Chem Biol Drug Des 2013; **81:** 577–582

Research Article

α-Substitution Effects on the Ease of *S* → *N*-Acyl Transfer in Aminothioesters

Bahaa El-Dien M. El-Gendy[1,2], Ebrahim H. Ghazvini Zadeh[1], Ania C. Sotuyo[1], Girinath G. Pillai[1] and Alan R. Katritzky[1,3,*]

[1]Department of Chemistry, Center for Heterocyclic Compounds, University of Florida, Gainesville, FL 32611-7200, USA
[2]Department of Chemistry, Faculty of Science, Benha University, PO Box 13511 Benha, Egypt
[3]Department of Chemistry, King Abdulaziz University, Jeddah 21589, Saudi Arabia
**Corresponding author: Alan R. Katritzky, katritzky@chem.ufl.edu*

In *S*-acylcysteines and homocysteines, the efficacy and rate of *S* → *N*-acyl transfer (5 and 6 cyclic TSs) vary with the size of *S*-acyl group. Conformational and quantum chemical calculations indicate that the spatial distance, b(N-C), between the terminal amine and the thioester carbon is shortened by α-C(O)X (X = OH, OMe, NH_2) substituents.

Key words: aminothioesters, conformational analysis, *S* → *N*-acyl transfer

Received 3 August 2012, revised 8 October 2012 and accepted for publication 17 October 2012

The chemical synthesis of peptides through selective ligations involving *O* → *N* or *S* → *N*-acyl transfer can circumvent problems during the expression and purification of small recombinant proteins (1) that often contain 'difficult sequences' in which hydrophobic amino acid side chains lead to aggregation and hence poor yields (Scheme 1) (2). Ongoing efforts to extend the applicability of chemical ligation to non-cysteine ligation sites have included the use of *O*-acyl isopeptides, which undergo a pH-dependent *O* → *N* intramolecular acyl migration; this can facilitate the synthesis of difficult peptide sequences including water-soluble antitumor taxoid prodrug derivatives (3–6) and HIV-1 protease inhibitors (7–11).

The ease of intramolecular acyl transfers *via* cyclic transition states depends significantly on the transition state size. Entropic forces greatly assist both 5- and 6-membered TS ligations (12), while ligation rates can be slow for 8-, 9-, 11-, and 12-membered ring transition states because of unfavorable ring strain (13). The work of Seitz suggests that internal-cysteine ligations of 8- and 11-membered TS can be disfavored processes (12). Molecular dynamic simulations of these systems (12) have computed distances between the N-terminal amino group and the sp^2-carbon of Cys thioester and indicated that the ligation rate varies inversely with the H_3N^+–C(O) distance (14). A study by Seitz used a modified NCL protocol where the reaction vessels were incubated in a thermo mixer at 35 °C (12). We showed that NCL *via* 11-membered transition state was achieved (~14%) when the reaction mixture was subjected to microwave irradiation of 50 W at 50 °C (15).

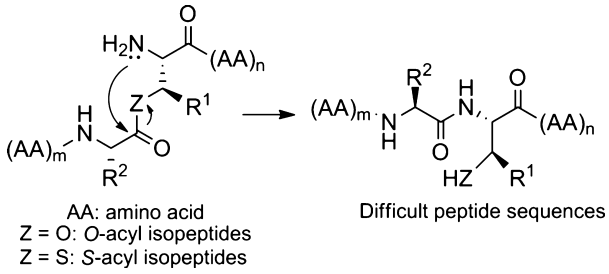
Larger ring (14, 17, 20, 23, 26, 29, and 32-membered) transition states ligations proceed at significant rates; thus, Wong and coworkers reported efficient sugar-assisted *O*- and *N*-glycopeptide ligation *via* 14- and 15-membered ring transition states, respectively (16,17). Brik *et al.* (18) examined peptide ligation using a side chain auxiliary, which involves a 15-membered transition state. A comprehensive report by Seitz concludes that internal-cysteine ligations take place via 14, 17, 20, and 23-membered cyclic transition states with the highest rate for the 17-membered TS (12). These results support the molecular dynamic simulations, which relate the rate of ligation to the ^+H_3N–C(O) distance (12). This depends on the ability of an optimal conformation of the peptide that can situate the thioacyl component in the immediate vicinity of the *N*-terminal amino group.

The present work investigates the ease of ligations in compounds with all carbon/oxygen (but no amidic group) backbones as compared with those found in peptide sequences with 5-, 6-, and 8-membered cyclic TSs, aiming initially to examine the differential effect of cysteine-free acid, α-ester, and α-amide groups on transition state conformation during the ligation process.

Methods and Materials

Melting points were determined on a capillary point apparatus equipped with a digital thermometer and are uncorrected. Column chromatography was conducted on flash silica gel (200–425 mesh). NMR spectra were recorded in $CDCl_3$ or DMSO-d_6 with TMS for ^1H (300 MHz) and ^{13}C (75 MHz) as an internal reference.

 doi: 10.1111/cbdd.12092



Scheme 1: Synthesis of difficult sequence peptides from *O*- and *S*-acyl isopeptides.

N-(2-Mercaptoethyl)-4-methyl benzamide (6a)

A solution of **5a** (0.231 g, 1 mmol) and Et_3N (0.279 mL, 2 mmol) in DCM (5 mL) was stirred at rt for 30 min. The mixture was washed with NH_4Cl, brine, dried over $MgSO_4$, and concentrated. The resulting solid was crystallized from DCM/hexanes to give **6a** as white needles (0.186 g, 95%); mp. 150.0–151.0 °C. ^1H NMR (300 MHz, $CDCl_3$) δ 7.74–7.70 (m, 2H), 7.23–7.16 (m, 2H), 7.05–6.93 (m, 1H), 3.78 (q, J = 6.2 Hz, 2H), 2.97 (t, J = 6.3 Hz, 2H), 2.39 (s, 3H), 1.70 (s, 1H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 167.9, 142.2, 131.5, 129.3, 127.2, 39.2, 38.2, 21.6. Anal. Calcd. For $C_{10}H_{13}NOS$ (195.29): C, 61.50; H, 6.71; N, 7.17. Found: C, 61.14; H, 6.14; N, 7.01.

N-(2-Mercaptoethyl)-1-naphthamide (6b)

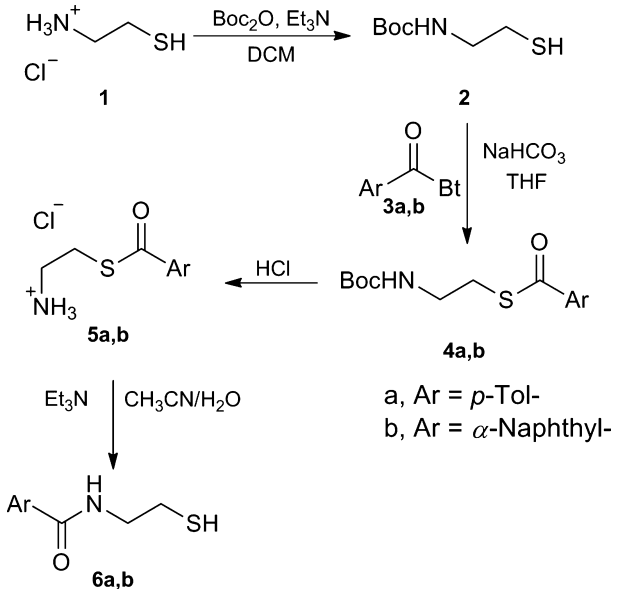
Pale yellow solid (0.18 g, 78%). ^1H NMR (300 MHz, $CDCl_3$) δ 8.28–8.09 (m, 1H), 7.93–7.75 (m, 2H), 7.61–7.40 (m, 3H), 7.34-7.29 (m, 1H), 6.94-6.88 (m, 1H), 3.75 (qd, J = 6.3, 1.9 Hz, 2H), 2.95 (td, J = 6.4, 1.8 Hz, 2H), 1.44 (br s, 1H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 1670.0, 134.0, 133.7, 130.8, 130.1, 128.3, 127.1, 126.4, 125.4, 125.3, 124.7, 39.0, 37.9. Anal. Calcd. For $C_{13}H_{13}NOS$ (231.32): C, 67.50; H, 5.66; N, 6.06. Found: C, 67.31; H, 5.31; N, 5.87.

N-(3-Mercaptopropyl)benzamide (13)

A solution of **12** (0.062 g, 0.2 mmol) and Et_3N in DCM (20 mL, 1 mM) was stirred at rt for 30 min. The mixture was washed with HCl (2 *N*, 3 × 15 mL), brine (15 mL), and dried over $MgSO_4$. The solvent was removed, and the crude solid was recrystallized (DCM:hexanes) to afford **13** as white solid (0.03 g, 85%). ^1H NMR (300 MHz, $CDCl_3$) δ 7.82–7.72 (m, 2H), 7.52–7.44 (m, 1H), 7.43–7.35 (m, 2H), 6.62 (br s, 1H), 3.57 (q, J = 6.6 Hz, 2H), 2.81 (t, J = 7.0 Hz, 2H), 2.05 (p, J = 7.0 Hz, 2H), 1.68 (s, 1H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 167.8, 134.6, 131.6, 128.7, 127.0, 38.8, 33.7, 22.4. Anal. Calcd. For $C_{10}H_{13}NOS$ (195.29): C, 61.50; H, 6.71; N, 7.17. Found: C, 61.32; H, 6.50; N, 7.03.

Results and Discussion

We examined $S \rightarrow N$-acyl transfer *via* 5-, 6-, and 8-membered TSs in substrates **5a, b**, **12**, and **18**.



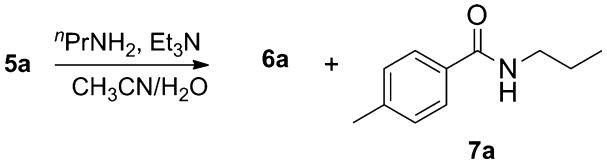
Scheme 2: Synthesis of **6a,b** as examples for $S \rightarrow N$-acyl transfer *via* 5-membered TS.

Study of S → N-acyl transfer *via* a 5-membered TS

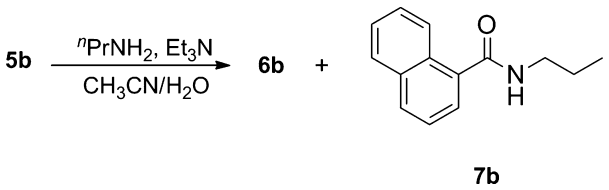
To prepare compounds **6a, b** (Scheme 2), 2-aminoethanethiol hydrochloride **1** was treated with Boc_2O to give compound **2**, which was acylated by 1-(aroyl)-1*H*-benzotriazoles **3a, b** under mild basic conditions to yield **4a,b**. Compounds **4a,b** were subsequently deprotected by HCl (4 *N*) in 1-4,dioxane to afford the HCl salts **5a,b**. Intermediates **5a,b** were converted into **6a,b** by Et_3N in mixed CH_3CN/H_2O. Interestingly, the aqueous workup of **5a** often afforded nearly 50% of the product **6a** through $S \rightarrow N$-acyl transfer. Thioesters **5a,b** were designed to resemble the chemical structure of *S*-acylated cysteine without the α-carboxylic group.

To determine whether $S \rightarrow N$-acyl transfer proceeds intramolecularly or intermolecularly, equimolar ratios of **5a**, *n*-propylamine, and Et_3N were mixed in CH_3CN/H_2O at rt for 3 h (Scheme 3). ^1H NMR spectral analysis showed that the major product obtained was **6a** (~97%), while **7a** was not detected, indicating that $S \rightarrow N$ tolyl transfer followed an intramolecular pathway.

When **5b** was treated with Et_3N in the presence of *n*-propylamine, a surprisingly different result was obtained



Scheme 3: Competition experiment between **5a** and *n*-propylamine.

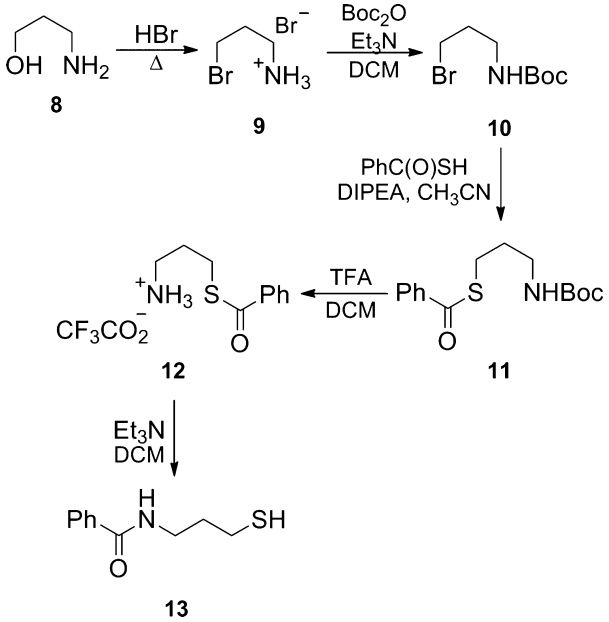


Scheme 4: Competition experiment between **5b** and *n*-propylamine.

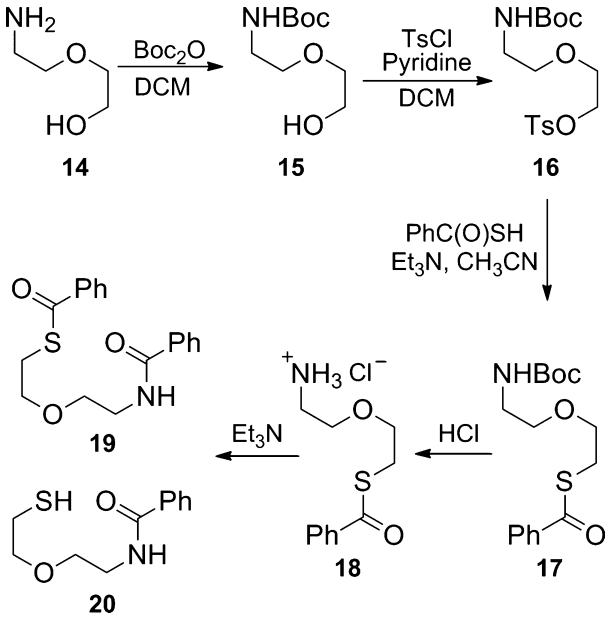
(Scheme 4). ^{1}H NMR spectra indicated that the reaction mixture was comprised of a 2:1 of **6b** and **7b**. In effect, the naphthoyl group sterically hindered the intramolecular *S* → *N*-acyl transfer, resulting in competitive acylation by *n*-propylamine.

Study of S → N-acyl transfer via a 6-membered TS

Aminopropanol **8** was heated under reflux with HBr (48%) to provide derivative **9** (55%). Boc-protection of the amine group then gave **10**, which reacted with thiobenzoic acid to afford the corresponding thioester **11** (Scheme 5). The Boc group was removed with TFA to give **12** (97%). Intermediate **12** has a structure similar to that of a decarboxylated homocysteine, which could undergo transacylation through a 6-membered TS. Homocysteine has been employed as a ligating moiety in several studies to prepare small proteins such as GstI protein of *Rhizobium leguminosarum*, the endogenous inhibitor of the glnII (glutamine synthetase II) gene expression (19,20). On stirring **12** with 2 equiv. of TEA in DCM, *S* → *N*-acyl transfer took place to give product **13** (85%).



Scheme 5: Synthesis of **12** as an example for *S* → *N*-acyl transfer *via* 6-membered TS.



Scheme 6: Synthesis of **18** as an example for *S* → *N* acyl transfer *via* 8-membered TS.

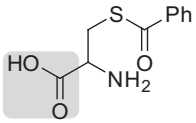
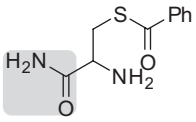
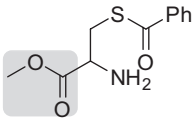
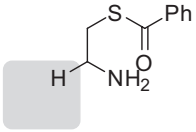
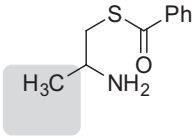
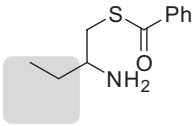
Study of S → N-acyl transfer via an 8-membered TS

N-Boc protection of 2-(-aminoethoxy)ethanol **14** afforded **15** (95%) and subsequent tosylation of the hydroxyl group furnished **16** (70–82%) (Scheme 6). Nucleophilic substitution of the OTs in **16** by thiobenzoic acid yielded thioester **17** (90%). Subsequent deprotection of the Boc group by HCl gave **18** as a white solid (quantitative). Compound **18** has the skeletal structure of C-terminal cysteine-containing dipeptide. Although it is reported (12) that *S*- → *N*-acyl transfer *via* 8-membered TS is not favored, we attempted to evaluate the effect of the backbone in compound **18**. We expected that in the absence of any directing structural motifs (turn-inducers, i.e. proline), the acyl transfer would be random. In fact, when **18** was treated with Et_3N in CH_3CN, a mixture of bis-acylated product **19** and the ligated product **20** was obtained, indicating that the intramolecular acylation *via* an 8-membered TS was not favored.

Computational assessment of substituent effects on S- → N-acyl transfer

A prerequisite for facile *S*- → *N*-acyl transfer is to bring the terminal amino group in close proximity to the thioester carbon atom. We therefore applied techniques previously employed (21) for similar ligation reactions including a full conformational search followed by scoring the conformers based on energies and spatial distances between relevant centers (b(N-C)). To justify the spatial distance b(N-C), quantum chemical calculations were carried out at the DFT/6-31G* level of theory (22) using HYPERCHEM 8.0 Software ([a]). The relevant energies and b(N-C) calculated for the different pre-organized structures are considered to

Table 1: Respective structure, energy, b(N-C) and AlogP of **5r**, **5s**, **5t**, **5x**, **5y**, and **5z**.

Entry	Thioester 5	Structure	Energy (kcal/mol)	b(N-C) (A°)	ALog P
1	**5r**		−101.85	2.927	1.19
2	**5s**		−59.69	2.915	0.33
3	**5t**		−98.49	3.059	1.22
4	**5x**		−21.83	3.325	1.79
5	**5y**		−22.86	3.189	1.74
6	**5z**		−25.00	3.172	2.29

prioritize the conformers. Thus, the conformation with the shortest geometrical distance between these centers (b(N-C)) afford the pre-organized structure through which the transfer was expected to occur.

A full conformational search considering both rotatable bonds and the phenyl ring of six related structures **5r**, **5s**, **5t**, **5x**, **5y**, and **5z** (Table 1) was implemented using the MMX force field, in PCMODEL v. 9.3 software ([b]). The resultant conformers were ranked in ascending order of b(N-C), and the best pre-organized structure, with the smallest b(N-C) values, is shown in Figure 1. In addition, the spatial distances b(N-C) and AlogP for five structures are shown in Table 1, respectively.

We compared the effect of the carboxylic acid moiety in **5r** against **5x** (no CO_2H), **5y** (+CH_3), **5z** (+C_2H_5), **5s** (+$CONH_2$), and **5t** (+CO_2Me) in Figure 1. The conformational analysis shows that the presence of the α-carboxylic acid, ester, or amide substituent brings the terminal amine and thioester centers closer to each other compared with the system with no α-substituents (**5x**). To determine whether this result is due to the congestion offered by the presence of α-substituents (Thorpe-Ingold effect), b(N-C)'s for **5y** and **5z** were computed. Interestingly, b(N-C)'s for **5y** and **5z** were considerably larger than that of **5r-t**. In addition, the energy levels of **5x-z** lie significantly higher than those of **5r-t**. The results indicate that a stabilization effect furnished by the α-carboxylic acid, ester, or amide substituent favors intramolecular *S*- → *N*-acyl transfer.

Conclusions

Competition experiments on *S* → *N*-acyl transfer *via* 5-membered TS compared with intermolecular attack showed that the size of the acyl group can present a steric hindrance that may slow the rate of intramolecular acylation. The results complement the findings of Dawson (23) that explains the role which the amino acid adjacent to the C-terminal cysteine ligation site plays in determining the rate of ligation. Similar reasoning may be used to explain *S* → *N*-acyl transfer *via* a 6-membered TS; however, molecules that undergo acyl transfer *via* an 8-membered TS must accommodate more strain and therefore unfavorable intramolecular acylation as found by Seitz *et al.*

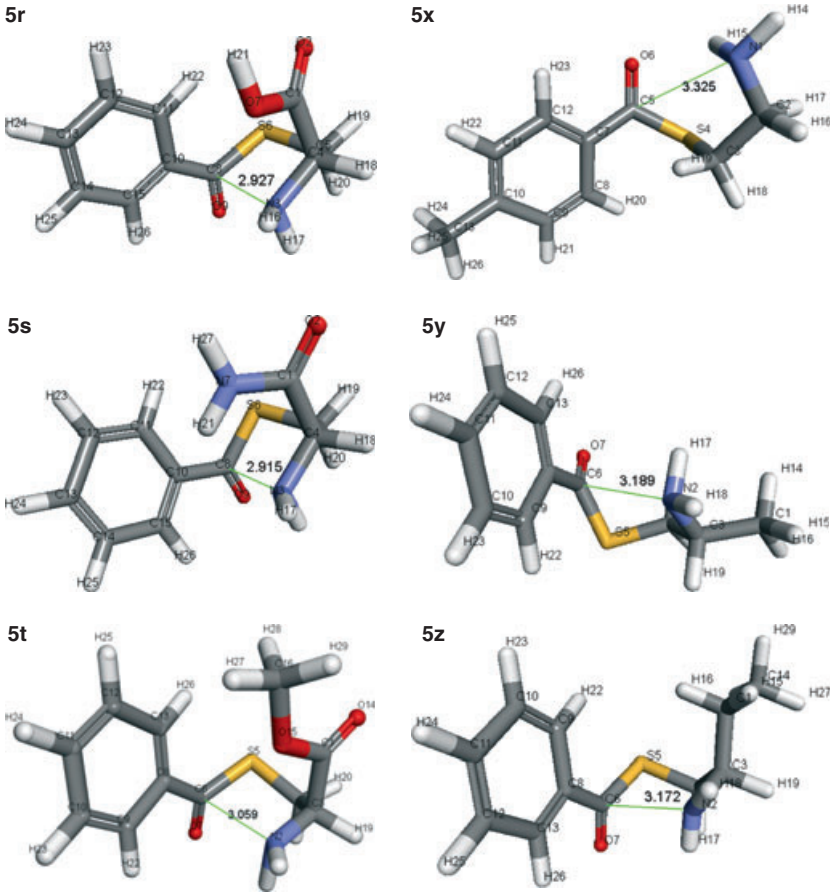


Figure 1: Best pre-organized conformers of **5r**, **5s**, **5t**, **5x**, **5y**, and **5z**. Green lines show the b(N-C) in Angstrom (A°). Accelrys Discovery Studio Visualizer 3.1(©) to generate 3D representations.

Conformational analysis and quantum chemical calculations showed that the spatial distance between the terminal amine and the thioester carbon b(N-C) is a central factor in controlling reaction rates and product yields. While the presence of either α-CO_2H, CO_2R, and $CONH_2$ shortens b(N-C), this shortening was not observed when the CO_2H group was replaced by Me or Et groups, suggesting that the Thorpe-Ingold effect is not pronounced in these structures.

References

1. Nilsson B.L., Soellner M.B., Raines R.T. (2005) Chemical synthesis of proteins. Annu Rev Biophys Biomol Struct;34:91–118.
2. Wohr T., Wahl F., Nefzi A., Rohwedder B., Sato T., Sun X., Mutter M. (1996) Pseudo-prolines as a solubilizing, structure-disrupting protection technique in peptide synthesis. J Am Chem Soc;118:9218–9227.
3. Hayashi Y., Skwarczynski M., Hamada Y., Sohma Y., Kimura T., Kiso Y. (2003) A novel approach of water-soluble paclitaxel prodrug with no auxiliary and no byproduct: design and synthesis of isotaxel. J Med Chem;46:3782–3784.
4. Skwarczynski M., Sohma Y., Kimura M., Hayashi Y., Kimura T., Kiso Y. (2003) O–N intramolecular acyl migration strategy in water-soluble prodrugs of taxoids. Bioorg Med Chem Lett;13:4441–4444.
5. Skwarczynski M., Sohma Y., Noguchi M., Kimura M., Hayashi Y., Hamada Y., Kimura T., Kiso Y. (2005) No auxiliary, no byproduct strategy for water-soluble prodrugs of taxoids: scope and limitation of O-N intramolecular acyl and acyloxy migration reactions. J Med Chem;48:2655–2666.
6. Skwarczynski M., Sohma Y., Noguchi M., Kimura T., Hayashi Y., Kiso Y. (2006) O-N intramolecular alkoxycarbonyl migration of typical protective groups in hydroxyamino acids. J Org Chem;71:2542–2545.
7. Kiso Y., Matsumoto H., Yamaguchi S., Kimura T. (1999) Design of small peptidomimetic HIV-1 protease inhibitors and prodrug forms. Lett Pept Sci;6:275–281.
8. Hamada Y., Ohtake J., Sohma Y., Kimura T., Hayashi Y., Kiso Y. (2002) New water-soluble prodrugs of HIV protease inhibitors based on O → N intramolecular acyl migration. Bioorg Med Chem;10:4155–4167.
9. Hamada Y., Matsumoto H., Kimura T., Hayashi Y., Kiso Y. (2003) Effect of the acyl groups on O → N-acyl migration in the water-soluble prodrugs of HIV-1 protease inhibitor. Bioorg Med Chem;13:2727–2730.

10. Hamada Y., Matsumoto H., Yamaguchi S., Kimura T., Hayashi Y., Kiso Y. (2004) Water-soluble prodrugs of dipeptide HIV protease inhibitors based on O → N intramolecular acyl migration: design, synthesis and kinetic study. Bioorg Med Chem;12:159–170.
11. Yoshiya T., Ito N., Kimura T., Kiso Y. (2008) Isopeptide method: development of S-acyl isopeptide method for the synthesis of difficult sequence-containing peptides. J Pept Sci;14:1203–1208.
12. Haase C., Seitz O. (2009) Internal cysteine accelerates thioester-based peptide ligation. Eur J Org Chem;13:2096–2101.
13. Brik A., Yang Y.-Y., Ficht S., Wong C.-H. (2006) Sugar-assisted glycopeptide ligation. J Am Chem Soc;128:5626–5627.
14. Payne R.J., Ficht S., Tang S., Brik A., Yang Y.-Y., Case D.A., Wong C.-H. (2007) Extended sugar-assisted glycopeptide ligations: development, scope, and applications. J Am Chem Soc;129:13527–13536.
15. Katritzky A.R., Abo-Dya N.E., Tala S.R., Abdel-Samii Z.K. (2010) The chemical ligation of selectively *S*-acylated cysteine peptides to form native peptides *via* 5-, 11- and 14-membered cyclic transition states. Org Biomol Chem;8:2316–2319.
16. Casadei M.A., Galli C., Mandolini L. (1984) Ring-closure reactions. Kinetics of cyclization of diethyl (ω-bromoalkyl)malonates in the range of 4- to 21-membered rings. Role of ring strain. J Am Chem Soc;106:1051–1056.
17. Brik A., Ficht S., Yang Y.-Y., Bennett C.S., Wong C.-H. (2006) Sugar-assisted ligation of N-linked glycopeptides with broad sequence tolerance at the ligation junction. J Am Chem Soc;128:15026–15033.
18. Lutsky M.-Y., Nepomniaschiy N., Brik A. (2008) Peptide ligation via side-chain auxiliary. Chem Commun;10:1229–1231.
19. Tam J.P., Yu Q. (1998) Methionine ligation strategy in the biomimetic synthesis of parathyroid hormones. Biopolymers;46:319–327.
20. Saporito A., Marasco D., Chambery A., Botti P., Monti S.M., Pedone C., Ruvo M. (2006) The chemical synthesis of the GstI protein by NCL on a x-met site. Biopolymers;5:508–518.
21. Oliferenko A.A., Katritzky A.R. (2011) Alternating chemical ligation reactivity of S-acyl peptides explained with theory and computations. Org Biomol Chem;9:4756–4759.
22. Levy M. (1979) Universal variational functionals of electron densities, first-order density matrices, and natural spin-orbitals and solution of the v-representability problem. Proc Natl Acad Sci USA;76:6062–6065.
23. Hackeng T.M., Griffin J.H., Dawson P.E. (1999) Protein synthesis by native chemical ligation: expanded scope by using straightforward methodology. Proc Natl Acad Sci USA;96:10068–10073.

Notes

[a]HyperChem 8.0.6 (2010) Hypercube, Inc., FL. USA.
[b]PC Model 9.3 (2011) Serena Software, IN, USA.
[c]Discovery Studio Visualizer 3.1 (2011) Accelrys Software Inc., USA.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Appendix S1. Synthetic procedures for the preparation of **2, 4ab, 5ab, 9, 10, 11, 12, 15, 16, 17, 18.**

Appendix S2. ^{1}H NMR and ^{13}C NMR spectra for **2, 4ab, 5ab, 6ab, 9, 10, 11, 12, 13, 15, 16, 17, 18.**