Organic & Biomolecular Chemistry

COMMUNICATION

View Article Online
View Journal | View Issue

CrossMark click for updates

Cite this: *Org. Biomol. Chem.*, 2015, **13**, 6922

Received 24th April 2015,
Accepted 8th May 2015

DOI: 10.1039/c5ob00825e

www.rsc.org/obc

Peptide 2-formylthiophenol esters do not proceed through a Ser/Thr ligation pathway, but participate in a peptide aminolysis to enable peptide condensation and cyclization†

Chun Ling Tung,‡[a,b] Clarence T. T. Wong‡[a,b] and Xuechen Li*[a,b]

Peptide thiol salicylaldehyde (SAL) esters unexpectedly do not follow a Ser/Thr ligation pathway to react with peptides containing N-terminal Ser/Thr, but proceed towards a peptide aminolysis in DMSO. The reaction takes place even at a low substrate concentration (1 mM). The method has been successfully used to synthesize several natural cyclic peptides, with a high ratio of monocyclic to dimeric products.

Chemoselective peptide ligation enables two side chain unprotected peptide segments to be joined together at the termini with the generation of the natural peptidic linkage at the ligation site, which has dramatically advanced the protein chemical synthesis.[1] Alternatively, some partially chemoselective aminolysis-based methods, which require the internal lysine side chain to be protected, have also been used in the convergent synthesis of peptides and proteins.[2] These aminolysis-mediated peptide condensation methods are often limited to condense the peptides at the C-terminal glycine and proline sites, due to the proneness to epimerization with other amino acids. Recently, our laboratory has developed a chemoselective Ser/Thr ligation (STL),[3] in which one side chain unprotected peptide segment with a C-terminal salicylaldehyde (SAL) ester reacts with another side chain unprotected peptide segment with N-terminal serine or threonine to form an *N*,*O*-benzylidene acetal intermediate, followed by acidolysis to reveal the natural Xaa-Ser/Thr linkage (Fig. 1). This method has been successfully applied in protein synthesis, protein semi-synthesis and cyclic peptide synthesis.[3–5]

A logical inquiry along the line of STL chemistry is to use peptide thiol-salicylaldehyde (2-formylthiophenol) esters

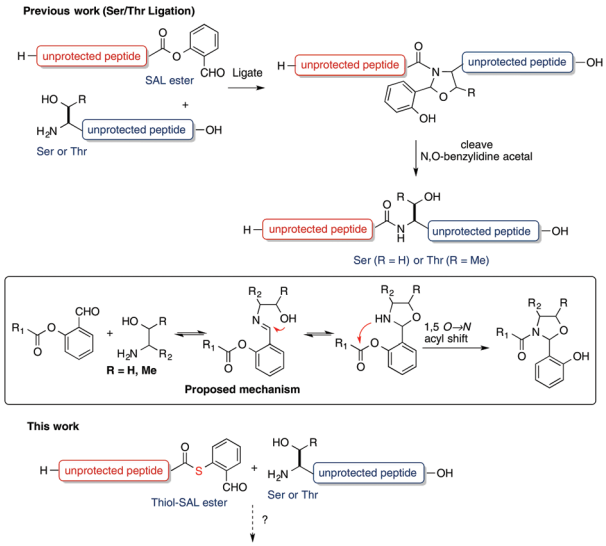


Fig. 1 Peptide (thiol) salicylaldehyde ester-mediated reactions.

under the Ser/Thr ligation conditions and is the subject of this communication. From the point of physical organic chemistry, the thioester is a better leaving group than the *O*-ester counterpart, thus we expect that a 1,5 *S* to *N* acyl transfer would be more feasible than a 1,5 *O* to *N* acyl transfer (Fig. 1). To this end, we first tested this idea by using Fmoc-Ala thiol-SAL ester to react with a serine derivative. These two components were dissolved in pyridine acetate buffer (AcOH : Pyr 1 : 1, mol : mol), and indeed the expected *N*,*O*-benzylidene acetal product was obtained, which upon acidolysis gave rise to Fmoc-Ala-Ser-Phe-OMe tripeptide, as analyzed by LC-MS (Fig. 2a). However, when a heptapeptide (Ac-SPKMVQG thiol-SAL ester) was reacted with a hexapeptide (NH_2-SFAVGA-CO_2H) with N-terminal Ser under the same conditions as above, we were surprised to see that no ligated product was observed and

[a]*Department of Chemistry, State Key Laboratory of Synthetic Chemistry, The University of Hong Kong, China. E-mail: xuechenl@hku.hk*
[b]*Chong Yuet Ming Chemistry Building, Pokfulam Road, Hong Kong, China*
†Electronic supplementary information (ESI) available. See DOI: 10.1039/c5ob00825e
‡Authors share equal contributions.

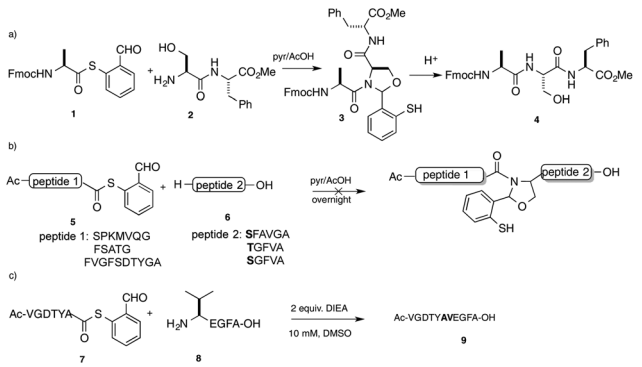


Fig. 2 Thiol-SAL ester mediated reactions.

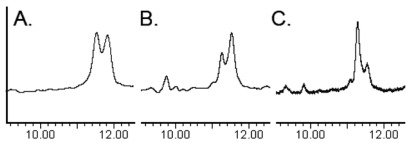
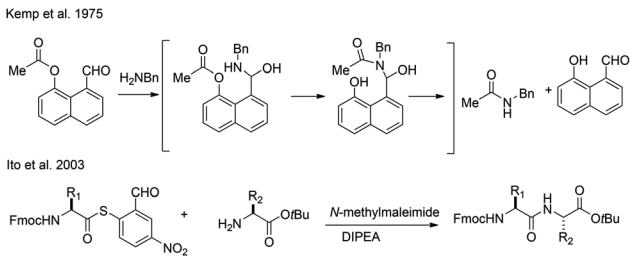


Fig. 3 RP-HPLC profile of epimerization determination between the ligation reaction of Ac-FVGFSDTYGA thiol-SAL ester (**10**)/Ac-FVGFSDTYG*a* thiol-SAL ester (**11**) with NH_2-AVYAAPYLAGG-CO_2H (**12**). A. Co-injection of ligated products. B. Ligation products from using C-terminal L-Ala thiol-SAL ester and C. Ligation products from using C-terminal D-Ala thiol-SAL ester.

the starting material remained intact as analyzed by LC-MS (Fig. 2b). We have varied the peptide sequences for the ligation study, none of which gave the ligated product. The underlying reason is unclear to us yet.

In the 1970s, Kemp had reported that aminolysis of 8-acetoxy-1-naphthaldehyde or 2-acetoxybenzaldehyde with primary amines generated amides. Under the conditions, an initial hemiaminal formation took place, followed by intramolecular 1,5 *O* to *N* acyl transfer to generate the amide.[6] Following this strategy, Ito and coworkers utilized 2-formyl-4-nitrothiophenol esters to facilitate the amide formation by hemiaminal-mediated acyl transfer.[7] These results were very promising, however, all these studies were limited to simple substrates (Scheme 1). These precedent examples prompted us to explore the utility of peptide thiol SAL esters for peptide condensation *via* hemiaminal formation-mediated aminolysis.

Initially, when peptide **7** (Ac-VGDTYA thiol-SAL ester) and peptide **8** (NH_2-VEGFA-CO_2H) were dissolved in DMSO (10 mM) with 2 equiv. of DIEA, a peptide condensation was indeed observed to give **9** within 6 h (Fig. 2c).[6] As a comparison, Agrigento *et al.* have used peptides with C-terminal *p*-chloro or *p*-nitro thiophenyl ester for the peptide aminolysis (DIEA, DMSO), in which the reaction needed 20–96 h at the substrate concentration of 60 mM.[2f] Thus, the hemiaminal formation-mediated reaction helped expedite the aminolysis.



Scheme 1 Previous examples using a hemiaminal-mediated acyl transfer strategy.

Next, we evaluated the epimerization issue under these conditions. Peptide **10** (Ac-FVGFSDTYGA thiol-SAL ester) and peptide **11** (Ac-FVGFSDTYG*a* thiol-SAL ester) were synthesized using the epimerization-free peptide hydrazine displacement approach.[8] These two peptide thiol SAL esters were then reacted with peptide **12** (H_2N-AVYAAPYLAGG-CO_2H), respectively. The ligation could go to completion within few hours. However, we observed the significant epimerized product, which was proved by comparing the retention time of C-terminal L- and D-alanine peptides (Fig. 3). Thus, like other aminolysis-mediated peptide condensation,[2a,c] this method will be limited to condense the peptide segment at the site with the C-terminal glycine or proline residue. In addition, this method will not tolerate the unprotected lysine and cysteine residues in the peptide sequence.

To further probe the optimal conditions, Ac-AFQIG thiol-SAL ester (**13**) was allowed to react with NH_2-GLVYA-CO_2H (**14**) under different conditions at a substrate concentration of 1 mM. Under neutral or basic aqueous solution, the hydrolysis product predominated (entries 2 and 3, Table 1).[9] Organic solvents (DMSO, DMF and 1-methyl-2-pyrrolidone) were more suitable. An increase of the amount of DIEA to 10 equiv. caused side reactions. In addition to DIEA, other bases including DBU and DEA were found to be unsuitable. Therefore, the

Table 1 Optimization of the reaction conditions

Entry	Solvent	Base	Conversion
1	PBS (pH 6)	—	<5%
2	PBS (pH 7.4)	—	<5%
3	PBS (pH 8.5)	—	<5%
4	DMSO	10 eq. DIEA	69%
5	DMSO	10 eq. Imid.	67%
6	DMSO	3 eq. DIEA	91%
7	DMSO–NMM	3 eq. DIEA	90%
8	DMSO	1 eq. DIEA	90%
9	DMSO	3 eq. DBU	<5%
10	DMSO	3 eq. DEA	<5%
11	DMF	1 eq. DIEA	82%
12	DMF	3 eq. DIEA	80%
13	DMF	10 eq. DIEA	38%
14	1-Methyl-2-pyrrolidone	3 eq. DIEA	77%
15	50% DMSO/H_2O	3 eq. DIEA	22%

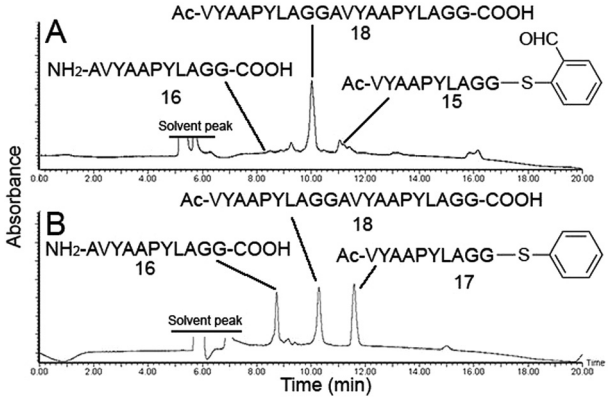
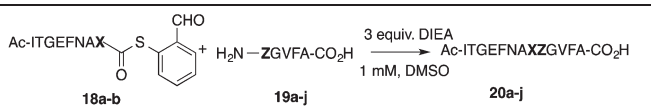


Fig. 4 Comparison of the aminolysis rate between a peptide thiol SAL ester (**15**) and peptide thiophenyl ester (**17**). Reaction **A** was performed for 5 h at 10 mM while **B** for 20 h at 10 mM.

Table 2 Examples of thiol SAL-mediated aminolysis of C-terminal Gly and Pro thiol-SAL ester with different N-terminal amino acids



Entry	X	Z	Product	Conversion 4 h	Conversion 8 h
1[a]	Gly	Asp	**12a**	77	78
2[a]	Gly	Glu	**12b**	80	89
3[a]	Gly	Pro	**12c**	75	77
4[a]	Gly	Ser	**12d**	82	82
5[a]	Gly	Gly	**12e**	85	85
6[b]	Pro	Asp	**12f**	65	83
7[b]	Pro	Glu	**12g**	60	90
8[b]	Pro	Pro	**12h**	61	71
9[b]	Pro	Ala	**12i**	79	85
10[b]	Pro	Gly	**12j**	90	98

[a] Reaction conditions: peptide thiol-SAL ester (**18a–b**) (1 equiv.), C-terminal peptides (**19a–j**) (1.2–1.3 equiv.), DIEA (3.0 equiv.), DMSO at a final conc. of 1 mM, r.t.; conversion was calculated by the ratio of the product area over the product and the hydrolyzed peptide. [b] Reaction conditions: peptide thiol-SAL ester (**18a–b**) (1.5 equiv.), C-terminal peptides (**19a–j**) (1 equiv.), DIEA (3.0 equiv.), DMSO at a final conc. of 1 mM, r.t.; conversion was calculated by the ratio of the product area over the product and the remaining C-terminal peptide.

optimal condition is to use 1–3 equiv. of DIEA in DMSO. Under the same condition, a peptide thiol SAL ester (**15**) reacted much faster than its peptide thiophenyl ester counterpart (**17**) (Fig. 4).

As the C-terminal residue was limited to the glycine or proline residue, we next explored the scope and limitations on the N-terminal residues. The synthesis of peptides containing C-terminal Gly/Pro thiol SAL esters was straightforward using the direct coupling conditions. We selected to use the peptide thiol-SAL ester of Ac-ITGEFNAX (X = Gly or Pro) (**18a–b**) as the N-terminal peptide and the peptide NH_2-ZGVFA-CO_2H (Z = amino acid) (**19a–j**) as the C-terminal peptide. The two peptide segments were dissolved at 1 mM concentration in DMSO with 3 equiv. DIEA. The reaction progress was analyzed by LC-MS at 4 h and 8 h. As seen in Table 2, the reactions with the peptide C-terminal Gly thiol-SAL ester could be completed or nearly completed within 4 h, while the peptide C-terminal Pro thiol-SAL ester required 8 h. We also observed a small amount of the imine product for the peptide C-terminal proline thiol-SAL ester, which was consistent with Kemp's observation that certain substrates stopped at the imine stage without inducing acyl transfer.

To demonstrate the effectiveness of this method, we next applied it to the synthesis of the natural cyclic peptides. Many natural cyclic peptides exhibit broad biological activities, including anti-cancer, anti-bacteria, and anti-virus; thus cyclic peptides serve as a useful scaffold for the development of new therapeutic agents.[10] As such, the development of different methods for the synthesis of natural and unnatural cyclic peptides is attracting much attention.[11]

We selected several natural cyclic peptides with rings made of 7 to 11 amino acid residues, containing Gly or Pro in the sequence. These peptides include phakellistatin-13,[12] cyclosquamosin D,[13] Dichotomin G,[14] antamanide[15] and Stelladelin D.[16] The cyclization was performed at Pro–Phe, Gly–Gly, Pro–Leu, Pro–Leu, Pro–Ala and Gly–Gly sites, respectively, *via* the thiol-SAL ester-mediated peptide cyclization. The cyclizations were performed at 1 mM in DMSO, which proceeded smoothly with completion in 4–8 h. The monocyclic products were obtained as major or sole products (Table 3, Fig. 5). Unfortunately, highly constrained tetrapeptides could not be cyclized using this method, in which only the dimerized cyclic products were observed.

Table 3 Synthesis of natural cyclic peptides *via* peptide thiol-SAL ester-mediated cyclization

Entry	Name	Sequence	Ring size	M : D	Yield[a]
1	Phakellistatin-13	Cyclo-[FGPTLWP]	7	8 : 1	41%
2	Cyclosquamosin D	Cyclo-[GVVSYYPG]	8	99 : 1	49%
3	Dichotomin G	Cyclo-[LPSTFPPIP]	9	9 : 1	50%
4	Antamanide	Cyclo-[AFFPPFFVPP]	10	99 : 1	48%
5	Stelladelin D	Cyclo-[VPSPYFPAAIG]	11	99 : 1	45%

[a] Isolated by HPLC. M: monocyclic product. D: dimerized product.

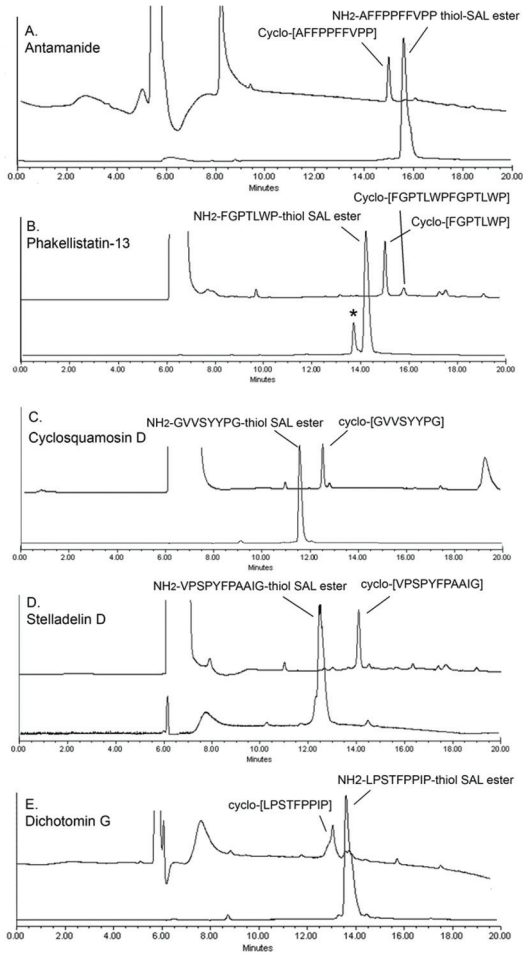


Fig. 5 RP-HPLC profiles of the crude reaction mixture of the thiol SAL-mediated cyclization. * = partial removal of Trp (Boc) protecting group.

Conclusions

In conclusion, the peptide thiol-SAL ester surprisingly could not participate in Ser/Thr ligation, but could undergo a direct aminolysis, likely through a hemiaminal-mediated acyl transfer.[17] This thiol-SAL ester mediated aminolysis method can serve as an alternative approach for the convergent synthesis of peptides and cyclic peptides. We have demonstrated the effectiveness of this method in the synthesis of several natural cyclic peptides. The application of this method towards protein chemical synthesis is ongoing.

Acknowledgements

This work was supported by the National Basic Research Program of China (973 program, 2013CB836900), the General Research Fund of the Research Grants Council of Hong Kong (HKU702813P), AoE/M-12/06 and the Peacock Program-Project Development Fund (KQC201109050074A).

Notes and references

1 (*a*) V. R. Pattabiraman and J. W. Bode, *Nature*, 2011, **480**, 471–479; (*b*) C. P. R. Hackenberger and D. Schwarzer, *Angew. Chem., Int. Ed.*, 2008, **47**, 10030–10047; (*c*) L. Raibaut, N. Ollivier and O. Melnyk, *Chem. Soc. Rev.*, 2012, **41**, 7001–7015; (*d*) C. T. T. Wong, C. L. Tung and X. Li, *Mol. BioSyst.*, 2013, **9**, 826–833; (*e*) P. E. Dawson, T. W. Muir, I. Clark-Lewis and S. B. H. Kent, *Science*, 1994, **266**, 776–779.

2 (*a*) S. Aimoto, *Curr. Org. Chem.*, 2001, **5**, 45–87; (*b*) R. J. Payne, S. Ficht, W. A. Greenberg and C. H. Wong, *Angew. Chem., Int. Ed.*, 2008, **47**, 4411–4415; (*c*) G. Chen, Q. Wan, Z. Tan, C. Kan, Z. Hua, K. Ranganathan and S. J. Danishefsky, *Angew. Chem., Int. Ed.*, 2007, **46**, 7383–7387; (*d*) D. Crich and I. Sharma, *Angew. Chem., Int. Ed.*, 2009, **48**, 2355–2358; (*e*) B. L. Wilkinson, C. K. Chun and R. J. Payne, *Biopolymers*, 2011, **96**, 137–146; (*f*) P. Agrigento, F. Albericio, S. Chamoin, I. Dacquigneis, H. Koc and M. Eberle, *Org. Lett.*, 2014, **16**, 3922–3925; (*g*) S. Popovic, H. Bieräugel, R. J. Detz, A. M. Kluwer, J. A. A. Koole, D. E. Streefkerk, H. Hiemstra and J. H. van Maarseveen, *Chem. – Eur. J.*, 2013, **19**, 16934–16937; (*h*) H. Hojo, Y. Murasawa, H. Katayama, T. Ohira, Y. Nakahara and Y. Nakahara, *Org. Biomol. Chem.*, 2008, **6**, 1808–1813.

3 (*a*) X. Li, H. Y. Lam, Y. Zhang and C. K. Chan, *Org. Lett.*, 2010, **12**, 1724–1727; (*b*) Y. Zhang, C. Xu, H. Y. Lam, C. L. Lee and X. Li, *Proc. Natl. Acad. Sci. U. S. A.*, 2013, **110**, 6657–6662; (*c*) C. T. Wong, T. Li, H. Y. Lam, Y. Zhang and X. Li, *Front. Chem. Biol.*, 2014, **2**, 28.

4 (*a*) C. L. Lee and X. Li, *Curr. Opin. Chem. Biol.*, 2014, **22**, 108–114; (*b*) H. Y. Lam, Y. Zhang, H. Liu, J. Xu, C. T. Wong, C. Xu and X. Li, *J. Am. Chem. Soc.*, 2013, **135**, 6272–6279; (*c*) C. T. T. Wong, H. Y. Lam, T. Song, G. Chen and X. Li, *Angew. Chem., Int. Ed.*, 2013, **52**, 10212–10215; (*d*) C. Xu, H. Y. Lam, Y. Zhang and X. Li, *Chem. Commun.*, 2013, **49**, 6200–6202; (*e*) Y. Zhang, T. Li and X. Li, *Org. Biomol. Chem.*, 2013, **11**, 5584–5587.

5 (*a*) P. M. Levine, T. W. Craven, R. Bonneau and K. Kirshenbaum, *Org. Lett.*, 2014, **16**, 512–515; (*b*) P. M. Levine, T. W. Craven, R. Bonneau and K. Kirshenbaum, *Chem. Commun.*, 2014, **50**, 6909–6912; (*c*) P. M. Levine, T. W. Craven, R. Bonneau and K. Kirshenbaum, *Org. Biomol. Chem.*, 2013, **11**, 4142–4146.

6 D. S. Kemp and F. Vellaccio Jr., *J. Org. Chem.*, 1975, **40**, 3003–3004.

7 A. Ishiwata, T. Ichiyanagi, M. Takatani and Y. Ito, *Tetrahedron Lett.*, 2003, **44**, 3187–3190. In this study, the substrate was limited to the Fmoc-Val-SAr.

8 G. M. Fang, Y. M. Li, F. Shan, Y. C. Huang, J. B. Li, Y. Lin, H. K. Cui and L. Liu, *Angew. Chem., Int. Ed.*, 2011, **50**, 7645–7649.

9 Different from the result of ref. 7, in which aqueous buffers could be used.

10 (*a*) A. T. Bockus, C. M. McEwen and R. S. Lokey, *Curr. Top. Med. Chem.*, 2013, **13**, 821–836; (*b*) S. H. Joo, *Biomol. Ther.*, 2012, **20**, 19–26.

11 (*a*) C. J. White and A. K. Yudin, *Nat. Chem.*, 2011, **3**, 509–524; (*b*) J. P. Tam and C. T. Wong, *J. Biol. Chem.*, 2012, **287**, 27020–27025.
12 W. L. Li, Y. H. Yi, H. M. Wu, Q. Z. Xu, H. F. Tang, D. Z. Zhou, H. W. Lin and Z. H. Wang, *J. Nat. Prod.*, 2003, **66**, 146–148.
13 A. Dellai, I. Maricic, V. Kumar, S. Arutyunyan, A. Bouraoui and A. Nefzi, *Bioorg. Med. Chem. Lett.*, 2010, **20**, 6020–6023.
14 H. Morita, A. Shishido, T. Kayashita, K. Takeya and H. C. Itokawa, *J. Nat. Prod.*, 1997, **60**, 404–407.
15 I. L. Karle, T. Wieland, D. Schermer and H. C. J. Ottenheym, *Proc. Natl. Acad. Sci. U. S. A.*, 1979, **76**, 1532–1536.
16 Y. R. Zhou, J. Zhou, X. K. Wang, X. L. Huang, H. M. Wu and X. Cheng, *Chin. Chem. Lett.*, 1996, **7**, 237–240.
17 We do not have direct evidence for the formation of a hemiaminal.