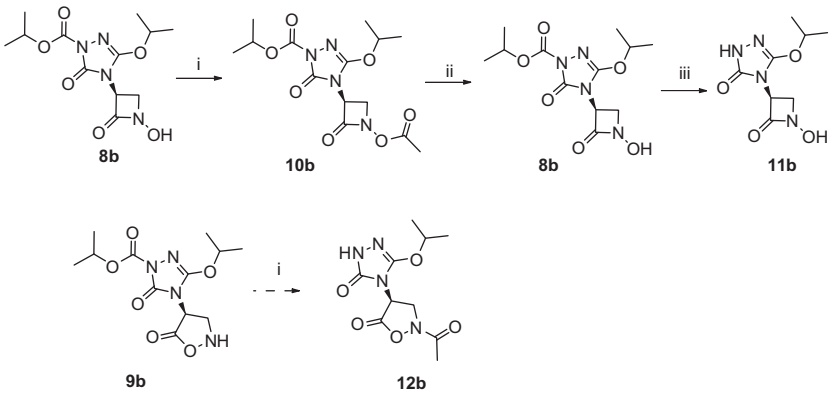
Due to the inherent instability of *N*-hydroxy-β-lactams,[12,13] especially upon exposure to strong acids and their potential rearrangement to five-membered ring isoxazolidin-5-ones **9** (Scheme 5), we acetylated product **8b** with acetic anhydride. The conversion to acetate **10b** proceeded quantitatively in 30 min (LC/MS analysis, rt 5.65 min, $[M+H]^+$ 357, $[M+NH_4]^+$ 374, $[2M+NH_4]^+$ 730, $[2M+Na]^+$ 735). Then, we adjusted the pH with sodium bicarbonate to pH 8. After overnight exposure the acetyl group was cleaved to allow clean regeneration of **8b** (LC/MS



Scheme 3. Polymer-supported synthesis of triazolinone derivatives. Reagents and conditions: (i) PPh_3, anhydrous THF, rt, 1 h; (ii) 50% TFA in DCM (*v*/*v*), rt, 1 h; (iii) 0.5 M NaOH, THF/MeOH (1:1; *v*/*v*), rt, 30 min.



Scheme 4. Concurrent synthesis of β-lactams using Miller's route and formation of triazolinones. Reagents and conditions: (i) Fmoc-Ser-OH (1 equiv), HOBt (1 equiv), *N*,*N*′-diisopropylcarbodiimide (DIC) (1 equiv), DCM/DMF (1:1, *v*/*v*), rt, 2 h; (ii) PPh_3, azodicarboxylate **2a** or **2b**, anhydrous THF, rt, 1 h; (iii) 50% TFA in DCM (*v*/*v*), rt, 1 h.

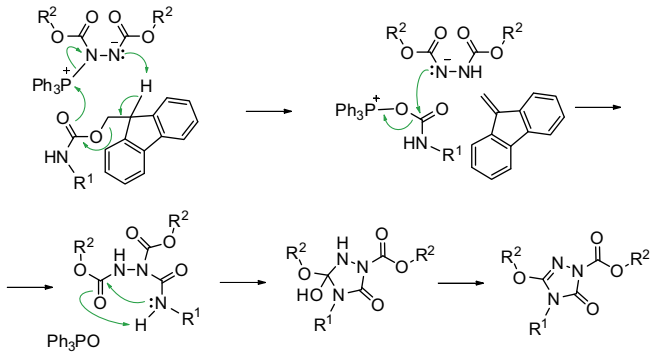


Scheme 5. Chemical evidence for structure confirmation. Reagents and conditions: (i) Ac_2O, THF, 30 min; (ii) sodium bicarbonate, water/THF, overnight; (iii) 0.5 M NaOH in MeOH/THF (1:1) to pH∼10, 15 min.

analysis, rt 3.22 min, $[M+H]^+$ 315, $[M+NH_4]^+$ 332, $[2M+NH_4]^+$ 646, $[2M+Na]^+$ 651). We also adjusted the pH of acetylated product **10b** to pH 10 with a 0.5 M solution of NaOH. After 15 min, the solution was acidified with acetic acid and analyzed. LC/MS traces revealed the presence of de-acetylated product **11b** that also had lost the *iso*-propyl carbamate group (LC/MS analysis, rt 0.91 min, $[M+H]^+$ 229, $[M+Na]^+$ 251, $[2M+Na]^+$ 479). The loss of the *iso*-propyloxycarbonyl group in NaOH has already been demonstrated in our previous experiments. This result provided evidence that product **8b** is the β-lactam, because the *N*-acetyl derivative of the rearranged compound, **12b**, would not be readily de-acetylated under these conditions.

A plausible mechanism for the formation of triazolinone is outlined in Scheme 6. The presence of PPh_3 is essential; in the absence of PPh_3 no transformation to triazolinone derivative was observed. Synthesis of analogous 1,2,4-triazole derivatives from isocyanates by Mitsunobu reagents by a similar mechanism has also been reported.[4,5]

Because of druglikeness[14] of synthesized heterocycles we considered it meaningful to evaluate their potential antibacterial and cytotoxic activities. Triazolinone derivatives and ciprofloxacin as a control were tested for their antibacterial activities against various strains of Gram-positive and Gram-negative bacteria using agar diffusion assays. None of the compounds exhibited significant inhibition at 2 mM concentration. Antiproliferative and cytotoxic activities were tested using the cell lines MCF7 (breast cancer cell line), and PC3 (prostate cancer cell line) for antiproliferative effects and HeLa (human cervix carcinoma) for cytotoxic effects. Only marginal inhibition was observed at 20 μM concentration (Table 2). Details of assays were described in our previous publication.[15]



Scheme 6. Consistent mechanism for triazolinone formation.

Table 2
Inhibition activity of synthesized compounds at 20 μM concentration

Entry	Compound	MCF7 (%)	PC3 (%)	HeLa (%)
1	**3a**	4	11	2
2	**3b**	25	12	0
3	**3e**	6	11	10
4	**4**	15	12	9
5	**5**	15	15	4
6	**8a**	10	16	1
7	**8b**	0	14	17

To conclude, Fmoc-amines were converted to 4-alkyl-3-alkoxy-5-oxo-4,5-dihydro-1*H*-1,2,4-triazole-1-carboxylates with an excess of Mitsunobu reagents azodicarboxylate and triphenylphosphine.[16] This transformation requires high excess of reagents and does not interfere with potential formation of traditional Mitsunobu products. Thus, control of stoichiometry allows controlled product variation and heterocycle generation.

Acknowledgements

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.tetlet.2013.06.115.

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16. Triazolinone synthesis: Resin **1** (250 mg) in a 5 mL plastic reaction vessel was washed 3× with anhydrous THF. A solution of PPh_3 (2 mmol, 525 mg) in 2.5 mL of anhydrous THF was then added to the reaction vessel followed by addition of azodicarboxylate (2 mmol). The resin slurry was shaken at rt for 1 h. The resin was washed 5× with THF and 3× with DCM. Cleavage and isolation: *Acid-mediated cleavage*: Triazolinones on Wang or Rink resins (250 mg) were treated with 3 mL of 50% TFA in DCM at rt for 1 h. The TFA solution was collected. The resin was washed 3× with 50% TFA in DCM, and the combined extracts were evaporated by a stream of nitrogen gas. The residual products were purified by semi-preparative reverse phase HPLC in a gradient of acetonitrile (10–40%) in 10 mM ammonium acetate buffer. *NaOH-mediated cleavage*: Triazolinones on Wang resin (250 mg) were treated with 2.5 mL of a 0.5 M solution NaOH in MeOH/THF (1:1; *v*/*v*) for 30 min. The solution was collected. The resin was washed 3× with MeOH and the combined extracts were neutralized by glacial acetic acid, concentrated and the products were purified by semi-preparative reverse phase HPLC in a gradient of (10–40%) acetonitrile in 10 mM ammonium acetate buffer.