Chemical ligation from O-acyl isopeptides via 8- and 11-membered cyclic transition states

Siva S. Panda, a Mohamed Elagawany, a,b Hadi M. Marwani, c Eray Çalışkan, a Mirna El Khatib, a Alexander Oliferenko, a Khalid A. Alamry, c and Alan R. Katritzky* a,c

a Center for Heterocyclic Compounds, Department of Chemistry, University of Florida, Gainesville, FL 32611-7200 (USA)
b Department of Organic Chemistry, College of Pharmacy, Misr University for Science and Technology, Al-Motamayez District, P.O. Box: 77, Egypt
c Department of Chemistry, King Abdulaziz University, Jeddah, 21589, Saudi Arabia
E-mail: Katritzky@chem.ufl.edu

DOI: http://dx.doi.org/10.3998/ark.5550190.p008.632

Abstract
Unprotected O-acylated serine and O-acylated threonine isopeptides have been synthesized, and their conversion to native tripeptides and tetrapeptides by O- to N-terminus transfer investigated. Ligations involving 8- and 11-membered cyclic transition states are shown experimentally and computationally to be more favorable than intermolecular cross-ligations.

Keywords: Ligation, serine, threonine, peptide, benzotriazole

Introduction

The crucial importance of peptides in biological systems and their potent therapeutic activity1–4 has increased the demand for efficient preparative methods. Synthetic access to proteins allows changes to be made in their covalent structure and enables specific labelling of a protein.5–7

Merrifield’s linear solid-phase peptide synthesis (SPPS) is commonly used in polypeptide synthesis9 but, linear SPPS of a large polypeptide can be tedious and costly. Thus, techniques to achieve convergent synthesis from smaller polypeptide fragments are critical in terms of reducing the production costs of peptide therapeutics and the synthesis of proteins.5–7

Chemoselective ligations are increasingly the ‘key to the success’ of protein synthesis.10–13 Native chemical ligation (NCL), uniquely joins two unprotected peptide segments chemoselectively and regioselectively to afford a native peptide bond.5,7 As reported in 1953 by Wieland et al.8 NCL can join two peptide fragments through a thioester transesterification step followed by an intramolecular rearrangement involving an S- to N-acyl transfer via a cyclic
transition state resulting in the native peptide bond.\textsuperscript{7,8} The bifunctional nature of the N-terminal cysteine 1,2-mercaptoamine moiety is responsible for the observed chemoselectivity in NCL.\textsuperscript{14}

NCL development as a synthetic tool for building peptides depends on additives to increase ligation rates and yields. Ligations depend on factors such as steric demand, the exogenous thiol reactivity and the nature of the solvent.\textsuperscript{14–17} In addition, the low abundance of cysteine (1.7\% of the residues in protein sequences) is a major drawback of this methodology since NCL is restricted to Cys residues.

Our focus has been on serine and threonine, each possessing the 1,2-hydroxylamine bifunctionality\textsuperscript{14} (corresponding to the SH/NH\textsubscript{2} in cysteine) thus affording chemoselective ligation by O- to N- acyl transfer without the need of cysteine residues. Acylation of the hydroxyl groups of serine and threonine, difficult without epimerization (especially in solid-phase synthesis)\textsuperscript{18,19} was recently simplified by a solution-phase entry to enantiopure O-acyl isopeptides using acyl-benzotriazoles.\textsuperscript{20}

Our group developed ligations of S-acylated Cys-peptides,\textsuperscript{21–23} and N-acylated Trp-peptides.\textsuperscript{24} Recently we demonstrated such classic O- to N-acyl migration via various transition states in O-acylated serine and tyrosine isopeptides.\textsuperscript{25,26}

We now report an experimental and computational study of “traceless” chemical ligation involving O- to N- acyl shift (at Ser and Thr sites) with 8- and 11-membered transition states, involving neither cysteine nor an auxiliary group at the ligation site.

Results and Discussion

Monoisotripeptides 5a–g were synthesized as starting materials to study the possibility of O- to N-acyl migrations via 8-membered cyclic transition state. Compounds 5a,f were used as starting material for the synthesis of monoisotetrapeptide 9a,b for ligation studies via an 11-membered cyclic transition state.

Preparation of the monoisotripeptides 5a–g

\textit{N-(Pg-\alpha-Aminoacyl)}benzotriazoles 1a–c were coupled with L-Ser-OH 2a/L-Thr-OH 2b using a previously reported method\textsuperscript{20} to give the corresponding protected dipeptides 3a–e. Dipeptides 3a–e were O-acylated by Cbz-L-Ala-Bt 1b, Boc-L-Phe-Bt 1c, Boc-Gly-Bt 1d or Boc-L-Val-Bt 1e in the presence of diisopropylethylamine (DIPEA) to provide N-protected monoisotripeptides 4a–g (71–86\%), which after deprotection by hydrogenation with Pd/C in methanol or HCl solution in 1,4-dioxane, yielded the free monoisotripeptides 5a–g (Scheme 1, Table 1). Peptides 5a,f were used both directly for ligation studies and also as intermediates to prepare the monoisotetrapeptides 9a,b.
Scheme 1. Preparation of monoisotripeptide 5a–g.

Table 1. Preparation of monoisotripeptides

| Product 4                              | Yield (%) | Mp (°C) | Product 5 | Yield (%) | Mp (°C) |
|----------------------------------------|-----------|---------|-----------|-----------|---------|
| Cbz-L-Phe-Ser(Boc-Gly)-OH, 4a          | 86        | 88–90   | 5a        | 80        | 170–172 |
| Cbz-L-Ala-Ser(Boc-L-Phe)-OH, 4b        | 73        | 66–68   | 5b        | 79        | 150–152 |
| Cbz-L-Ala-Ser(Boc-Gly)-OH, 4c          | 75        | 58–60   | 5c        | 85        | 108–110 |
| Cbz-L-Ala-Ser(Boc-L-Val)-OH, 4d        | 71        | 73–75   | 5d        | 75        | gum     |
| Boc-L-Phe-Ser(Cbz-L-Ala)-OH, 4e        | 79        | 72–73   | 5e        | 95        | 103–104 |
| Cbz-L-Phe-Thr(Boc-Gly)-OH, 4f          | 80        | oil     | 5f        | 79        | 148–154 |
| Boc-L-Phe-Thr(Cbz-L-Ala)-OH, 4g        | 83        | 72–74   | 5g        | 92        | 93–95   |

Study of the feasibility of O→N acyl migrations via 8-membered cyclic transition state
The O- to N-acyl migration for monoisotripeptides 5a–g were studied under microwave irradiation in piperidine-DMF (20 v/v%), 50 °C, 50 W, 1 h to generate native peptides. Anhydrous conditions were chosen to minimize ester hydrolysis. HPLC–MS indicated the formation the ligated products 6a–g along with the bis-acylated product 7a–g. The results show that the nature of amino acids used has a profound effect on the yield of ligated product (Scheme 2, Table 2).

The desired O- to N-acyl transfer migration was only successful for of 5a–c, e. The retention times and fragmentation patterns of starting material and ligated products were also studied in
control experiments (HPLC−MS of pure monoisotripeptide). Thus HPLC−MS, via (−)ESI-MS/MS, confirmed that compounds 5a–c, e have different fragmentation patterns than 6a–c,e proving the formation of the intramolecular ligated product.

The effect of O- to N-acyl transfer rearrangement using serine versus threonine for the same amino acid sequence was noted, with a 57% yield of native peptide 6a (L-Ser) but none from 6f (L-Thr). Microwave irradiation for 18 h at 50 °C, 50 W in piperidine-DMF (20 v/v%) did not alter 5g significantly and gave no 6g. The addition of salt, such as NaCl, and microwave irradiation 5g in piperidine-DMF 20 v/v%, 70 °C, 50 W for 10 h ineffective in the formation of 6g.

Scheme 2. Acyl migration of O-acyl isotripeptides 5a–g.

Table 2. Chemical ligation of O-acyl isotripeptides 5a–g

| Entry | Reactant 5 | Ligated product 6 (%)a | Bis-acylated 7 (%)a |
|-------|------------|------------------------|--------------------|
| 1     | L-Phe-Ser(Boc-Gly)-OH, 5a | 57                     | 0                  |
| 2     | L-Ala-Ser(Boc-L-Phe)-OH, 5b | 78                     | 0                  |
| 3     | L-Ala-Ser(Boc-Gly)-OH, 5c | 23                     | 7                  |
| 4     | L-Ala-Ser(Boc-L-Val)-OH, 5d | 0                      | 4                  |
| 5     | L-Phe-Ser(Cbz-L-Ala)-OH, 5e | 8                      | 1                  |
| 6     | L-Phe-Thr(Boc-Gly)-OH, 5f | 0                      | 0.5                |
| 7     | L-Phe-Thr(Cbz-L-Ala)-OH, 5g | 0                      | 0                  |

aSemiquantitative determination HPLC−MS. The area of ion-peak resulting from the sum of the intensities of the [M+H]⁺ and [M+Na]⁺ ions for each compound was integrated (corrected for starting material).
Computational rationalization

Conformational preorganization has been shown\textsuperscript{27} to be important for internal ligation. In previous publications\textsuperscript{20,23} we have demonstrated this principle and shown that sterically hindered and thus poorly preorganized isopeptides gave lower yields.

The reactivity of O-acyl isopeptides 5a–g also varies with the protecting group and the nature of amino acid adjacent to the protecting group. To rationalize the reactivity patterns of 5a–g, a previously developed computational protocol\textsuperscript{27} were applied, which included a full conformational search and virtual screening based on a purposefully defined scoring function. This function $b$(N-C) is the geometrical distance between the amine nucleophile and the target ester carbon atom. Conformational searches were performed using the MMX force field (as implemented in PCModel v.9.3 software). The $b$(N-C) values are given in Table 3 for all the isopeptides. Generally the scoring function values are in qualitative but not perfect agreement with the yields. This can be explained by the presence of additional degrees of freedom. By analyzing the preorganized structures we found that the nucleophilic attack can be attenuated by hydrogen bond contact between one of the amino group protons and the oxygen atom belonging to the target carbonyl group. These contacts are characterized by bond separations $b$(H-O), which are also listed in Table 3. There is a clear relationship between the yield and the hydrogen bond strength characterized by $b$(H-O): the shorter the $b$(H-O) the lower the yield. The preorganized structures of 5b and 5f are shown in Figure 1: structure 5f forms a distinct hydrogen bond contact (designated with a dashed line) both in terms of $b$(H-O) and the N-H-O angle, whereas 5b is definitely not in a hydrogen bond configuration, since the NH bonds face away from the acceptor atom (Figure 1).

The ratio of $b$(N-C):$b$(H-O) gives an important measure of conformational preorganization for binding, as the best performing compounds 5a–c have lower $b$(N-C)/$b$(H-O) values. This NH…O=C is not the only contact that can alter the reactivity: the optimized isopeptide structures disclose additional hydrogen bond contacts capable of locking the structure in an unfavorable conformation. Such conformationally locked structures were found for 5c–g. The locking hydrogen bonds were characterized not only by bond separations, but also by the H-A…D angles (where A and D stand for acceptor and donor, respectively) are listed in Table 3. Table 3 suggests that hydrogen bond contacts are particularly strong in structures 5c,d,f. Apparently, such hydrogen bond may be supportive, as, for example, in 5c, which locks the structure in a favorable conformation, but this is not always the case, as evident from the zero yields found with 5d and 5f.

Replacement of Ser by Thr clearly reduces reactivity. This can be deduced by comparing the yields of the target product 6a–g in 5a against 5f (57\% vs. 0) and 5e against 5g (8\% vs. 0). In conformational terms, it follows that the Thr methyl group (absent in Ser) drives the N-terminus slightly farther away from the target carbonyl, which is confirmed by larger $b$(N-C) values in 5f,g compared with 5a and 5e, respectively.

Evidently, the preorganization of the starting O-acyl isotripeptides is an important factor in
the success of intramolecular long range 8-membered transition acyl transfers.

**Figure 1.** Putative NH⋯O=C hydrogen bond contact in preorganized structure 5f (right); no hydrogen bond in 5b (left).

**Table 3. Structural characteristics of preorganized conformers 5a–g**

| Structure 5 | b(N-C) (Å) | b(H-O) (Å) | Ratio | Yield 6 (%) | Acceptor | Donor | b(D-A) (Å) | θHAD (deg.) |
|------------|------------|------------|-------|-------------|----------|-------|------------|-------------|
| 5a         | 3.53       | 2.38       | 1.48  | 57          | -        | -     | -          | -           |
| 5b         | 3.48       | 2.47       | 1.41  | 78          | -        | -     | -          | -           |
| 5c         | 3.34       | 2.33       | 1.43  | 23          | Ala N    | Boc NH| 1.81       | 174         |
| 5d         | 3.26       | 2.17       | 1.50  | -           | Ala N    | Boc NH| 1.86       | 162         |
| 5e         | 3.85       | 2.21       | 1.74  | 8           | Phe O    | Cbz NH| 2.06       | 148         |
| 5f         | 3.75       | 2.06       | 1.82  | -           | Boc O    | Thr NH| 1.98       | 156         |
| 5g         | 4.25       | 2.80       | 1.52  | -           | Cbz O    | Phe NH| 2.05       | 135         |

**Preparation of the O-acyl isosotetrapeptides 9a,b**

Cbz protected monoisotetrapeptides 9a,b were synthesized in solution phase, by coupling the benzotriazolides of Cbz protected Glycine 1f with the unprotected monoisotripeptides 5a,f at 20 °C in 75–85% yield. The protecting groups of 8a,b were removed by stirring each with Pd/C in methanol in hydrogen atmosphere for 1 h to afford the unprotected monoisotetrapeptides 9a,b (Scheme 3). Compounds 8a,b and 9a,b were fully characterized by 1H, 13C NMR analysis.
Scheme 3. Synthesis of O-acyl isotetrapeptides 9a,b.

Study of the feasibility of O→N acyl migrations via an 11-membered cyclic transition state

Intermediates 9a,b underwent ligation (Scheme 4, Table 4) under basic conditions (piperidine 20 v/v% in DMF, MW 50 °C, 50 W, 1 h for 9a and 1 h, 3 h, and 6 h for 9b). Unlike the challenges of steric hindrance and poor organization for binding eight-membered cyclic transition state, the expanded eleven transition state afforded O- to N-acyl transfer to give the desired native peptide 10a. However, threonine remained a challenge in both eight and eleven ring transition states. HPLC-MS showed the formation of the expected intramolecular ligated products 10a. The retention times and fragmentation patterns of 9a was studied by control experiments (HPLC-MS of pure 9a). Different fragmentation patterns in HPLC-MS, via (−)ESI-MS/MS, were found for 9a and 10a, each of MW 466. In addition, product 10a was isolated and its structure further confirmed by HRMS.

The ligation of 9a (eleven transition state) under aqueous conditions (pH 7.6, 1 M buffer strength, MW 50 °C, 50 W, 1 h). HPLC-MS gave a small amount of the corresponding ligated product 10a, together with a major peak having MW 366 corresponding to the removal of the Boc-group either from 9a or from the ligated product 10a.
Scheme 4. Acyl migration of O-acyl isopeptides 9a,b.

Table 4. Chemical ligation of O-acyl isotetrapeptides 9a,b

| Entry | Reactant 9                  | Ligated product 10 (%)a | Bis-acylated 11 (%)a |
|-------|-----------------------------|-------------------------|----------------------|
| 1     | Gly-L-Phe-L-Ser(Boc-Gly)-OH, 9a | 99                      | 0                    |
| 2     | Gly-L-Phe-Thr(Boc-Gly)-OH, 9b | 0                       | 0                    |

*aSemiquantitative determination HPLC−MS. The area of ion-peak resulting from the sum of the intensities of the [M+H]+ and [M+Na]+ ions for each compound was integrated (corrected for starting material).

Conclusions

In summary, the chemical ligation of peptides affording O-acyl isopeptides occurs successfully without the use of cysteine or an auxiliary group. However, conformation preorganization of such peptides is critical for successful O- to N-acyl transfer to afford the corresponding native peptides.
Experimental Section

**General.** Melting points were determined on a capillary point apparatus equipped with a digital thermometer and are uncorrected. NMR spectra were recorded with TMS for $^1$H (300 MHz) and $^{13}$C (75 MHz) as an internal reference. Starting materials are available commercially and used without further purification. Reaction progress was monitored by thin-layer chromatography (TLC) and visualized by UV light. Elemental analyses were performed on a Carlo Erba EA 1108 instrument. HPLC-MS analyses were performed on reverse phase gradient Phenomenex Synergi Hydro-RP (C18): (2 x 150 mm; 4 um) + C18 guard column (2 x 4 mm), wavelength = 254 nm; flow rate 0.2 mL/min; and mass spectrometry was done with electro spray ionization (ESI).

**General procedure for the preparation of dipeptides (3a–e).** $N$-(Pg-$\alpha$-Aminoacyl)benzotriazoles (1.0 mmol) in MeCN (5 mL) was added dropwise to a solution of free amino acid (1.5-2.0 equiv) and DIPEA (3.0 equiv) in MeCN (sometimes MeCN/H$_2$O 9:1, 15 mL) at the room temperature and stirred until all the $N$-(Pg-$\alpha$-aminoacyl)benzotriazoles were consumed (12 h). MeCN was evaporated and the residue dissolved in EtOAc (50 mL) and washed with 3 N HCl (5 x 50 mL). The organic portion was dried over anhyd. Na$_2$SO$_4$, filtered and concentrated to give the desired peptide fragment. No further purification was required in all cases.

**((Benzyloxy)carbonyl)-L-phenylalanyl-L-serine (Cbz-L-Phe-L-Ser-OH, 3a).** White solid (85%); mp 156–157 °C; $^1$H NMR (CD$_3$OD) $\delta$ 8.16 (d, J 7.8 Hz, 1H), 7.38–7.20 (m, 10 H), 5.05–4.80 (m, 2H), 4.52–4.42 (m, 2H), 3.95–3.80 (m, 2H), 3.23–3.16 (m, 1H), 2.90–2.81 (m, 1H); $^{13}$C NMR (CD$_3$OD) $\delta$ 174.3, 173.2, 158.4, 138.7, 138.2, 130.5, 129.6, 129.0, 128.8, 127.8, 67.7, 63.0, 57.9, 56.2, 39.3. Anal. Calcd for C$_{20}$H$_{22}$N$_2$O$_6$: C, 62.47; H, 5.74; N, 7.25; Found: C, 62.47; H, 5.82; N, 7.21.

**(Benzyloxy)carbonyl-L-alanyl-L-serine (Cbz-L-Ala-L-Ser-OH, 3b).** White solid (81%); 195–197 °C; (lit.$^{28}$ mp 192–194 °C); $^1$H NMR (DMSO-$d_6$) $\delta$ 7.99 (d, J 7.8 Hz, 1H), 7.46 (d, J 7.8 Hz, 1H), 7.36–7.29 (m, 5H), 5.02 (s, 2H), 4.29–4.23 (m, 1H), 4.17–4.10 (m, 1H), 3.72 (dd, J 11, 5 Hz, 1H) 3.62 (dd, J 11, 4 Hz, 1H), 1.21 (d, J 7.1 Hz, 3H); $^{13}$C NMR (DMSO-$d_6$) $\delta$ 172.5, 171.9, 155.6, 137.0, 128.3, 127.8, 127.7, 65.4, 61.3, 54.6, 49.8, 18.3.

**(tert-Butyloxycarbonyl)-L-phenylalanyl-L-serine (Boc-L-Phe-L-Ser-OH, 3c).** White solid (80%); mp 63–65 °C; $^1$H NMR (CDCl$_3$) $\delta$ 7.47–7.28 (m, 5H), 5.02 (br s, 2H), 4.29–4.22 (m, 1H), 4.17–4.10 (m, 1H), 3.76–3.71 (m, 1H), 3.66–3.59 (m, 1H), 1.23–1.20 (m, 9H); $^{13}$C NMR (CDCl$_3$) $\delta$ 172.7, 172.1, 156.1, 136.3, 129.4, 128.5, 126.9, 80.8, 62.6, 55.5, 54.7, 38.7, 28.2, 28.0. Anal. Calcd for C$_{17}$H$_{24}$N$_2$O$_6$: C, 57.94; H, 6.86; N, 7.95; Found: C, 57.83; H, 7.34; N, 7.47.

**(Benzyloxy)carbonyl-L-phenylalanyl-L-threonine (Cbz-L-Phe-L-Thr-OH, 3d).** White solid (89%); mp 51–53 °C; $^1$H NMR (CD$_3$OD) $\delta$ 7.40–7.20 (m, 10H), 4.55–4.46 (m, 2H), 4.34 (br s, 1H), 4.10–4.06 (m, 1H), 3.30–3.18 (m, 1H), 2.95–2.88 (m, 1H), 1.96 (br s, 1H), 1.20–1.15 (m, 3H); $^{13}$C NMR (CD$_3$OD) $\delta$ 174.1, 173.1, 157.8, 138.1, 137.7, 130.1, 129.0, 128.5, 128.3, 127.3, 71.14, 65.4, 61.3, 54.6, 49.8, 18.3.
68.3, 67.2, 58.7, 57.4, 38.7, 20.2. HRMS m/z for C$_{21}$H$_{24}$N$_2$O$_6$ [M+Na]$^+$ calcd. 423.1527, found 423.1514.

*(tert-Butoxy carbonyl)*-L-phenylalanyl-L-threonine (Boc-L-Phe-L-Thr-OH, 3e). Yellow gel$^{29}$ (78%); $^1$H NMR (CDCl$_3$) $\delta$ 7.57 (d, $J$ 7.9 Hz, 1H), 7.25–7.19 (m, 5H), 5.70 (d, $J$ 7.5 Hz, 1H), 4.75–4.25 (m, 3H), 3.25–3.08 (m, 1H), 2.99–2.81 (m, 1H), 1.33 (s, 9H), 1.17 (d, $J$ 5.8 Hz, 3H); $^{13}$C NMR (CDCl$_3$) $\delta$ 173.4, 173.1, 156.1, 136.5, 129.5, 128.5, 126.9, 80.5, 68.1, 57.6, 28.3, 19.5.

**General procedure for the preparation of O-acetyl isopeptides 4a–g.** Compound 3 (1.0 mmol) was added to a solution of N-(Pg-α-aminoacyl)benzotriazoles (1.0 mmol) (1.0 mmol) and DIPEA (3.0 mmol) in MeCN (20 mL) at room temperature and stirred for 12 h. MeCN was evaporated and the residue dissolved in EtOAc (50 mL) and washed with 2 N HCl (3 x 50 mL). The organic portion was dried over anhyd. Na$_2$SO$_4$, filtered and concentrated to give 4a–g.

*N-((Benzoxy)carbonyl)*-L-phenylalanyl-O-((tert-butoxy carbonyl)glycyl)-L-serine (Cbz-L-Phe-L-Ser(Boc-Gly)-OH, 4a). White solid. (86%); mp 86–88 °C; $^1$H NMR (CD$_3$OD) $\delta$ 8.35 (d, $J$ 8.1Hz, 1H), 7.33–7.17 (m, 10 H), 5.01–4.98 (m, 2H), 4.77–4.73 (m, 1H), 4.57 (dd, $J$ 11.4, 3.6 Hz, 1H), 4.48–4.36 (m, 2H), 3.78 (s, 2H), 3.18 (dd, $J$ 13.8, 4.5 Hz, 1H), 2.89–2.80 (m, 1H), 1.42 (s, 9H); $^{13}$C NMR (CD$_3$OD) $\delta$ 174.3, 171.8, 158.6, 158.3, 138.6, 138.2, 130.5, 129.5, 129.0, 128.8, 127.8, 80.9, 67.7, 65.0, 57.8, 53.0, 43.0, 39.2, 28.9. Anal. Calcd for C$_{27}$H$_{35}$N$_3$O$_9$: C, 59.66; H, 6.12; N, 7.73; Found C, 59.62; H, 6.13; N, 6.96.

*N-((Benzoxy)carbonyl)*-L-alanayl-O-((tert-butoxy carbonyl)-L-phenylalanyl)-L-serine (Cbz-L-Ala-L-Ser(Boc-L-Phe)-OH, 4b). White solid (73%); mp 66–68 °C; $^1$H NMR (CDCl$_3$) $\delta$ 7.89 (br s, 2H), 7.40–7.16 (m, 10 H), 5.66–5.56 (m, 1H), 5.21–5.10 (m, 2H), 5.08–4.98 (m, 2H), 4.81–4.23 (m, 3H), 3.22–2.88 (m, 2H), 1.40–1.28 (m, 9H); $^{13}$C NMR (CDCl$_3$) $\delta$ 172.4, 172.1, 171.3, 156.4, 155.7, 136.7, 135.8, 129.3, 128. 5, 128.2, 128.1, 126.7, 80.3, 67.3, 63.7, 56.8, 51.8, 48.9, 38.3, 28.2, 17.5. Anal. Calcd for C$_{28}$H$_{35}$N$_3$O$_9$: C, 60.31; H, 6.33; N, 7.54; Found C, 60.34; H, 6.74; N, 7.37.

*N-((Benzoxy)carbonyl)*-L-alanayl-O-((tert-butoxy carbonyl)glycyl)-L-serine (Cbz-L-Ala-L-Ser(Boc-Gly)-OH, 4c). White solid (75%); mp 58–60 °C; $^1$H NMR (DMSO-d$_6$) $\delta$ 8.24 (d, $J$ 7.2 Hz, 1H), 7.45 (d, $J$ 7.5 Hz, 1H), 7.36–7.30 (m, 5H), 7.21 (t, $J$ 12.0 Hz, 1H), 5.01 (s, 2H), 4.5 (s, 1H), 4.32–4.24 (m, 2H), 4.13–4.08 (m, 1H), 3.68 (t, $J$ 10.5 Hz, 2H), 1.45–1.23 (m, 9H), 1.20 (d, $J$ 7.2 Hz, 3H); $^{13}$C NMR (DMSO-d$_6$) $\delta$ 172.7, 170.5, 170.2, 155.8, 155.6, 137.0, 128.3, 127.8, 78.3, 65.4, 63.5, 50.9, 49.8, 41.7, 28.2, 18.2. Anal. Calcd for C$_{22}$H$_{29}$N$_3$O$_9$: C, 53.96; H, 6.25; N, 8.99; Found C, 53.28; H, 6.49; N, 7.81.

*N-((Benzoxy)carbonyl)*-L-alanayl-O-((tert-butoxy carbonyl)-L-valyl)-L-serine (Cbz-L-Ala-L-Ser(Boc-Val)-OH, 4d). White solid (71%); mp 73–75 °C; $^1$H NMR (CDCl$_3$) $\delta$ 7.33–7.28 (m, 5H), 5.24 (d, 1H), 5.15–5.03 (m, 2H), 4.84 (br s, 1H), 4.54 (br s, 1H), 4.42–4.07 (m, 2H), 2.13–2.04 (m, 1H), 1.43–1.38 (m, 12H), 0.89 (dd, $J$ 16.4, 6.6 Hz, 6H); $^{13}$C NMR (CDCl$_3$) $\delta$ 173.3, 172.3, 171.5, 156.3, 136.3, 128.6, 128.3, 128.2, 80.5, 67.2, 63.8, 58.9, 51.9, 50.7, 30.9, 28.4, 18.8, 17.8. Anal. Calcd for C$_{24}$H$_{35}$N$_3$O$_9$: C, 56.57; H, 6.92; N, 8.25; Found C, 56.92; H, 7.35; N, 8.02.
O-(((Benzyloxy)carbonyl)-L-alanyl)-N-((tert-butoxycarbonyl)-L-phenylalanyl)-L-serine (Boc-L-Phe-L-Ser(Cbz-L-Ala)-OH, 4e). White solid. (79%); mp 72–73 °C; 1H NMR (CDCl3) δ 7.85 (br s, 2H), 7.36–7.16 (m, 10H), 5.65 (br s, 1H), 5.21–4.98 (m, 2H), 4.80–4.69 (m, 2H), 4.53–4.23 (m, 3H), 3.24–2.88 (m, 2H), 1.39–1.28 (m, 12H); 13C NMR (CDCl3) δ 172.4, 172.1, 171.3, 156.4, 155.7, 136.7, 135.8, 129.3, 128.5, 128.2, 128.1, 126.7, 80.3, 67.3, 63.7, 55.8, 51.8, 49.9, 38.3, 28.2, 17.5. Anal. Calcd for C28H35N3O7: C, 60.31; H, 6.33; N, 7.54; Found C, 60.05; H, 6.77; N, 7.39.

N-(((Benzyloxy)carbonyl)-L-phenylalanyl)-O-((tert-butoxycarbonyl)glycyl)-L-threonine (Cbz-L-Phe-L-Thr(Cbz-Gly)-OH, 4f). Yellow oil (80%); 1H NMR (CDCl3) δ 7.30–7.17 (m, 10H), 6.28 (br s, 3H), 5.80–5.75 (m, 1H), 5.44–5.40 (m, 1H), 5.32–5.30 (m, 1H), 5.10–4.95 (m, 2H), 4.80–4.75 (m, 1H), 4.70–4.54 (m, 2H), 3.80–3.78 (m, 2H), 3.15–3.13 (m, 1H), 3.07–2.98 (m, 1H), 2.04 (br s, 2H), 1.41 (br s, 9H), 1.27–1.23 (m, 3H); 13C NMR (CDCl3) δ 172.4, 172.2, 171.7, 171.3, 156.4, 136.3, 129.4, 128.6, 128.5, 128.2, 128.0, 127.0, 80.5, 67.2, 60.5, 56.2, 55.7, 42.3, 28.3, 17.1, 14.2. Anal. Calcd for C28H35N3O7: C, 59.04; H, 6.43; N, 7.38; Found C, 59.40; H, 6.76; N, 7.00.

O-(((Benzyloxy)carbonyl)-L-alanyl)-N-((tert-butoxycarbonyl)-L-phenylalanyl)-L-threonine (Boc-L-Phe-L-Thr(Cbz-L-Ala)-OH, 4g). White solid (83%); mp 72–74 °C; 1H NMR (CDCl3) δ 10.24 (s, 1H), 7.30–7.16 (m, 10H), 5.44 (dd, J 6.4, 3.8 Hz, 1H), 5.14–5.07 (m, 2H), 4.80 (dd, J 8.9, 3.5 Hz, 1H), 4.60–4.47 (m, 1H), 4.32–4.24 (m, 1H), 3.23–2.94 (m, 2H), 1.43–1.23 (m, 15H); 13C NMR (CDCl3) δ 173.1, 173.0, 171.8, 156.5, 156.3, 136.9, 136.3, 129.6, 128.7, 128.3, 127.0, 80.5, 71.7, 67.4, 55.8, 49.9, 38.1, 28.4, 18.6, 17.9, 17.3. Anal. Calcd for C29H37N3O7: C, 60.93; H, 6.52; N, 7.35; Found C, 61.24; H, 6.64; N, 7.55.

General Papers

For deprotection of the Boc-protecting group. Compound 4a–d, 4f and 4g (1.0 mmol) was dissolved in anhydrous MeOH (30 mL) and stirred under an atmosphere of hydrogen in the presence of a catalytic amount of Pd/C for 4 h. Filtration through a bed of celite and evaporation afforded 5a–d, 5f and 5g. Compound 4f was deprotected and used as crude to make compound 8b.

For deprotection of the Boc-protecting group. Compounds 4e and 4h (1.0 mmol) was dissolved in either HCl-dioxane (4.0 M HCl in dioxane, 15 mL) or freshly prepared HCl-MeOH (prepared by bubbling HCl in MeOH) (15 mL) and stirred for 2 h. Solvent is evaporated, and ether was added to the residue and stirred for 2 h. Filtration gave a white solid 5e and 5h (when sticky solid resulted, decantation of ether several times was performed instead).

N-(L-Phenylalanyl)-O-((tert-butoxycarbonyl)glycyl)-L-serine (L-Phe-L-Ser(Cbz-Gly)-OH 5a). White solid (80%); mp 170–172 °C; 1H NMR (CD3OD) δ 7.35–7.24 (m, 5H), 4.60–4.50 (m, 1H), 4.39 (s, 2H), 4.25–4.18 (m, 1H), 3.77 (s, 2H), 3.30–3.00 (m, 2H), 1.38 (s, 9H); 13C NMR (CD3OD) δ 174.2, 172.8, 169.5, 158.8, 135.5, 130.7, 130.3, 129.0, 81.5, 66.5, 55.9, 55.6, 43.1, 38.2, 28.8. HRMS m/z for C19H28N3O7 [M+H]+ calcd. 410.1922, found 410.1909.
N-(L-Alanyl)-O-((tert-butoxycarbonyl)-L-phenylalanyl)-L-serine (L-Ala-L-Ser(Boc-L-Phe)-OH, 5b). White solid (79%); mp 150–152 °C; 1H NMR (CD3OD) δ 7.28–7.17 (m, 5H), 4.53–4.33 (m, 4H), 3.18 (dd, J 13.9, 4.7 Hz, 1H), 2.87 (dd, J 13.9, 9.5 Hz, 1H), 1.53 (d, J 6.7 Hz, 3H), 1.36 (s, 9H); 13C NMR (CD3OD) δ 174.2, 173.4, 170.8, 157.8, 138.5, 130.5, 130.3, 129.4, 127.7, 80.6, 66.5, 56.5, 55.6, 50.4, 38.4, 28.7, 17.5. Anal. Calcd for C20H29N3O7: C, 56.73; H, 6.90; N, 9.92; Found C, 56.61; H, 7.33; N, 9.18.

N-(L-Alanyl)-O-((tert-butoxycarbonyl)glycyl)-L-serine (L-Ala-L-Ser(Boc-Gly)-OH, 5c). White solid (85%); mp; 108–110; 1H NMR (CD3OD) δ 4.42–4.30 (m, 2H), 3.85–3.83 (m, 1H), 3.63 (br s, 2H), 3.20–3.14 (m, 1H), 1.38 (d, J 7.2 Hz, 3H), 1.29 (s, 9H); 13C NMR (CD3OD) δ 174.5, 172.3, 171.0, 157.9, 80.8, 66.5, 55.7, 43.1, 28.9, 17.7. Anal. Calcd for C13H23N3O7: C, 46.84; H, 6.95; N, 12.61; Found C, 46.36; H, 7.34; N, 12.08.

N-(L-Alanyl)-O-((tert-butoxycarbonyl)-L-valyl)-L-serine (L-Ala-L-Ser(Boc-L-Val)-OH, 5d). Gum (75%); 1H NMR (CD3OD) δ 4.67–4.38 (m, 3H), 4.10–3.28 (m, 2H), 2.21–2.03 (m, 1H), 1.56 (d, J 6.1 Hz, 3H), 1.44 (s, 9H), 0.92 (dd, J 11.3, 6.8 Hz, 6H); 13C NMR (CD3OD) δ 173.6, 170.8, 158.4, 80.7, 66.2, 60.7, 50.5, 42.2, 31.8, 28.9, 19.8, 18.5. HRMS m/z for C16H29N3O7 [M+H]+ calcd. 376.2010, found 376.2039.

N-(L-Phenylalanyl)-O-(((benzyloxy)carbonyl)-L-alanyl)-L-serine (L-Phe-L-Ser(Cbz-L-Ala)-OH, 5e). White microcrystals (95%); mp 103–104 °C; 1H NMR (DMSO-d6) δ 9.15 (d, J 8.1 Hz, 1H), 8.37 (br s, 3H), 7.82 (d, J 7.2 Hz, 1H), 7.39–7.23 (m, 10H), 4.99 (dd, J 15.7, 12.6 Hz, 2H), 4.65–4.59 (m, 1H), 4.36 (dd, J 11.3, 4.7 Hz, 1H), 4.27 (dd, J 11.3, 5.9 Hz, 1H), 4.16–4.05 (m, 2H), 3.20 (dd, J 14.3, 5.7 Hz, 1H), 3.03 (dd, J 14.3, 7.5 Hz, 1H), 1.29 (d, J 7.4 Hz, 3H); 13C NMR (DMSO-d6) δ 172.6, 170.0, 168.2, 155.9, 136.9, 134.8, 129.7, 128.5, 128.4, 127.8, 127.1, 66.4, 65.6, 53.2, 51.2, 49.3, 36.7, 16.9. Anal. Calcd for C46H58N6O15: C, 54.93; H, 5.81; N, 8.35; Found C, 54.63; H, 6.27; N, 8.02.

N-(L-Phenylalanyl)-O-((tert-butoxycarbonyl)glycyl)-L-threonine (L-Phe-L-Thr(Boc-Gly)-OH, 5f). Used as crude to make compound 8b. See 8b.

N-(L-Phenylalanyl)-O-(((benzyloxy)carbonyl)-L-alanyl)-L-threonine (L-Phe-L-Thr(Cbz-L-Ala)-OH, 5g). White solid (92%); mp 93–95 °C; 1H NMR (CD3OD) δ 7.35–7.28 (m, 10H), 5.46–5.38 (m, 1H), 5.07 (s, 2H), 4.70 (d, J 5.0 Hz, 1H), 4.30 (dd, J 8.3, 5.7 Hz, 1H), 4.23–4.15(m, 1H), 3.34 (dd, J 9.8, 4.4 Hz, 1H), 3.08 (dd, J 14.3, 8.4 Hz, 1H), 1.37 (d, J 7.3 Hz, 3H), 1.31 (d, J 6.4 Hz, 3H); 13C NMR (CD3OD) δ 173.8, 171.6, 170.3, 158.6, 138.2, 135.5, 130.8, 130.2, 129.6, 129.2, 129.0, 128.8, 72.0, 67.8, 57.5, 55.7, 51.2, 38.6, 17.6, 17.5. Anal. Calcd for C48H62Cl2N6O15: C, 55.76; H, 6.04; N, 8.13; Found C, 55.59; H, 6.35; N, 7.98.

General procedure for the preparation of unprotected O-acyl isopeptides 8a–c
Compounds 8a–c were prepared by following the same procedure followed for 4a–g.

N-(Benzylxoy)carbonyl)glycyl-L-phenylalanyl-O-((tert-butoxycarbonyl)glycyl)-L-serine (Cbz-Gly-L-Phe-L-Ser(Boc-Gly)-OH, 8a). White solid (89%), converted to compound 9a after checking NMR. 8a: mp 180–181 °C (decomposed). 1H NMR (CD3OD) δ 7.39–7.22 (m, 10H), 5.08 (s, 2H), 4.75–4.63 (m, 2H), 4.57–4.49 (m, 1H), 4.43–4.37 (m, 1H), 3.79–3.66 (m, 4H),
3.30–3.06 (m, 1H), 2.97–2.77 (m, 4H), 1.42 (s, 9H); $^{13}$C NMR (CD$_3$OD) δ 177.0, 173.6, 173.4, 172.1, 171.9, 171.8, 159.1, 138.3, 130.5, 129.6, 129.1, 129.0, 127.9, 127.2, 80.9, 74.3, 68.0, 65.0, 55.7, 53.0, 45.0, 44.0, 43.0, 38.7, 28.6. HRMS m/z for C$_{20}$H$_{36}$N$_{4}$O$_{10}$ [M+H]$^+$ calcd. 601.2441, found 601.2402.

_N-(Benzzyloxy)carbonylglycyl-L-alanyl-O-((tert-butoxycarbonyl)-L-phenylalanyl)-L-serine (Cbz-Gly-L-Ala-L-Ser(Boc-L-Phe)-OH, 8b)._ Colorless oil (89%); converted to compound 9b after checking NMR. 8b: $^1$H NMR (CDCl$_3$) δ 9.70 (br s, 2H), 7.34 (d, $J$ 7.4 Hz, 1H), 7.25–7.07 (m, 10H), 6.03 (d, $J$ 7.4 Hz, 1H), 5.07–4.57 (m, 4H), 4.29–4.14 (m, 2H), 3.84–3.55 (m, 3H), 3.12–2.89 (m, 2H), 1.36 (d, $J$ 2.8 Hz, 3H), 1.32 (s, 9H); $^{13}$C NMR (CDCl$_3$) δ 171.5, 170.3, 170.0, 169.4, 155.5, 155.1, 135.1, 134.9, 128.2, 127.5, 127.4, 127.1, 127.0, 125.9, 124.4, 66.1, 62.6, 53.0, 50.9, 48.8, 42.8, 36.5, 29.3, 27.2, 16.4. HRMS m/z for C$_{30}$H$_{38}$N$_{4}$O$_{10}$ [M+H]$^+$ calcd. 615.2584, found 615.2601.

_N-(Benzzyloxy)carbonylglycyl-L-phenylalanyl-O-((tert-butoxycarbonyl)glycyl)-L-threonine (Cbz-Gly-L-Phe-L-Thr(Boc-Gly)-OH, 8c)._ Colorless oil (82%); $^1$H NMR (CD$_3$OD) δ 7.37–7.20 (m, 10H), 5.10–5.08 (m, 2H), 4.13–4.08 (m, 1H), 3.83–3.29 (m, 3H), 2.01–1.97 (m, 8H), 1.44 (br s, 9H), 1.23 (t, $J$ 7.2 Hz, 3H); $^{13}$C NMR (CD$_3$OD) δ 175.4, 174.5, 174.0, 173.8, 173.2, 138.3, 130.5, 129.6, 129.1, 129.0, 128.0, 80.7, 68.0, 67.8, 61.7, 55.1, 45.0, 43.3, 42.9, 38.9, 38.5, 28.8, 21.0, 20.9, 14.6. HRMS m/z for C$_{21}$H$_{30}$N$_{4}$O$_{8}$Na [M+Na]$^+$ calcd. 489.1956, found 489.1965. HRMS m/z for C$_{30}$H$_{38}$N$_{4}$O$_{10}$ [M+H]$^+$ calcd. 615.2584, found 615.2596.

**General procedure for the preparation of unprotected O-acyl isopeptides 9a,b**

Compounds 9a,b were prepared by following the same procedure followed for 5a–g.

**O-((tert-Butoxycarbonyl)glycyl)-N-glycyl-L-phenylalanyl-L-serine (Gly-L-Phe-L-Ser(Boc-Gly)-OH, 9a).** White solid (85%) yield; mp 168–173 °C; $^1$H NMR (CD$_3$OD) δ 7.41–7.11 (m, 5H), 4.78–4.63 (m, 1H), 4.62–4.49 (m, 2H), 4.43–4.33 (m, 1H), 3.93–3.63 (m, 3H), 3.27–3.18 (m, 1H), 3.01–2.63 (m, 4H), 1.43 (s, 9H); $^{13}$C NMR (CD$_3$OD) δ 174.9, 173.2, 172.2, 167.8, 158.7, 138.5, 130.5, 129.7, 128.0, 80.9, 66.0, 56.6, 54.7, 44.8, 43.1, 38.8, 28.9. HRMS m/z for C$_{21}$H$_{30}$N$_{4}$O$_{8}$Na [M+Na]$^+$ calcd. 489.1956, found 489.1965.

**O-((tert-Butoxycarbonyl)glycyl)-N-glycyl-L-phenylalanyl-L-threonine (Gly-L-Phe-L-Thr(Boc-Gly)-OH, 9b).** Sticky yellow solid (75%); $^1$H NMR (CDCl$_3$) δ 7.30–7.13 (m, 5H), 5.14–4.49 (m, 1H), 3.75–3.70 (m, 2H), 3.10–3.06 (m, 1H), 1.80–1.50 (m, 3H), 1.45–1.10 (m, 12H); $^{13}$C NMR (CDCl$_3$) δ 171.7, 170.1, 168.5, 152.0, 151.4, 132.0, 125.5, 125.3, 124.6, 123.1, 76.3, 52.0, 50.5, 50.3, 48.3, 38.3, 34.4, 33.9, 29.6, 24.3, 22.2. HRMS m/z for C$_{22}$H$_{32}$N$_{4}$O$_{8}$ [M+H]$^+$ calcd. 481.2320, found 481.2334.

(tert-Butoxycarbonyl)glycyl-L-phenylalanyl-L-serine (Boc-Gly-L-Phe-L-Ser-OH, 6a). Compound 5a (20 mg, 0.05 mmol) was dissolved in piperidine 20 v/v% in DMF (1 mL) and stirred at 50 °C and 50 W for 1h. The mixture was then evaporated and purified by HPLC to give ligated product 6a (57%); The sample was analyzed via reverse phase gradient C18 HPLC/UV/(-)ESI-MSn to give a retention time of 23.07 min. To confirm structure, HRMS for 6a m/z for C$_{19}$H$_{26}$N$_{3}$O$_{7}$ [M-H]$^-$ calcd. 408.1776, found 408.1794.
(tert-Butoxycarbonyl)glycylglycyl-L-phenylalanyl-L-serine (Boc-Gly-Gly-L-Phe-L-Ser-OH, 10a). Compound 9a (20 mg, 0.04 mmol) was dissolved in piperidine 20 v/v% in DMF (1 mL) and stirred at 50 °C and 50 W for 1h (3h for 9b). The mixture was then evaporated and purified by HPLC to give ligated product 10a (99.39%). The sample was analyzed via reverse phase gradient C18 HPLC/UV (254 nm/ESI-MSn gave a retention time of 21.67 min (for 10a). To confirm structure, HRMS for 11a m/z for C_{21}H_{29}N_{4}O_{8} [M-H]^{+} calcd. 465.2064, found 465.1992.

Acknowledgements

We thank the University of Florida and the Kenan Foundation for financial support. This paper was also funded by the Deanship of Scientific Research (DSR), King Abdulaziz University, Jeddah, under Grant no. (24-3-1432/HiCi). The authors, therefore, acknowledge with thanks DSR technical and financial support. The authors, therefore, acknowledge the technical and financial support of KAU. We also thank to Dr. C. D. Hall for helpful suggestions.

References

1. Fjell, C. D.; His, J. A.; Hancock, R. E. W.; Schneider, G. Nat. Rev. Drug Discov. 2012, 11, 37–51.
2. Castanho, M.; Santos, N. C. Peptide Drug Discovery and Development: Translational Research in Academia and Industry, 1st edn., Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim, 2011.
3. Groner, B. Peptides as Drugs. Discovery and Development, Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim, 2009.
4. White, C. J.; Yudin, A. K. Nat. Chem. 2011, 3, 509–524. http://dx.doi.org/10.1038/nchem.1062
5. Dawson, P. E.; Muir, T. W.; Clark-Lewis, I.; Kent, S. B. H. Science 1994, 266, 776–779. http://dx.doi.org/10.1126/science.7973629
6. Kent, S. B. H. Chem. Soc. Rev. 2009, 38, 338–351. http://dx.doi.org/10.1039/b700141j
7. Yeo, D.S. Y.; Srinivasan, R.; Chen, G. Y. J.; Yao, S. Q. Chem. Eur. J. 2004, 10, 4664–4672. http://dx.doi.org/10.1002/chem.200400414
8. Wieland, T.; Bokelmann, E.; Bauer, L.; Lang, H. U.; Lau, H.; Schafer, W. Justus Liebig’s Ann. Chem. 1953, 583, 129–149. http://dx.doi.org/10.1002/jlac.19535830110
9. Merrifield, B. Science 1986, 232, 341–347. http://dx.doi.org/10.1126/science.3961484
10. Heapy, A. M.; Williams, G. M.; Fraser, J. D.; Brimble, M. A. Org. Lett. 2012, 14, 878–881.
11. Sakamoto, I.; Tezuka, K.; Fukae, K.; Ishii, K.; Taduru, K.; Maeda, M.; Ouchi, M.; Yoshida, K.; Nambu, Y.; Igarashi, J.; Hayashi, N.; Tsuji, T.; Kajihara, Y. J. Am. Chem. Soc. 2012, 134, 5428–5431.
   [http://dx.doi.org/10.1021/ja2109079](http://dx.doi.org/10.1021/ja2109079)

12. Mandal, K.; Kent, S. B. H. Angew. Chem., Int. Ed. 2011, 50, 8029–8033.
   [http://dx.doi.org/10.1002/anie.201103237](http://dx.doi.org/10.1002/anie.201103237)

13. Luisier, S.; Avital-Shmilovici, M.; Weiss, M. A.; Kent, S. B. H. Chem. Comm. 2010, 46, 8177–8179.
   [http://dx.doi.org/10.1039/c0cc03141k](http://dx.doi.org/10.1039/c0cc03141k)

14. Li, X.; Lam, H. Yung; Z.; Yinfeng; C., Chun K. Org. Lett. 2010, 12, 1724–1727.
   [http://dx.doi.org/10.1021/ol1003109](http://dx.doi.org/10.1021/ol1003109)

15. Hackenberger, C. P. R.; Schwarzer, D. Angew. Chem., Int. Ed. 2008, 47, 10030–10074.
   [http://dx.doi.org/10.1002/anie.200801313](http://dx.doi.org/10.1002/anie.200801313)

16. Dirksen, A.; Dawson, P. E. Curr. Opin. Chem. Biol. 2008, 12, 760–766.
   [http://dx.doi.org/10.1016/j.cbpa.2008.10.009](http://dx.doi.org/10.1016/j.cbpa.2008.10.009)

17. Johnson, E. C. B.; Kent, S. B. H. J. Am. Chem. Soc. 2006, 128, 6640–6646.
   [http://dx.doi.org/10.1021/ja058344i](http://dx.doi.org/10.1021/ja058344i)

18. Bacsa, B.; Bősze, S.; Kappe, C. O. J. Org. Chem. 2010, 75, 2103–2106.
   [http://dx.doi.org/10.1021/jo100136r](http://dx.doi.org/10.1021/jo100136r)

19. Sohma, Y.; Yoshiya, T.; Taniguchi, A.; Kimura, T.; Hayashi, Y.; Kiso, Y. Biopolymers 2007, 88, 253–262.
   [http://dx.doi.org/10.1111/bip.20683](http://dx.doi.org/10.1111/bip.20683)

20. El Khatib, M.; Jauregui, L.; Tala, S. R.; Khelashvili, L.; Katritzky, A. R. MedChemComm 2011, 2, 1087–1092.
   [http://dx.doi.org/10.1039/c1md00130b](http://dx.doi.org/10.1039/c1md00130b)

21. Katritzky, A. R.; Tala, S. R.; Abo-Dya, N. E.; Ibrahim, T. S.; El-Feky, S. A.; Gyanda, K.; Pandya, K. M. J. Org. Chem. 2011, 76, 85–96.
   [http://dx.doi.org/10.1021/jo1015757](http://dx.doi.org/10.1021/jo1015757)

22. Ha, K.; Chahar, M.; Monbaliu, J.-C. M.; Todadze, E.; Hansen, F. K.; Oliferenko, A. A.; Ocampo, C. E.; Leino, D.; Lillicotch, A.; Stevens, C. V.; Katritzky, A. R. J. Org. Chem. 2012, 77, 2637–2648.
   [http://dx.doi.org/10.1021/jo2023125](http://dx.doi.org/10.1021/jo2023125)

23. Panda, S. S.; El-Nachef, C.; Bajaj, K.; Al-Youbi, A. O.; Oliferenko, A.; Katritzky, A. R. Chem. Biol. Drug Des. 2012, 80, 821–827.
   [http://dx.doi.org/10.1111/cbdd.12053](http://dx.doi.org/10.1111/cbdd.12053)

24. Popov, V.; Panda, S. S.; Katritzky, A. R. Org. Biomol. Chem. 2013, 11, 1594–1597.
   [http://dx.doi.org/10.1039/c3ob27421g](http://dx.doi.org/10.1039/c3ob27421g)

25. Popov, V.; Panda, S. S.; Katritzky, A. R. J. Org. Chem. 2013, 78, 7455–7461.
   [http://dx.doi.org/10.1021/jo4009468](http://dx.doi.org/10.1021/jo4009468)
26. El Khatib, M.; Elagawany, M.; Jabeen, F.; Todadze, E.; Bol'shakov, O.; Oliferenko, A.; Khelashvili, L.; El-Feky, S. A.; Asiri, A.; Katritzky, A. R. *Org. Biomol. Chem.* **2012**, *10*, 4836–4838.  
   [http://dx.doi.org/10.1039/c2ob07050b](http://dx.doi.org/10.1039/c2ob07050b)

27. Oliferenko, A. A.; Katritzky, A. R. *Org. Biomol. Chem.* **2011**, *9*, 4756–4759.  
   [http://dx.doi.org/10.1039/c1ob05536d](http://dx.doi.org/10.1039/c1ob05536d)

28. Katritzky, A. R.; Shestopalov, A. A.; Suzuki, K. *Synthesis* **2004**, 1806–1813.  
   [http://dx.doi.org/10.1055/s-2004-829126](http://dx.doi.org/10.1055/s-2004-829126)

29. Meneses, C.; Nicoll, S. L.; Trembleau, L. *J. Org. Chem.* **2010**, *75*, 564–569.  
   [http://dx.doi.org/10.1021/jo902116p](http://dx.doi.org/10.1021/jo902116p)