Combinatorial approach toward synthesis of small molecule libraries as bacterial transglycosylase inhibitors†

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Received 15th January 2010, Accepted 19th February 2010
First published as an Advance Article on the web 29th March 2010
DOI: 10.1039/c000622j

The development of iminocyclitol-based small molecule libraries against a bacterial TGase is described. An iminocyclitol was conjugated with a pyrophosphate mimic using either a 1,3-dipolar cycloaddition or reductive amination reaction, which was then condensed with a variety of lipophilic carboxylic acids in an amide bond coupling to generate a desired molecular library. With assistance of microtiter plate-based combinatorial chemistry and *in situ* screening, a potential inhibitor, the first potent iminocyclitol-based inhibitor against bacterial TGases was efficiently developed.

Introduction

The crisis of multi-drug resistance requires the urgent discovery of new antibacterial drugs and targets.[1] Inhibition of bacterial cell wall biosynthesis receives much attention as the lack of a eukaryotic counterpart reduces the potential for side effects.[2] Among the several enzymes involved in cell-wall biosynthesis, transglycosylase (TGase), a class of glycosyltransferases (GTases) (EC 2.4), catalyzes the transfer of the sugar moiety from the activated polymeric peptidoglycan (a glycosyl donor) to the specific hydroxyl group (4-OH) of Lipid II (**1**, a glycosyl acceptor), with concomitant release of a C55 undecaprenyl pyrophosphate carrier (Fig. 1).[3] This bacterial enzyme (TGase) is considered a promising antibiotic target, due to its essential function and ready accessibility, it is located on the external surface of bacterial membrane, within easy reach of inhibitors.[4]

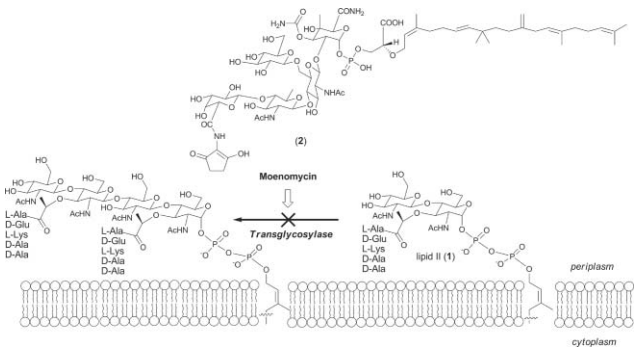

Fig. 1 Chemical structures of lipid II (**1**) and moenomycin A (**2**) and general function of transglycosylase (TGase).

To date, only moenomycin complexes such as moenomycins A (**2**), A12, C1, C3, and C4, are known to inhibit TGases. However, due to their poor bioavailability, moenomycins are currently used only as growth promoters in animal feeds.[5] The main impediments to the progress of TGase inhibitor discovery are the lack of both a convenient high throughput screening (HTS) assay, and an efficient synthetic protocol for the small molecule library preparation.

Recently, we have developed a HTS TGase assay called Fluorescence Anisotropy (FA)-based Moenomycin A-binding assay (also called fluorescence polarization binding assay (FP-binding assay)).[6] This assay can be used as a primary assay to quickly identify potential hits from compound libraries, the activity of which would be confirmed using the HPLC-based lipid II polymerizing activity analysis (lipid II-based functional assay).[7]

The design and preparation of a TGase inhibitor library is therefore the present challenge. To the best of our knowledge, few TGase inhibitors have been reported, all of which are based on the chemical structures of **1** or **2**.[8]

We posited that a molecule comprising an iminocyclitol (also called an azasugar), a pyrophosphate mimic and a lipophilic moiety would have the potential to impart inhibitory activity, since azasugars are thought to mimic the shape and charge distribution of the oxonium ion transition state during the sugar transfer process;[9] the lipophilic part would interact with TGases or cell membrane; and the pyrophosphate-like moiety would mimic the pyrophosphate group or be a tether (Fig. 2).

As neither the undecaprenyl moiety (C55) of **1** nor moenocinol (C25) moiety of **2** are easily accessible starting materials, we adopted a combinatorial approach to identify a new, simple lipophilic moiety. To simplify the library preparation, it was decided to conjugate an adduct (iminocyclitol-pyrophosphate mimic) with a lipophilic part by means of an amide bond (temporary linker Z) rather than the native ether bond (linker Y) because the amide bond is much easier than the ether bond to form (Fig. 2). These simplified libraries are called 'primary libraries'. Each member of a given primary library was screened in the FP-binding assay, and the bioactivity of the hits was confirmed by using the lipid II-based functional assay. The structures of hits were turned by, for example, synthesizing analogues containing the native ether linker (Fig. 3). This technique allows rapid screening of lipid moieties, as well as optimization of the orientation and identities of the other fragments. Herein, a new TGase inhibitor discovered using this strategy is disclosed.

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† Electronic supplementary information (ESI) available: Experimental details and NMR spectra. See DOI: 10.1039/c000622j

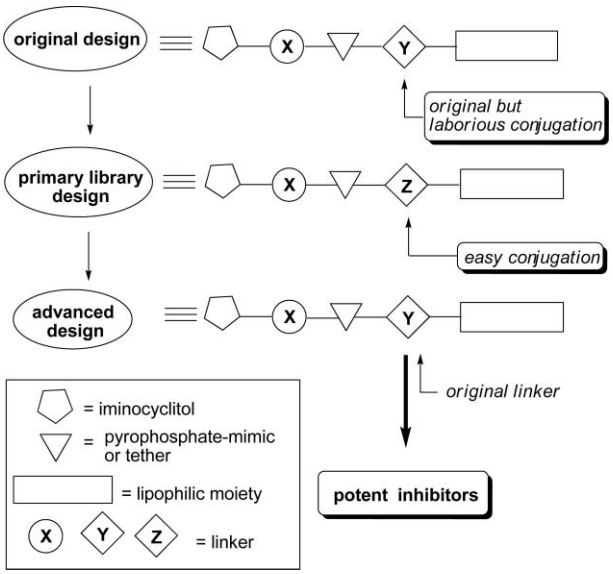


Fig. 2 General structures and principles for a primary library design.

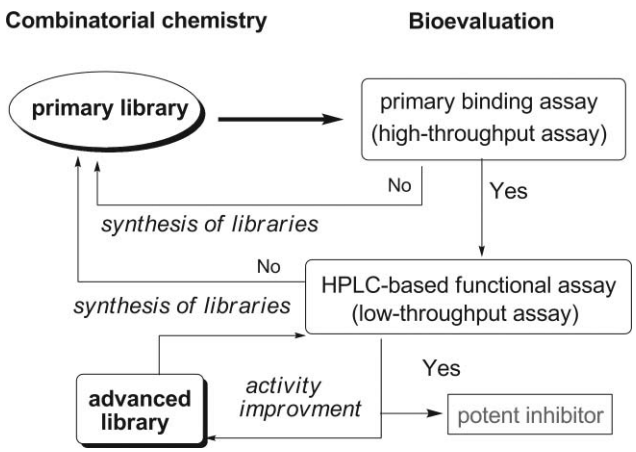


Fig. 3 The relationship between library synthesis and bio-evaluation.

Results and discussion

Preparation of the primary library A

Inspired by uridine diphosphono-β-Gal*f* mimics,[10] our initial desired structure consists of an iminocyclitol, a lipophilic part, and a 2-hydroxypropyl tether as shown in Fig. 4. In our retrosynthetic analysis (Fig. 4), **3** is conjugated with **4** *via* a 1,3-dipolar cycloaddition to give an adduct, which is linked to members of a diverse acid library **5** in an amide-bond forming reaction to generate our first primary library.

Cyclic nitrone **3** was prepared in four steps from tri-*O*-benzyl D-arabinose according to our previous report.[11] For the preparation of **4**, dibenzyl allylphosphonate (**6**)[12] was treated with oxalyl chloride to give phosphonochloridate **7**, which was reacted with benzyl 3-hydroxypropylcarbamate in the presence of triethylamine to obtain **4** in overall 61% yield for two steps (Scheme 1).

As illustrated in Scheme 2, the cycloaddition of **3** and **4** gave **8** as a single product in 42% yield with high region- and stereoselectivity. The configuration of the newly established chiral

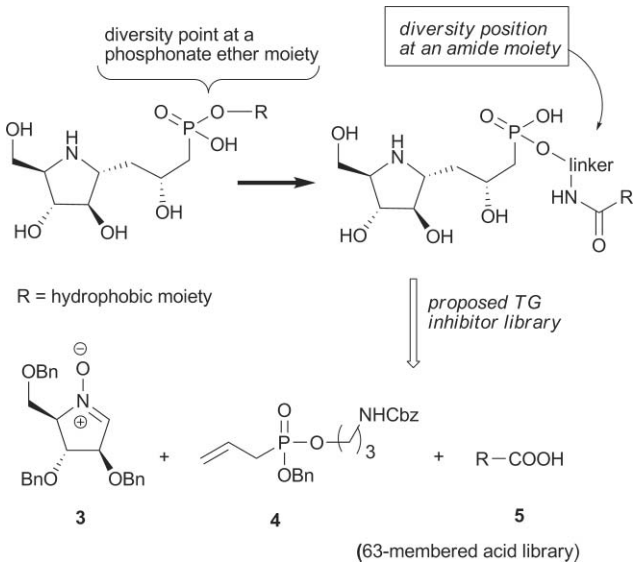
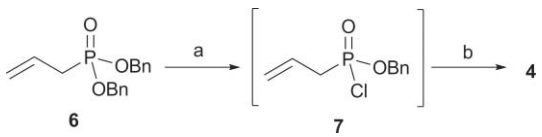
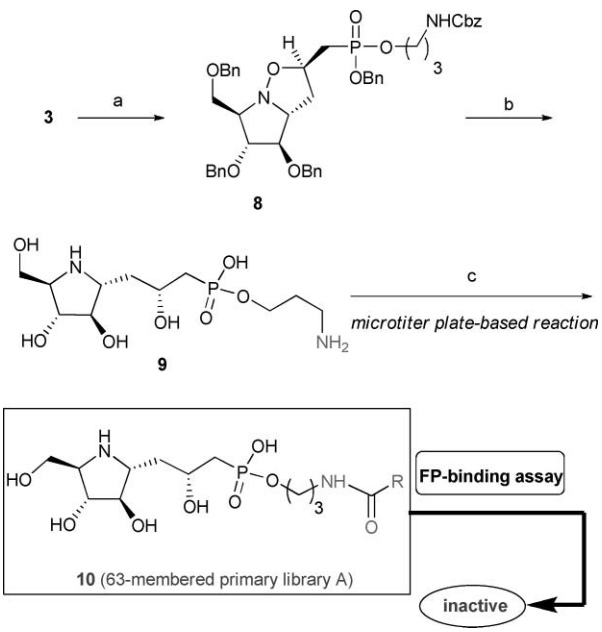


Fig. 4 Proposed TGase inhibitor library and retro-synthetic analysis.



Scheme 1 Preparation of unsaturated phosphonate diester **4**. *Reagents and conditions*: (a) $(COCl)_2$, CH_2Cl_2; (b) benzyl 3-hydroxypropylcarbamate, Et_3N, CH_2Cl_2.

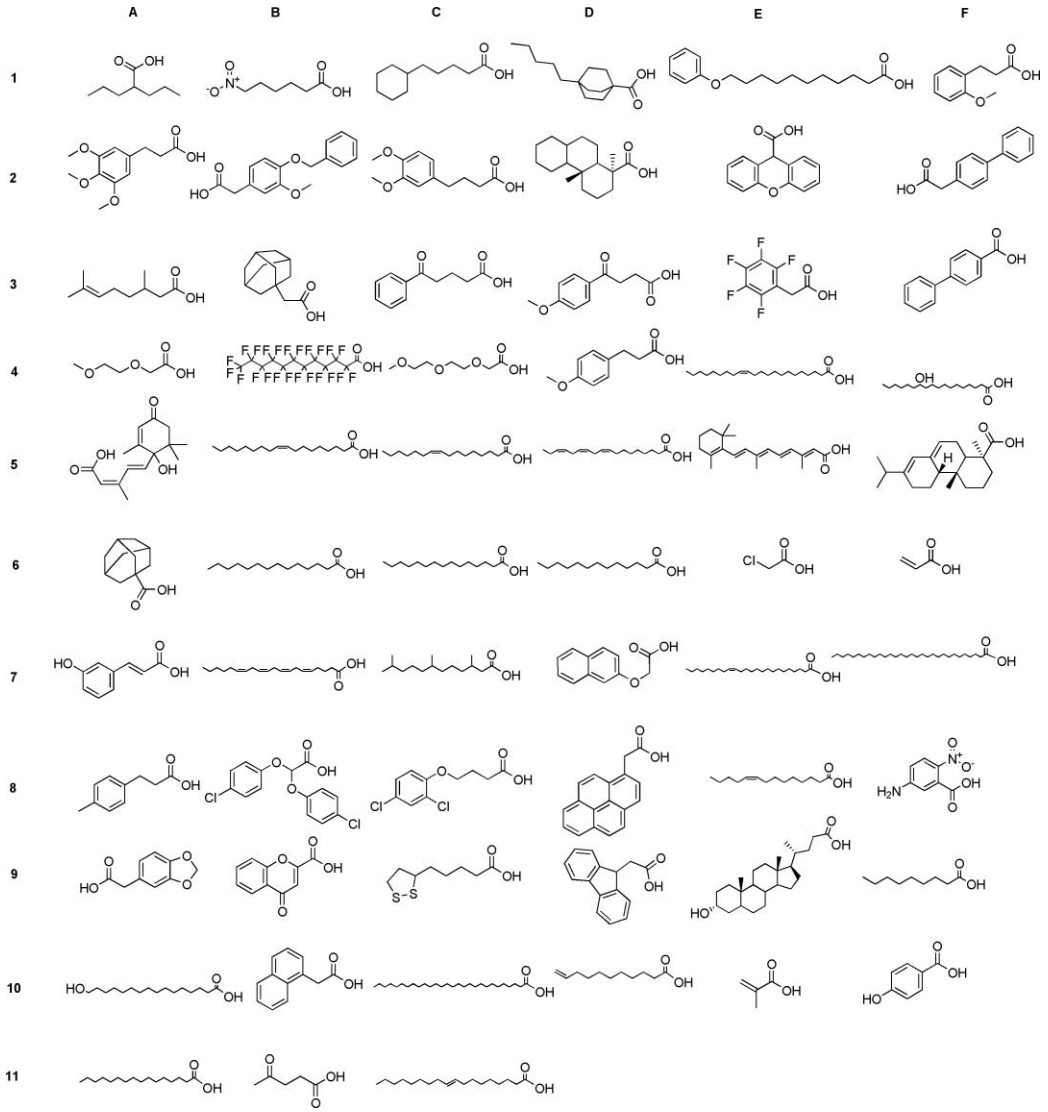


Scheme 2 Preparation of the primary library A (**10**). *Reagents and conditions*: (a) **4**, TCE, 80 °C; (b) H_2, $Pd(OH)_2$, MeOH; (c) acid library **5**, HBTU, HOBt, DIEA, DMF.

centers in **8** was determined by the NMR analysis and similar literature reports.[10]

Hydrogenation of **8** gave amine **9**, which was coupled with a randomly selected 63-membered acid library **5** (Table 1) by prior activation of the carboxylic acids in the presence of HBTU

Table 1 Diverse carboxylic acid library **5** for amide bond formation

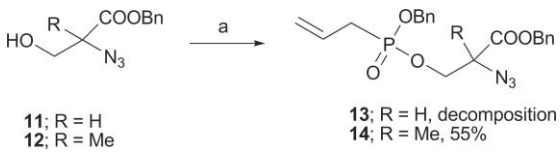


(1.5 equiv), HOBt (1.5 equiv) and DIEA (3 equiv) in DMF. After 24 h, the reactions were analyzed by LC-MS. It was found that amine **9** had been completely consumed and significant amount of **10** formed (estimate conversion). The 63 members (10 mM) in the primary library A (**10**) were directly evaluated in the HTS FP-binding assay without further purification.[13] Unfortunately, at an inhibitor concentration of 1 mM, no hits were identified.

Preparation of the primary library B

Our re-design was inspired from the phosphoglycerate moiety in moenomycin A (**2**). Initial attempts to conjugate alcohol **11** with phosphonochloridate **7** were not successful, perhaps due to decomposition of **13** *via* β-elimination.[14] In contrast, the stable, unsaturated phosphonomethylserine **14** could be prepared from **12**[15] in a reasonable yield (55%) (Scheme 3).

In a similar procedure to that depicted in Scheme 2, amine **16**, prepared from **3** *via* 1,3-dipolar cycloaddition with **14**, N–O bond reduction, and debenzylation, was coupled with the acid

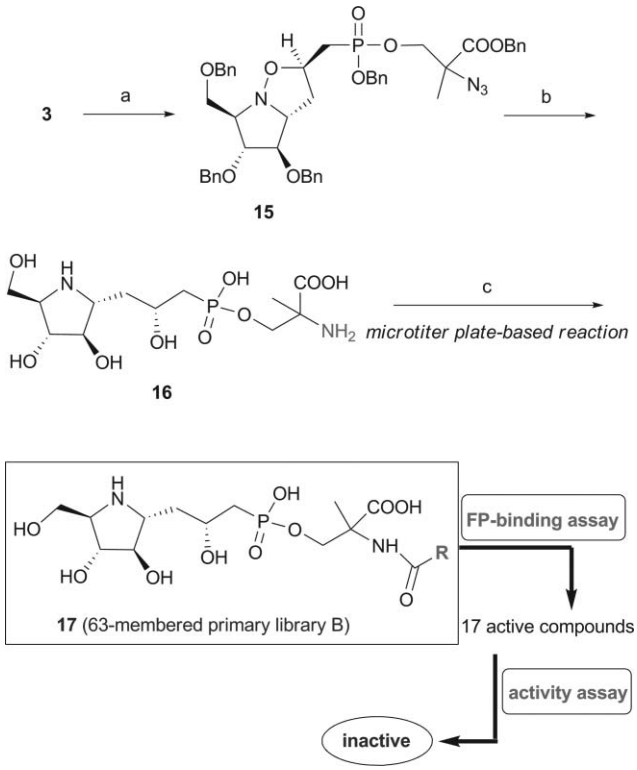


Scheme 3 Preparation of unsaturated phosphono methylserine **14**. *Reagents and conditions*: (a) **7**, Et_3N, CH_2Cl_2.

library **5** to generate the primary library B (**17**) in a microtiter plate (Scheme 4). After the FP-binding assay screening, 17 hits were found at 1 mM, none of which was potent in the further functional assay screening at the same final concentration (1 mM). This disappointing result suggested that a fundamental re-design of the inhibitor was required.

Preparation of the primary library C

After detailed studies of the chemical structures of the proposed transition state, we designed a new iminocyclitol-based structure



Scheme 4 Preparation of the primary library B (**17**). *Reagents and conditions*: (a) **14**, TCE, 80 °C; (b) H_2, $Pd(OH)_2$, MeOH; (c) acid library **5**, HBTU, HOBt, DIEA, DMF.

bearing an *N*-acetyl methyl group at the C-2 position and linking with other fragments at the nitrogen on the ring system *via* reductive amination (Fig. 5).

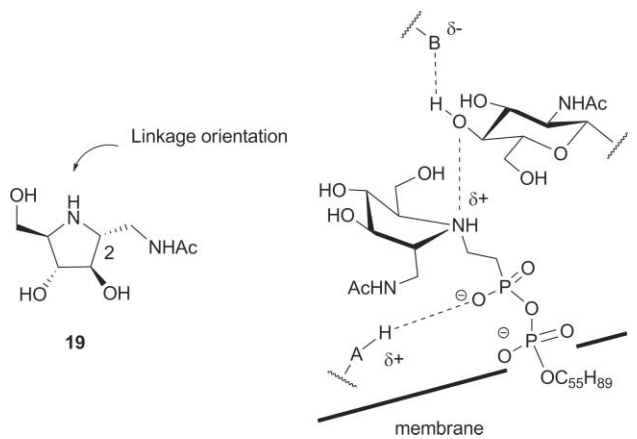
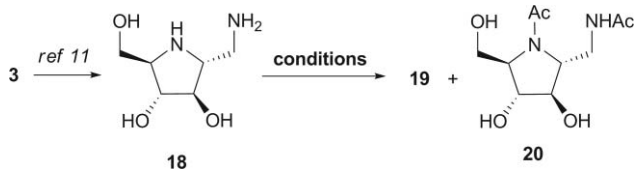


Fig. 5 Proposed transition state and desired iminocyclitol fragment **19**.

According to our previous report,[11] **18** (ADMDP) was conveniently prepared from cyclic nitrone **3** *via* a four-step procedure in 77% overall yield. Unfortunately, selective mono-acetylation of **18** on a multi-gram scale was not successful.[16] Various conditions were screened (Table 2), and it was found that the use of acetic anhydride (1.5 equiv) in a buffer solution (pyridine and aqueous HCl, pH ~ 4) could obtain **19** in satisfactory yield (73%; see entry 4).

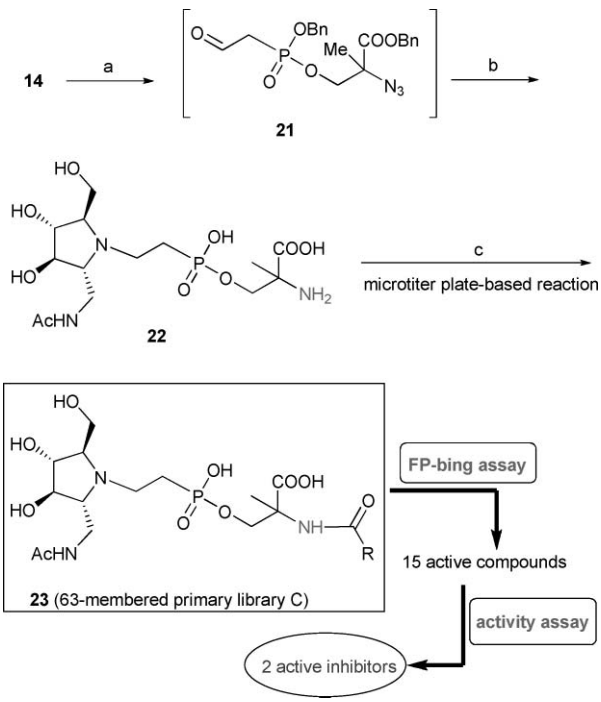
Table 2 *N*-Acetylation of **18** under various conditions



Entry	conditions	time	*T*/°C	**19** (%)[a]	**20** (%)[a]	**18** (%)[a]
1	Ac_2O	10 min	4 to rt	18	42	33
2	Ac_2O/py.	2 h	4 to rt	13	33	42
3	AcOH/EDCI	8 h	rt	8	47	33
4	Ac_2O/py./2N HCl	3 h	rt	73	8	8

[a] Isolated yield after column chromatography.

Dihydroxylation of alkene **14** using OsO_4 and NMO followed by oxidative cleavage with $NaIO_4$ gave **21**. Since aldehyde **21** was not stable to chromatography or even when left to stand at room temperature, it was freshly prepared and used immediately without purification. Condensation of **21** with **19** in the presence of $NaCNBH_3$, followed by hydrogenolysis gave **22** in 54% overall yield over the four steps from **14** (Scheme 5). After rapid library generation and *in situ* screening, 15 hits were found out at 1 mM. These were submitted for the lipid II-based functional assay (activity assay) and two compounds **24** and **25** were found as inhibitors (~60% inhibition) (Fig. 6). Our screening data indicated the structure of the lipid moiety dramatically influences the inhibition: lipophilic moieties in both active molecules were branched not linear, biphenyl, or cholesterol-related.



Scheme 5 Preparation of the primary library C (**23**). *Reagents and conditions*: (a) (i) OsO_4, NMO, acetone; (ii) $NaIO_4$, THF; (b) (i) **19**, $NaCNBH_3$, MeOH; (ii) H_2, $Pd(OH)_2$, MeOH; (c) acid library **5**, HBTU, HOBt, DIEA, DMF.

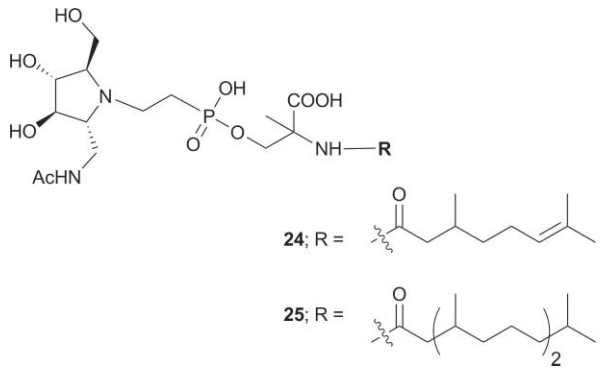


Fig. 6 Chemical structures of **24** and **25**.

Table 3 TGase inhibition study

Compound	Inhibition% [a](1 mM)	Inhibition% (100 μM)
22	—[b]	—
24	69	11
25	73	15
31	100	>80
32	38	< 10
33	71	< 10
34	82	< 10
35	—	—
36	—	—
37	41	13
38	77	42
Moe A (**2**)[c]	100	100

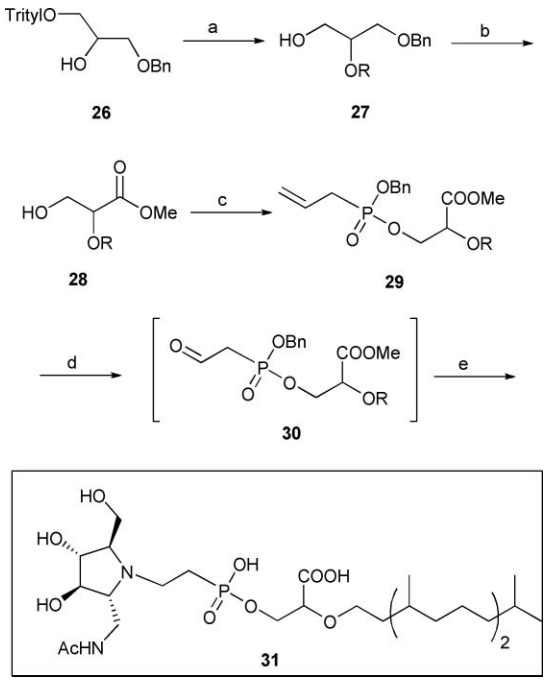
[a] 1. Inhibition % calculated by comparing the reaction rate in the presence of the compound to the rate in the absence of any inhibitory compound. 2. *E. Coli.* PBP1b test system. [b] — refers to no inhibition. [c] IC_{50} of moenomycin A (**2**) was around 40 nM in our HPLC activity assay system.

After re-synthesis of **24** and **25**, followed by purification, the inhibitory activities were re-examined (Table 3). It can be seen that they are slightly higher than that of the unpurified samples, synthesized in the microtiter plate-based parallel synthesis.

Next, the amide linkage of **24** was replaced by the original ether linkage and the inhibitory potency was investigated again (Scheme 6). Alkylation of **26**[17] with 3,6,9-trimethyldodecanyl bromide, followed by acid medicated hydrolysis gave **27** (60%). Alcohol **27** was converted to the corresponding **28** in 47% overall yield for three steps: oxidation (bis-acetoxyiodobenzene (BAIB), 2,2,6,6-tetramethylpiperidine-1-oxyl radical (TEMO)),[18] esterification, and debenzylation. Notably, debenzylation in acid mediated conditions (BCl_3/CH_2Cl_2) was found to be more efficient and practical than catalytic hydrogenolysis, even after a prolonged reaction time (3 days) under 70 psi of hydrogen.

Following the similar transformations (**14** → **22**) in Scheme 5, compound **31**, containing an ether linkage instead of amide group, was generated in 38% overall yield for four steps from **29**. Similarly, compound **32** was prepared from **26** using dodecanyl bromide as an alkylation reagent. For comparison purposes, several interesting molecules, shown in Fig. 7, were prepared such as two iminocyclitol-based compounds **33** and **34**, lipid I (**37**), and lipid II analogue **38**.[19]

All of the compounds described above were tested for inhibitory activity against the *E. coli* transglycosylase PBP1b (Table 3). The



Scheme 6 Preparation of **31** bearing the ether linkage. *Reagents and conditions*: (a) (i) NaH, 3,7,11-trimethyldodecane bromide, DMF; (ii) CSA, MeOH; (b) (i) BAIB, TEMPO, acetone; (ii) DCC, MeOH; (iii) BCl_3, CH_2Cl_2; (c) **7**, Et_3N, CH_2Cl_2; (d) (i) OsO_4, NMO, acetone; (ii) $NaIO_4$, THF; (e) (i) **19**, $NaCNBH_3$, MeOH; (ii) H_2, $Pd(OH)_2$, MeOH.

results showed the lipophilic moiety to be essential for inhibition of TGase activity. For example, compounds **24** and **25** exhibited the inhibitory potency but **22**, the truncated form without a lipophilic part, did not. Similar observations have been previously reported in studies of Moenomycin A (**2**), when the lipophilic moiety (moenocinol) was removed from **2**, the inhibitory activity was completely lost.[20] Our results also indicated that the branched lipid-linked **31** was a more potent inhibitor (80% inhibition at 100 μM) than the linear lipid-linked **32** (almost no inhibition at 100 μM). The linkage between the lipophilic moiety and the pyrophosphate-mimic moiety was also found to affect the inhibitory activity, **31** (with an ether linkage) was five times more potent than **25** (with an amide linkage) (Table 3).

In this study, **31** exhibited the best inhibitory potency against *E. coli* transglycosylase PBP1b. When presented at concentrations of 1 mM and 100 μM, the inhibition activities were 100% and 80%, respectively, two and six times more potent than the lipid II-based inhibitor **38** or lipid I (**37**), respectively. In contrast, **35** and **36**, with no iminocyclitol moiety, exhibited no inhibitory activity. Presumably, **31** is a transition-state mimic inhibitor and its lipophilic part can interact both with the enzyme and also the cell membrane, increasing its local concentration.[21] In addition, the *N*-acetyl methyl moiety at the C2 position on the pyrrolidine ring played a crucial role for inhibition (Table 3; **31** *v.s.* **33**). Compound **31** was also tested against other TGases including *C. difficile*, *S. aureus* and *H. pylori*, and found to exhibit an inhibitory effect against all three (>80% inhibition at 100 μM). Although the MIC value of **31** against *S. aureus* was lower (125 μM), it still constituents a useful lead in the search for more potent and selective inhibitors.

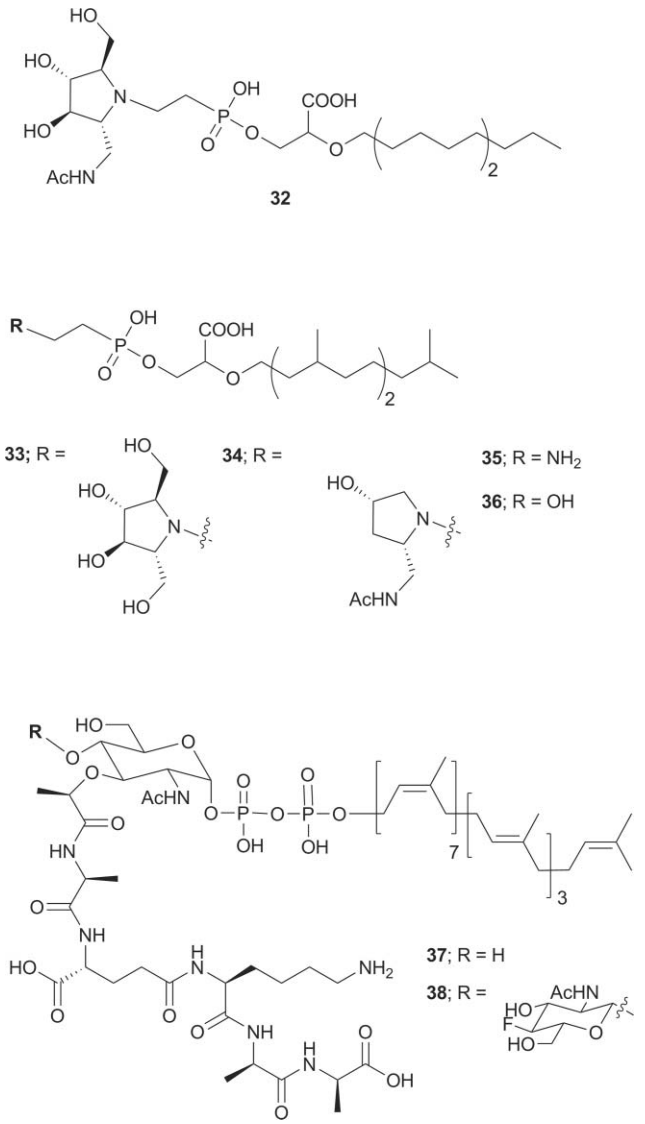


Fig. 7 Chemical structures of **32–38**.

Conclusion

We have successfully developed a strategy to efficiently assemble an iminocyclitol, a pyrophosphate mimic, and a lipophilic moiety for the discovery of bacterial TGase inhibitors, and used it to discover a novel, broad spectrum TGase inhibitor. Several primary libraries were rapidly synthesized and evaluated, and the optimized moieties and their orientations were disclosed. The phosphono methylserine moiety in **22** can be considered a preliminary surrogate of the pyrophosphate mimic, and the amino group is the diversity point, by virtue of its capacity to be easily, quickly, and directly conjugated with various acids. We are currently using this approach to synthesize and identify other potent glycosyltransferase inhibitors and also explore more potent bacterial TGase inhibitors.

Experimental section

General information. All the solvents and reagents were obtained commercially and used without further purification. NMR spectra (^{1}H at 600 MHz; ^{13}C at 150 MHz) were recorded on a spectrometer in d-solvent at ambient temperature. Mass spectra were obtained by BRUKER Daltonics Bio-TOF III. CC refers to column chromatography.

19. Ac_2O (0.12 mL, 1.2 mmol) in water (1 mL) was added dropwise to a solution of diamine **18** (0.2 g, 1.2 mmol) in a buffer solution of 2 N HCl (0.6 mL, 1.2 mmol) and pyridine (0.2 mL, 2.6 mmol) in water (5 mL). The mixture was stirred for 3 h. After the reaction was completed (monitored by TLC), Dowex 550A resin was added to adjust the pH value to 8. The resin was removed by filtration and filtrate was evaporated to dryness. The residue was purified by CC (n-propanol : 2 N NH_4OH = 7 : 3) to give oil compound in 73% yield. ^{1}H NMR (600 MHz, $CDCl_3$) δ 1.87 (s, 3H), 3.40–3.60 (m, 4H), 3.79 (dd, 1H, J = 6.1 and 12.4 Hz), 3.87 (dd, 1H, J = 3.7 and 12.4 Hz), 3.97 (t, 1H, J = 6.6 Hz), 4.01 (t, 1H, J = 7.2 Hz); ^{13}C NMR (150 MHz, $CDCl_3$) δ 170.7, 76.2, 74.7, 62.4, 60.9, 58.6, 39.1, 21.6; HRMS calcd for $[C_8H_{16}N_2O_4+H]^+$ 205.1188, found 205.1198.

22. To a solution of **14** (1.5 g, 3.5 mmol) in acetone (30 mL) and water (5 mL) was added *N*-methylmorpholine *N*-oxide (NMO) (1 g, 7.7 mmol) and catalytic amount of osmium tetraoxide (OsO_4). The solution was stirred overnight. The reaction was quenched by Na_2SO_3 (2 g) and stirred for another 1 h. The solution was evaporated to dryness and the residue was washed with EtOAc then filtrated. The organic layer was dried with $MgSO_4$ and concentrated. The residue was purified by CC (10% MeOH in EA) to give unseparable diastereomers as a yellow oil in 77% yield. ^{1}H NMR (600 MHz, $CDCl_3$) δ 1.43 (s, 3H), 2.13–2.28 (m, 2H), 3.44 (m, 1H), 3.86–4.44 (m, 5H), 4.94–5.07 (m, 4H), 7.36–7.44 (m, 10H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 169.9, 136.3, 134.9, 128.6 (× 2), 128.5 (× 2), 128.2 (× 2), 127.2 (× 4), 71.6, 69.1, 67.6, 67.4, 67.1, 60.1, 37.7, 20.1; HRMS calcd for $[C_{21}H_{26}N_3O_7P+H]^+$ 464.1587, found 464.1593.

The diol (1 g, 2 mmol) in THF (5 mL) and water (5 mL) was added by sodium periodate ($NaIO_4$) (0.64 g, 3 mmol) and stired 1 h at RT. The precipitate was filtrated and washed with THF. The organic layer was evaporated to dryness and the aldehyde was used directly without further purification. The amine **19** (0.27 g, 1.3 mmol) in MeOH (10 mL) was added to the aldehyde and stirred 10 min at RT, then $NaCNBH_3$ (0.2 g, 2.6 mmol) was added in one portion and the mixture was stirred overnight. After the reaction was complete, the solution was evaporated to dryness. The residue was purified by CC (10% MeOH in DCM) to give unseparable diastereomers as an oil in 72% yield. ^{1}H NMR (600 MHz, $CDCl_3$) δ 1.36 (s, 3H), 1.98 (s, 3H), 2.06–2.18 (m, 2H), 2.88–3.11 (m, 5H), 3.66–3.72 (m, 6H), 3.91–3.93 (m, 1H), 4.96–5.25 (m, 4H), 7.31–7.41 (m, 10H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 170.1, 136.3, 134.9, 128.6 (× 2), 128.5 (× 2), 128.2 (× 2), 127.2 (× 4), 79.1, 69.3, 68.2, 67.9, 67.7, 67.6, 67.5, 66.8, 65.9, 65.1, 59.4, 39.5, 37.1, 21.3, 18.3; HRMS calcd for $[C_{28}H_{38}N_5O_9P+H]^+$ 620.2485, found 620.2491.

A solution of azide (0.2 g, 0.5 mmol), $Pd(OH)_2$ and 1 drop AcOH in MeOH (10 mL) was stirred 24 h under hydrogen atmosphere. After the reaction was complete, the solution was filtrated and evaporated to dryness to give the title compound as unseparable diastereomers in 89% yield. ^{1}H NMR (600 MHz, $CDCl_3$) δ 1.45 (s, 3H), 1.82–2.01 (m, 5H), 2.95–3.01 (m, 2H), 3.11 (br, 1H), 3.15 (br, 1H), 3.40–3.47 (m, 1H), 3.55 (dd, 1H, J = 4.2 and 14.4 Hz), 3.78 (d, 2H, J = 4.2 Hz), 3.86 (t, 1H J = 3 Hz), 3.93–3.95 (m, 1H), 3.99 (br, 1H), 4.11 (dd, 1H, J = 4.2 and 10.8 Hz); ^{13}C NMR (150 MHz,

$CDCl_3$) δ 174.5, 174.4, 78.6, 78.0, 67.7, 66.8, 66.7, 66.7, 66.2, 63.4, 61.2, 61.1, 58.4, 58.3, 41.7, 37.1, 37.0, 24.7, 24.4, 23.8, 21.8, 18.4; HRMS calcd for $[C_{14}H_{28}N_3O_9P+H]^+$ 414.1641, found 414.1649.

24. A solution of acid (0.08 g, 0.54 mmol), HBTU (0.2 g, 0.54 mmol), HOBT (0.07 g, 0.54 mmol) and DIEA (0.18 mL, 1.1 mmol) in dried DMF was stirred 15 min, then amine **22** (0.15 g, 0.36 mmol) was added. The reaction was stirred at RT overnight. The solution was evaporated to dryness and residue was purified by CC (n-propanol : 2 N NH_4OH = 7 : 3) to give title compound as unseparable diastereomers in 68% yield. ^{1}H NMR (600 MHz, $CDCl_3$) δ 0.84 (d, 3H, J = 6.6 Hz), 0.96 (br, 1H), 1.09–1.28 (m, 2H), 1.40 (s, 3H), 1.52 (s, 3H), 1.59 (s, 3H), 1.67 (br, 1H), 1.78–1.82 (m, 1H), 1.88–2.07 (m, 10H), 2.19 (dd, 1H, J = 6.0 and 14.4 Hz), 3.31–3.37 (m, 2H), 3.52–3.62 (m, 5H), 3.88–3.91 (m, 3H), 3.99 (br, 1H), 4.06–4.14 (m, 2H), 5.04 (t, 1H, J = 6.6 Hz); ^{13}C NMR (150 MHz, $CDCl_3$) δ180.9, 176.2, 176.2, 175.6, 175.5, 133.3, 125.8, 78.1, 18.0, 77.9, 77.8, 71.7, 71.5, 70.1, 68.7, 62.7, 62.7, 62.6, 58.9, 58.8, 46.2, 46.1, 44.6, 37.9, 31.5, 26.5, 26.3, 23.2, 20.3, 19.9, 18.1; HRMS calcd for $[C_{23}H_{42}N_3O_{10}P+H]^+$ 552.2686, found 552.2695.

25. The procedure used for the synthesis of **25** was similar to **24**. Compound **25** was obtained as unseparable diastereomers.^{1}H NMR (600 MHz, $CDCl_3$) δ 0.77–0.90 (m, 12H), 1.01–1.33 (m, 12H), 1.44 (s, 3H), 1.88–1.85 (m, 1H), 1.98–2.23 (m, 1H), 3.28–3.35 (m, 2H), 3.45–3.55 (m, 3H), 3.61–3.66 (m, 1H), 3.86–3.97 (m, 4H), 4.06–4.08 (m, 1H), 4.15–4.19 (m, 1H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 178.6, 174.2, 174.1, 174.0, 173.9, 77.3, 77.2, 77.0, 70.0, 69.8, 68.4, 67.3, 65.7, 61.3, 61.2, 57.8, 57.6, 48.3, 48.1, 44.1, 43.1, 43.0, 39.3, 39.1, 37.3, 37.2, 37.1, 37.0, 37.0, 36.9, 36.6, 32.7, 32.4, 30.4, 30.1, 27.8, 27.7, 24.7, 24.4, 24.3; HRMS calcd for $[C_{29}H_{56}N_3O_{10}P+H]^+$ 638.3782, found 638.3788.

26. To a solution of diol (5.7 g, 31 mmol) and triphenylmethyl chloride (8.7 g, 31 mmol) in DCM (100 mL) at 0 °C was slowly added TEA (6.5 mL, 47 mmol). The reaction was slowly warmed to RT and stirred for 2 h. The mixture was poured into water, diluted with DCM and extracted with 1 N HCl, water and $NaHCO_{3(aq)}$. The organic layer was dried with $MgSO_4$ and concentrated. The residue was purified by CC (DCM with 1% TEA) to give unseparable enantiomers as yellow oil in 87% yield. ^{1}H NMR (600 MHz, $CDCl_3$) δ 3.21 (dd, 2H, J = 5.4 and 9.6 Hz), 3.57 (dd, 2H, J = 6.0 and 9.6 Hz), 3.98 (m, 1H), 4.53 (s, 2H), 7.18–7.42 (m, 20H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 144.1 (× 3), 138.3, 128.7 (× 6), 128.4 (× 2), 128.3 (× 2), 127.7 (× 6), 127.5, 126.9 (× 3), 86.5, 78.3, 73.2, 70.7, 70.5; HRMS calcd for $[C_{29}H_{28}O_3+H]^+$ 425.2116, found 425.2122.

27. To a solution of alcohol **26** (5.1 g, 12 mmol) and 3,7,11-trimethyldodecan-1-ol (4.5 mL, 18 mmol) in dried DMF (150 mL) at 0 °C was added NaH (0.58 g, 14 mmol) in one portion. The reaction was slowly warmed to RT and stirred for 5 h. The mixture was poured into water, diluted with ether and extracted with water and $NaHCO_{3(aq)}$. The organic layer was dried with $MgSO_4$ and concentrated. The residue was used without further purification. A solution of alkane (5.1 g, 7.6 mmol) and camphorsulfonic acid (CSA) (0.53 g, 2.2 mmol) in MeOH (100 mL) was reflux for 1 h. The reaction was quenched with TEA and evaporated to dryness. The mixture was extracted with water and $NaHCO_{3(aq)}$. The organic layer was dried with $MgSO_4$ and concentrated. The residue was purified by CC (30% EtOAc in hexane) to give unseparable enantiomers as yellow oil. Yield : 82%. ^{1}H NMR (600 MHz, $CDCl_3$) δ 0.81–0.87 (m, 12H), 1.03–1.48 (m, 13H), 1.50–1.61 (m, 4H), 3.51–3.56 (m, 4H), 3.60–3.71 (m, 3H), 5.08 (d, 2H, J = 2.4 Hz), , 7.27–7.32 (m, 5H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 137.9, 128.4 (× 2), 127.7, 127.6 (× 2), 78.4, 73.5, 69.9, 68.6, 62.7, 39.3, 37.4, 37.3, 37.2, 37.1, 37.0, 32.7, 29.8, 27.9, 24.8, 24.3, 22.6, 19.6, 19.5; HRMS calcd for $[C_{25}H_{44}O_3+H]^+$ 393.3369, found 393.3363

28. To a solution of alcohol **27** (2.6 g, 6.3 mmol) in acetone (30 mL) and water (5 mL) at 0 °C was added (bis-acetoxyiodobenzene) (BAIB) (5.4 g, 16 mmol) and TEMPO (0.2 g, 1.3 mmol). The reaction was slowly warmed to RT and stirred for 3 h. The mixture was poured into water, diluted with EtOAc and extracted with water. The aqueous layer was extracted with EtOAc twice and the combined EtOAc was further extracted with $Na_2S_2O_3$ and water. The organic layer was dried with $MgSO_4$ and concentrated. The residue was used directly without further purification. To a solution of acid in DCM (20 mL) was added DCC (2.7 g, 13 mmol), MeOH (5 mL) and catalytic amount of DMAP. The mixture was stirred at RT overnight. The reaction was concentrated, washed with hexane and filtrated. The filtrate was concentrated and used directly without further purification. A solution of benzyl protected glycerate (1.3 g, 2.9 mmol) in DCM (50 mL) was cooled to −50 °C and BCl_3 (1M in hexane, 4.5 mL, 4.5 mmol) was added dropwisely. After the reaction was complete (~ 1h), the solution was quenched with MeOH (10 mL) and TEA (10 mL) and evaporated to dryness. The residual was purified by CC (20% EtOAc in hexane) to give unseparable enantiomers Yield : 88%. ^{1}H NMR (600 MHz, $CDCl_3$) δ 0.81–1.58 (m, 29H), 3.40–3.46 (m, 1H), 3.70–3.86 (m, 5H), 3.96 (dd, 1H, J = 3.0 and 6.0 Hz); ^{13}C NMR (150 MHz, $CDCl_3$) δ 171.4, 79.5, 69.7, 63.4, 52.0, 39.3, 37.7 (× 4), 32.8, 29.7, 27.9, 24.7, 24.3, 22.6 (× 2), 19.6 (× 2); HRMS calcd for $[C_{19}H_{38}O_4+H]^+$ 331.2848, found 331.2855.

29. The procedure used for the synthesis of **29** was similar to **4** (see supporting information). Yield : 71%. ^{1}H NMR (600 MHz, $CDCl_3$) δ 0.81–1.32 (m, 27H), 1.50 (br, 1H), 1.63 (br, 1H), 2.62 (m, 2H), 3.44 (m, 1H), 3.66 (m, 1H), 3.71 (m, 3H), 4.04 (br, 1H), 4.17–4.33 (m, 2H), 5.04 (m, 2H), 5.17 (m, 2H), 5.74 (m, 1H), 7.30–7.35 (m, 5H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 170.2, 136.2, 128.6 (× 2), 128.5, 127.8, 126.9 (× 2), 120.3, 78.2, 69.7, 67.2, 65.8, 52.1, 39.3, 37.7 (× 4), 37.3, 32.8, 29.7, 27.9, 24.7, 24.3, 22.6 (× 2), 19.6 (× 2); HRMS calcd for $[C_{29}H_{49}O_6P+H]^+$ 525.3345, found 525.3351.

31. The procedure used for the synthesis of **31** was similar to **22**. Yield : 92%. ^{1}H NMR (600 MHz, MeOH-d4) δ 0.85–0.90 (m, 12H), 1.07–1.68 (m, 17H), 2.01 (s, 3H), 2.08 (m, 2H), 3.45–3.76 (m, 8H), 3.92–3.97 (m, 2H), 4.05 (t, 1H, J = 2.8 Hz), 4.09–4.16 (m, 4H); ^{13}C NMR (150 MHz, MeOH-d4) δ 176.6, 172.4, 81.7, 81.5, 79.3, 78.5, 69.6, 69.1, 68.9, 68.4, 68.2, 66.1, 64.8, 63.4, 58.5, 58.4, 48.5, 40.7, 39.1, 37.4 (× 4), 37.3, 37.2, 37.0, 36.4, 36.3, 32.6, 29.8, 29.7, 27.7, 24.5, 24.1, 24.0, 21.8 (× 2), 19.6 (× 2); HRMS calcd for $[C_{28}H_{55}N_2O_{10}P+H]^+$ 611.3673, found 611.3682.

32. The procedure used for the synthesis of **32** was similar to **22**. Yield : 88%. ^{1}H NMR (600 MHz, MeOH-d4) δ 0.88 (t, 3H, J = 7.0 Hz), 1.27 (br, 18H), 1.60 (br, 2H), 2.02 (s, 3H), 2.12 (br, 2H), 3.44 (br, 1H), 3.51–3.79 (m, 7H), 3.94 (br, 1H), 3.97 (t, 1H, J = 3.8 Hz), 4.08 (br, 1H), 4.12 (br, 3H); ^{13}C NMR (150 MHz, MeOH-d4) δ 173.8, 173.3, 78.9, 77.0, 76.9, 71.0, 69.9 (× 2), 65.1, 63.8, 57.8, 48.8, 32.0, 29.7 (× 4), 29.6, 29.4, 26.1, 24.2, 22.7, 21.7, 13.4 (× 2); HRMS calcd for $[C_{25}H_{49}N_2O_{10}P+H]^+$ 569.3203, found 569.3211.

Acknowledgements

This work was supported by National Science Council and Academic Sinica.

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