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Direct Synthesis of *N*-Functionalized Dipropargylamine Linkers as Models for Use in Peptide Stapling

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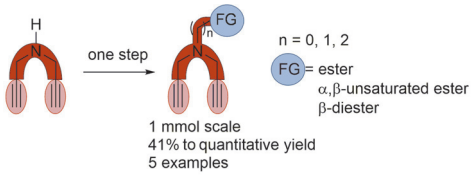

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Abstract *N*-Substituted dipropargylamines that are suitable as functionalized linkers for peptide stapling can be synthesized in one step under mild conditions from commercially available starting materials (41% to quantitative yield).

Key words peptides, stapling, linkers, amines, alkynes, click chemistry

Cyclic peptides are emerging as a new class of drugs, alongside small molecules and proteins, because of their ability to inhibit protein–protein interactions (PPI).[1] The conformational rigidity created by cyclization often confers cyclic peptides better pharmacological properties than their linear analogues such as higher binding affinity for the biological target, higher resistance to proteolytic degradation, and higher cell permeability. One of the most versatile techniques available for peptide cyclization is two-component peptide stapling, in which the side chains of two amino acid residues in a linear peptide are covalently linked by a bifunctional linker (Figure 1, a).[2–10] Stapling usually results in the generation of an α-helix[2–6] which is responsible for the increased biological activity,[11] although biologically active stapled peptides having non-helical conformation are known.[7–10] A widely used reaction that allows two-component peptide stapling is the Cu-catalyzed azide–alkyne cycloaddition between two side-chain azido groups and a terminal dialkyne (Figure 1, b).[12] Linear dialkynyl linkers of various length and conformational flexibility can be used, based on the distance of the amino acid residues to be linked on the peptide chain (Figure 1, c). The introduction of a branching point along the dialkyne chain provides a chemical handle to which additional functionality can be attached (Figure 1, d).[4] For instance, fluorescent tags, affinity tags, and cell-permeable peptide chains can be added to branched linkers, improving the properties of the stapled peptide.

In this way, by reacting a single linear peptide with various functionalized linkers it is possible to obtain a library of stapled peptides with diverse properties.

Further diversity can be achieved using dialkynyl linkers bearing nitrogen in the place of carbon at the branching point. The stereochemical lability of sp^3-hybridized nitrogen atom at room temperature provides an additional degree of conformational control of the peptide. Furthermore, nitrogen protonation under physiological conditions (pH ca. 7.5) confers cationic nature to the peptide, increasing its binding affinity for the phospholipid bilayer[13] and hence cell uptake[5,9] and biological activity.[14] *N*-Substituted dipropargylamines are suitable for use in Cu-catalyzed azide–alkyne cycloadditions, because they are symmetrical and achiral and therefore avoid the formation of regioisomers and diastereomers during peptide stapling. *N*-Substituted dipropargylamines have been used as functionalized linkers for the stapling of *i*,*i*+4,[9,10] *i*,*i*+6,[8] and *i*,*i*+7[6] diazido peptides; however, they remain largely underexploited in peptide stapling. Most of the dipropargylamines available by chemical synthesis are unsuitable for two-component peptide stapling because their side chain bears either unsaturated C=C or C≡C bonds,[15] which pose a chemoselectivity issue during stapling, or highly reactive functional groups incompatible with the stapling conditions (acyl halides[16] and organotrialkoxysilanes[17]). Other dipropargylamines are compatible with stapling but have multiple functional groups on the side chain, which compromise the selectivity of linker's functionalization.[18] In this work we report the synthesis of five *N*-functionalized dipropargylamine linkers suitable for two-component peptide stapling (Figure 2).[19] Spacers of various length (0–2 carbon atoms)

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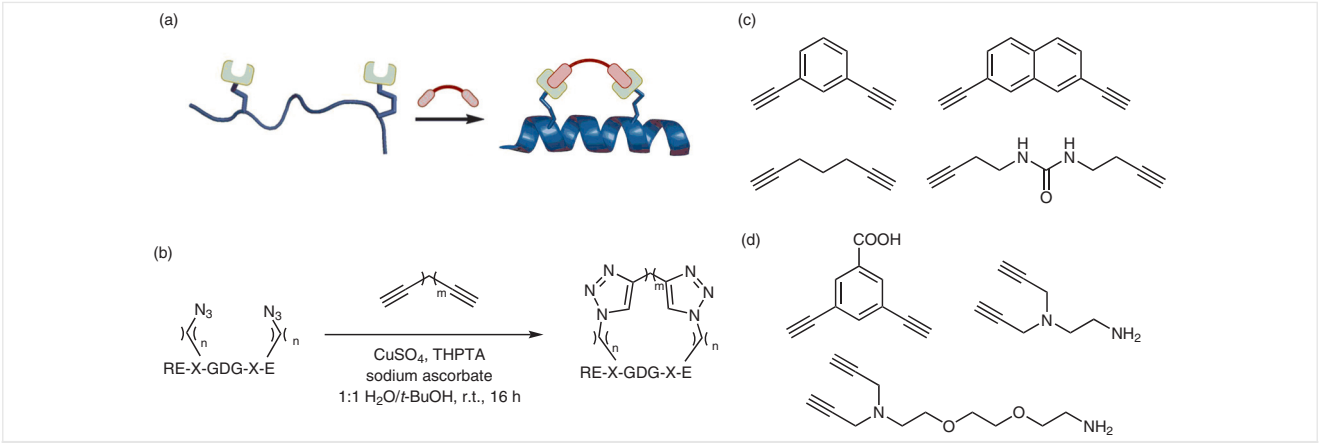


Figure 1 (a) Two-component peptide stapling. (b) Example of Cu(I)-catalyzed azide–alkyne cycloaddition reaction between peptide and linker.[9] (c) Linear dialkyne linkers. (d) Branched dialkyne linkers.

are incorporated between the nitrogen atom and the functional group on the side chain. All compounds can be obtained in one step from commercially available starting materials. This work provides a new method for the synthesis of *N*-substituted dipropargylamines, expanding the number of dialkyne linkers and hence the diversity of stapled peptides, with a significant impact on the development of new peptide PPI inhibitors.

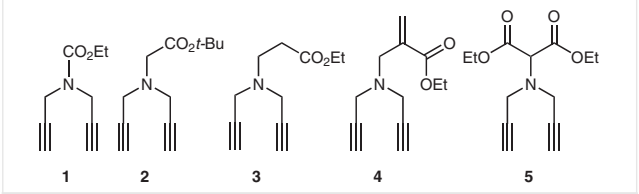
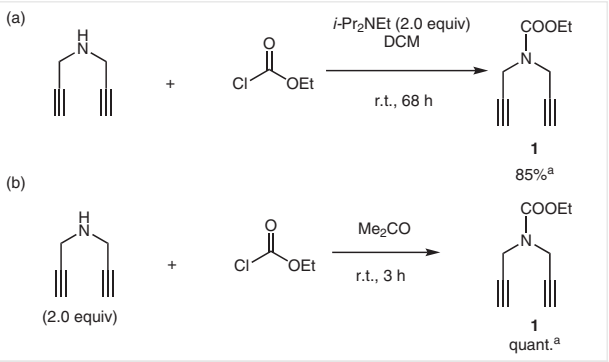


Figure 2 *N*-Substituted dipropargylamines studied in this work.

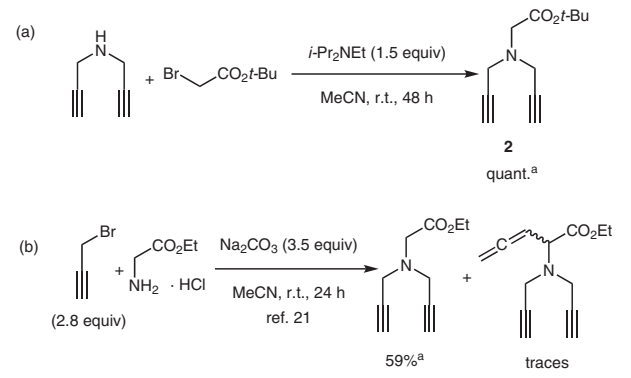
Compound **1** features the amine group protection as a carbamate, which may confer additional biological activity to the peptide or turn the peptide into a prodrug.[20] After stapling, acidic hydrolysis of carbamate **1** would regenerate the free amine, allowing either prodrug bioactivation or further functionalization through amine chemistry. Esters **2** and **3** can be further functionalized by nucleophilic acyl substitution reactions, whereas α,β-unsaturated ester **4** may undergo 1,2-addition or 1,4-conjugate addition reactions. β-Diester **5** offers the opportunity of C_α quaternization due to the H_α acidity (pK_a β-diesters = 13). Compound **5** may also undergo symmetrical chain extension by reaction of both ester groups. Alternatively, hydrolysis of a single ester moiety followed by decarboxylation would provide a monoester analogous to **2**.

Compound **1** was obtained by nucleophilic acyl substitution of ethyl chloroformate with dipropargylamine at room temperature (Scheme 1). The use of a stoichiometric amount of dipropargylamine and two equivalents of *i*-Pr_2NEt in dichloromethane resulted in limited conversion of starting materials (85% yield), even after prolonged reaction time (68 h; Scheme 1, a). The reaction could be made quantitative in a shorter time (3 h) by using two equivalents of dipropargylamine in the absence of tertiary base (Scheme 1, b). The use of acetone (polar aprotic solvent) ensured the precipitation of the ammonium salt by-product, shifting the reaction equilibrium to completion.



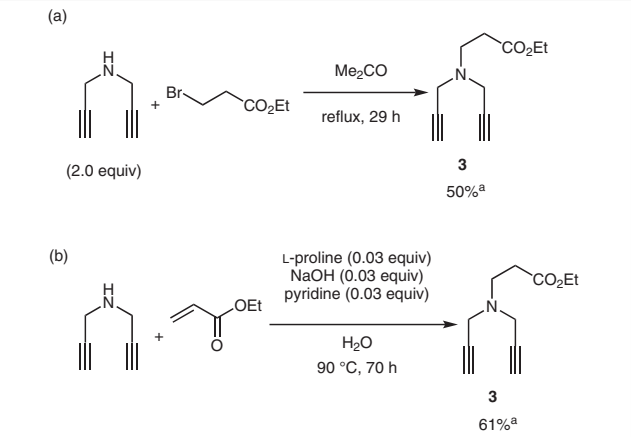
Scheme 1 Synthesis of **1** from dipropargylamine and ethyl chloroformate (a) in the presence and (b) in the absence of *i*-Pr_2NEt. [a] Isolated yield.

Compound **2** was obtained by nucleophilic substitution of *t*-butyl bromoacetate with dipropargylamine in the presence of *i*-Pr_2NEt (1.5 equiv) in acetonitrile at room temperature (Scheme 2, a). An alternative methodology, based on alkylation of glycine ester with propargyl bromide (Scheme 2, b),[21] uses cheaper starting materials but affords lower yield, partly due to the formation of a trilakylated byproduct containing an allene group attached at the α-position. The present methodology affords the product quantitatively under similar conditions in a comparable time.[22]



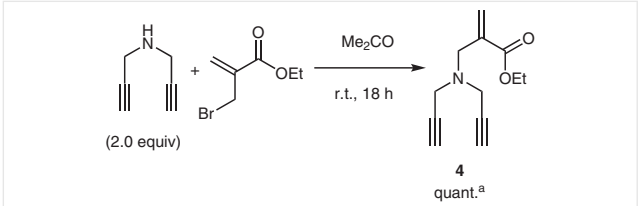
Scheme 2 Synthesis of **2** by (a) alkylation of dipropargylamine, and (b) dipropargylation of glycine ester. [a] Isolated yield.

Treatment of ethyl 3-bromopropanoate with dipropargylamine under the same conditions used for **1** (1.5 equiv tertiary base in acetonitrile) resulted into no reaction, even under prolonged reflux (66 h). An alternative methodology based on the Zn-mediated alkylation of secondary amines in THF, which has been applied successfully to piperazines and morpholine,[23] yielded no reaction in the case of dipropargylamine. The use of 2.0 equivalents of nucleophile in acetone in the absence of other bases afforded product **3** in 50% yield, leaving the amine mostly unreacted (Scheme 3, a). No improvement was obtained by addition of KI (0.1 equiv) in an attempt to convert the 3-bromoester into a more reactive 3-iodoester. Slightly higher yield (61%) was obtained by 1,4-conjugate addition of dipropargylamine to ethyl acrylate in water at 90 °C, using catalytic amounts of pyridine and sodium L-prolinate (Scheme 3, b).[24] The lower yield of **3** with respect to **2** can be attributed to the lower reactivity of 3-haloesters with respect to 2-haloesters in nucleophilic substitutions due to the longer distance of the electrophilic center from the electron-withdrawing carboxylate group.



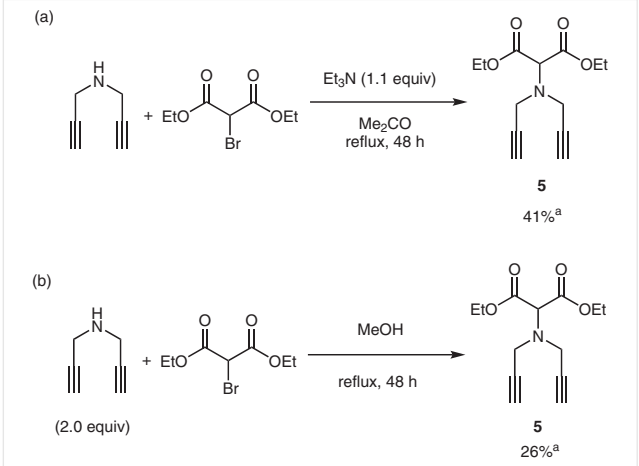
Scheme 3 Synthesis of **3** by (a) nucleophilic substitution of ethyl 3-bromopropanoate with dipropargylamine, and (b) 1,4-conjugate addition of dipropargylamine to ethyl acrylate. [a] Isolated yield.

In line with this trend, ethyl 2-(bromomethyl)acrylate, which bears an additional positive center on the β-carbon of the α,β-unsaturated system, afforded product **4** quantitatively in acetone at room temperature (Scheme 4).



Scheme 4 Synthesis of **4** by nucleophilic substitution of ethyl 2-(bromomethyl)acrylate with dipropargylamine. [a] Isolated yield.

Diethyl 2-bromomalonate proved to be less reactive than *t*-butyl bromoacetate, despite the presence of two ester groups, likely due to the larger steric hindrance at C_α (Scheme 5, a). Acetone proved to be a better solvent than acetonitrile (41% vs. 13% yield). The amination of methyl α-bromomalonate with dimethylamine (a secondary amine smaller than dipropargylamine) in methanol at 60 °C for 1 hour affords the product in 76% yield;[25] in our case, higher temperature (reflux) and longer reaction time (48 h) provided product **5** in 26% only (Scheme 5, b).



Scheme 5 Synthesis of **5** by nucleophilic substitution of diethyl 2-bromomalonate with dipropargylamine. [a] Isolated yield.

In summary, this work provides a new method for the synthesis of *N*-substituted dipropargylamines, expanding the number of dialkyne linkers and hence the diversity of stapled peptides accessible by chemical synthesis, with a significant impact on the development of new peptide drugs.

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Supporting Information

Supporting information for this article is available online at https://doi.org/10.1055/s-0039-1690217.

References and Notes

(1) Zorzi, A.; Deyle, K.; Heinis, C. *Curr. Opin. Chem. Biol.* **2017**, *38*, 24.
(2) Iegre, J.; Ahmed, N. S.; Gaynord, J. S.; Wu, Y.; Herlihy, K. M.; Tan, Y. S.; Lopes-Pires, M. E.; Jha, R.; Lau, Y. H.; Sore, H. F.; Verma, C.; O'Donovan, D. H.; Pugh, N.; Spring, D. R. *Chem. Sci.* **2018**, *9*, 4638.
(3) (a) Iegre, J.; Gaynord, J. S.; Robertson, N. S.; Sore, H. F.; Hyvönen, M.; Spring, D. R. *Adv. Ther.* **2018**, *1*, 1800052. (b) Li, X.; Zou, Y.; Hu, H.-G. *Chin. Chem. Lett.* **2018**, *29*, 1088.
(4) Lau, Y. H.; Wu, Y.; de Andrade, P.; Galloway, W. R. J. D.; Spring, D. R. *Nat. Protoc.* **2015**, *10*, 585.
(5) Lau, Y. H.; de Andrade, P.; Quah, S.-T.; Rossmann, M.; Laraia, L.; Sköld, N.; Sum, T. J.; Rowling, P. J. E.; Joseph, T. L.; Verma, C.; Hyvönen, M.; Itzhaki, L. S.; Venkitaraman, A. R.; Brown, C. J.; Lane, D. P.; Spring, D. R. *Chem. Sci.* **2014**, *5*, 1804.
(6) Zhang, L.; Navaratna, T.; Liao, J.; Thurber, G. M. *Bioconjugate Chem.* **2015**, *26*, 329.
(7) Wiedmann, M. M.; Tan, Y. S.; Wu, Y.; Aibara, S.; Xu, W.; Sore, H. F.; Verma, C. S.; Itzhaki, L.; Stewart, M.; Brenton, J. D.; Spring, D. R. *Angew. Chem. Int. Ed.* **2017**, *56*, 524.
(8) Wu, Y.; Villa, F.; Maman, J.; Lau, Y. H.; Dobnikar, L.; Simon, A. C.; Labib, K.; Spring, D. R.; Pellegrini, L. *Angew. Chem. Int. Ed.* **2017**, *56*, 12866.
(9) Xu, W.; Lau, Y. H.; Fischer, G.; Tan, Y. S.; Chattopadhyay, A.; de la Roche, M.; Hyvönen, M.; Verma, C.; Spring, D. R.; Itzhaki, L. S. *J. Am. Chem. Soc.* **2017**, *139*, 2245.
(10) Torres, O.; Yüksel, D.; Bernardina, M.; Kumar, K.; Bong, D. *ChemBioChem* **2008**, *9*, 1701.
(11) Haridas, V. *Eur. J. Org. Chem.* **2009**, 5112.
(12) Moses, J. E.; Moorhouse, A. D. *Chem. Soc. Rev.* **2007**, *36*, 1249.
(13) Hädicke, A.; Blume, A. *Biochim. Biophys. Acta, Biomembr.* **2017**, *1859*, 415.
(14) (a) Zhang, L.; Falla, T. J. *Expert Opin. Invest. Drugs* **2004**, *13*, 97. (b) Giuliani, A.; Pirri, G.; Nicoletto, S. *Cent. Eur. J. Biol.* **2007**, *2*, 1.
(15) (a) Chauvelier, J. *Bull. Soc. Chim. Fr.* **1954**, 736. (b) Walker, F. H. (Stauffer Chemical Co) FR2250740, **1975**. (c) Dai, H.; Liu, G.; Zhang, X.; Yan, H.; Lu, C. *Organometallics* **2016**, *35*, 1488. (d) Nelson, J. H.; Jonassen, H. B.; Roundhill, D. M. *Inorg. Chem.* **1969**, *8*, 2591. (e) Trofimov, B. A.; Parshina, L. N.; Lavrov, V. I.; Sobenina, L. N.; Mikhaleva, A. I.; Shevchenko, S. G.; Korostova, S. E.; Vins, V. V.; Fel'dman, V. D.; Shishov, N. I.; Solodovnikov, Yu. A. *J. Appl. Chem. USSR (Engl. Transl.)* **1992**, *65*, 337–340. (f) Jin, P.-Y.; Jin, P.; Ruan, Y.-A.; Ju, Y.; Zhao, Y.-F. *Synlett* **2007**, 3003. (g) Yamada, M.; Aoki, K. (Nippon Paint Co Ltd) US5196583, **1993**. (h) Carr, B. A.; Jadhav, V. R.; Kenski, D. M.; Tellers, D. M.; Willingham, A. T. (Sirna Therapeutics, Inc.) WO2013/165816, **2013**. (i) Tellers, D.; Colletti, S. L.; Dudkin, V.; Aaronson, J.; Momose, A.; Tucker, T. J.; Yuan, Y.; Calati, K. B.; Tian, L.; Parmar, R. G.; Shaw, A. W.; Wang, W.; Storr, R. A.; Busuek, M.; Kowtoniuk, R. A. (Sirna Therapeutics, Inc.) WO2013/166155, **2013**. (j) Tellers, D.; Colletti, S. L.; Dudkin, V.; Ikemoto, N.; Liao, H.; Zhu, M.; Yuan, Y.; Wang, L.; Truong, Q.; Shaw, A.; Pei, T.; Parish, C. (Sirna Therapeutics, Inc.) WO2013/166121, **2013**. (k) Colletti, S. L.; Tucker, T. J.; Tellers, D. M.; Kim, B.; Burke, R.; Calati, K. B.; Stanton, M. G.; Parmar, R. G.; Aaronson, J. G.; Wang, W. (Merck Sharp & Dohme Corp.) WO2015/069587, **2015**. (l) Colletti, S. L.; Tucker, T. J.; Tellers, D. M.; Kim, B.; Burke, R.; Calati, K. B.; Stanton, M. G.; Parmar, R. G.; Aaronson, J. G.; Wang, W. (Merck Sharp & Dohme Corp.) WO2015/069586, **2015**.
(16) Yanagi, A.; Watanabe, Y.; Narabu, S.-i. (Nihon Bayer Agrochem K. K.) US5767286, **1998**.
(17) Bürglová, K.; Moitra, N.; Hodacová, J.; Cattoën, X.; Man, M. W. C. *J. Org. Chem.* **2011**, *76*, 7326.
(18) (a) Hayashi, K.; Hiki, S.; Kumagai, T.; Nagao, Y. *Heterocycles* **2002**, *56*, 433. (b) Lee, K. C.; Lee, J.; Kim, J.-S.; Park, J.; Lee, Y. J.; Kim, B. I.; An, G.; Lim, S. KR101494429, **2015**. (c) Tóth, G.; Varga, Z. (Pozitron-Diagnosztika Kft.) WO2018/189560, **2018**.
(19) ***N*-Ethoxycarbonyldipropargylamine (1):** Ethyl chloroformate (56 µL, 0.54 mmol, 1.0 equiv) was added to a solution of dipropargylamine (103 µL, 1.08 mmol, 2.0 equiv) in acetone (10 mL) at room temperature. A white precipitate formed almost immediately. The mixture was stirred at room temperature for 2.5 h. After this time, TLC analysis (silica gel; PE/EtOAc = 4:1, $KMnO_4$ stain) indicated complete consumption of starting materials. The mixture was filtered over Celite® and solvent was removed under reduced pressure. The residue was dissolved in dichloromethane (20 mL) and extracted with water (2 × 20 mL). The organic layer was separated, dried over $MgSO_4$, filtered and evaporated to afford **1** (144 mg, 0.87 mmol, quant.) as a pale-yellow liquid. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.28 (t, *J* = 7.2 Hz, 3 H, CH_2CH_3), 2.24 (t, *J* = 2.4 Hz, 2 H, 2 × C≡CH), 4.19 (q, *J* = 7.2 Hz, 2 H, CH_2CH_3), 4.22 (br s, 4 H, 2 × CH_2C≡C). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 13.2, 36.3, 62.4, 73.6, 79.2, 155.2. HRMS (ESI+): *m*/*z* [M + 1] calcd for $C_9H_{12}O_2N$: 166.0863; found: 166.0865.
(20) (a) Carey, J. S.; Laffan, D.; Thomson, C.; Williams, M. T. *Org. Biomol. Chem.* **2006**, *4*, 2337. (b) Ghosh, A. K.; Brindisi, M. *J. Med. Chem.* **2015**, *58*, 2895.
(21) Lau, N. Y. H. *Dissertation*; University of Cambridge: UK, **2014**, 34.
(22) Synthesis of **2** from glycine *tert*-butyl ester and propargyl bromide is also known, but the yield was not reported, see: Mock, J. N.; Taliaferro, J. P.; Lu, X.; Patel, S. K.; Cummings, B. S.; Long, T. E. *Bioorg. Med. Chem. Lett.* **2012**, *22*, 4854.
(23) Murty, M. S. R.; Jyothirmai, B.; Krishna, P. R.; Yadav, J. S. *J. Synth. Commun.* **2003**, *33*, 2483.
(24) Pawar, S. S.; Dekhane, D. V.; Thore, S. N.; Shingare, M. S. *J. Heterocycl. Chem.* **2008**, *45*, 1869.
(25) Kral, V.; Arnold, Z. *Collect. Czech. Chem. Commun.* **1980**, *45*, 92.

