ORGANIC LETTERS

2004
Vol. 6, No. 21
3659−3662

Asymmetric Synthesis and Translational Competence of L-α-(1-Cyclobutenyl)glycine

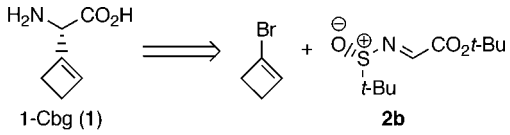
Lasanthi P. Jayathilaka, Mahua Deb, and Robert F. Standaert*

Department of Chemistry (M/C 111), University of Illinois at Chicago, 845 West Taylor Street, Room 4500 Chicago, Illinois 60607

rfs@uic.edu

Received May 26, 2004

ABSTRACT



Substitutes for Val and Ile in protein translation *in vitro*

L-α-(1-Cyclobutenyl)glycine (1-Cbg) was targeted as a potentially translatable analogue of isoleucine and valine and as a useful building block for peptides. An enantioselective synthesis was executed in which the key step was diastereoselective addition of 1-cyclobutenylmagnesium bromide to the sulfinimine 2b derived from (*S*)-*t*-butanesulfinimide and *tert*-butyl glyoxylate. 1-Cbg was found to substitute efficiently for isoleucine and valine, but not leucine, in the translation of green fluorescent protein in vitro.

Unnatural amino acids are important tools for the preparation of novel peptides, proteins, and derived materials such as bioconjugates and polymers. Among the amino acids, an interesting subset comprises translatable ones, that is, analogues of the standard 20 amino acids that are accepted by the protein biosynthetic machinery.[1] Such analogues can allow for the production of large quantities of analogue-labeled polypeptides by microbial expression, and the introduction of unique functional groups can allow for selective posttranslational chemistry. For the full potential of this approach to be realized, it will be necessary to expand the pool of functionalized amino acids that are translationally competent, an effort that entails both modification of the biosynthetic machinery[2] and synthesis of novel amino acids.

Cyclobutenes have recently attracted heightened interest because of their high reactivity in olefin metathesis, making them valuable as substrates for ring-opening metathesis polymerization[3] and as synthetic intermediates.[4] However, little effort has been directed toward their applications in peptide and protein chemistry. The first cyclobutene-containing amino acid, (±)-β-(1-cyclobutenyl)alanine, was prepared in 1961[5] but has not appeared subsequently in the literature. In addition, there is one citation to the isomeric β-(3-cyclobutenyl)alanine, which was found to be a translationally competent analogue of leucine (Leu).[6]

This laboratory maintains an interest in translatable analogues of isoleucine (Ile) such as the natural product furanomycin.[7,8] Recognizing the chemical value of cyclobutenes and that the cyclobutenyl group is a reasonable isostere for the *sec*-butyl side chain of Ile or the isopropyl side chain of Val, we targeted α-(1-cyclobutenyl)glycine (1-Cbg, **1**) as an analogue of potentially high value. Especially in light of the translational competence of (3-cyclobutenyl)-alanine as a substitute for Leu, it seemed plausible that 1-Cbg

(1) Reviews: (a) Link, A. J.; Mock, M. L.; Tirrell, D. A. *Curr. Opin. Biotechnol.* **2003**, *14*, 603−609. (b) Wang, L.; Schultz, P. G. *Chem. Commun. (Cambridge)* **2002**, 1−11. (c) van Hest, J. C. M.; Tirrell, D. A. *Chem. Commun. (Cambridge)* **2001**, 1897−1904. (d) Budisa, N.; Minks, C.; Alefelder, S.; Wenger, W.; Dong, F.; Moroder, L.; Huber, R. *FASEB J.* **1999**, *13*, 41−51. (e) Kiick, K. L.; Tirrell, D. A. *Mater. Sci. Technol.* **1999**, *20*, 571−594. (f) Kothakota, S.; Dougherty, M. J.; Fournier, M. J.; Mason, T. L.; Yoshikawa, E.; Tirrell, D. A. *Macromol. Symp.* **1995**, *98*, 573−583. (g) Wilson, M. J.; Hatfield, D. L. *Biochim. Biophys. Acta* **1984**, *781*, 205−215. (h) Sykes, B. D.; Weiner, J. H. In *Magnetic Resonance in Biology*; Cohen, J. S., Ed.; John Wiley & Sons: New York, 1980; Vol. 1. (i) Pine, M. J. *Antimicrob. Agents Chemother.* **1978**, *13*, 676−685. (j) Richmond, M. H. *Bacteriol. Rev.* **1962**, *26*, 398−420. (k) Dittmer, K. *Ann. N. Y. Acad. Sci.* **1950**, *52*, 1274−1301.

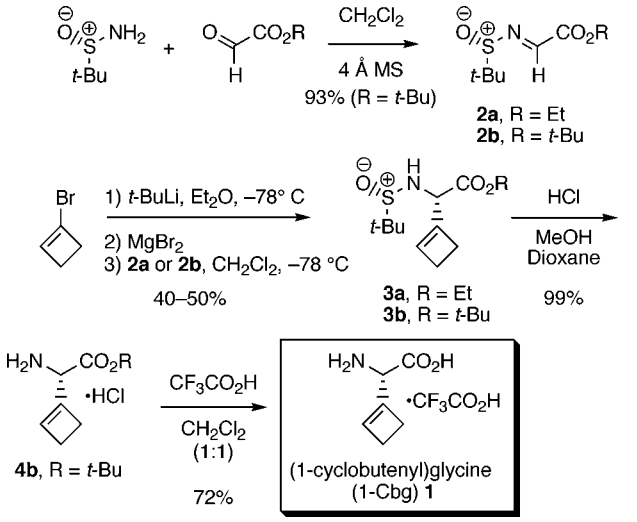
10.1021/ol049026b CCC: $27.50
Published on Web 09/11/2004

would likewise substitute for Ile and perhaps Val. Even if this proved not to be the case, we expected that 1-Cbg would be of high value in chemically synthesized peptides.

An asymmetric synthesis of 1-Cbg was designed around a stereoselective addition of the cyclobutenyl component to a chiral sulfinimine.[9] Davis[10] has demonstrated high diastereoselectivity in the addition of Grignard reagents to sulfinimine **2a** derived from condensation of *t*-butanesulfinamide[11] with ethyl glyoxylate. In accord with his findings, we found that 1-cyclobutenylmagnesium bromide (prepared by lithiation of 1-bromocyclobutene[12] and transmetalation with $MgBr_2$) likewise added smoothly to provide a 9:1 mixture of diastereomers. Unfortunately, attempted saponification of the ethyl ester led to extensive epimerization and decomposition.

To circumvent the need for strong base in deprotection, we sought an acid-labile ester and accordingly prepared the corresponding sulfinimine **2b** from *tert*-butyl glyoxylate, easily obtained through ozonolysis of di-*tert*-butyl fumarate.[13] As with the ethyl glyoxylate-derived sulfinimine **2a**, addition to **2b** proceeded smoothly to afford a 9:1 mixture of diastereomers, from which the major isomer **3b** could be crystallized in 40−50% yield and >97% de from hexane.

Scheme 1. Synthesis of l-(1-Cyclobutenyl)glycine



To obtain the parent amino amino acid for use in ribosomal protein translation (and potentially other applications), both N- and C-protecting groups had to be removed. The simplest path appeared to be simultaneous deprotection of both the *tert*-butylsulfinamide and the *tert*-butyl ester. In practice, the *tert*-butyl ester proved to be much more resistant to acid, and best results were obtained via two-stage deprotection; thus, treatment of **3b** with a 1:1 mixture of methanol and 4

(2) (a) de Prat Gay, G.; Duckworth, H. W.; Fersht, A. R. *FEBS Lett.* **1993**, *318*, 167−171. (b) Ibba, M.; Hennecke, H. *FEBS Lett.* **1995**, *364*, 272−275. (c) Furter, R. *Protein Sci.* **1998**, *7*, 419−426. (d) Sharma, N.; Furter, R.; Kast, P.; Tirrell, D. A. *FEBS Lett.* **2000**, *467*, 37−40. (e) Doring, V.; Mootz, H. D.; Nangle, L. A.; Hendrickson, T. L.; de Crecy-Lagard, V.; Schimmel, P.; Marliere, P. *Science (Washington, DC)* **2001**, *292*, 501−504. (f) Wang, L.; Brock, A.; Herberich, B.; Schultz, P. G. *Science (Washington, DC)* **2001**, *292*, 498−500. (g) Chin, J. W.; Schultz, P. G. *ChemBioChem* **2002**, *3*, 1135−1137. (h) Chin, J. W.; Martin, A. B.; King, D. S.; Wang, L.; Schultz, P. G. *Proc. Natl. Acad. Sci. U.S.A.* **2002**, *99*, 11020−11024. (i) Datta, D.; Wang, P.; Carrico, I. S.; Mayo, S. L.; Tirrell, D. A. *J. Am. Chem. Soc.* **2002**, *124*, 5652−5653. (j) Kiick, K. L.; Saxon, E.; Tirrell, D. A.; Bertozzi, C. R. *Proc. Natl. Acad. Sci. U.S.A.* **2002**, *99*, 19−24. (k) Sakamoto, K.; Hayashi, A.; Sakamoto, A.; Kiga, D.; Nakayama, H.; Soma, A.; Kobayashi, T.; Kitabatake, M.; Takio, K.; Saito, K.; Shirouzu, M.; Hirao, I.; Yokoyama, S. *Nucl. Acids Res.* **2002**, *30*, 4692−4699. (l) Tang, Y.; Tirrell, D. A. *Biochemistry* **2002**, *41*, 10635−10645. (m) Zhang, D.; Vaidehi, N.; Goddard, W. A., III; Danzer, J. F.; Debe, D. *Proc. Natl. Acad. Sci. U.S.A.* **2002**, *99*, 6579−6584. (n) Chin, J. W.; Cropp, T. A.; Anderson, J. C.; Mukherji, M.; Zhang, Z.; Schultz, P. G. *Science (Washington, DC)* **2003**, *301*, 964−967. (o) Dedkova, L. M.; Fahmi, N. E.; Golovine, S. Y.; Hecht, S. M. *J. Am. Chem. Soc.* **2003**, *125*, 6616−6617. (p) Frankel, A.; Roberts, R. W. *RNA* **2003**, *9*, 780−786. (q) Kwon, I.; Kirshenbaum, K.; Tirrell, D. A. *J. Am. Chem. Soc.* **2003**, *125*, 7512−7513. (r) Mehl, R. A.; Anderson, J. C.; Santoro, S. W.; Wang, L.; Martin, A. B.; King, D. S.; Horn, D. M.; Schultz, P. G. *J. Am. Chem. Soc.* **2003**, *125*, 935−939. (s) Wang, L.; Zhang, Z.; Brock, A.; Schultz, P. G. *Proc. Natl. Acad. Sci. U.S.A.* **2003**, *100*, 56−61. (t) Zhang, Z.; Smith, B. A. C.; Wang, L.; Brock, A.; Cho, C.; Schultz, P. G. *Biochemistry* **2003**, *42*, 6735−6746.

(3) (a) Brunthaler, J. K.; Stelzer, F.; Leising, G. *J. Mol. Catal.* **1985**, *28*, 393−402. (b) Wu, Z.; Wheeler, D. R.; Grubbs, R. H. *J. Am. Chem. Soc.* **1992**, *114*, 146−151. (c) Alder, R. W.; Allen, P. R.; Khosravi, E. *Chem. Commun. (Cambridge)* **1994**, 1235−1236. (d) Perrott, M. G.; Novak, B. M. *Macromolecules* **1995**, *28*, 3492−3494. (e) Wu, Z.; Grubbs, R. H. *Macromolecules* **1995**, *28*, 3502−3508. (f) Perrott, M. G.; Novak, B. M. *Macromolecules* **1996**, *29*, 1817−1823. (g) Maughon, B. R.; Weck, M.; Mohr, B.; Grubbs, R. H. *Macromolecules* **1997**, *30*, 257−265. (h) Maughon, B. R.; Grubbs, R. H. *Macromolecules* **1997**, *30*, 3459−3469. (i) Weck, M.; Mohr, B.; Maughon, B. R.; Grubbs, R. H. *Macromolecules* **1997**, *30*, 6430−6437. (j) Thorn-Csanyi, E.; Ruhland, K. *Macromol. Chem. Phys.* **1999**, *200*, 1662−1671. (k) Thorn-Csanyi, E.; Ruhland, K. *Macromol. Chem. Phys.* **1999**, *200*, 2245−2249. (l) Thorn-Csanyi, E.; Ruhland, K. *Macromol. Chem. Phys.* **1999**, *200*, 2606−2611. (m) Charvet, R.; Novak, B. M. *Macromolecules* **2001**, *34*, 7680−7685.

(4) (a) Randall, M. L.; Tallarico, J. A.; Snapper, M. L. *J. Am. Chem. Soc.* **1995**, *117*, 9610−11. (b) Zuercher, W. J.; Hashimoto, M.; Grubbs, R. H. *J. Am. Chem. Soc.* **1996**, *118*, 6634−6640. (c) Snapper, M. L.; Tallarico, J. A.; Randall, M. L. *J. Am. Chem. Soc.* **1997**, *119*, 1478−1479. (d) Tallarico, J. A.; Randall, M. L.; Snapper, M. L. *Tetrahedron* **1997**, *53*, 16511−16520. (e) Cuny, G. D.; Cao, J.; Sidhu, A.; Hauske, J. R. *Tetrahedron* **1999**, *55*, 8169−8178. (f) Bassindale, M. J.; Hamley, P.; Harrity, J. P. A. *Tetrahedron Lett.* **2001**, *42*, 9055−9057. (g) Feng, J.; Szeimies, G. *Eur. J. Org. Chem.* **2002**, 2942−2947. (h) White, B. H.; Snapper, M. L. *J. Am. Chem. Soc.* **2003**, *125*, 14901−14904.

(5) Heyns, K.; Molge, K.; Walter, W. *Chem. Ber.* **1961**, *94*, 1015−25.

(6) Tirrell, D. A.; Tang, Y.; Carrico, I. S. *Abstracts of Papers*, 222nd National Meeting of the American Chemical Society, Chicago, IL, August 26−30, 2001; American Chemical Society: Washington, DC, 2001; ORGN-019.

(7) Isolation and biochemistry: (a) Katagiri, K.; Tori, K.; Kimura, Y.; Yoshida, T.; Nagasaki, T.; Minato, H. *J. Med. Chem.* **1967**, *10*, 1149−1154. (b) Tanaka, K.; Tamaki, M.; Watanabe, S. *Biochim. Biophys. Acta* **1969**, *195*, 244−245. (c) Parry, R. J.; Buu, H. P. *J. Am. Chem. Soc.* **1983**, *105*, 7446−7447. (d) Parry, R. J.; Turakhia, R.; Buu Hanh, P. *J. Am. Chem. Soc.* **1988**, *110*, 4035−4036. (e) Kohno, T.; Kohda, D.; Haruki, M.; Yokoyama, S.; Miyazawa, T. *J. Biol. Chem.* **1990**, *265*, 6931−6935. (f) Vanbrunt, M. P. Ph.D. Dissertation, Texas A&M University, College Station, TX, 2001.

(8) Total syntheses: (a) Joullié, M. M.; Wang, P. C.; Semple, J. E. *J. Am. Chem. Soc.* **1980**, *102*, 887−889. (b) Kang, S. H.; Lee, S. B. *Chem. Commun. (Cambridge)* **1998**, 761−762. (c) Zhang, J.; Clive, D. L. J. *J. Org. Chem.* **1999**, *64*, 1754−1757. (d) VanBrunt, M. P.; Standaert, R. F. *Org. Lett.* **2000**, *2*, 705−708. (e) Zimmermann, P. J.; Blanarikova, I.; Jager, V. *Angew. Chem., Int. Ed.* **2000**, *39*, 910−912.

(9) For reviews on the use of sulfinimines in the synthesis of amino acids, see: (a) Ellman, J. A. *Pure Appl. Chem.* **2003**, *75*, 39−46. (b) Ellman, J. A.; Owens, T. D.; Tang, T. P. *Acc. Chem. Res.* **2002**, *35*, 984−995. (c) Davis, F. A.; Chen, B.-C. *Chem Soc. Rev.* **1998**, *27*, 13−18.

(10) Davis, F. A.; McCoull, W. *J. Org. Chem.* **1999**, *64*, 3396−3397.

(11) (a) Liu, G.; Cogan, D. A.; Ellman, J. A. *J. Am. Chem. Soc.* **1997**, *119*, 9913−9914. (b) Weix, D. J.; Ellman, J. A. *Org. Lett.* **2003**, *5*, 1317−1320.

(12) (a) Weber, J.; Haslinger, U.; Brinker, U. H. *J. Org. Chem.* **1999**, *64*, 6085−6086. (b) Feng, J.; Szeimies, G. *Tetrahedron* **2000**, *56*, 4249−4252.

(13) Bishop, J. E.; O'Connell, J. F.; Rapoport, H. *J. Org. Chem.* **1991**, *56*, 5079−5091.

M HCl in dioxane[14] removed the *tert*-butylsulfinyl group exclusively, providing *tert*-butyl ester **4b** in 99% yield.

The enantiomeric purity and absolute configuration of the amino ester were confirmed by converting it to both (*R*)- and (*S*)-MTPA (Mosher) amides.[15] The diastereomeric amides had well-resolved resonances for the vinylic hydrogen and methoxy protons in the ^{1}H NMR spectrum and for the trifluoromethyl group in the ^{19}F NMR. In both derivatives, the diastereomeric excess was judged to be >95% from the ^{19}F NMR, indicating an ee of >95% in the parent amino ester. Further, the chemical shifts were in excellent accord with the findings of Kusumi et al. for L-amino acid methyl esters, specifically the strong upfield shift of the OCH_3 protons and slight upfield shift of H_α in the (*R*)-MTPA-L-Val-OMe diastereomer. [16]

Subsequent removal of the *tert*-butyl ester was effected with 50% TFA in dichloromethane to afford 1-Cbg (**1**) as its trifluoroacetic acid salt in 72% yield. The stereochemical integrity of the amino acid was confirmed by converting the amino acid into both diastereomeric Mosher amides; NMR confirmed no loss of stereochemical purity in the amino acid. Use of the *tert*-butyl glyoxylate-derived sulfinimine **2b** should therefore also prove to be useful in the synthesis of other highly racemization-prone amino acids.

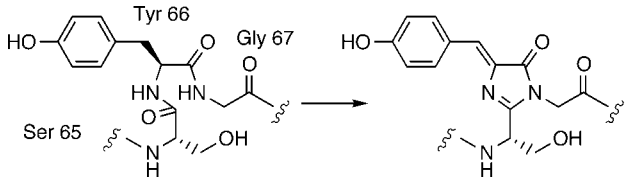
Having the free amino acid in hand, we performed preliminary tests of 1-Cbg's usefulness in its intended applications. First among these was its ability to support ribosomal protein translation. As mentioned previously, 1-Cbg is a reasonable isostere for either of the β-branched amino acids Val and Ile, but not the γ-branched Leu (isobutyl side chain). Thus, we tested for the ability of 1-Cbg to substitute for these amino acids in protein translation, with the expectation that substutition might be observed for Val or Ile but not Leu.

Translatability demands two properties of the amino acid. First, it must be a substrate for an aminoacyl tRNA synthetase (AARS), the enzyme that couples an amino acid with its corresponding tRNA. Second, the aminoacylated tRNA must be accepted by the ribosome. Of these two, the former is more important, as AARSs are highly selective among the standard amino acids,[17] whereas the ability of the ribosome to accept a vast array of unnatural amino acids has been amply demonstrated.[18]

To test the translational competence of 1-Cbg, we employed a novel approach in which the expression of green fluorescent protein (GFP) in vitro was monitored. In vitro translation has been used previously to assess the translational competence of amino acid analogues and was found to provide a useful indicator of the analogues' abilities to substitute in vivo.[19] The in vitro approach offers several advantages over in vivo expression. One is that it allows for determination of an analogue's ability to substitute for any amino acid of interest, as the amino acids are added at known concentrations, and it is a simple matter to substitute the analogue for one of the standard amino acids or to perform direct competition experiments. In addition, the method is operationally simple, obviates the need for expensive microchemical analysis of expressed proteins, and avoids a variety of complications associated with living cells (discussed further below).

One drawback to the in vitro approach as originally implemented was the need for electrophoretic separation, blotting, and radioisotopic detection of the expressed protein. These steps were circumvented in the present work by using GFP as the reporter for translation. GFP is a small (27 kDa) protein in which a fluorescent chromophore forms spontaneously and without assistance by enzymes or other catalysts (Scheme 2).[20,21] The natural fluorescence of the protein

Scheme 2. Spontaneous Formation of the GFP Chromophore



allows its relative concentration to be monitored quantitatively over time; in addition, it has been demonstrated that chromophore formation occurs cotranslationally, so that the rate of development of fluorescence is a useful proxy for the rate of protein synthesis and folding.[22]

Using in vitro-translated GFP as the reporter, 1-Cbg was tested as a substitute for Ile, Val, and Leu. To our gratification, the rate and maximal accumulation of fluorescence was essentially the same using either 1-Cbg or Ile in the translation mixture; when 1-Cbg was substituted for Val, approximately half as much expression was obtained, and when it was substituted for Leu, no expression above background was obtained.[23]

The origin of the reduced fluorescence observed with Cbg substituted for Val vs Ile is uncertain. One possibility we

(14) Cogan, D. A.; Ellman, J. A. *J. Am. Chem. Soc.* **1999**, *121*, 268−269.

(15) (a) Dale, J. A.; Mosher, H. S. *J. Am. Chem. Soc.* **1973**, *95*, 512−519. (b) Sullivan, G. R.; Dale, J. A.; Mosher, H. S. *J. Org. Chem.* **1973**, *38*, 2143-2147.

(16) Kusumi, T.; Fukushima, T.; Ohtani, I.; Kakisawa, H. *Tetrahedron Lett.* **1991**, *32*, 2939−42.

(17) Reviews: (a) Sankaranarayanan, R.; Moras, D. *Acta Biochim. Pol.* **2001**, *48*, 323−335. (b) Carter, C. W., Jr. *Annu. Rev. Biochem.* **1993**, *62*, 715−748. (c) Soll, D. *Experientia* **1990**, *46*, 1089−1096. (d) Schimmel, P. R.; Söll, D. *Annu. Rev. Biochem.* **1979**, *48*, 601−648.

(18) Reviews: (a) Sisido, M. *Biopolymers* **2003**, *7*, 25−49. (b) Steward, L. E.; Chamberlin, A. R. *Methods Mol. Biol. (Clifton, NJ)* **1998**, *77*, 325−354. (c) Hohsaka, T.; Sisido, M. *Curr. Opin. Chem. Biol.* **2002**, *6*, 809−815. (d) Gilmore, M. A.; Steward, L. E.; Chamberlin, A. R. *Top. Curr. Chem.* **1999**, *202*, 77−99. (e) Sisido, M.; Hohsaka, T. *Bull. Chem. Soc. Jpn.* **1999**, *72*, 1409−1425. (f) Thorson, J. S.; Cornish, V. W.; Barrett, J. E.; Cload, S. T.; Yano, T.; Schultz, P. G. *Methods Mol. Biol. (Clifton, NJ)* **1998**, *77*, 43−73. (g) Mendel, D.; Cornish, V. W.; Schultz, P. G. *Annu. Rev. Biophys. Biomol. Struct.* **1995**, *24*, 435−462.

(19) Kothakota, S.; Yoshikawa, E.; Murphy, O. J.; Mason, T. L.; Tirrell, D. A.; Fournier, M. J. *J. Polym. Sci., Part A: Polym. Chem.* **1995**, *33*, 1267−1274.

(20) Cody, C. W.; Prasher, D. C.; Westler, W. M.; Prendergast, F. G.; Ward, W. W. *Biochemistry* **1993**, *32*, 1212−1218.

(21) Chalfie, M.; Tu, Y.; Euskirchen, G.; Ward, W. W.; Prasher, D. C. *Science (Washington, DC)* **1994**, *263*, 802−805.

(22) Waldo, G. S.; Standish, B. M.; Berendzen, J.; Terwilliger, T. C. *Nat. Biotechnol.* **1999**, *17*, 691−695.

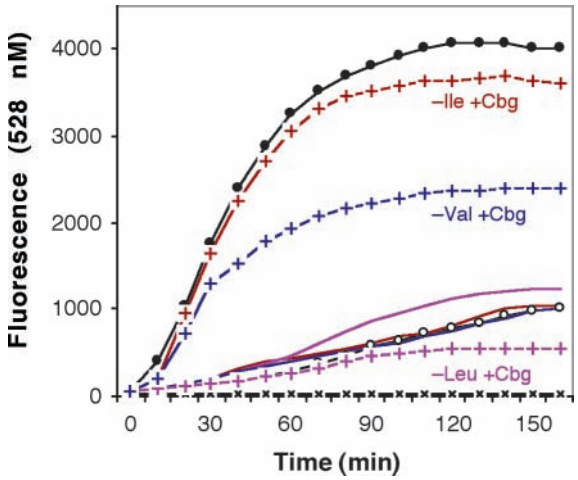


Figure 1. In vitro translation of GFP. Translations were performed using *E. coli* T7 S30 Extract System (Promega), a plasmid encoding an enhanced-fluorescence mutant of GFP (pQBI-T7-GFP, QBiogene), and amino acids (aa) at 0.1 mM concentration. Reactions were performed at 37 °C according to Promega's protocol and, with exceptions as noted, included 1 μg of plasmid and all standard aa. Fluorescence measurements were made with an excitation filter selecting for 485 ± 10 nm and an emission filter selecting for 528 ± 10 nm. Data shown are averages of triplicate runs. Black lines: ×, −DNA/−aa; open circles, −aa; filled circles, no deletions. Red lines: +,−Ile/+1-Cbg; no symbol, −Ile. Blue lines: +,−Val/+1-Cbg; no symbol, −Val. Purple lines: +,−Leu/+1-Cbg; no symbol, −Leu.

considered was that expression of the protein or formation of the chromophore was slower for the Val-labeled protein; however, the rate of fluorescence increase is essentially identical in both cases. The overall reaction progress displays pseudo-first-order kinetics, and the rate constants ($k \pm s_k$) calculated for the 10−120 min window are 0.029 ± 0.001 m^{-1} for the Ile substitution and 0.031 ± 0.001 for the Val substitution at 37 °C.[24] Therefore, the reduced fluorescence is not the result of a difference in the rate of biosynthesis, folding, or chromophore formation. Moreover, the three proteins (wild-type, Ile→1-Cbg, and Val→1-Cbg) have identical fluorescence emission and excitation spectra, with an excitation maximum at 470 nm and an emission maximum at 504 nm (not shown). Our current hypothesis is that the substitution of 1-Cbg for Val leads to a lower quantum yield for fluorescence. That 1-Cbg was able to subsitute in vitro for Ile and Val shows that it is a substrate for the corresponding Ile and Val aminoacyl tRNA synthetases and that the aminoacylated tRNAs translate efficiently. However, for the potential of 1-Cbg in protein engineering to be realized, it must also substitute in vivo, which imposes several additional requirements. First, it must enter cells; second, it must have reasonable metabolic stability; third, it must compete with a background of endogeneous amino acid (even in auxotrophic strains); finally, it must not be toxic. By using in vitro translation, the intrinsic translational competence of the amino acid can be assessed without interference.

In summary, we have completed an efficient, enantioselective synthesis of the title compound using the *tert*-butylsulfinamide chiral auxiliary and have shown that it substitutes effectively for Val and Ile in the translation of GFP in vitro. Efforts to introduce 1-Cbg into microbially expressed proteins are currently underway in this laboratory, as are efforts to explore its reactivity in synthetic peptides.

Acknowledgment. We are grateful to Dr. Jing Feng and Prof. Günther Szeimies for their experimental procedure for the preparation of 1-bromocyclobutene. We thank the University of Illinois at Chicago and the National Institutes of Health (GM 57543) for generous support of this work.

Supporting Information Available: Experimental procedures for the synthesis of 1-Cbg (**1**); ^{1}H NMR spectra for 1-Cbg (**1**) and intermediates **2b**, **3b**, and **4b**; ^{1}H and ^{19}F NMR spectra for the (*R*)- and (*S*)-MTPA derivatives of **1** and **4b**; and experimental procedures for in vitro transcription−translation. This material is available free of charge via the Internet at http://pubs.acs.org.

OL049026B

(23) Commercial S30 extracts contain varying amounts of residual amino acids that lead to background expression even in the absence of added amino acids. The experiments reported herein were performed with a batch of S30 extract from Promega (Madison, WI) selected for low background expression. Some batches were found to be unsuitable.

(24) Rate constants were calculated with the KORE program: Swain, C. G.; Swain, M. S.; Berg, L. F. *J. Chem. Inf. Comput. Sci.* **1980**, *20*, 47−51.