Tetrahedron Letters,Vol.27,No.16,pp 1819-1822,1986 0040-4039/86 $3.00 + .00
Printed in Great Britain

NEW DEVELOPMENT OF A COMMON GLUCOSAMINE DISACCHARIDE INTERMEDIATE WITH CHEMICALLY DIFFERENTIATED TWO AMINO AND SIX HYDROXYL GROUPS FOR LIPID A SYNTHESES AND A FORMAL SYNTHESIS OF *SALMONELLA* MUTANT LIPID A.[1)]

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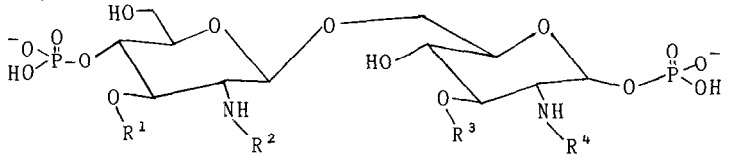
Abstract: A formal synthesis of *Salmonella* mutant lipid A via the novel disaccharide intermediate bearing chemically differentiated two amino and six hydroxyl groups was described.

Lipid A, a biologically active constituent of LPS of Gram-negative bacteria, was found recently to contain a β-1,6-linked D-glucosamine disaccharide substituted by two phosphates and four ester- and amide-bonded fatty acid.[2,3)] On the basis of these results, *Salmonella* mutant (**1a**)[4a)] and *Escherichia coli* lipid As (**1b**)[4c)] were synthesized by Shiba group using the elegant two fragment condensation method.[4)]

We wish to describe here new development of the common key disaccharide intermediate (**2**) bearing chemically differentiated two amino and six hydroxyl groups which is capable of direct conversion into several lipid As (**1a-e**),[2,5)] and a formal synthesis of **1a** from **2**.



1a; $R^1=R^2=R^3=R^4=C_{14}$-OH

1b; $R^1=C_{14}$-O-C_{14}, $R^2=C_{14}$-O-C_{12}, $R^3=R^4=C_{14}$-OH

1c; $R^1=C_{14}$-O-C_{14}, $R^2=C_{14}$-O-C_{12}, $R^3=C_{14}$-OH, $R^4=C_{14}$-O-C_{16}

1d; $R^1=C_{14}$-O-C_{14}, $R^2=C_{14}$-O-C_{14}, $R^3=R^4=C_{14}$-OH

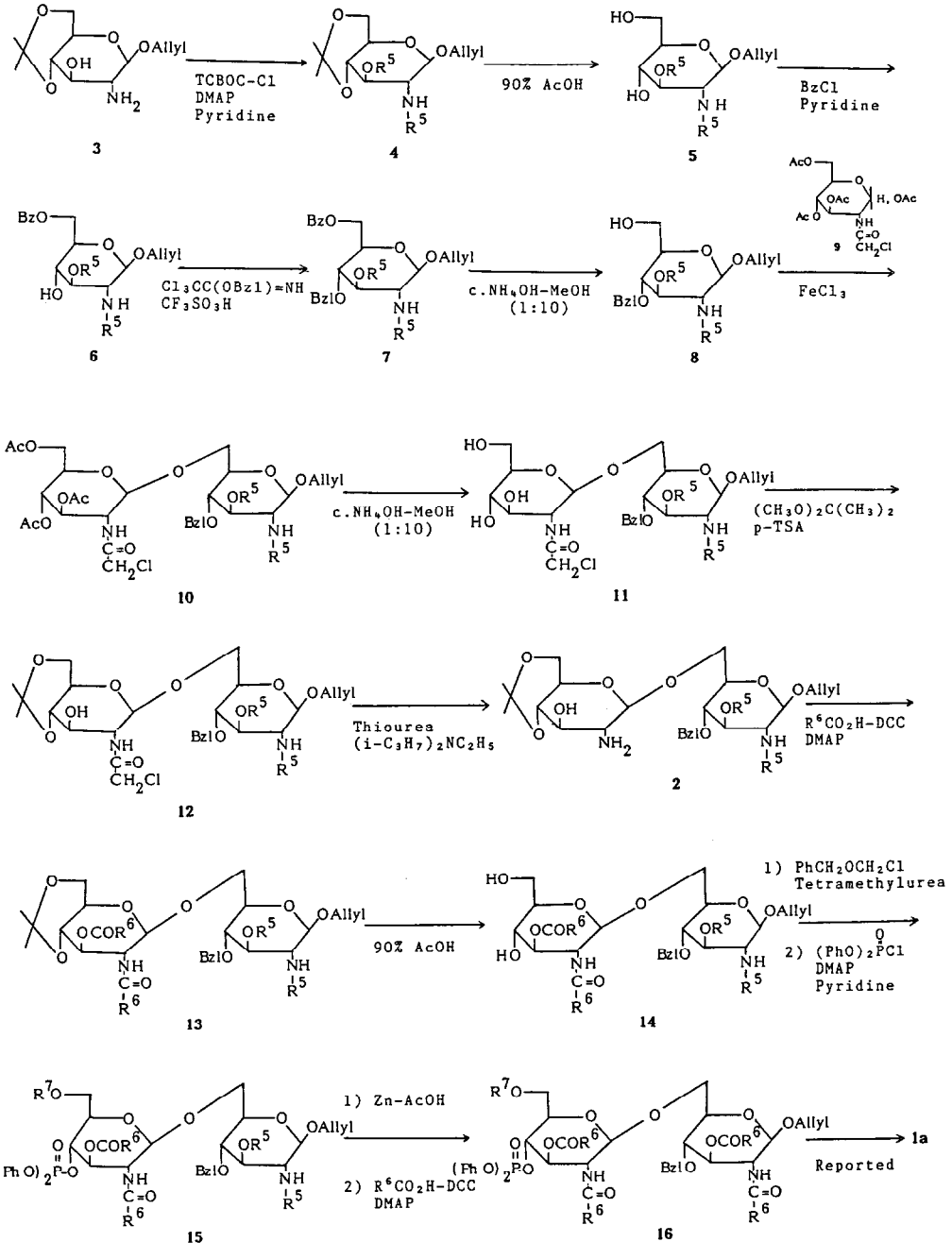
1e; $R^1=C_{14}$-O-C_{14}, $R^2=C_{14}$-O-C_{14}, $R^3=C_{14}$-OH, $R^4=C_{14}$-O-C_{16}

C_{14}-OH: (R)-3-hydroxytetradecanoyl, C_{14}-O-C_{12}: (R)-3-dodecanoyloxytetradecanoyl, C_{14}-O-C_{14}: (R)-3-tetradecanoyloxytetradecanoyl, C_{14}-O-C_{16}: (R)-3-hexadecanoyloxytetradecanoyl.

The compound (**4**)[6] was prepared by treating the free amino and hydroxyl groups of allyl 2-amino-2-deoxy-4,6-isopropylidene-β-D-glucopyranoside (**3**)[4d] with 2,2,2-trichloro-1,1-dimethyl-ethyl chloroformate (TCBOC chloride), an acid and base resistant protecting group, in pyridine containing a catalytic amount of 4-dimethylaminopyridine at room temperature for 12 hr [95%, mp 85-87°C, $[\alpha]_D^{21}$ -18.1° (c=1.00, $CHCl_3$)]. The isopropylidene group of **4** was then removed by hydrolysis with 90% acetic acid at 90°C for 15 min to yield **5**[6] [90%, mp 106°C, $[\alpha]_D^{21}$ -10.7° (c=1.00, $CHCl_3$)]. Treatment of the diol (**5**) with benzoyl chloride in pyridine-THF at 0°C for 9 hr afforded 6-benzoyl compound (**6**)[6] [75%, mp 84°C, $[\alpha]_D^{20}$ -9.97° (c=1.00, $CHCl_3$)]. The following benzylation of **6** was carried out with benzyl 2,2,2-trichloroacetimidate and a catalytic amount of trifluoromethanesulfonic acid at room temperature for 15 hr to yield **7**[6] [49%, mp 69°C, $[\alpha]_D^{23}$ +9.73° (c=1.04, $CHCl_3$)]. Debenzoylation of **7** was then carried out with aqueous NH_4OH-MeOH (1:10) to give **8**[6] [84%, mp 75-77°C, $[\alpha]_D^{24}$ -9.98° (c=1.04, $CHCl_3$)].

Condensation of the two components, **8** and **9** (used in 1:2 molar ratio), proceeded in the presence of $FeCl_3$ and N,N,N',N'-tetramethylurea in CH_2Cl_2 at room temperature for 24 hr to give a simple disaccharide (**10**)[6] [72%, mp 100-102°C, $[\alpha]_D^{21}$ -6.97° (c=1.13, $CHCl_3$)]. The 3',4',6'-acetyl groups of **10** were then removed with aqueous NH_4OH-MeOH (1:10) at room temperature for 15 hr to give **11**[6] [86%, mp 174-175°C, $[\alpha]_D^{24}$ -21.6° (c=1.00, MeOH)]. The compound (**11**) was converted into 4',6'-isopropylidene derivative (**12**)[6] with 2,2-dimethoxypropane-TsOH in DMF at room temperature for 5 hr [71%, mp 126-128°C, $[\alpha]_D^{26}$ -23.7° (c=0.27, $CHCl_3$)]. Successful removed of the N-chloroacetyl group was effected by refluxing a mixture of **12**, thiourea and diisopropylethylamine (1:5:5) in THF for 12 hr to afford the general key intermediate (**2**)[6] which bears chemically differentiated two amino and six hydroxyl groups suitable for syntheses of several lipid As [93%, mp 118-120°C, $[\alpha]_D^{21}$ -20.0° (c=1.11, $CHCl_3$)].

Next, to justify the utility of the key intermediate (**2**), a formal synthesis of *Salmonella* mutant lipid A was carried out as follows. The free amino and hydroxyl groups of **2** were acylated with (R)-3-benzyloxytetradecanoic acid in the presence of dicyclohexylcarbodiimide and a catalytic amount of 4-dimethylaminopyridine in CH_2Cl_2 at room temperature for 12 hr to give **13**[6] [88%, mp 95-97°C, $[\alpha]_D^{27}$ -13.0° (c=0.64, $CHCl_3$)]. Removal of the acetonide from **13** with 90% acetic acid at 90°C for 15 min afforded **14**[6] [94%, mp 65-67°C, $[\alpha]_D^{20}$ -13.3° (c=1.09, $CHCl_3$)]. Benzyloxymethylation with benzyloxymethyl chloride and tetramethylurea in CH_2Cl_2 followed by phosphorylation with diphenylphosphoryl chloride in the presence of pyridine and 4-dimethylaminopyridine in CH_2Cl_2 gave the fully substituted compound (**15**)[6] [55%, mp 53-55°C, $[\alpha]_D^{21}$ -4.72° (c=1.09, $CHCl_3$)]. Replacement of the TCBOC group into an acyl group was carried out as follows. Treatment of **15** with Zn-acetic acid at room temperature for 5 hr and the following acylation of the deprotected compound with (R)-3-benzyloxytetra-



Ac : CH_3CO, Bz : C_6H_5CO, Bzl : $C_6H_5CH_2$, Allyl : $CH_2=CHCH_2$, R^5= TCBOC = $Cl_3CC(CH_3)_2OCO$,
R^6= $CH_3(CH_2)_{10}CHCH_2$, R^7= $C_6H_5CH_2OCH_2$
$C_6H_5CH_2O$

decanoic acid, dicyclohexylcarbodiimide and 4-dimethylaminopyridine in CH_2Cl_2 at room temperature gave **16**[6], the intermediate for *Salmonella* mutant lipid A synthesis reported by Shiba group[7] [81%, mp 50-52°C, $[\alpha]_D^{19}$ -1.65° (c=1.29, $CHCl_3$)].

It should be also noted that the present work offered the common key intermediate (**2**) convertible into several lipid As.

Acknowledgment: We are grateful to professor T. Shiba for his kindness to send us the ^{1}H-NMR spectrum of **17**[7] for identification.

References and Notes

1) Lipid A and related compounds. V., Part IV; ref 4g.

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6) Satisfactory analytical and spectral data were obtained for this compound.

7) Identification was carried out with the authentic ^{1}H-NMR spectrum of the deallylated compound (**17**) derived from **16**, because of no recording the authentic spectral data.

(Received in Japan 13 February 1986)