Pergamon

Tetrahedron Letters 43 (2002) 8215–8218

TETRAHEDRON LETTERS

Synthesis of the methyl glycoside of a branched octasaccharide fragment specific for the *Shigella flexneri* serotype 2a *O*-antigen†

Frédéric Bélot,[a] Corina Costachel,[a,b,‡] Karen Wright,[a,§] Armelle Phalipon[b] and Laurence A. Mulard[a,*]

[a]*Unité de Chimie Organique, URA CNRS 2128, Institut Pasteur, 28 rue du Dr Roux, 75 724 Paris Cedex 15, France*
[b]*Unité de Pathogénie Microbienne Moléculaire, INSERM U 389, Institut Pasteur, 28 rue du Dr Roux, 75 724 Paris Cedex 15, France*

Received 31 August 2002; revised 17 September 2002; accepted 19 September 2002

Abstract—An efficient synthesis of the methyl glycoside of a branched octasaccharide representative of *Shigella flexneri* serotype 2a *O*-specific polysaccharide is described. The synthesis is based on the use of the trichloroacetimidate methodology, and involves the condensation of a pentasaccharide acceptor and a trisaccharide donor as the key step.

Shigella flexneri serotype 2a is a common infective agent in humans that is responsible for the endemic form of shigellosis or bacillary dysentery.[2] Shigellosis is a priority target as defined by the World Health Organization since this disease is a major cause of mortality in developing countries, especially among children and in the immunocompromised population. Several programs targetting the eradication of this bacterial infection are under development. Amongst those, the design of glycoconjugate vaccines based on the use of the surface *O*-specific polysaccharide (O-SP) is of particular interest. Indeed, analogously to the use of bacterial capsular polysaccharide conjugates as efficacious vaccines against infections caused by *Haemophilus influenzae* type b or *Neisseria meningitidis* Group C,[3–5] it was hypothesized that protein conjugates of detoxified lipopolysaccharides (LPS) may protect humans against infections caused by non-capsulated bacteria.[6,7] There is evidence that conjugates incorporating oligosaccharide fragments of the native bacterial polysaccharides may be immunogenic as well.[8] As part of a program aimed at the design of optimal vaccine conjugates based on the use of synthetic fragments of the O-SP of *S. flexneri* 2a, the study of the interaction between the bacterial surface polysaccharide and homologous protective monoclonal antibodies is under investigation.

The O-SP of *S. flexneri* 2a is a heteropolysaccharide defined by the branched pentasaccharide repeating unit **I**.[9,10] Besides the known methyl glycoside of the **EC** disaccharide,[11,12] a set of di- to pentasaccharides representative of fragments of *S. flexneri* 2a O-SP have been synthesized recently in this laboratory.[13–15] The use of these compounds as molecular probes for mapping at the molecular level the binding characteristics of a set of protective antibodies against *S. flexneri* 2a infection indicated that access to larger oligosaccharides would help the characterization of the carbohydrate epitopes. For this purpose, methodologies allowing a straightforward access to *S. flexneri* 2a oligosaccharides of larger size are under study in the laboratory. As part of this study, we now report the synthesis of the first octasaccharide in the series, namely the **B(E)CDAB(E)C** fragment, which was prepared as its methyl glycoside (**1**).

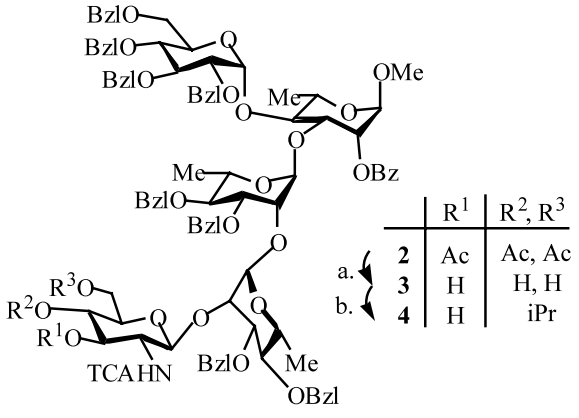
A **B** **E** **C** **D**
2)-α-L-Rha*p*-(1→2)-α-L-Rha*p*-(1→3)-[α-D-Glc*p*-(1→4)]-α-L-Rha*p*-(1→3)-β-D-GlcNAc*p*-(1→ **I**

Keywords: bacterial oligosaccharides; *Shigella flexneri*; chemical glycosylation.
* Corresponding author. Tel.: +33 1-40-61-36-77; fax: +33 1-45-68-84-04; e-mail: lmulard@pasteur.fr
† See Ref. 1.
‡ Present address: Unité des Aspergillus, Institut Pasteur, 25 rue du Dr. Roux, 75 724 Paris Cedex 15, France.
§ SIRCOB, Université de Versailles, 45 avenue des Etats-Unis, 78 035 Versailles Cedex, France.

0040-4039/02/$ - see front matter
PII: S0040-4039(02)02055-5

As the linear construction of the target **1** is not practical, one of the challenges was to define the building blocks of interest in the design of an efficient convergent synthesis. Based on previous work in the *S. flexneri* 2a series[15] as well as in the related *S. flexneri* Y series,[16,17] a retrosynthetic analysis of **1** showed that the most advantageous disconnection would be at the **CD** linkage. Such a strategy would involve a trisaccharide donor bearing a 2-*O*-acyl group at the reducing residue that could direct the glycosylation towards the desired stereochemistry and a pentasaccharide acceptor bearing a free hydroxyl group at position 3 of a potential *N*-acetyl glucosaminyl residue.

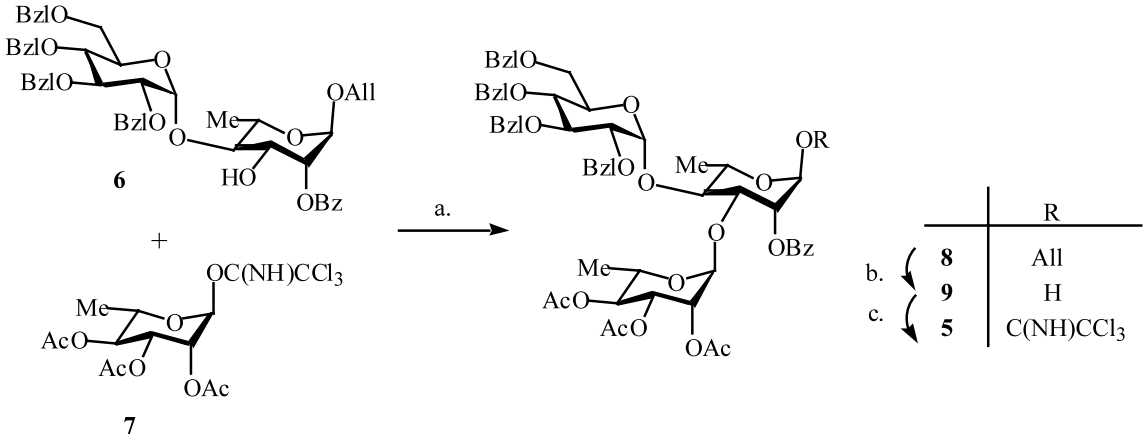
The fully protected pentasaccharide **2**, which was described recently,[14] was chosen as a suitable precursor to the latter. Previous work in the series,[15] supported by independent observations,[18] indicated that the benzoyl group at position 2_C was particularly stable and reacted poorly under Zemplén conditions. In addition, mild transesterification conditions were expected to prevent the migration of the trichloroacetyl (TCA) group to the vicinal 3_D-OH. Taking advantage of these observations, compound **2** was, indeed, selectively de-*O*-acetylated into the triol **3** (Scheme 1), which was subsequently blocked at positions 4_D and 6_D by an isopropylidene acetal to give the required pentasaccharide acceptor **4** (72%, two steps). The use of the isopropylidene acetal, despite its rather high lability under acidic conditions compared to that of the more common benzylidene protecting group, was a crucial element in the strategy. In fact, the choice of the former derived from previous work[13] in the series demonstrating the lack of reactivity under glycosylation conditions of similar acceptors bearing a benzylidene-protected glucosaminyl residue and confirmation in our hands of these observations (not described). Next, the readily available trichloroacetimidate **5** was selected as an efficient trisaccharide donor (Scheme 2). Condensation of the known disaccharide acceptor **6**[15] and rhamnopyranosyl donor **7**[19] gave the fully protected trisaccharide intermediate **8** (95%). A conventional two-step deallylation allowed the selective removal of the anomeric protecting group and consequently the smooth conversion of compound **8** into the corresponding hemiacetal **9**. Standard activation of the latter resulted in the trichloroacetimidate donor **5** (77% from **8**).



	R^1	R^2, R^3
2	Ac	Ac, Ac
3	H	H, H
4	H	iPr

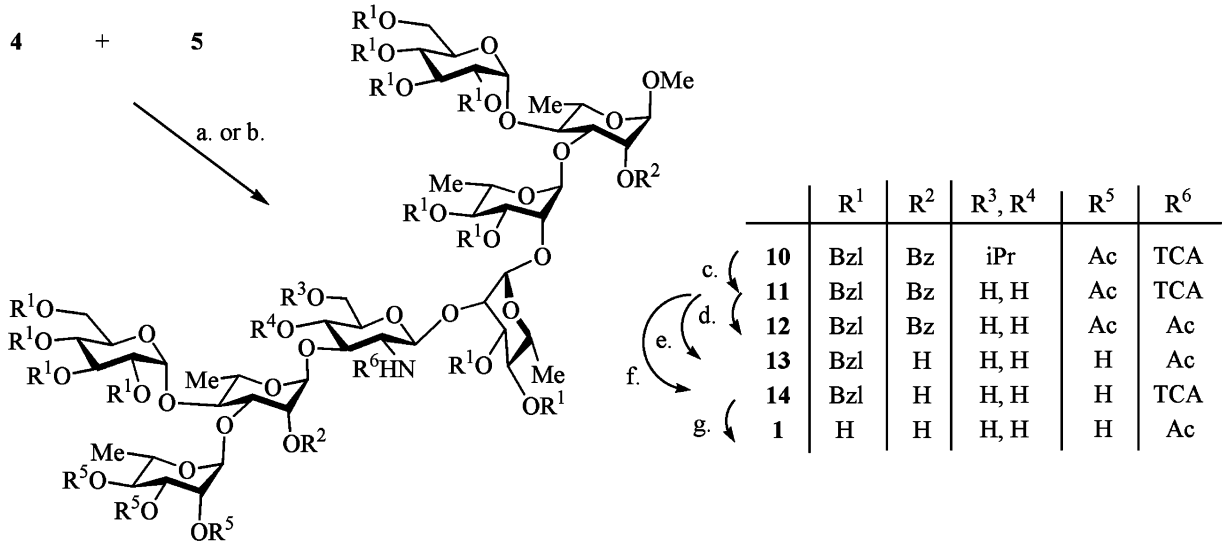
Scheme 1. (a) cat. MeONa, MeOH, 30 min; (b) 2-methoxypropene, CSA, DMF (72% from **2**).

At this stage, the key glycosylation step involving acceptor **4** and donor **5** was investigated with the aim of finding standard and reproducible conditions allowing easy access to the fully protected octasaccharide **10** (Scheme 3). The demonstration in this laboratory of the efficiency of the $BF_3{\cdot}OEt_2$ complex as an alternative to the less satisfactory TMSOTf catalyst in the synthesis of related oligosaccharides[15] encouraged the use of the former in the present work. However, reaction of **4** and **5** in the presence of the $BF_3{\cdot}OEt_2$ complex led to a major condensation product (82%) lacking the isopropylidene group as seen from the ^1H and ^{13}C NMR spectra. This side-reaction was avoided when using triflic acid as the catalyst. In the latter case, the fully protected octasaccharide **10** was isolated in a satisfactory yield (85%).[20] Acidic hydrolysis of the isopropylidene group gave access to the diol **11** (90%), the availability of which allowed further investigation of the $BF_3{\cdot}OEt_2$-mediated condensation of **4** and **5**. Comparison of the NMR data for **11** and the unknown diol was in favor of identity for the two octasaccharides. This assumption was further supported by analysis of



	R
8	All
9	H
5	$C(NH)CCl_3$

Scheme 2. (a) cat. TMSOTf, anhydrous Et_2O, 4 h, −50°C (95%); (b) i. cat. $[Ir(COD)\{PCH_3(C_6H_5)_2\}_2]^+PF_6^-$, THF, rt, 20 h, ii. HgO, $HgCl_2$, acetone/water, rt, 2 h; (c) CCl_3CN, DBU, CH_2Cl_2, 0°C, 1 h (77% from **8**).



	R^1	R^2	R^3, R^4	R^5	R^6
10	Bzl	Bz	iPr	Ac	TCA
11	Bzl	Bz	H, H	Ac	TCA
12	Bzl	Bz	H, H	Ac	Ac
13	Bzl	H	H, H	H	Ac
14	Bzl	H	H, H	H	TCA
1	H	H	H, H	H	Ac

Scheme 3. (a) cat. TfOH, 4 Å MS, 1,2-DCE, −35→+10°C, 2.5 h (85%); (b) $BF_3{\cdot}OEt_2$, 4 Å MS, DCM, −60°C→rt, 24 h (**11**, 82%); (c) 50% aq. TFA, CH_2Cl_2, 0°C, 4 h (90%); (d) Bu_3SnH, AIBN, $PhCH_3$, 80°C, 2 h; (e) i. MeONa, MeOH, ii. H_2O, 60°C, 16 h, iii. Ac_2O, rt, 16 h; (f) cat. MeONa, MeOH, 55°C, 24 h (80%); (g) i. H_2, Pd/C, EtOH/AcOEt/1 M aq. HCl (2 equiv.), 72 h, ii. H_2, Pd/C, Et_3N, MeOH, 24 h (77%).

the acetylation product of the unknown diol. A switch from 3.3 to 3.7 ppm, respectively, was observed for both H-6_D in the corresponding ^1H NMR spectra. Thus, it is assumed that the isolation of **11** in the $BF_3{\cdot}OEt_2$-mediated glycosylation is a two-step process involving first the condensation of **4** and **5**, and second the hydrolysis of the acetal protecting group. It is expected that a better control of the kinetics of the reaction would avoid the deblocking step. Conversion of the *N*-TCA participating group into the required *N*-acetyl group was found to be a rather challenging task. As observed previously in the series, the use of radical conditions[21,22] yielding a mixture of partially dechlorinated products failed to give the expected **12**. Attempts to convert **11** into **13** by a two-step process involving alkaline removal of the TCA group and in situ *N*-acetylation following a recently described procedure,[23] yielded again an unworkable mixture of compounds. The outcome of the latter reaction was tentatively blamed on the presence of the benzoyl groups at position 2_C and $2_{C'}$ which, as shown earlier in analogous compounds, were expected to be rather resistant to hydrolysis due to steric hindrance. Consequently, diol **11** was engaged in a controlled *O*-deacylation process to give the heptaol **14** (80%). The latter was next submitted to an efficient, two-step, in-house process involving first, hydrogenolysis under acidic conditions which allowed the removal of the benzyl groups and second, basic hydrochlorination which resulted in the conversion of the trichloroacetamide intermediate into the target octasaccharide **1** (78%).

The structure of all intermediates and of the target **1** were unambiguously confirmed by ^1H and ^{13}C NMR analysis as well as by mass spectrometry and elemental analysis. For selected data see Ref. 24. The results described herein support our hypothesis that, starting from readily available precursors, a strategy based on a disconnection at the CD linkage and the use of standard glycochemistry is appropriate for the synthesis of complex oligosaccharides in the *S. flexneri* 2a series.

Acknowledgements

The authors thank Professor P. J. Sansonetti who is a scholar of the Howard Hughes Medical Institute for his key input in the project. The authors are grateful to J. Ughetto-Monfrin (Unité de Chimie Organique, Institut Pasteur) for recording all the NMR spectra. The authors thank the CANAM, the Fondation pour la Recherche Médicale, the Bourses Mrs. Frank Howard Foundation and the Bourses Roux foundation for financial support.

References

1. Part 9 of the series, Synthesis of ligands related to the O-SP of *Shigella flexneri* serotype 2a and *Shigella flexneri* serotype 5a. For part 8, see Ref. 15.
2. Sansonetti, P. *Rev. Infect. Dis.* **1991**, *13*, S285–S292.
3. Mulard, L.; Alonso, J. M.; Fournier, J. M. In *La Vaccinologie*; Annales de l'Institut Pasteur/actualités; Cohen, G. N.; Sansonetti, P. Eds.; Éditions scientifiques et médicales; Elsevier SAS: Paris, 2002; pp. 39–54.
4. Wenger, J. D.; Booy, R.; Health, P. T.; Moxon, R. In *New Generation Vaccines*; 2nd ed., revised and expanded ed.; Levine, M. M., Woodrow, G. C., Kaper, J. B., Cobon, G. S., Eds.; Marcel Dekker: New York, 1997; pp. 489–502.
5. MacLennan, J. M.; Shackley, F.; Heath, P. T.; Deeks, J. J.; Flamank, C.; Herbert, M.; Griffith, H.; Hatzmann, E.; Goilav, C.; Moxon, E. R. *J. Am. Med. Assoc.* **2000**, *283*, 2795–2801.

6. Robbins, J. B.; Chu, C.; Watson, D. C.; Szu, S. C.; Daniels, E. M.; Lowe, C. U.; Schneerson, R. *Rev. Infect. Dis.* **1991**, *13*, S362–365.
7. Robbins, J. B.; Schneerson, R.; Szu, S. C. In *New Generation Vaccines*; Levine, M. M.; Woodrow, G. C.; Kaper, J. B.; Cobon, G. S., Eds.; Marcel Dekker, 1997; pp. 803–815.
8. Pozsgay, V. In *Adv. Carbohydr. Chem. Biochem.*; Horton, D., Ed.; Academic Press: San Diego, 2000; Vol. 56, pp. 153–199.
9. Simmons, D. A. R. *Bacteriol. Rev.* **1971**, *35*, 117–148.
10. Lindberg, A. A.; Karnell, A.; Weintraub, A. *Rev. Infect. Dis.* **1991**, *13*, S279–S284.
11. Berry, J. M.; Dutton, G. G. S. *Can. J. Chem.* **1974**, *54*, 681–683.
12. Lipkind, G. M.; Shashkov, A. S.; Nikolaev, A. V.; Mamyan, S. S.; Kochetkov, N. K. *Bioorg. Khim.* **1987**, *13*, 1081–1092.
13. Mulard, L. A.; Costachel, C.; Sansonetti, P. J. *J. Carbohydr. Chem.* **2000**, *19*, 849–877.
14. Costachel, C.; Sansonetti, P. J.; Mulard, L. A. *J. Carbohydr. Chem.* **2000**, *19*, 1131–1150.
15. Segat, F.; Mulard, L. A. *Tetrahedron: Asymmetry* **2002**, in press.
16. Pinto, B. M.; Reimer, K. B.; Morissette, D. G.; Bundle, D. R. *J. Chem. Soc. Perkin Trans. 1* **1990**, 293–299.
17. Pinto, B. M.; Reimer, K. B.; Morissette, D. G.; Bundle, D. R. *J. Org. Chem.* **1989**, *54*, 2650–2656.
18. Varga, Z.; Bajza, I.; Batta, G.; Liptak, A. *Tetrahedron Lett.* **2001**, *42*, 5283–5286.
19. Kitagawa, I.; Baek, N. I.; Ohashi, K.; Sakagami, M.; Yoshikawa, M.; Shibuya, H. *Chem. Pharm. Bull.* **1989**, *37*, 1131–1133.
20. Detailed procedure: A mixture of **5** (150 mg, 0.12 mmol) and **4** (150 mg, 83 μmol), 4 Å molecular sieves and dry 1,2-DCE was stirred for 1 h then cooled to −35°C. Triflic acid (5 μl) was added. The stirred mixture was allowed to reach 10°C in 2 h 30 min. Et_3N was added and the mixture filtered. After evaporation, chromatography of the residue afforded **10** (208 mg, 85%). Selected data for **10**: ^{1}H NMR (400 MHz, $CDCl_3$): δ ^{1}H: δ 6.82 (d, 1H, $J_{2,NH}=8.2$ Hz, NH_D), 5.38 (d, 1H, $J_{1,2}<1.0$ Hz, $J_{2,3}=2.8$ Hz, H-2_C), 5.27 (d, 1H, $J_{1,2}<1.0$ Hz, $J_{2,3}=2.8$ Hz, H-2_C), 5.15 (m, 2H, $J_{1,2}=1.0$ Hz, $J_{2,3}=2.8$ Hz, H-2_B and H-1_B), 5.08 (dd, 1H, $J_{2,3}=2.8$ Hz, $J_{3,4}=10.0$ Hz, H-3_B), 5.01 (d, 1H, $J_{1,2}<1.0$ Hz, H-1_C), 4.96 (d, 1H, $J_{1,2}<1.0$ Hz, H-1_A), 4.94 (d, 1H, $J_{1,2}<1.0$ Hz, H-1_C), 4.90 (d, 1H, $J_{1,2}=3.3$ Hz, H-1_E), 4.85 (d, 1H, $J_{1,2}=3.3$ Hz, H-1_E), 4.82 (dd, 1H, $J_{3,4}=J_{4,5}=10.0$ Hz, H-4_B), 4.80 (d, 1H, $J_{1,2}=8.5$ Hz, H-1_D), 4.68 (d, 1H, $J_{1,2}<1.0$ Hz, H-1_B), 3.29 (s, 3H, OCH_3), 1.98, 1.78, 1.68 (3s, 9H, OAc), 1.26–0.85 (m, 15H, H-6_A, 2 H-6_B, 2 H-6_C), 1.18, 1.16 (2s, 6H, $(CH_3)_2$). ^{13}C: δ 168.5, 168.2, 168.1, 164.4, 164.1, 160.3 (6C, C=O), 101.4 (bs, 2C, 2 C-1_C), 101.2 (C-1_A), 101.0 (C-1_D), 99.8 ($C(CH_3)_2$), 98.4 (bs, 2C, 2 C-1_E), 98.2 (C-1_B), 97.4 (C-1_B), 91.0 (CCl_3), 62.1 (C-6_D), 59.3 (C-2_D), 55.5 (OCH_3), 29.4 ($C(CH_3)_2$), 21.3, 21.0, 20.9 (3C, OAc), 19.46 ($C(CH_3)_2$), 19.2, 19.1, 18.6, 18.2, 17.5 (5C, C-6_A, 2 C-6_B, 2 C-6_C). FAB MS for $C_{158}H_{174}Cl_3NO_{41}$ (M, 2849.4), m/z 2872.5 ($[M+Na]^+$). Anal. calcd for $C_{158}H_{174}Cl_3NO_{41}$ C, 66.60; H, 6.16; N, 0.49; found: C, 66.53; H, 6.28; N, 0.43.
21. Blatter, G.; Beau, J.-M.; Jacquinet, J.-C. *Carbohydr. Res.* **1994**, *260*, 189–202.
22. Bélot, F.; Jacquinet, J.-C. *Carbohydr. Res.* **2000**, *326*, 88–97.
23. Scherman, A. A.; Yudina, O. A.; Minorov, Y. V.; Sukhova, E. V.; Shashkov, A. S.; Menshov, V. M.; Nifantiev, N. E. *Carbohydr. Res.* **2001**, *336*, 13–46.
24. Selected data for **1**: ^{1}H NMR (400 MHz, D_2O): δ 5.12 (d, 1H, $J_{1,2}=3.5$ Hz, H-1_E), 5.09 (d, 1H, $J_{1,2}=3.5$ Hz, H-1_E), 5.06 (d, 1H, $J_{1,2}<1.0$ Hz, H-1_A), 4.98 (d, 1H, $J_{1,2}<1.0$ Hz, H-1_B), 4.88 (d, 1H, $J_{1,2}<1.0$ Hz, H-1_C), 4.76 (d, 1H, $J_{1,2}<1.0$ Hz, H-1_B), 4.65 (d, 1H, $J_{1,2}=8.5$ Hz, H-1_D), 4.58 (d, 1H, $J_{1,2}=2.0$ Hz, H-1_C), 3.32 (s, 3H, OCH_3), 2.00 (s, 3H, AcNH), 1.35–1.19 (m, 15H, H-6_A, 2 H-6_B, 2 H-6_C). ^{13}C NMR: δ 103.3 (C-1_C), 102.6 (C-1_D), 101.7 (C-1_A), 101.4 (C-1_B), 101.1 (C-1_B), 100.8 (C-1_C), 98.6 (C-1_E), 98.4 (C-1_E), 61.0, 60.9, 60.8 (3C, C-6_D, 2 C-6_E), 55.9 (C-2_D), 55.3 (OCH_3), 22.7 (AcNH), 18.3, 18.0, 17.1, 16.9, 16.8 (5C, C-6_A, 2 C-6_B, 2 C-6_C). HRMS: calcd for $C_{51}H_{87}NO_{36}$+Na: 1312.4906. Found: 1312.4902.