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Preorganised Dipeptide Mimics as Foldamer Building Blocks

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Preorganised dipeptide mimics containing a 3,4-disubstituted oxazolidin-2-one, a flexible methylene group, and a 1,4-disubstituted triazole have been prepared in a few steps and high yield by copper-catalysed azide–alkyne cycloaddition (CuAAC). The combination of these three moieties allows the formation of a new dipeptide mimic that favours the formation of bent conformations, as we could demonstrate by IR and ROESY analysis.

Introduction

Peptidic oligomers that contain local constrains are interesting building blocks in foldamer research.[1] In particular, cyclic constrains are useful in stabilising specific secondary structures in a wide variety of foldamers. Several examples have been reported, including 1-amino-2-carboxycycloalkanes[2] and 1-amino-2-carboxypyrrolinones.[3] We have demonstrated that the introduction of a 4-carboxy-5-methyloxazolidin-2-one (Oxd) unit into an oligomer causes a local constraint that favours the formation of secondary structures (Figure 1).[4]

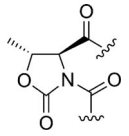

Figure 1. Preferential conformation of the imide bond in the Oxd moiety.

The oxazolidin-2-one nitrogen is connected to two carbonyl groups, and so it forms an imide moiety that is a rigid spacer. The endocyclic carbonyl group strictly imparts a *trans* conformation to the adjacent peptide bond, as the two carbonyls must lie apart from one another. As a consequence of this remarkable property, imido-type oligomers containing such monomers are forced to fold in ordered conformations, that, in combination with other kinds of interactions (hydrogen bond, non-polar interactions, etc.), lead to the formation of foldamers.[5]

Our group has extensively studied the conformational behaviour of oxazolidin-2-one homo-oligomers[6] that fold in an helical structure, similar to that adopted by poly-(L-Pro)$_n$, with *trans* tertiary peptide bonds (type II). We have also demonstrated that a wide variety of new foldamers with interesting properties may be obtained if the oxazolidin-2-one moiety is alternated with an α- or a β-amino acid from either the L or the D series.[7] For instance, a β-bend ribbon spiral structure can be attributed to Boc-(L-Ala-D-Oxd)$_5$-OH and Boc-(L-Ala-D-Oxd)$_6$-OH (Boc = *tert*-butyloxycarbonyl). It may be considered to be a subtype of the polypeptide 3_{10} helix, being stabilised by alternate 1←4 intramolecular C=O···H–N hydrogen bonds, and having approximately the same fold of the peptide chain.[8]

This outcome may be ascribed to the cooperative effect of many factors: (i) the rigidity of the –CO–N(CH<)–CO– moiety, which always tends to assume a *trans* conformation; (ii) the formation of C=O···H-$^{\alpha}$C hydrogen bonds; (iii) the alternate formation of C=O···H–N hydrogen bonds. Thus, the combination of these three effects can hold the oligomers in a well-defined, rigid conformation. This effect is possible only when a D-Oxd moiety is alternated with an L-amino acid, as an L-Oxd-L-Xaa sequence leads to the formation of random coils or other secondary structures.[7b,7d]

Herein, we describe the preparation of new preorganised dipeptide units that mimic a constrained α,β-dipeptide moiety, and also the results of some preliminary studies of their conformational behaviour. A general synthetic method leading to the formation of compounds containing two heterocyclic units, an oxazolidin-2-one (Oxd) and a 1,2,3-triazole (Tri), has been developed. Both heterocycles impart a rigid *trans* conformation to the chain, but they differ in their spatial arrangements, as the Oxd moiety is chiral and 3,4-disubstituted, whereas the Tri moiety is flat, achiral, and 1,4-disubstituted (Figure 2).

Triazoles bearing 1,4- and 1,5-substituents provide good analogues of peptide bonds because they are planar, have a strong dipole, and have hydrogen-bond accepting and donating properties that mimic those of a peptide bond.[9] Several research groups have recently reported the formation of 1,4-triazoles that are mimics of *trans* peptide bonds,[10]

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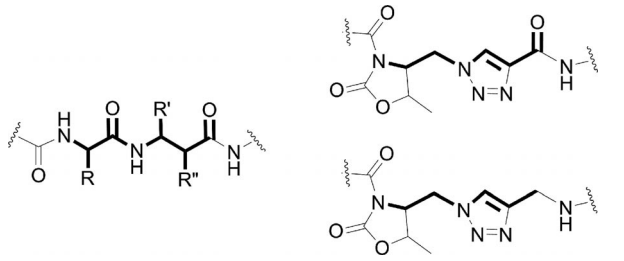


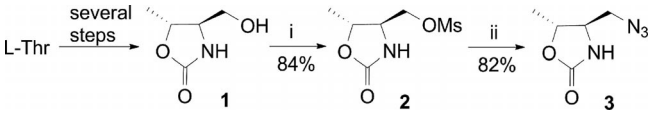
Figure 2. General structure of the L-Oxd-Tri scaffolds described in this work and a comparison with an α,β-dipeptide.

and, less frequently, the preparation of 1,5-triazoles that are mimics of *cis* peptide bonds.[11]

Results and Discussion

Synthesis

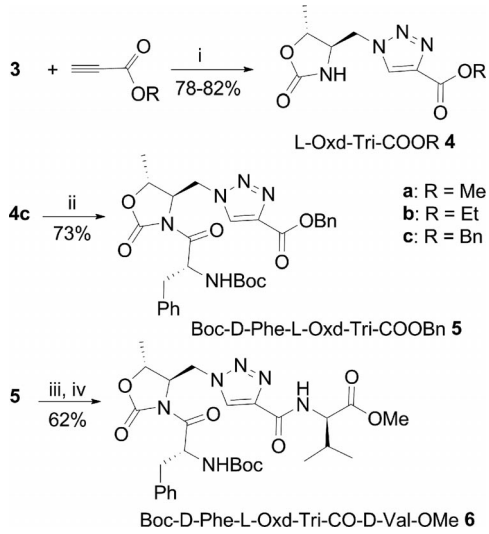
The starting material for the synthesis of both scaffolds was (4*R*,5*R*)-4-(azidomethyl)-5-methyloxazolidin-2-one **3**, which was prepared in a few steps from L-threonine (Scheme 1). (4*R*,5*R*)-4-(Hydroxymethyl)-5-methyl-oxazolidin-2-one **1** was obtained in high yield following a reported method,[12] then the hydroxy group was transformed into the corresponding azide (in **3**) by classical methods (mesylation and reaction of the derivative **2** with NaN_3 in DMF) with no need to protect the nitrogen of the carbamate moiety.



Scheme 1. Reagents and conditions: (i) Et_3N (1.5 equiv.), MsCl (1.3 equiv.), dry CH_2Cl_2, 0 °C, 2 h; (ii) NaN_3 (1.3 equiv.), dry DMF, 80 °C, 30 h.

The preparation of Boc-L-Oxd-Tri-COOR derivatives **4a**–**c** was achieved by reaction of azide **3** with three propiolate esters (methyl, ethyl and benzyl) (Scheme 2). The triazole ring could easily be prepared by Huisgen cycloaddition.[13] This reaction was first developed under thermal conditions, but mixtures of 1,4- and 1,5-substituted triazoles were usually obtained. Recently, the copper-catalysed azide–alkyne cycloaddition (CuAAC)[14] and the ruthenium-catalysed azide–alkyne cycloaddition (RuAAC)[15] have been reported; these approaches exclusively give 1,4-disubstituted or 1,5-disubstituted triazoles, respectively, in high yield.

After several attempted reactions under different conditions, we found that the reagents of choice were copper(I) iodide, diisopropylethylamine (DIEA), and lutidine.[16] Under these conditions, the reaction was quite versatile, as it

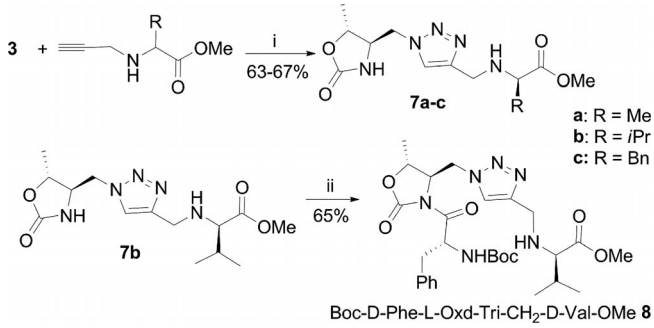


Scheme 2. Reagents and conditions: (i) propiolate (1.0 equiv.), DIEA (2.0 equiv.), lutidine (2.0 equiv.), CuI (0.1 equiv.), dry acetonitrile, room temp., 2 h; (ii) Boc-Xaa-OH (1.0 equiv.), HBTU [*O*-(benzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate; 1.1 equiv.], Et_3N (1.0 equiv.), dry acetonitrile, room temp., 50 min; (iii) H_2, Pd/C (10%) (0.1 equiv.), MeOH, room temp., 16 h; (iv) HCl·H-D-Val-OMe (1.0 equiv.), HBTU (1.1 equiv.), Et_3N (2.0 equiv.), dry acetonitrile, room temp., 50 min.

gave good yields of the products in every case. As expected, the reaction was totally regioselective, and only the 1,4-triazole derivatives **4a**–**c** were obtained in high yield.

To apply these compounds to the preparation of new oligomers, we analysed their reactivity towards acylation and acyl substitution. In a first attempt, we chose to introduce two amino acids of the D series, specifically, to alternate the L-Oxd moiety with two D-Xaa moieties to obtain the D-Xaa-L-Oxd-Tri-D-Oxd sequence. As in the study of the Oxd-Xaa series, we obtained interesting secondary structures with the L-Xaa-D-Oxd series, as mentioned above. Thus, **4c** was derivatised at both ends by standard coupling methods in high yield. It was acylated at the nitrogen with Boc-D-Phe-OH to give Boc-D-Phe-L-Oxd-Tri-CO-OBn **5**. Then the benzyl ester of **5** was quantitatively reduced to the corresponding acid, which was coupled with D-Val-OMe to give Boc-D-Phe-L-Oxd-Tri-CO-D-Val-OMe **6**.

Encouraged by these interesting results, we prepared other compounds containing the Oxd and the Tri moieties, but we replaced the carbonyl group close to the triazole ring with a more flexible methylene group. This was achieved by simply using *N*-propargyl amino acids, obtained following a known procedure from *O*-protected α-amino acids and propargyl bromide.[17] The results for the CuAAC reaction between (4*R*,5*R*)-4-(azidomethyl)-5-methyloxazolidin-2-one **3** and three *O*-methyl-*N*-propargyl amino acids under the previously reported reaction conditions are shown in Scheme 3. Compounds **7a**–**c** were obtained in good yield. Moreover, we prepared Boc-D-Phe-L-Oxd-Tri-CH_2-D-Val-OMe **8**, by acylation of **7b** with Boc-D-Phe-OH, to check the tendency of this compound to form folded structures.



Scheme 3. Reagents and conditions: (i) DIEA (2.0 equiv.), lutidine (2.0 equiv.), CuI (0.1 equiv.), dry acetonitrile, room temp., 2 h; (ii) Boc-D-Phe-OH (1.0 equiv.), HBTU (1.1 equiv.), Et_3N (1.0 equiv.), dry acetonitrile, room temp., 50 min.

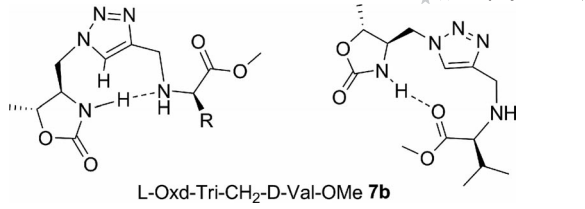


Figure 3. Two possible conformations of compound **7b** that account for the formation of a NH hydrogen bond.

Conformational Analysis

Some preliminary results were obtained from the analysis of compounds **4a**–**c** and **7a**–**c**. From the ^{1}H NMR spectra of these compounds, we observed that the proton of the NH groups of **4a**–**c** and **7a**–**c** always had chemical shifts between 6.40 and 6.90 ppm, that is, much further downfield than their usual chemical shifts, centred around 5 ppm (see for instance compounds **5**, **6** and **8**) (Table 1). This could be ascribed to the formation of an intramolecular NH hydrogen bond or to the dipolar effect of the triazole ring.

Table 1. List of the ^{1}H NMR chemical shifts and of the IR NH stretching bands of the oxazolidin-2-one NH moieties of compounds **4a–c** and **7a–c**.

Compound	^{1}H NMR NH chemical shift [ppm]	IR NH stretch [cm^{-1}]
4a	6.57	3440
4b	6.83	3440
4c	6.86	3436
7a	6.40	3444
7b	6.52	3440 and 3338
7c	6.57	3440 and 3338

Moreover, analysis of the IR spectra of these compounds can also give information about whether intramolecular hydrogen bonds are formed, since non-hydrogen-bonded amide NH bonds show stretching signals above 3400 cm^{-1}, while hydrogen-bonded amide NH bonds produce stretching bands below 3400 cm^{-1}. The FTIR spectra of **4a**–**c** and **7a**–**c** were recorded using 3 mM solutions in dichloromethane, as this low concentration avoids possible self-aggregation (Table 1).

The IR spectra of **4a**–**c** and **7a** showed only the presence of non-hydrogen-bonded amide N–H bands at about 3440 cm^{-1}, but the IR spectra of **7b**–**c** showed the presence of two bands at 3440 and 3340 cm^{-1}, which suggests that an equilibrium takes place, involving the hydrogen-bonded NH of the oxazolidin-2-one moiety and the C=O of the methyl ester or the nitrogen of the amino group. In Figure 3, the two possible conformations of **7b** consistent with the reported data are shown.

Subsequently, we analysed the preferred conformations of compounds **6** and **8** by ^{1}H NMR spectroscopy. After complete characterisation, the most interesting information was obtained from ROESY experiments using a mixing time of 0.500 s. Besides the trivial couplings, we found for both compounds a cross-peak between the C–H of the triazole ring and the *tert*-butyl group of the Boc moiety. Figures 4 and 5 show enlargements of the ROESY spectra of **6** and **8**, with the cross-peaks accounting for the interactions between the triazole C–H and the Boc *tert*-butyl group highlighted. Proposed preferred conformations of **6** and **8**, together with all the correlations from the ROESY spectra of **6** and **8**, are shown in Figure 6.

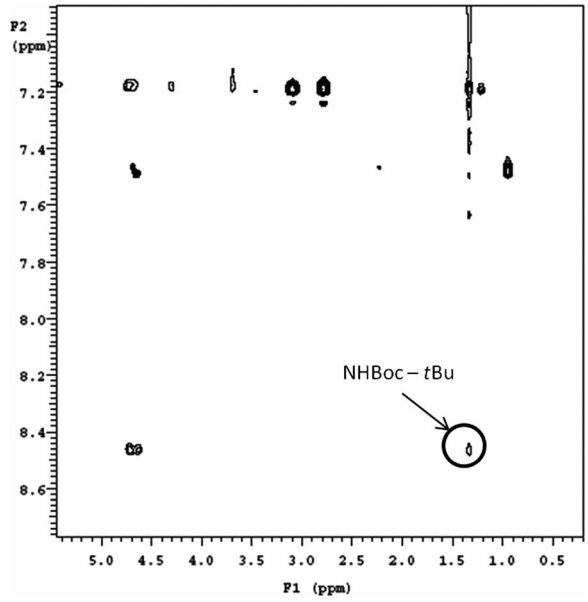


Figure 4. Enlargement of the ROESY spectrum of **6** (mixing time 0.500 s), showing the cross-peak between the triazole C–H and the Boc *tert*-butyl group.

The correlation between the triazole CH and the *tert*-butyl group in both **6** and **8**, together with the presence of an NH hydrogen bond in compounds **7b** and **7c**, suggest that the two rigid heterocycles are connected by a flexible methylene group that favours a bent conformation. This moiety can mimic a turn if it is introduced into a polypeptide chain (Figure 7). Furthermore, the formation of new foldamers may be envisaged, if homo or hybrid oligomers containing these preorganised units are prepared.

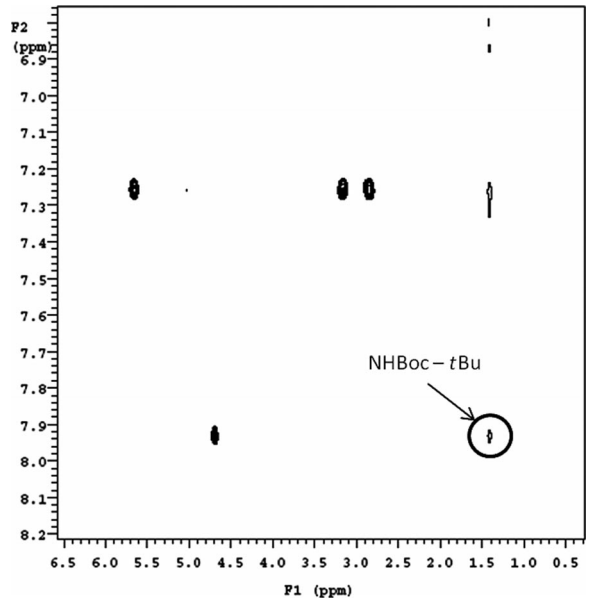


Figure 5. Enlargement of the ROESY spectrum of **8** (mixing time 0.500 s), showing the cross-peak between the triazole C–H and the Boc *tert*-butyl group.

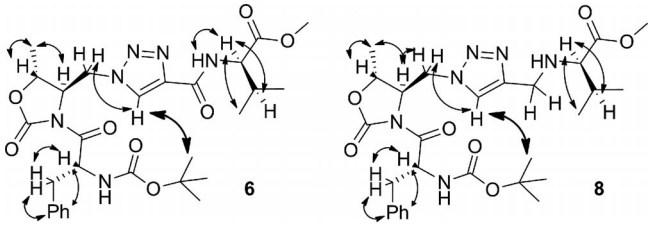


Figure 6. NOE enhancements obtained from the ROESY spectrum of **6** (10 mM solution in $CDCl_3$, mixing time 0.500 s) and the ROESY spectrum of **8** (10 mM solution in $CDCl_3$, mixing time 0.500 s).

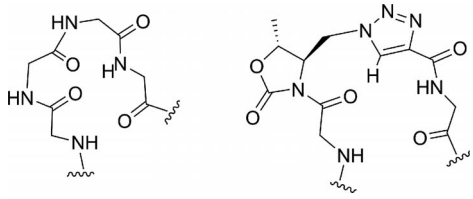


Figure 7. Proposed preferred confomation of Oxd-Tri (right), compared with a β-turn conformation (left).

Conclusions

In this work, we have reported the preparation of a new class of preorganised dipeptide mimics that are highly tunable and are easily obtained in enantiomerically pure form in a few steps and in high yield. The compounds all contain two heterocycles, a 3,4-disubstituted oxazolidin-2-one ring and a 1,4-disubstituted triazole ring, connected by a flexible methylene group. The combination of these three moieties allowed the formation of a new dipeptide mimic that favoured the formation of bent conformations, as we could demonstrate with IR and ROESY analysis.

To check whether these compounds may form new foldamers, the preparation of longer homo and hybrid oligomers containing these new building blocks is currently ongoing in our laboratory.

Experimental Section

General Methods: Melting points were determined in open capillaries. High quality infrared spectra (64 scans) were obtained at 2 cm^{-1} resolution using a 1 mm NaCl solution cell and a Nicolet 210 FTIR spectrometer. Spectra were obtained in 3 mM solutions in dry CH_2Cl_2 at 297 K. All compounds were dried in vacuo and sample preparations were performed under a nitrogen atmosphere. Routine NMR spectra were recorded with a Varian Inova 400 spectrometer at 400 (^1H) or 100 (^{13}C) MHz. The measurements were carried out in CD_3OD or $CDCl_3$. The proton signals were assigned using gCOSY spectra. Chemical shifts are reported on the δ scale, using the solvent (CD_3OD or $CDCl_3$) peak as an internal reference.

(4*R*,5*R*)-4-(Hydroxymethyl)-5-methyloxazolidin-2-one (1): For the preparation see ref.[12]. Yellow oil. $[\alpha]_D^{20}$ = +58.8 (c = 1.5, $CHCl_3$). IR (CH_2Cl_2, 3 mM): $\tilde{\nu}$ = 3452, 3280, 1760 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$): δ = 1.44 (d, J = 7.2 Hz, 3 H), 3.49–3.60 (m, 2 H, CHN-Oxd, CHN-C*H*H), 3.65–3.79 (m, 1 H, CHN-CH*H*), 4.51 (dq, J = J = 5.6 Hz, 1 H, CHO-Oxd), 6.73 (br. s, 1 H, NH) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 20.3, 60.9, 62.8, 75.6, 160.3 ppm.

[(4*R*,5*R*)-5-Methyl-2-oxooxazolidin-4-yl]methyl Methanesulfonate (2): A solution of methanesulfonyl chloride (0.3 mL, 3.5 mmol) in CH_2Cl_2 (10 mL) was added dropwise to a stirred solution of L-Oxd-CH_2-OH (0.35 g, 2.7 mmol) and triethylamine (0.55 mL, 4 mmol) in dry dichloromethane at 0 °C. Stirring was maintained for 2 h. After the end of the reaction, checked by TLC, the mixture was directly washed with brine (3 × 30 mL). The organic phase was dried with sodium sulfate and then concentrated under reduced pressure. After silica gel chromatography (cyclohexane/ethyl acetate, 40:60 → cyclohexane/ethyl acetate, 10:90 as eluant), the pure product (0.47 g, 84%) was obtained as a white solid. $[\alpha]_D^{20}$ = +48.4 (c = 1.3, $CHCl_3$). IR (CH_2Cl_2, 3 mM): $\tilde{\nu}$ = 3624, 3505, 3444, 1765 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$): δ = 1.49 (d, J = 6.4 Hz, 3 H, CHC*H*$_3$), 3.10 (s, 3 H, SO_2CH_3), 3.8 (dt, J = J = 5.2 Hz, 1 H, CHN), 4.23 (ABX, J_{AB} = 10.8, J_{AX} = 4.8, J_{BX} = 5.2 Hz, 2 H, CHN-CH*H*), 4.51 (dq, J = 6.4, J = 3.6 Hz, 1 H), 6.60 (br. s, 1 H, NH) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 20.4, 37.6, 57.8, 68.7, 75.1, 158.8 ppm. $C_6H_{11}NO_5S$ (209.22): calcd. C 34.44, H 5.30, N 6.69; found C 34.48, H 5.28, N 6.73.

(4*R*,5*R*)-4-(Azidomethyl)-5-methyloxazolidin-2-one (3): Sodium azide (0.13 g, 2 mmol) was added to a stirred solution of compound **2** (0.3 g, 1.5 mmol) in dry DMF. The mixture was stirred at reflux (80 °C) for 30 h. After the end of the reaction, checked by TLC, the DMF was replaced with ethyl acetate, and the organic phase was washed with brine (2 × 30 mL). The organic phase was separated, dried with sodium sulfate, and concentrated under reduced

pressure. After silica gel chromatography (cyclohexane/ethyl acetate, 40:60 → cyclohexane/ethyl acetate, 10:90 as eluant), the pure product (82%) was obtained as a yellow oil. $[\alpha]_D^{20}$ = +37.5 (*c* = 0.9, $CHCl_3$). IR (CH_2Cl_2, 3 mM): $\tilde{\nu}$ = 3440, 2120, 1764 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$): δ = 1.44 (d, *J* = 6.4 Hz, 3 H, CHC*H*$_3$), 3.44 (ABX, J_{AB} = 12.4, J_{AX} = 5.2, J_{BX} = 5.6 Hz, 2 H, CHN-CH*H*), 3.55 (ddd, *J* = 0.8, *J* = 5.2, *J* = 12.0 Hz, 1 H, CHN), 4.44 (dq, *J* = 6.8, *J* = 0.8 Hz, 1 H), 6.87 (br. s, 1 H, NH) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 20.4, 53.5, 58.4, 76.3, 159.2 ppm. $C_5H_8N_4O_2$ (156.14): calcd. C 38.46, H 5.16, N 35.88; found C 38.42, H 5.19, N 35.85.

General Method for the Preparation of L-Oxd-Tri-COOR (4): Diisopropylethylamine (0.56 mL, 3.2 mmol), lutidine (0.37 mL, 3.2 mmol), CuI (0.03 g, 0.16 mmol), and azide **3** (0.25 g, 1.6 mmol) were added in that order to a stirred solution of methyl, ethyl, or benzyl propiolate (1.6 mmol) in dry acetonitrile (10 mL) at room temperature. Stirring was maintained for about 2 h at room temperature, then, after checking the reaction by TLC, the acetonitrile was removed under reduced pressure and replaced with ethyl acetate. The resulting solution was washed with brine (3 × 30 mL). The organic phase was separated, dried with sodium sulfate, and concentrated under reduced pressure. The product was obtained pure after silica gel chromatography (cyclohexane/ethyl acetate, 30:70 → ethyl acetate 100% as eluant).

L-Oxd-Tri-COOMe (4a): White solid, yield: 0.30 g, 78%. M.p. = 132 °C. $[\alpha]_D^{20}$ = +62.0 (*c* = 0.9, MeOH). IR (CH_2Cl_2, 3 mM): $\tilde{\nu}$ = 3440, 1773, 1723 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$): δ = 1.48 (d, *J* = 6.4 Hz, 3 H, CHC*H*$_3$), 3.96 (s, 3 H, OCH_3), 4.05 (q, *J* = 5.2 Hz, 1 H, CHN), 4.52–4.62 (m, 3 H, CHO, CHC*H*$_2$), 6.57 (br. s, 1 H, NH), 8.28 (s, 1 H, CH-triazole) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 20.3, 52.4, 53.2, 53.4, 58.4, 75.7, 128.7, 158.0, 160.8 ppm. $C_9H_{12}N_4O_4$ (240.22): calcd. C 45.00, H 5.04, N 23.32; found C 45.07, H 5.01, N 23.35.

L-Oxd-Tri-COOEt (4b): White solid, yield: 0.33 g, 82%. M.p. = 165 °C. $[\alpha]_D^{20}$ = +60.0 (*c* = 0.9, MeOH). IR (CH_2Cl_2, 3 mM): $\tilde{\nu}$ = 3440, 1769, 1736, 1712 cm^{-1}. ^1H NMR (400 MHz, CD_3OD): δ = 1.37 (d, *J* = 8.0 Hz, 3 H, CHC*H*$_3$), 1.38 (t, *J* = 8.0 Hz, 3 H, CH_2CH_3), 3.97 (q, *J* = 5.2 Hz, 1 H, CHN), 4.37 (q, *J* = 7.2 Hz, 1 H, C*H*$_2$CH$_3$), 4.59 (dt, *J* = 9.2, *J* = 6.0 Hz, 1 H, CHO), 4.64 (d, *J* = 5.6 Hz, 2 H, C*H*$_2$CH), 8.56 (s, 1 H, CH-triazole) ppm. ^{13}C NMR (100 MHz, CD_3OD): δ = 14.5, 20.4, 53.9, 59.8, 62.3, 77.3, 130.6, 140.7, 141.0, 160.7, 161.9 ppm. $C_{10}H_{14}N_4O_4$ (254.25): calcd. C 47.24, H 5.55, N 22.04; found C 47.28, H 5.53, N 22.01.

L-Oxd-Tri-COOBn (4c): White solid, yield: 0.39 g, 78%. M.p. = 167 °C. $[\alpha]_D^{20}$ = +53.0 (*c* = 1.6, $CHCl_3$). IR (CH_2Cl_2, 3 mM): $\tilde{\nu}$ = 3436, 3284, 1765 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$): δ = 1.36 (d, *J* = 6.4 Hz, 3 H, CHC*H*$_3$), 3.95 (m, 1 H, CHN), 4.41–4.55 (m, 3 H, CHO, C*H*$_2$CH), 5.31 (AB, *J* = 12.8 Hz, 2 H, CH_2Ph), 7.03 (br. s, 1 H, NH), 7.25–7.47 (m, 5 H, Ph), 8.26 (s, 1 H, CH-triazole) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 20.2, 53.1, 58.5, 67.0, 75.7, 128.6, 128.7, 129.0, 135.2, 158.4, 160.3 ppm. $C_{15}H_{16}N_4O_4$ (316.32): calcd. C 56.96, H 5.10, N 17.71; found C 56.91, H 5.14, N 17.65.

Boc-D-Phe-L-Oxd-Tri-COOBn (5): A solution of Boc-Phe-OH (0.28 g, 1.06 mmol) and HBTU (0.44 g, 1.17 mmol) in dry acetonitrile (20 mL) was stirred under a nitrogen atmosphere for 10 min at room temperature. Then a solution of L-Oxd-Tri-OBn **4c** (0.33 g, 1.06 mmol) and Et_3N (0.3 mL, 1.06 mmol) in dry acetonitrile (15 mL) was added at room temperature. The resulting solution was stirred for 50 min under a nitrogen atmosphere, then the acetonitrile was removed under reduced pressure and replaced with ethyl acetate. The mixture was washed with brine (30 mL), HCl (1 N aq.; 30 mL), and $NaHCO_3$ (conc. aq.; 30 mL), dried with sodium sulfate, and concentrated in vacuo. After silica gel chromatography (cyclohexane/ethyl acetate, 30:70 → ethyl acetate 100% as eluant), the pure product (0.45 g, 73%) was obtained as a white solid. M.p. = 174–175 °C. $[\alpha]_D^{20}$ = –75.7 (*c* = 2.7, $CHCl_3$). IR (CH_2Cl_2, 3 mM): $\tilde{\nu}$ = 3432, 1785, 1740, 1699, 1675 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$): δ = 1.25 (d, *J* = 6.4 Hz, 3 H), 1.35 (s, 9 H, *t*Bu), 2.30 (dq, *J* = 5.2, *J* = 6.8 Hz, 1 H, *i*Pr), 2.81 (dd, *J* = 9.2, *J* = 13.6 Hz, 1 H, C*H*H-Ph), 3.12 (dd, *J* = 5.6, *J* = 13.6 Hz, 1 H, CH*H*-Ph), 4.32–4.37 (m, 1 H, CHN), 4.67–4.80 (m, 3 H, CH_2O, CHO), 4.98 (d, *J* = 6.0 Hz, 1 H, NH), 5.37 (AB, *J* = 14.0 Hz, 2 H, OC*H*$_2$Ph), 5.58–5.66 (m, 1 H, CHα-Phe), 7.21–7.44 (m, 10 H, Phe), 8.75 (s, 1 H, CH-triazole) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 20.3, 28.2, 37.2, 49.3, 54.3, 59.6, 66.7, 73.3, 80.6, 127.3, 128.2, 128.3, 128.5, 128.8, 129.3, 135.2, 140.6, 155.6, 160.2, 173.9 ppm. $C_{29}H_{33}N_5O_7$ (563.61): calcd. C 61.80, H 5.90, N 12.43; found C 61.76, H 5.87, N 12.47.

Boc-D-Phe-L-Oxd-Tri-CO-D-Val-OMe (6): Compound **5** (40 mg, 0.08 mmol) was dissolved in MeOH (20 mL) under nitrogen. Pd/C (10% w/w; 4 mg) was added under nitrogen. Vacuum was created inside the flask using the vacuum line. The flask was then filled with hydrogen using a balloon (1 atm). The solution was stirred for 16 h under a hydrogen atmosphere. After filtration through filter paper and concentration in vacuo, the free acid (35 mg, 98% yield) was obtained pure as a white solid. This compound was dissolved in dry acetonitrile (20 mL) with HBTU (33 mg, 0.09 mmol). The resulting solution was stirred under a nitrogen atmosphere for 10 min at room temperature. Then a solution of D-Val-OMe chlorohydrate (13 mg, 0.08 mmol) and Et_3N (0.03 mL, 0.24 mmol) in dry acetonitrile (15 mL) was added dropwise at room temperature. The resulting solution was stirred for 50 min under a nitrogen atmosphere, then the acetonitrile was removed under reduced pressure and replaced with ethyl acetate. The mixture was washed with brine (30 mL), HCl (1 N aq.; 30 mL), and $NaHCO_3$ (conc. aq.; 30 mL), dried with sodium sulfate, and concentrated in vacuo. After silica gel chromatography (cyclohexane/ethyl acetate, 10:90 → ethyl acetate 100% as eluant), the pure product (29 mg, 62%) was obtained as a white solid. M.p. = 130 °C. $[\alpha]_D^{20}$ = –50.0 (*c* = 1.6, CH_3OH). IR (CH_2Cl_2, 3 mM): $\tilde{\nu}$ = 3428, 1785, 1740, 1699, 1675 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$): δ = 1.01 (d, *J* = 6.8 Hz, 3 H, *i*Pr), 1.03 (d, *J* = 6.8 Hz, 3 H, *i*Pr), 1.24 (d, *J* = 7.2 Hz, 3 H, d), 1.50 (s, 9 H, *t*Bu), 2.30 (dq, *J* = 5.2, *J* = 6.8 Hz, 1 H, *i*Pr), 2.85 (dd, *J* = 8.8, *J* = 13.2 Hz, 1 H, C*H*H-Ph), 3.16 (dd, *J* = 4.4, *J* = 13.2 Hz, 1 H, CH*H*-Ph), 3.87 (s, 3 H, OCH_3), 4.34–4.40 (m, 1 H, CHN), 4.65–4.86 (m, 5 H, CH_2O, CHO, NH, CHα-Val), 5.61–5.68 (m, 1 H, CHα-Phe), 7.24–7.37 (m, 5 H, Phe), 7.55 (d, *J* = 9.2 Hz, 1 H, NH), 8.54 (s, 1 H, CH-triazole) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 17.8, 19.0, 20.4, 28.2, 31.4, 49.6, 51.2, 54.3, 56.9, 59.7, 65.8, 73.8, 127.3, 128.7, 129.3, 135.3, 143.4, 151.3, 155.5, 159.4, 171.9, 173.8 ppm. $C_{28}H_{38}N_6O_8$ (586.64): calcd. C 57.33, H 6.53, N 14.33; found C 57.28, H 6.57, N 14.29.

General Method for the Preparation of L-Oxd-Tri-CH$_2$-Xaa-OMe (7): Diisopropylethylamine (0.56 mL, 3.2 mmol), lutidine (0.37 mL, 3.2 mmol), CuI (0.03 g, 0.16 mmol), and azide **3** (0.25 g, 1.6 mmol) were added in that order to a stirred solution of *N*-propargyl amino acids **8a**–**d** (1.6 mmol) in dry acetonitrile (10 mL) at room temperature. Stirring was maintained for about 2 h at room temperature, then, after checking the reaction by TLC, the acetonitrile was removed under reduced pressure and replaced with ethyl acetate. The resulting solution was washed twice with brine (3 × 30 mL). The organic phase was separated, dried with sodium sulfate, and concentrated under reduced pressure. The product was obtained pure after silica gel chromatography (cyclohexane/ethyl acetate, 30:70 → ethyl acetate 100% as eluant).

L-Oxd-Tri-CH_2-L-Ala-OMe (7a): Yellow oil, yield: 0.31 g, 65%. $[\alpha]_D^{20}$ = –32.7 (*c* = 1.1, $CHCl_3$). IR (CH_2Cl_2, 3 mM): $\tilde{\nu}$ = 3444, 1769, 1708 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$): δ = 1.33–1.46 (m, 6 H, $CHCH_3$, $CHCH_3$), 3.59–3.7 (m, 5 H, $CHNH$, OCH_3, CH_α-Ala), 3.86–4.13 (m, 2 H, $CHHNH$, CHN), 4.39–4.60 (m, 3 H, CH_2CH, CHO), 6.40 (s, 1 H, NH), 7.78 (s, 1 H, CH-triazole) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 18.6, 19.0, 20.3, 31.5, 43.6, 51.7, 52.9, 58.8, 66.8, 75.9 ppm. $C_{12}H_{19}N_5O_4$ (297.31): calcd. C 48.48, H 6.44, N 23.56; found C 48.53, H 6.49, N 23.59.

L-Oxd-Tri-CH_2-L-Val-OMe (7b): Yellow oil, yield: 0.33 g, 63%. $[\alpha]_D^{20}$ = –15.8 (*c* = 0.7, $CHCl_3$). IR (CH_2Cl_2, 3 mM): $\tilde{\nu}$ = 3440, 3338, 1773, 1728 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$): δ = 0.90 [s, 6 H, $CH(CH_3)_2$], 1.40 (d, *J* = 6.4 Hz, 3 H, $CHCH_3$), 1.91 [m, 1 H, $CH(CH_3)_2$], 2.34 (br. s, 1 H, CH_2NH), 3.05 (br. s, 1 H, CH_α-Val), 3.59–3.98 (m, 6 H, OMe, CH_2NH, CHN), 4.38–4.54 (m, 3 H, CH_2CH, CHO), 6.52 (s, 1 H, NH), 7.59 (s, 1 H, CH-triazole) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 18.6, 19.1, 20.2, 31.5, 43.6, 51.6, 52.9, 58.8, 66.8, 75.9, 123.0, 147.1, 158.2, 175.1 ppm. $C_{14}H_{23}N_5O_4$ (325.37): calcd. C 51.68, H 7.13, N 21.52; found C 51.70, H 7.19, N 21.49.

L-Oxd-Tri-CH_2-L-Phe-OMe (7c): Yellow oil, yield: 0.40 g, 67%. $[\alpha]_D^{20}$ = –16.9 (*c* = 0.8, $CHCl_3$). IR (CH_2Cl_2, 3 mM): $\tilde{\nu}$ = 3440, 3338, 1768, 1736 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$): δ = 1.38 (d, *J* = 6.4 Hz, 3 H, $CHCH_3$), 2.36 (br. s, 1 H, CH_2NH), 2.80–3.04 (m, 2 H, CH_2Ph), 3.40–3.59 (m, 1 H, $CHNH$), 3.63 (s, 3 H, OCH_3), 3.70–4.00 (m, 3 H, CH_α-Phe, $CHHNH$, CHN), 4.30–4.50 (m, 3 H, CH_2CH, CHO), 6.57 (s, 1 H, NH), 7.11–7.38 (m, 6 H, CH-triazole, Ph) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 29.7, 39.5, 43.1, 52.0, 52.8, 62.2, 75.8, 123.4, 126.8, 128.4, 129.3, 137.1, 146.7, 158.3, 174.6 ppm. $C_{18}H_{23}N_5O_4$ (373.41): calcd. C 57.90, H 6.21, N 18.76; found C 57.86, H 6.23, N 18.79.

Boc-D-Phe-L-Oxd-Tri-CH_2-D-Val-OMe (8): Boc-D-Phe-OH (21 mg, 0.08 mmol) was dissolved in dry acetonitrile (20 mL) with HBTU (33 mg, 0.09 mmol). The resulting solution was stirred under a nitrogen atmosphere for 10 min at room temperature. Then a solution of L-Oxd-Tri-CH_2-D-Val-OMe **7b** (26 mg, 0.08 mmol) and Et_3N (0.03 mL, 0.16 mmol) in dry acetonitrile (15 mL) was added dropwise at room temperature. The resulting solution was stirred for 50 min under a nitrogen atmosphere, then the acetonitrile was removed under reduced pressure and replaced with ethyl acetate. The mixture was washed with brine (30 mL), HCl (1 N aq.; 30 mL), and $NaHCO_3$ (conc. aq.; 30 mL), dried with sodium sulfate, and concentrated in vacuo. After silica gel chromatography (cyclohexane/ethyl acetate, 10:90 → ethyl acetate 100% as eluant), the pure product (30 mg, 65%) was obtained as an oil. $[\alpha]_D^{20}$ = –43.2 (*c* = 1.2, $CHCl_3$). IR (CH_2Cl_2, 3 mM): $\tilde{\nu}$ = 3436, 1785, 1699, 1634 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$): δ = 0.93 (d, *J* = 6.8 Hz, 3 H, $CHCH_3$), 0.95 (d, *J* = 6.8 Hz, 3 H, $CHCH_3$), 1.27 (d, *J* = 6.4 Hz, 3 H, $CHCH_3$), 1.40 (s, 9 H, *t*Bu) 1.94–2.08 [m, 1 H, $CH(CH_3)_2$], 2.24–2.53 (br. s, 1 H, CH_2NH), 2.84 (dd, *J* = 9.2, *J* = 13.6 Hz, 1 H, CHH-Ph), 3.10–3.14 (m, 1 H, CH_α-Val), 3.17 (dd, *J* = 4.2, *J* = 13.6 Hz, 1 H, CHH-Ph), 3.69 (s, 3 H, OMe), 3.79 (d, *J* = 14.0 Hz, 1 H, $CHHNH$), 4.01 (d, *J* = 14.0 Hz, 1 H, $CHHNH$), 4.30–4.38 (m, 1 H, CHN), 4.69 (ABX, *J* = 3.2, *J* = 5.2, *J* = 14.4 Hz, 2 H, CH_2CH), 4.82 (dq, *J* = 2.6, *J* = 6.4 Hz, CHO), 5.07 (s, 1 H, NH), 5.59–5.71 (m, 1 H, CH_α-Phe), 7.22–7.36 (m, 5 H, Ph), 7.94 (s, 1 H, CH-triazole) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 18.6, 19.0, 20.4, 28.2, 31.3, 37.6, 43.3, 49.0, 51.7, 54.4, 59.9, 66.2, 73.8, 80.4, 124.0, 127.3, 128.7, 129.3, 135.5, 151.5, 174.0 ppm. $C_{28}H_{40}N_6O_7$ (572.66): calcd. C 58.73, H 7.04, N 14.68; found C 58.69, H 7.09, N 14.70.

Supporting Information (see footnote on the first page of this article): Copies of the ^1H NMR spectra of compounds **1**–**8**, copies of the IR spectra of compounds **1**–**8**, copies of the ROESY spectra of **6** and **8**.

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[1] a) D.-W. Zhang, X. Zhao, J.-L. Hou, Z.-T. Li, *Chem. Rev.* **2012**, *112*, 5271–5316; b) T. A. Martinek, F. Fülöp, *Chem. Soc. Rev.* **2012**, *41*, 687–702; c) I. Saraogi, A. D. Hamilton, *Chem. Soc. Rev.* **2009**, *38*, 1726–1743; d) G. Guichard, I. Huc, *Chem. Commun.* **2011**, *47*, 5933–5941.

[2] a) F. Fülöp, *Chem. Rev.* **2001**, *101*, 2181–2204; b) F. Fülöp, G. Bernath, K. Pihlaja, *Adv. Heterocycl. Chem.* **1998**, *69*, 349–477; c) T. A. Martinek, F. Fülöp, *Chem. Soc. Rev.* **2012**, *41*, 687–702; d) F. Fülöp, T. A. Martinek, G. K. Toth, *Chem. Soc. Rev.* **2006**, *35*, 323–334; e) W. S. Horne, S. H. Gellman, *Acc. Chem. Res.* **2008**, *41*, 1399–1408; f) R. P. Cheng, S. H. Gellman, W. F. DeGrado, *Chem. Rev.* **2001**, *101*, 3219–3232.

[3] A. B. Smith III, A. K. Charnley, R. Hirschmann, *Acc. Chem. Res.* **2011**, *44*, 180–193.

[4] C. Tomasini, G. Angelici, N. Castellucci, *Eur. J. Org. Chem.* **2011**, 3648–3669.

[5] a) F. Bernardi, M. Garavelli, M. Scatizzi, C. Tomasini, V. Trigari, M. Crisma, F. Formaggio, C. Peggion, C. Toniolo, *Chem. Eur. J.* **2002**, *8*, 2516–2525; b) C. Tomasini, M. Villa, *Tetrahedron Lett.* **2001**, *42*, 5211–5214.

[6] a) S. Lucarini, C. Tomasini, *J. Org. Chem.* **2001**, *66*, 727–732; b) C. Tomasini, V. Trigari, S. Lucarini, F. Bernardi, M. Garavelli, C. Peggion, F. Formaggio, C. Toniolo, *Eur. J. Org. Chem.* **2003**, 259–267.

[7] a) G. Luppi, C. Soffrè, C. Tomasini, *Tetrahedron: Asymmetry* **2004**, *15*, 1645–1650; b) C. Tomasini, G. Luppi, M. Monari, *J. Am. Chem. Soc.* **2006**, *128*, 2410–2420; c) G. Angelici, G. Luppi, B. Kaptein, Q. B. Broxterman, H.-J. Hofmann, C. Tomasini, *Eur. J. Org. Chem.* **2007**, 2713–2721; d) G. Longhi, S. Abbate, F. Lebon, N. Castellucci, P. Sabatino, C. Tomasini, *J. Org. Chem.* **2012**, *77*, 6033–6042.

[8] a) B. V. Venkataram Prasad, P. Balaram, *Int. J. Biol. Macromol.* **1982**, *4*, 99–102; b) B. Di Blasio, V. Pavone, M. Saviano, A. Lombardi, F. Nastri, C. Pedone, E. Benedetti, M. Crisma, M. Anzolin, C. Toniolo, *J. Am. Chem. Soc.* **1992**, *114*, 6273–6278; c) I. Segalas, Y. Prigent, D. Davoust, B. Bodo, S. Rebuffat, *Biopolymers* **1999**, *50*, 71–85; d) I. L. Karle, J. Flippen-Anderson, M. Sukumar, P. Balaram, *Proc. Natl. Acad. Sci. USA* **1987**, *84*, 5087–5091.

[9] a) D. S. Pedersen, A. Abell, *Eur. J. Org.* **2011**, 2399–2411; b) M. Meldal, C. W. Tornoe, *Chem. Rev.* **2008**, *108*, 2952–3015.

[10] a) N. G. Angelo, P. S. Arora, *J. Org. Chem.* **2007**, *72*, 7963–7967; b) C.-F. Wu, X. Zhao, W.-X. Lan, C. Cao, J.-T. Liu, X.-K. Jiang, Z.-T. Li, *J. Org. Chem.* **2012**, *77*, 4261–4270; c) Z. Zhang, E. Fan, *Tetrahedron Lett.* **2006**, *47*, 665–669; d) Y. L. Angell, K. Burgess, *Chem. Soc. Rev.* **2007**, *36*, 1674–1689; e) Y. Li, K. Burgess, *J. Org. Chem.* **2005**, *70*, 9595–9598; f) K. Oh, Z. Guan, *Chem. Commun.* **2006**, 3069–3071.

[11] a) J. K. Pokorski, L. M. Miller Jenkins, H. Feng, S. R. Durell, Y. Bai, D. H. Appella, *Org. Lett.* **2007**, *9*, 2381–2383; b) A. Tam, U. Arnold, M. B. Soellner, R. T. Raines, *J. Am. Chem. Soc.* **2007**, *129*, 12670–1267; c) W. S. Horne, C. A. Olsen, J. M. Beierle, A. Montero, M. R. Ghadiri, *Angew. Chem.* **2009**, *121*, 4812–4818; *Angew. Chem. Int. Ed.* **2009**, *48*, 4718–472.

[12] G. S. Lemen, J. P. Wolfe, *Org. Lett.* **2010**, *12*, 2322–2325.

[13] a) R. Huisgen, *Pure Appl. Chem.* **1989**, *61*, 613–628; b) R. Huisgen, G. Szeimies, L. Moebius, *Chem. Ber.* **1967**, *100*, 2494–2507.

[14] a) C. W. Tornoe, C. Christensen, M. Meldal, *J. Org. Chem.* **2002**, *67*, 3057–3064; b) L. Zhang, X. G. Chen, P. Xue, H. H. Y.

Sun, I. D. Williams, K. B. Sharpless, V. V. Fokin, G. C. Jia, *J. Am. Chem. Soc.* **2005**, *127*, 15998–15999.
[15] a) M. M. Majireck, S. M. Weinreb, *J. Org. Chem.* **2006**, *71*, 8680–8683; b) L. K. Rasmussen, B. C. Boren, V. V. Fokin, *Org. Lett.* **2007**, *9*, 5337–5339; c) B. C. Boren, S. Narayan, L. K. Rasmussen, L. Zhang, H. Zhao, Z. Lin, G. Jia, V. V. Fokin, *J. Am. Chem. Soc.* **2008**, *130*, 8923–8930.
[16] T.-S. Hu, R. Tannert, H.-D. Arndt, H. Waldmann, *Chem. Commun.* **2007**, *38*, 3942–3944.
[17] a) Q. Yang, Y.-Y. Lai, W.-J. Xiao, H. Alper, *Tetrahedron Lett.* **2008**, *49*, 7334–7336; b) J. H. Cho, B. M. Kim, *Tetrahedron Lett.* **2002**, *43*, 1273–1276.

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