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# SYNTHESIS AND PROPERTIES OF 2-(NAPHTHOSULTAMYL)METHYL-CARBAPENEMS WITH POTENT ANTI-MRSA ACTIVITY: DISCOVERY OF L-786,392

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**Abstract**: A series of 1β-methyl-2-(naphthosultamyl)methyl-carbapenems bearing dicationic groups on the naphthosultamyl moiety was prepared and evaluated for activity against resistant gram-positive bacteria. Based on a combination of excellent in vitro antibacterial activity, acceptable mouse acute toxicity, and a desirable fragmentation pattern on β-lactam ring opening, the analog **2g** (L-786,392) was selected for extended evaluation.


## Introduction

The carbapenem antibiotics imipenem[1] and meropenem[2] are unrivaled with regard to breadth of spectrum and are often the drugs of choice for the treatment of severe, life-threatening infections. These agents, however, are noticeably deficient against clinically important isolates of methicillin-resistant staphylococci (MRS), *Enterococcus faecium*, and some penicillin-resistant pneumococci. The resistance of gram-positive cocci to carbapenems[3] and other β-lactam antibiotics is generally attributed to the production of new or modified target enzymes, the penicillin-binding proteins (PBPs), that exhibit extremely low binding affinity to β-lactams. More specifically, methicillin-resistant *Staphylococcus aureus* (MRSA) and methicillin-resistant coagulase-negative staphylococci (MRCNS) express a new, inducible, low-affinity PBP2a[4] that maintains peptidoglycan synthesis in the presence of β-lactams; and enterococcal resistance is associated with the overproduction of normal or modified forms of a low-affinity PBP5.[5] These resistant gram-positive pathogens, besides representing a threat to the clinical efficacy of carbapenem antibiotics, are a growing problem of worldwide concern[6] due to the acquisition of resistance mechanisms to most other therapeutic agents.

In recent years, a number of research groups have succeeded in using the intrinsic activity of the carbapenem nucleus for the design of potent, anti-MRSA agents. This has been accomplished by appending novel PBP2a-binding side-chains at the 2-position, notably aryl,[7] thiazolylthio,[8] benzothiazolylthio,[9] aminothiocarbonylthio,[10] and aryloxymethyl[11] groups. Unfortunately, no clinically useful agents have been reported from this effort to date. Several advanced candidates were identified in our laboratories and ultimately abandoned for reasons of immune-based toxicity.[12] Contemplation of the immunotoxicity problem led to the releasable hapten hypothesis,[13] which proposed that β-lactam ring-opening would be chemically linked to expulsion of the immunogenic, anti-MRSA pharmacophore. Exploration of this concept in a series of 2-(sulfonamido)methyl carbapenems resulted in the discovery of the novel 2-(naphthosultamyl)methyl analog **1**.[13] In this paper, we describe the further elaboration of compound **1** with the goal of identifying a safe, parenteral agent for the treatment of infections caused by resistant gram-positive pathogens.

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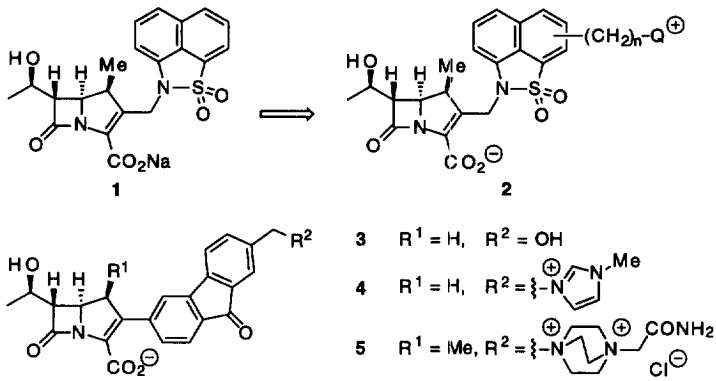
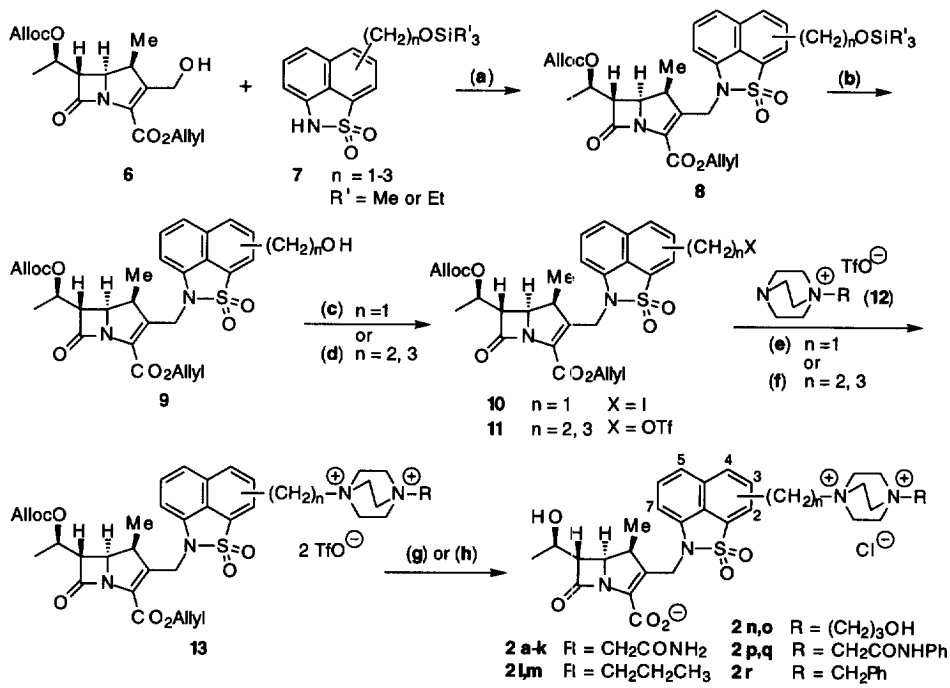



## Chemistry

The cationic substituted 2-(naphthosultamyl)methyl carbapenems **2** were targeted for synthesis based primarily on structure–activity relationships[14] developed during our 2-aryl carbapenem program. For example, progression in the 2-fluorenonyl carbapenem series from anionic analog **3** to the zwitterionic analog **4** resulted in lowered serum protein binding and concomitant improvement in in vivo activity. Further progression to the 1β-methyl, cationic zwitterion analog **5** resulted in improvements in water solubility, pharmacokinetics, and chemical stability. With these considerations in mind, emphasis was placed on preparing analogs of type **2** in which the distal substituent was a dicationic DABCO group (see Scheme 1).

**Scheme 1**




(a) DEAD or DIAD, $PPh_3$, THF, rt; (b) TfOH, THF-$H_2O$, rt; (c) MsCl, $Et_3N$, $CH_2Cl_2$, 0 °C then NaI, $Me_2CO$, rt; (d) $Tf_2O$, 2,6-lutidine, $CH_2Cl_2$, -20 °C; (e) AgOTf, MeCN, rt; (f) MeCN, rt; (g) $Pd(PPh_3)_4$, $PPh_3$, Bu-CHEt-$CO_2Na$, Bu-CHEt-$CO_2H$, DMF, 0 °C to rt; (h) $Pd(PPh_3)_4$, $PPh_3$, dimedone, $iPr_2NEt$, DMF, rt.

The Mitsunobu reaction[15] of the bis(allyl)-protected 2-hydroxymethyl carbapenem **6**[13] with a series of homologous silyloxyalkyl-1,8-naphthosultams **7**[16] produced the esters **8** in good yield. Removal of the silyl protection with catalytic triflic acid proceeded in nearly quantitative yield to give the alcohols **9**. The hydroxymethyl intermediates **9** were converted in two steps to the iodides **10**, which were reacted with substituted DABCO salts **12** in the presence of silver triflate to give esters **13**. Similarly, the hydroxyethyl and hydroxypropyl intermediates were converted into their triflate derivatives **11** and reacted with DABCO salts **12** to give **13**. Removal of the protecting groups was accomplished using the palladium catalyzed transallylation procedure of McCombie[17] or, preferably, by using dimedone[15,18] as the allyl scavenger. The cationic zwitterion products **2a–r** were purified by tandem ion-exchange and reversed-phase chromatography. Representative yields for the sequence **6** to **9**, **9** to **13**, and **13** to **2** were 65%, 62%, and 75% for **2c**, 57%, 77%, and 27% for **2f**, 58%, 86%, and 55% for **2d**, and 66%, 91% and 82% for **2g**.

**Table 1**. In Vitro Antibacterial Activity and DHP-I Susceptibility of Carbapenems **2a–k** (R = $CH_2CONH_2$)

| Compound | | MIC values (μg/mL)[a] | | | | | | DHP-I[b] |
|---|---|---|---|---|---|---|---|---|
| No. | Linker | MSSA (1) -/+ HSA Fr. V | MRSA (12) | MRCNS (5) | VREFs (1) | MDREFm (3) | PRSP (2) | Rate (x IPM) |
| **1** | | 0.016 / 0.25 | 0.7 | 3.5 | 8 | 25.4 | 0.25 | 0.99 |
| **2a** | 2-$CH_2$ | 0.016 / 0.031 | 2.5 | 4.0 | 16 | 20.2 | 0.71 | 0.01 |
| **2b** | 2-$(CH_2)_2$ | 0.016 / 0.016 | 0.9 | 1.2 | 8 | 10.1 | 0.35 | 0.00 |
| **2c** | 3-$CH_2$ | 0.016 / 0.031 | 2.0 | 3.0 | 16 | 8.0 | 0.50 | 0.21 |
| **2d** | 3-$(CH_2)_2$ | 0.016 / 0.016 | **0.5** | **0.6** | **8** | **3.2** | **0.17** | 0.75 |
| **2e** | 3-$(CH_2)_3$ | 0.016 / 0.031 | 2.2 | 4.0 | 16 | 6.4 | 0.50 | 1.25 |
| **2f** | 4-$CH_2$ | 0.031 / 0.031 | 1.0 | 1.5 | 16 | 8.0 | 0.50 | 0.74 |
| **2g** | 4-$(CH_2)_2$ | 0.016 / 0.031 | **0.7** | **1.0** | **8** | **4.0** | **0.17** | 1.77 |
| **2h** | 4-$(CH_2)_3$ | 0.016 / 0.031 | 2.0 | 3.5 | 8 | 4.0 | 0.25 | 2.64 |
| **2i** | 5-$CH_2$ | 0.031 / 0.125 | 4.2 | 12.1 | 32 | 32.0 | 1.0 | 0.81 |
| **2j** | 5-$(CH_2)_2$ | 0.062 / 0.062 | 8.0 | 12.1 | 32 | 16.0 | 1.0 | 0.74 |
| **2k** | 7-$(CH_2)_2$ | 32 / 64 | >64 | >64 | >64 | >64 | >64 | -- |
| Imipenem | | 0.016 / 0.016 | >40 | >64 | 8 | >64 | 0.25 | 1.00 |
| Vancomycin | | 1 / 1 | 0.9 | 1.7 | >64 | 20.2 | 0.35 | -- |

[a]See reference13, Table 2 for a description of the assay. The effect of 43 mg/mL of Human Serum Albumin, Fraction V (HSA Fr. V) on antimicrobial activity was assessed against MSSA strain MB 2985 (Smith isolate).

[b]Susceptibility to hog kidney dehydropeptidase-I (DHP-I) is expressed as a reaction rate normalized to the rate observed with imipenem (IPM); see Kropp, H.; Sundelof, J. G.; Hajdu, R.; Kahan, F. M. *Antimicrob. Agents Chemother.* **1982**, *22*, 62.

## Results and Discussion

The in vitro antibacterial activities and DHP-I susceptibilities of the carbamoylmethyl-DABCO substituted derivatives **2a–k** are presented in Table 1. The data reveal both profound and subtle influences of the length and location of the alkylene spacer on activity against the resistant gram-positive pathogens. First and foremost, substitution at positions 2, 3, and 4 of the naphthosultamyl moiety is clearly preferred over substitution at positions 5 and 7. The 5-substituted analogs **2i** and **2j** exhibit moderate to poor activity and the 7-substituted analog **2k** is inactive. By contrast, analogs **2a–h** range from highly potent (**2d** and **2g**) to moderately active (**2a**). With regard to alkylene length, the $CH_2CH_2$ spacer is generally preferred over $CH_2$ and $CH_2CH_2CH_2$,

although the distinction is not always clearcut. The activity of the 3-$CH_2CH_2$ analog **2d** is clearly superior to that of homologs **2c** and **2e** against all of the resistant bacteria. The 4-$CH_2CH_2$ analog **2g** is slightly more active than **2f** and approximately 3-fold more active than **2h** against MRS, and twice as active as **2f** and equipotent to **2h** against the resistant enterococci. The best compounds in terms of overall potency are analogs **2d** and **2g**, which are nearly equivalent. Both of these compounds are superior to the parent naphthosultam **1** and vancomycin, especially in terms of enterococcal activity. A comparison among **1**, **2d** and **2g** also reveals that addition of the dicationic group is most effective at enhancing activity against MRCNS and resistant *E. faecium*.

The location of the cationic substituent also had a dramatic effect on DHP-I susceptibility. The hydrolysis rate increases as the charged group is moved from position 2 to 3 to 5 to 4, which roughly correlates with the distance separating the cationic center from the carbapenem nucleus. This trend is further reflected in the tether lengths at the 3- and 4-positions, where susceptibility increases as the alkylene chain becomes longer. A comparison of the two most potent compounds shows that analog **2g** is more than two times as susceptible to DHP-I mediated hydrolysis as analog **2d**.

The effect of added human serum albumin (HSA Fr. V) on the in vitro activity of a methicillin-sensitive *S. aureus* (MSSA) isolate was used as an indicator for serum protein binding. As shown in Table 1, the parent naphthosultam is 16-fold less active in the presence of HSA whereas all cationic substituted analogs, except for **2i**, show a maximal twofold reduction in activity. The data suggest that the in vivo efficacy of the cationic derivatives will not be adversely affected by serum protein binding.

**Table 2.** In Vitro Antibacterial Activity and DHP-I Susceptibility of Carbapenems **2l–r**

| Compound | | | MIC values (μg/mL) | | | | | DHP-I |
|---|---|---|---|---|---|---|---|---|
| No. | Linker | R | MRSA (12) | MRCNS (5) | VREFs (1) | MDREFm (3) | PRSP (2) | Rate (x IPM) |
| **2l** | 3-$(CH_2)_2$ | $CH_2CH_2CH_3$ | 1.0 | 1.5 | 8 | 3.2 | 0.24 | -- |
| **2m** | 4-$(CH_2)_2$ | $CH_2CH_2CH_3$ | 2.2 | 4.0 | 16 | 6.4 | 0.25 | 1.93 |
| **2n** | 3-$(CH_2)_2$ | $(CH_2)_3OH$ | 1.1 | 1.5 | 8 | 4.0 | 0.17 | 0.85 |
| **2o** | 4-$(CH_2)_2$ | $(CH_2)_3OH$ | 0.6 | 0.9 | 16 | 6.4 | 0.50 | 1.90 |
| **2p** | 3-$(CH_2)_2$ | $CH_2CONHPh$ | 0.2 | 0.3 | 8 | 3.2 | 0.17 | 0.64 |
| **2q** | 4-$(CH_2)_2$ | $CH_2CONHPh$ | 0.8 | 1.5 | 4 | 4.0 | 0.12 | 1.40[a] |
| **2r** | 4-$(CH_2)_2$ | $CH_2Ph$ | 0.9 | 1.3 | 8 | 6.4 | 0.35 | 1.85 |

[a]Due to limited water solubility, the DHP-I susceptibility of this compound was determined in 10% aqueous DMSO.

Based on the SAR uncovered in the **2a–k** series, the 3-$CH_2CH_2$ and 4-$CH_2CH_2$ substituted naphthosultams were examined in greater detail with respect to the terminal DABCO substituent. The antibacterial activities and DHP-I susceptibilities of representative modifications are presented in Table 2. The data, while revealing no specific patterns, does indicate that MRS activity (~12-fold variation) is more significantly affected than enterococcal activity (~twofold variation) by changes in the distal substituent. The lipophilic *N*-phenylcarbamoylmethyl analog **2p**, the most active compound tested, is two to threefold more potent against MRS and equivalent in enterococcal activity when compared to the carbamoylmethyl analogs **2d** and **2g**.

Several of the compounds presented in Tables 1 and 2 were evaluated for their ability to bind to a soluble form of PBP2a. Analogs **2g**, **2o**, **2q** and **2r** exhibited $IC_{50}$ values of 0.9, 1.3, 2.5 and 2.1 μg/mL in a competition assay[19] with [$^3$H]-benzylpenicillin, whereas imipenem had an $IC_{50}$ value of 188 μg/mL. The finding that PBP2a binding affinities approach the MIC values for the MRS-active analogs confirms that PBP2a is a primary target for this class of compounds.

An acceptable level of mouse toxicity was a significant limiting factor in our selection process for advancing compounds for extended in vitro and in vivo evaluation. Acute toxicity data for several of the more active compounds are presented in Table 3. In the series of analogs bearing a terminal $CH_2CONH_2$ group (**2b**, **2d**, **2f**, **2g**, **2h**), the lowest toxicity occurred with the 4-$CH_2CH_2$ analog **2g**. A comparison of the $(CH_2)_3OH$ substituted analogs **2n** and **2o** also revealed that the 4-$CH_2CH_2$ positional isomer was less toxic. Incorporation of more lipophilic terminal subtituents, such as $CH_2CONHPh$ (**2p**, **2q**) and $CH_2Ph$ (**2r**), afforded compounds with the highest levels of toxicity regardless of the location of the cationic group.

**Table 3.** Mouse Acute Toxicity of Selected (Naphthosultamyl)methyl-Carbapenems

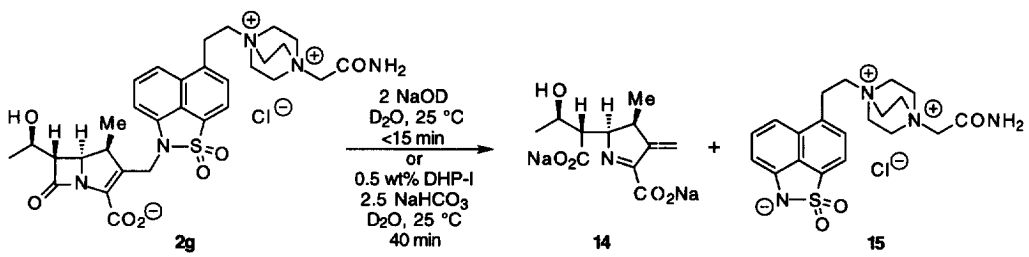
| | Dose (mg/kg)[a] | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | **2b** | **2d** | **2f** | **2g** | **2h** | **2n** | **2o** | **2p** | **2q** | **2r** |
| Lethal | 63 | 500 | 500 | **>500** | 500 | 500 | **>500** | 245[b] | 63[b] | 250 |
| NoEL | 16 | 125 | 250 | **250** | 125 | 125 | **250** | <61[b] | 31[b] | 63 |

[a]The test compound in 0.01M pH 7.1 MOPS buffer was administered via the tail vein to 20 g female mice at an upper dose of 500 mg/kg and serially diluted thereafter to determine the NoEL. The lethal dose is defined as the lowest dose resulting in the death of a single animal and the NoEL is the dose showing no visible effects (ataxia, somnolence) in two animals. All animal procedures were performed in accordance with the highest standards for the humane handling, care and treatment of research animals and were preapproved by the Merck Institutional Animal Care and Use Committee.

[b]Due to limited water solubility, this compound was dosed IP as a suspension in 5:95 DMSO:MOPS buffer.

Based on a favorable combination of in vitro antibacterial activity and acceptable mouse acute toxicity, the analog **2g** was selected for further evaluation. To insure that this compound exhibited a chemical fragmentation pattern consistent with the releasable hapten hypothesis,[13] its chemical and enzymatic hydrolysis was examined by $^1H$ NMR methods (see Scheme 2). Titration of **2g** with 2 equiv of NaOD in $D_2O$ resulted in rapid β-lactam ring opening accompanied by quantitative expulsion of the side chain to afford a mixture of pyrroline **14** and naphthosultam **15**. Similarly, exposure of **2g** to 0.5 wt% of purified porcine DHP-I in $D_2O$ buffered with $NaHCO_3$ resulted in first-order hydrolysis ($t_{1/2}$ ~10 min) to give exclusively **14** and **15**. A ring-opened, nonfragmented intermediate was not observed in either experiment. From these results, we predict that compound **2g** should not suffer from the immunotoxicity that plagued our previous candidates.

**Scheme 2**




## Summary

A series of 1β-methyl-2-(naphthosultamyl)methyl-carbapenems bearing dicationic, DABCO-based substituents on the naphthosultamyl residue were prepared and evaluated for in vitro antibacterial activity, DHP-I susceptibility, PBP2a binding, and mouse acute toxicity. The analog **2g** (L-786,392) was selected for extended evaluation based primarily on a favorable combination of antibacterial activity and acceptable mouse acute toxicity, and was shown to exhibit a desirable fragmentation pattern on β-lactam ring opening. Concerns regarding the

DHP-I susceptibility of this compound were allayed by chimpanzee pharmacokinetic studies[20] that predict twice-daily dosing and adequate urinary levels in humans. Detailed reports on the in vitro and in vivo properties of L-786,392 will be the subject of future communications from these laboratories.[21]

**Acknowledgment**: We thank Joe Leone for large-scale preparations of carbapenem **6** and its ylide precursor.

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